Determination of Absorption and Endogenous Excretion of Iron in Man by Monitoring Fecal Excretion of a Stable Iron Isotope ($^{58}$Fe)

Fumio HASHIMOTO, Yasuhiro FUJII, Masamichi TOBA, Hiroshi OKAMATSU, and Hideaki KOHRI

Saga Research Institute, Otsuka Pharmaceutical Co., Ltd., Higashisefuri-mura, Kanzaki-gun, Saga 842-01, Japan

(Received July 2, 1990)

Summary The absorption and endogenous excretion of iron in man was studied by monitoring the fecal excretion of a stable iron isotope ($^{58}$Fe). The study was carried out for 12 healthy volunteers who were divided into two groups. Group I received $^{58}$Fe-labeled ferric ammonium citrate (III) ($^{58}$FeAC) equivalent to 6 mg of iron as a control, and group II received a combination of 500 mg of vitamin C and $^{58}$FeAC. A new formula was used to calculate the $^{58}$Fe absorption ratio reflecting the pool of iron in the intestinal cells, and the ratio was compared with that obtained from Janghorbani's formula, which has been used as one of the common methods. As a result, the $^{58}$Fe absorption ratio in group II was statistically significantly higher than that of group I (34.4±6.1% vs. 15.0±5.5%, M±SD) using Janghorbani's formula. The similar absorption ratio (34.1±6.0% vs. 14.8±5.5%) was also obtained by our new formula. Our results confirmed the previous findings that the availability of iron is stimulated by the supplementation of vitamin C. Both formulae agreed in the absorption of iron, indicating that the endogenous excretion of iron (caused by the desquamated cells) in the intestine does not disguise the iron absorption.

Key Words iron absorption, stable iron isotope, ferric ammonium citrate, vitamin C

The use of stable isotopes in mineral nutrition research is considered to be an important and valuable tool from the point of view that they have no exposure to radiation and no decay over time (1). Since 1963, the availability and metabolism of iron in man have been thoroughly studied through the standard method of fecal monitoring using $^{58}$Fe (2–7). To date, there have been some calculation methods to determine the iron absorption (2, 7). Estimating iron absorption using the formula developed by Janghorbani and his co-workers has become one of the common methods (2). However, this method does not consider the interaction of food with
stable iron in the intestine. Thus, it has been pointed out that this method may not yield valid data on the absorption of dietary iron from food intake unless the foods were intrinsically labeled with $^{56}$Fe (2, 7). In these reports, the possibility of endogenous excretion of iron caused by the desquamation of intestinal cells and by the bile released from the gall bladder is not considered. Therefore, it is important to determine the level of iron excreted in the gastrointestinal tract in order to evaluate the net absorption of iron in the intestine. In the present study, we attempted to resolve the above problems possibly occurring in previous methods, and our new formula in Materials and Methods are described in detail.

Vitamin C is well-known as a nutrient that enhances the absorption of iron from food fortified with iron in such forms as FeAC (8), ferrous sulfate (9), ferric orthophosphate (5, 10), and heme-iron (11, 12). Cook and Monsen tried to identify the relationship between iron absorption and the dietary level of vitamin C supplementation using a meal labeled with both $^{55}$Fe and $^{59}$Fe. They found a good correspondence between the absorption of iron and the addition of vitamin C; the higher the vitamin C content in food, the higher the iron absorption (13).

We have previously demonstrated that the supplementation of vitamin C improves iron-deficiency anemia in female college students who took 6 mg of iron with the simultaneous supplementation of 500 mg vitamin C (14). Therefore, 6 mg of iron was thought to be enough to meet the guideline level of the Ministry of Health and Welfare in Japan, which recommends a daily intake of 12 mg iron for premenopause women (15). In the present study, we describe the availability of iron supplements given simultaneously with vitamin C by estimating the efficacy of FeAC. The iron absorption calculated by our formula showed good agreement with that estimated by Janghorbani's formula.

MATERIALS AND METHODS

Preparation of $^{56}$FeAC for administration. $^{56}$Fe-enriched salt was purchased as a powder of Fe$_2$O$_3$ (218.5 mg) from Oak Ridge National Laboratory (Oak Ridge, TN) and was dissolved in 6 N HCl (20 ml) by heating. The isotope composition of the metal Fe was $^{54}$Fe, 0.39; $^{56}$Fe, 13.78; $^{57}$Fe, 1.25; and $^{58}$Fe, 84.58 (atomic %). A solution of FeCl$_3$·6H$_2$O (525.8 mg) was added to the enriched $^{56}$Fe solution, and H$_2$O was added to a total amount of 1.6 l. A 10% NH$_4$HCO$_3$ solution (50 ml) was added stepwise at 50–60°C to the acidic mixture, and the ferric hydroxide precipitates were collected and washed extensively with 0.25% NH$_4$HCO$_3$ solution (21) until the solution indicated neutrality. The resultant precipitates were then dissolved in a solution of citric acid (1244 mg) in H$_2$O (150 ml), followed by the addition of 0.5 N aqueous ammonium solution (19.9 ml). The reaction mixture was concentrated by evaporation under reduced pressure to yield a pale yellow amorphous powder (1.624 g). The elemental constituents of the final product were measured as C (25.75%), H (4.56%), and N (7.13%) by elemental analysis (Central Analysis Room of Kyushu University, Fukuoka, Japan), and as Fe

J. Nutr. Sci. Vitaminol.
(15.45%) by flame atomic absorption spectrophotometry (FAA) (AA-845 Atomic Absorption & Flame Emission Spectrophotometer, Nippon Jarrell-Ash Co., Ltd., Kyoto, Japan). The product obtained is nearly identical with commercial FeAC, the composition of which is 25.25% C, 4.06% H, 6.83% N, and 15.60% Fe. The quantitative analysis of stable iron isotope ($^{58}$Fe) was carried out by neutron activation analysis (NAA).

Subjects and experimental design. Eleven men and one woman in the Tokushima and Saga Research Institute of Otsuka Pharmaceutical Co., Ltd. who were apparently healthy adults volunteered to participate in this study. Informed consent was obtained from all volunteers, and this study was approved by the Human Ethical Committee, Otsuka Pharmaceutical Co., Ltd. The subjects were divided into two groups as shown in Table 1, based on four parameters of iron status: serum iron (SI), transferrin (Tf), total iron binding capacity (TIBC) and serum ferritin (FER). During the study, each subject consumed the same amount and the same kind of diets, and they were neither permitted to play any sports nor to take any extra foods or beverages other than water. The foods they ingested were recorded and subjected to measurement for nutritional value. Each meal was served in the Tokushima Laboratory at 8:00, 12:00 and 18:00 every day from day 1 to day 10 with one exception: breakfast on day 4 was skipped in order to take $^{58}$FeAC preparation, which was administered at 10:00. Each subject in group I received the iron tablet containing $^{58}$FeAC equivalent to 6 mg of total iron, and each one in group II received an additional supplementation of 500 mg vitamin C with the iron tablet. The tablets were dissolved in H$_2$O (150 ml) and were given to each subject on day 4 as mentioned above. In the present study, the administration of $^{58}$Fe with and without vitamin C did not affect the other biochemical parameters such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), albumin (ALB) and triglyceride (TG) (data not shown). Carmine red dye (0.5 g) was given as a marker to all subjects at 12:00 on day 2 for starting and at 7:30 on day 9 for ending fecal collection.

Analytical methods and NAA. Feces were collected in plastic boxes, frozen and stored at $-30^\circ$C prior to analysis. They were dried in an oven at 110°C for 2 days. All samples were ground finely and divided into 200–300 mg groups, and then reduced to ash in silica crucibles at 600°C for 20 h (Electric Muffle Furnace; Operusor, Toyo Seisakusho, Co., Ltd., Chiba, Japan). The total amount of iron in these dried samples was analyzed by FAA. Additionally, 30–90 mg of dried sample seamlessly enclosed in a polyethylene irradiation pouch was subjected to NAA to calculate the total amount of $^{58}$Fe. The NAA was carried out at Kyushu Environmental Evaluation Association (Fukuoka, Japan). The conditions used for NAA are shown in Table 2. To evaluate the iron status, blood was drawn into tubes with EDTA at 8:00 on day 1 for a pre-treatment value, and at 11:00, 12:00, 14:00, 18:00 on day 4 and 1 week after administration of each preparation. Serum iron (SI) and other biochemical parameters were determined by automatic analyzer (Olympus AU 550), and concentrations of ferritin (FER), transferrin (Tf) and total iron
Table 1. Details of physical characteristics and iron parameters of each subject at the beginning of the study.$^1$

| Groups | Subjects | Sex | Age (yr) | Height (cm) | Body weight (kg) | Iron parameters$^2$ |   |   |   |   |
|--------|----------|-----|----------|-------------|------------------|----------------------|---|---|---|---|
|        |          |     |          |             |                  | SI (µg/100 ml)       | FER (ng/ml) | TIBC (µg/100 ml) | Tf (mg/100 ml) |
| I      | KM       | M   | 20       | 175         | 55               | 96                   | 14.0        | 350.6         | 346           |
|        | MO       | M   | 28       | 175         | 72               | 138                  | 26.2        | 377.3         | 394           |
|        | KY       | M   | 28       | 170         | 59               | 71                   | 107.7       | 342.4         | 300           |
|        | MI       | F   | 24       | 153         | 45               | 127                  | 32.5        | 269.0         | 257           |
|        | HI       | M   | 40       | 164         | 65               | 76                   | 66.0        | 296.7         | 285           |
|        | FH       | M   | 28       | 173         | 82               | 114                  | 256.5       | 251.0         | 257           |
|        | Mean ± SD|     | 28 ± 7   | 168 ± 9     | 63 ± 13          | 104 ± 27             | 83.8 ± 91.1 | 314.5 ± 49.9 | 307 ± 54     |
| II     | HI       | M   | 45       | 163         | 61               | 165                  | 54.8        | 316.5         | 330           |
|        | TT       | M   | 33       | 174         | 80               | 111                  | 90.9        | 371.9         | 378           |
|        | KA       | M   | 24       | 165         | 53               | 93                   | 13.2        | 350.7         | 378           |
|        | HH       | M   | 36       | 170         | 58               | 121                  | 87.9        | 288.7         | 300           |
|        | KY       | M   | 33       | 170         | 65               | 65                   | 25.0        | 318.5         | 315           |
|        | TN       | M   | 25       | 172         | 61               | 107                  | 82.1        | 282.6         | 300           |
|        | Mean ± SD|     | 33 ± 8   | 169 ± 4     | 63 ± 9           | 110 ± 33             | 59.0 ± 33.6 | 321.5 ± 34.7 | 334 ± 36     |

$^1$The measurements were carried out 1 week before the study. $^2$SI, serum iron; FER, ferritin; TIBC, total iron binding capacity; Tf, transferrin.
Table 2. Condition of NAA.1

| Standard | NIES No. 1 (Leaves of Clethra herbinervis) |
|----------|------------------------------------------|
| Nuclear reactor | Japan Atomic Laboratory |
| Neutron flux (n/cm²·s) | $8 \times 10^{13}$ |
| Irradiation time (min) | 10 |
| γ spectrometry system | Ge(Li) semiconductor detector |
| Period of decay (week) | 3-4 |
| Peak area (keV) | 1,099 |
| Measuring time (s) | 10,000 |
| Method | Total peak area |

1 NAA was carried out at Kyushu Environmental Evaluation Association, Fukuoka, Japan.

binding capacity (TIBC) were immunochemically analyzed (16). The measurement of the dietary intake of iron from the foods ingested by volunteers was carried out at Japan Food Research Laboratory (Osaka, Japan).

Calculation of iron absorption. In the present study, the ratios of $^{56}\text{Fe}$ and mass iron absorption were calculated by the following formulae [1] and [2], respectively, where $A$ (50.77 mg) corresponds to the total amount of iron obtained from the diet (mg), $B$ the total amount of iron identified in feces (mg), $k$ (=0.0028) the mass natural abundance of isotopic iron ratio, $X$ the total amount of endogenously excreted iron (mg), $C$ (=6.04 mg) and $D$ (=3.28 mg) the total amount of iron and $^{56}\text{Fe}$ content in the $^{56}\text{FeAC}$ preparation, respectively, and $E$ the total amount of $^{56}\text{Fe}$ content in the fecal collection (mg).

Iron absorption from stable isotope:

$$\frac{k(A+X)+D-E}{k(A+X)+D} \times 100\%$$  \[1\]

Mass iron absorption from labeled and unlabeled:

$$\frac{A+X+C-B}{A+X+C} \times 100\%$$  \[2\]

From these formulae [1] and [2], the total iron absorption ratio which does not include the $^{56}\text{Fe}$ absorption ratio is obtained with formula [3], and the iron absorption ratio obtained from these three formulae have a relationship as shown in equation [4]. Solution of this equation was attempted and led to the simple quadratic equation [5], which showed the answer $A+X$ in the equation [6], where the contents of $a$, $b$ and $c$ were shown as the formulae [7-9].

Iron absorption from unlabeled:

$$\frac{(1-k)(A+X)+C-D-(B-E)}{(1-k)(A+X)+C-D} \times 100\%$$  \[3\]

$$[2] \times (A+X+C-B) = [1] \times \{k(A+X)+D-E\}$$

Vol. 38, No. 5, 1992
The total amount of iron absorbed from the $^{58}$FeAC preparation (indicated as F) was calculated by the following formula [10]. This formula was used over the period of fecal collection of $^{58}$FeAC orally administered until the stable isotope content decreased to less than 1% of the natural isotopic ratio (Table 3).

$$F = \frac{C}{D} \times \{k(A+X)+D-E\}$$ [10]

The net iron absorption ratio from the whole diet was calculated by the formula [11], where G corresponds to the total amount of iron absorbed from the whole diets including the endogenously excreted iron as shown in the formula [12] (mg). (C−F) corresponds to the unabsorbed iron of the $^{58}$FeAC preparation, thus \{B−(C−F)\} shows the net unabsorbed iron from the whole diet.

$$G = A+X - \{B-(C-F)\}$$ [12]

The formula of Janghorbani et al. (2) was also used in order to compare with ours.

**Statistics.** Statistical analysis was done by a paired t-test using the difference between the pre-treatment and post-treatment values and by Student's t-test between the numbers obtained in each group as described in the figures and tables.

**RESULTS**

An example of Fe and $^{58}$Fe contents obtained from subject KM is shown in Table 3. The cumulative amount of iron and of $^{58}$Fe excreted was calculated in two different ways during overlapping periods, a period between two markers of carmine red dye and a period of $^{58}$Fe administration. As we described in the methods, 10 day fecal collection overestimated the iron accumulation because of endogenous excretion. Therefore, we calculated the iron and $^{58}$Fe absorption from 2-4 day fecal collection based on the abundance of the isotopic ratio (less than 1%). The cumulative sum of the daily amount of $^{58}$Fe and of the daily amount of
Table 3. Fecal collection in subject KM.

| Time (day) | Between two red markers | Period of $^{58}$Fe administration |
|------------|--------------------------|-------------------------------------|
|            | Fe¹ (mg) | $^{58}$Fe² (µg) | Abundance (%) | Fe¹ (mg) | $^{58}$Fe² (µg) |
| 1          | 4.16     | 11               | 0.27          |          |                  |
| 2          | 4.89     | 28               | 0.57          |          |                  |
| 3          | 10.42    | 2,706            | 25.98         | 10.42    | 2,706            |
| 4          | 1.64     | 66               | 4.02          | 1.64     | 66               |
| 5          | 5.02     | 26               | 0.52          |          |                  |
| 6          | 6.40     | 26               | 0.40          |          |                  |
| 7          | 2.16     | 9                | 0.43          |          |                  |
| 8          |          |                  |              |          |                  |
| 9          | 9.54     | 26               | 0.27          |          |                  |
| 10         | 2.32     | 6                | 0.25          |          |                  |
|            | 3.06     | 12               | 0.40          |          |                  |
| Total      | 49.61    | 2,917            | 12.06         | 2,772    |

¹ The analysis of the amount of iron in each feces by atomic absorption spectrophotometer was carried out in duplicate. ² The $^{58}$Fe concentration in each feces was measured by NAA.

iron over a period between two red markers was also indicated. The data on each subject were obtained by the same accumulation as shown in Table 4. The sum of the daily amount of $^{58}$Fe excreted in feces in group I in days 2–4 and 10 days of fecal collection were statistically higher than those found in group II (3,025 µg and 2,846 µg vs. 2,433 µg and 2,216 µg, respectively). On the other hand, the total excreted amount of iron in feces over the period between two red markers was found to be significantly different (60.46 mg vs. 53.03 mg), while the total excreted amount of iron in feces found in the period of $^{58}$Fe administration in both groups showed no significant difference between the groups (20.44 mg vs. 22.23 mg).

Every subject in each group was supplemented with 6.04 mg (=C) of iron as a $^{58}$FeAC preparation, in which 3.28 mg of $^{58}$Fe (=D) was measured by NAA. The percent ratio of $^{58}$Fe/total Fe in the $^{58}$FeAC preparation was calculated as 54.3%. The daily intake of iron and vitamin C from the diet in this study was measured and summarized in Table 5, together with the other nutrients, showing that the total iron intake in relation to the fecal collection during the experimental period was calculated as 50.77 mg (=A). The values of the total excreted amount of $^{58}$Fe and iron obtained over the period of $^{58}$Fe administration (Table 4) were applied in formula [1] and Janghorbani's formula (2) for all subjects. Comparisons of the $^{58}$Fe absorption ratio calculated by two different formulae are shown in Table 6; the similar values were obtained in each group. Moreover, the values found in the
Table 4. Cumulative sum of the daily amount of iron and $^{58}$Fe identified in feces in each subject.¹

| Groups | Subjects | Between two red markers | Period of $^{58}$Fe administration |
|--------|----------|-------------------------|-----------------------------------|
|        |          | Total iron (mg) | Total $^{58}$Fe ($\mu$g) | Total iron (mg) | Total $^{58}$Fe ($\mu$g) |
| I      | KM       | 49.60        | 2,917                      | 12.06           | 2,772                      |
|        | MO       | 67.45        | 2,991                      | 25.52           | 2,865                      |
|        | KY       | 77.23        | 2,917                      | 18.33           | 2,684                      |
|        | MI       | 53.59        | 2,873                      | 31.08           | 2,731                      |
|        | HI       | 55.89        | 3,055                      | 18.00           | 2,852                      |
|        | FH       | 59.02        | 3,396                      | 17.67           | 3,174                      |
|        | Mean±SD  | 60.46±10.18² | 3,025±193³                | 20.44±6.74      | 2,846±175³                |
| II     | HI       | 48.83        | 2,067                      | 19.50           | 1,920                      |
|        | TT       | 52.13        | 2,724                      | 23.34           | 2,538                      |
|        | KA       | 62.54        | 2,469                      | 24.90           | 2,162                      |
|        | HH       | 50.59        | 2,377                      | 25.51           | 2,161                      |
|        | KY       | 54.44        | 2,411                      | 14.37           | 2,186                      |
|        | TN       | 49.75        | 2,552                      | 25.74           | 2,326                      |
|        | Mean±SD  | 53.03±5.02²  | 2,433±218                  | 22.23±4.49      | 2,216±205                  |

¹Data are obtained by calculating the sum of the amount of iron and $^{58}$Fe in each feces by the same way as shown in Table 3. ²Different from corresponding group II at $p<0.05$. ³Different from corresponding group II at $p<0.001$.

Table 5. Daily nutrient intake from the diets.¹

| Day | Fe (mg) | Vitamin C (mg) | Weight (g) | Energy (kcal) | Water (%) | Protein (g) | Ash (g) |
|-----|---------|----------------|------------|---------------|-----------|-------------|---------|
| 1   | 8.0179  | 128.3          | 2,141.1    | 2,672.9       | 73.0      | 109.6       | 19.9    |
| 2   | 6.6852  | 86.0           | 1,873.3    | 2,104.3       | 76.0      | 90.6        | 14.5    |
| 3   | 8.7823  | 88.6           | 1,948.5    | 2,743.7       | 70.5      | 109.2       | 23.4    |
| 4   | 4.8021  | 51.5           | 1,507.3    | 1,665.1       | 73.3      | 88.2        | 13.7    |
| 5   | 7.5374  | 143.5          | 1,976.1    | 2,279.0       | 75.0      | 90.3        | 13.5    |
| 6   | 6.7173  | 116.8          | 1,972.5    | 2,362.2       | 71.9      | 104.6       | 12.2    |
| 7   | 6.8456  | 98.3           | 2,078.7    | 2,118.1       | 77.6      | 85.6        | 16.2    |
| 8   | 5.4226  | 97.5           | 2,266.7    | 2,222.2       | 77.3      | 93.0        | 17.5    |
| 9   | 4.4114  | 132.8          | 2,127.4    | 2,214.1       | 65.0      | 80.8        | 17.7    |
| 10  | 4.9927  | 98.3           | 2,135.1    | 2,188.9       | 75.6      | 88.9        | 19.3    |

¹Each subject was supplied the same contents of the diets. ²The measurement of the ingredients was carried out at Japan Food Research Laboratory, Osaka, Japan.

vitamin C-supplemented group were significantly higher than those found in the non vitamin C-supplemented group in both formulae. The total absorbed amount of iron from the $^{58}$FeAC preparation calculated by formula [10] and Janghorbani's
Table 6. Comparison of the $^{58}$Fe absorption ratio.\(^1\)

| Group I | Group II |
|---------|---------|
| (%)\(^2\) | (%)\(^3\) | (%)\(^2\) | (%)\(^3\) |
| KM      | 16.4    | 16.8    | HI      | 43.0    | 43.4 |
| MO      | 15.2    | 15.3    | TT      | 24.8    | 25.0 |
| KY      | 19.8    | 20.1    | KA      | 36.2    | 36.6 |
| MI      | 19.9    | 19.9    | HH      | 36.3    | 36.7 |
| HI      | 14.7    | 14.9    | KY      | 34.5    | 34.8 |
| FH      | 4.9     | 5.1     | TN      | 31.4    | 31.7 |
| Mean±SD | 15.2±5.5 | 15.4±5.5 | Mean±SD | 34.4±6.0\(^4\) | 34.7±6.1\(^4\) |

\(^1\)The values are obtained in the period of $^{58}$Fe administration. \(^2\)The values are obtained by substituting the data for the formula [1]. \(^3\)The values are obtained by substituting the data for the formula of reference 2. \(^4\)Different from corresponding group I at $p<0.001$.

Table 7. Comparison of the total absorbed amount of iron from $^{58}$FeAC preparation.\(^1\)

| Group I | Group II |
|---------|---------|
| Formula [10] (mg)\(^2\) | Janghobani’s formula (mg)\(^3\) | Formula [10] (mg)\(^2\) | Janghobani’s formula (mg)\(^3\) |
| KM      | 1.02    | 1.00    | HI      | 2.62    | 2.67 |
| MO      | 0.92    | 0.95    | TT      | 1.51    | 1.54 |
| KY      | 1.22    | 1.22    | KA      | 2.21    | 2.26 |
| MI      | 1.20    | 1.25    | HH      | 2.22    | 2.27 |
| HI      | 0.90    | 0.91    | KY      | 2.10    | 2.12 |
| FH      | 0.31    | 0.30    | TN      | 1.92    | 1.96 |
| Mean±SD | 0.93±0.33 | 0.94±0.34 | Mean±SD | 2.10±0.37\(^4\) | 2.14±0.37\(^4\) |

\(^1\)The values are obtained in the period of $^{58}$Fe administration. \(^2\)The values are obtained by substituting the data for the formula [10]. \(^3\)The values are obtained by substituting the data for Janghobani’s formula. \(^4\)Different from corresponding group I at $p<0.001$.

The iron absorption ratio from the diets, the total absorbed amount of iron and the endogenous excretion of iron in each subject as calculated by formulae [11], [12] and [6], respectively, are summarized in Table 8. Although we found a significant difference in the iron absorption ratio (28.8±1.0% vs. 32.2±1.6%) from the diets including the endogenously excreted iron when we calculated the 10 day fecal collection, the numbers in both groups were quite similar. This suggests
Table 8. Iron absorption ratio, the total absorbed amount of iron from the diets, and the endogenous excretion of iron in each subject.1

|         | Group I (%) | Absorbed (mg) | Excreted (mg) | Group II (%) | Absorbed (mg) | Excreted (mg) |
|---------|-------------|---------------|---------------|-------------|---------------|---------------|
| KM      | 29.6        | 18.70         | 12.49         | HI          | 34.5          | 23.90         | 18.58         |
| MO      | 27.9        | 24.21         | 35.85         | TT          | 30.1          | 20.49         | 17.34         |
| KY      | 27.5        | 27.50         | 49.14         | KA          | 30.4          | 25.59         | 33.49         |
| MI      | 29.3        | 20.20         | 18.22         | HH          | 32.0          | 22.02         | 18.08         |
| HI      | 28.3        | 20.05         | 20.03         | KY          | 31.6          | 23.37         | 23.11         |
| FH      | 26.8        | 19.46         | 21.97         | TN          | 31.0          | 20.53         | 15.43         |
| Mean    | 28.2        | 21.69         | 26.28         | Mean        | 31.65         | 22.65         | 21.01         |
| ±SD     | ±1.1        | ±3.43         | ±13.62        | ±SD         | ±1.6           | ±2.02         | ±6.62         |

1 The values are obtained during the 10 d's experiment. 2 The values are obtained from the formula [11]. 3 The values are obtained from the formula [12]. 4 The values are obtained from the equation [6]. 5 Different from corresponding group I at p<0.001. 6 Not significant from corresponding group I.

that one dose of vitamin C increases the iron absorption from the diets. However, no difference between the total absorbed and excreted amount of iron was found between the groups (22.30mg and 26.87mg vs. 23.34mg and 21.69mg, respectively). The daily absorption of approx. 2mg of iron and the excretion of approx. 2mg of iron per day were then observed during the 10 day experimental period. However, the individual values of the total absorbed and excreted amount of iron showed a fluctuation.

The change in serum iron concentration in each group is illustrated in Fig. 1. Comparing the pre-treatment and post-treatment values along with the time course in each respective group, a significantly higher difference at 4h after 58FeAC administration was obtained. When the appearance of iron in the blood was compared between the groups, no significant difference was found.

DISCUSSION

The observation that the fecal excretion of iron by four subjects (MO, KY, FH and KA, Table 4) was greater than that orally ingested iron (50.77mg from diet and 6.04mg from the iron preparation; total iron intake is 56.81mg) suggests individual variation in iron metabolism. Since these subjects did not suffer from iron-deficiency anemia (Table 1), there seems to be a daily fluctuation in iron absorption. The possibility of excretion of endogenous iron caused by the desquamation of intestinal cells and by the bile released from the gall bladder should be considered as this leads to evaluate the net absorption of iron in the intestine. Thus, the formulae [1-12] appear to calculate the absorption and excretion of endoge-
ABSORPTION AND ENDOGENOUS EXCRETION OF IRON

Fig. 1. Change in venous serum iron concentration after administration of $^{58}$FeAC. Group I, ($\circ\cdots\circ$); group II, ($\bullet\cdots\bullet$). Values are mean $\pm$ SEM of six subjects per group. Significant differences are indicated at *$p<0.05$ and **$p<0.01$ when each value is compared with the respective pre-treatment value.

Endogenous iron from $^{58}$FeAC preparation and diets.

The total iron (labeled and unlabeled), $^{58}$Fe and the unlabeled iron of the $^{58}$FeAC preparation are absorbed similarly and simultaneously in the matter of the amount and timing from the intestinal mucosa, indicating that the ratios from the $^{58}$FeAC preparation obtained with the formulae [1] ($^{58}$Fe absorption), [2] (mass iron absorption) and [3] (the unlabeled iron absorption) shall become the same percentage. Thus, the three formulae form an equation [1] = [2] = [3] by which the answer $X'$ results from all conjunctions as shown in formula [13]; where $X'$ corresponds to the total amount of apparent excretion of endogenous iron.

$$X' = \frac{B(kA+D)-E(A+C)}{E-kB}$$  \[13\]

The answer $X'$ was substituted for each formula and then the same absorption ratio was obtained as formula [14].

$$\left\{1 - \frac{E-kB}{D-kC}\right\} \times 100(\%)$$  \[14\]

In Fig. 2, the data obtained over the period of $^{58}$Fe administration were substituted into the formulae [1, 14] and Janghorbani's formula (2). As a result, the values obtained by each formula predictively gave the similar values since the labeled and unlabeled iron of the $^{58}$FeAC preparation as mentioned above was similarly and simultaneously absorbed. Though the equality of our formulae to Janghorbani's formula (2) was not obtained statistically, there seems to be a relationship between these three formulae. This examination suggests that:

Vol. 38, No. 5, 1992
Fig. 2. Comparison of the $^{58}$Fe absorption ratio from the supplement of $^{58}$FeAC preparation calculated by A: the formula [1] loading the answer of A + X from equation [6]; B: formula [14]; and C: Janghorbani's formula (2), in which data for 2-4 days from each subject was put into each formula, respectively. Subjects ($n=6$) in group I were administered the $^{58}$FeAC preparation as a control, and in group II ($n=6$), an additional 500mg of vitamin C was administered with the control preparation. Values are the mean±SD; those marked with an asterisk are significantly different at $p<0.001$ from the respective ratio in each case of calculation.

1. It is possible to calculate iron absorption using our formulae similarly as Janghorbani's formula (2).
2. These formulae can reasonably calculate the absorption of iron from the diets which include the endogenously excreted iron.
3. The amount of endogenous iron excreted in the intestine can be calculated by equation [6].

Our results demonstrated that a higher $^{58}$Fe absorption ratio was obtained in subjects administered $^{58}$FeAC with simultaneous supplementation of vitamin C than in subjects administered $^{58}$FeAC alone, indicating the availability of vitamin C. The percent ratio of $^{58}$Fe absorption was doubled (34.1% vs. 14.8%) when iron tablets were supplemented with vitamin C. These numbers were obtained using our new formula, which showed a good agreement with the results obtained from Janghorbani's formula (2) (34.4% vs. 15.0%). The total amount of absorbed iron was also increased two-fold with vitamin C-enriched tablets (2.11mg vs. 0.92mg). Thus, the total amount of absorbed unlabeled and labeled iron from the $^{58}$FeAC preparation was approximately 2mg out of 6mg of iron supplementation. This uptake (2mg), which cannot be achieved without vitamin C, is sufficient enough to supply the required daily intake of iron since it is estimated that 1.4mg of absorbed iron a day is required by menstruating women (17). As the subjects received a low iron diet (6mg/d) during the experimental period (Table 5), iron tablets supplementing 6mg of iron satisfies the Recommended Daily Allowance (12mg). It
fulfills the guidelines of the Ministry of Health and Welfare in Japan, according to which the recommended daily intake of iron for adult women and men is 12 mg (15), assuming that the net absorption rate is ~10%. This supports the fact that iron tablets are effective in improving iron-deficiency anemia in college women as we reported in a previous paper (14).

Under the conditions in our study where a high dose of vitamin C was administered, the 58Fe absorption ratio obtained in this study (34.1%) was considered to be much higher than that obtained from Cook and Monsen’s study (approximately 4.6%) (13), though the content of vitamin C (500 mg) and iron (6 mg) was similar to that used in the study (500 mg and approximately 4 mg, respectively). The discrepancy might be due to a different diet used in these two studies. Since different diets were used and the iron preparations used in the two studies were provided in completely different formulations, the difference in the absorption ratio was understandable. However, our results indicated that an iron and vitamin C tablet can yield a fair amount of iron which is close to the maximum dietary iron absorption since the mucosal iron transfer is limited as a maximal dietary iron absorption of 3–5 mg/d (18).

Iron absorption from food has been studied in recent years using intrinsically and/or extrinsically labeled foods (18–21), and the mechanism by which iron is absorbed from the diet has been discussed (22, 23). Since our formulae enable us to calculate the iron absorption ratio from the diets without using radio-labeled iron isotopes, our findings question the belief that fecal monitoring will not yield valid data on iron absorption from food intake unless foods are intrinsically labeled with 58Fe (2, 7). However, further studies are required to examine whether the iron absorption ratio from the diet obtained in this study represents an authentic value by conducting fecal monitoring with foods intrinsically labeled with 58Fe and by calculating the iron absorption with our formula. If our formula can give the absolute amount of iron that is absorbed and excreted, it can be used to obtain the nutritional status of iron in iron dynamics and in iron balance.

First, approximately 2 mg/d iron uptake in each group is a reasonable level of iron absorption from the dietary intake (24–26). Second, the total amount of excreted iron in each group was also found to be almost the same (26.87 ± 13.84 mg vs. 21.69 ± 6.79 mg) as the total amount of absorbed iron (22.30 ± 3.68 mg vs. 23.34 ± 2.16 mg) (Table 8), though there was an individual variation. Moreover, the study agreed with the previous findings that the iron balance on uptake and excretion is maintained within a narrow range (1–2 mg) (27–29). Third, since no 58Fe absorbed after administration was detected in the feces on days 7–10, which indicates the 58Fe was not easily excreted by desquamation of the intestinal cells and/or by the bile released from the gall bladder once 58Fe was absorbed from the brush border membrane. It is suggested that the endogenously excreted iron is derived from a different iron pool in which the recirculation of iron is possible in the intestine. However, the study to find out the possibility of the different source of iron is required in the future.

Vol. 38, No. 5, 1992
Taken together, vitamin C enhanced the iron absorption, and the iron absorption ratio can be easily calculated through fecal collection without measuring the intake of iron from the diet. Our formulae showed the calculation of $^{56}$Fe and the iron absorption ratio, which also indicated the endogenously excreted iron.

The authors are grateful to Drs. Masayoshi Takashima and Yonezo Maeda, Faculty of Science, Kyushu University, for preparing $^{56}$FeAC, and to the staff of the Central Analysis Room of Kyushu University for elemental analyses. Thanks are also due to Dr. Nobuaki Matsuoka, and the staff of Kyushu Environmental Evaluation Association for NAA, and to Ms Chiemi Umekawa, Ms Takako Kamata and Ms Kaoru Masui for their technical assistance.

REFERENCES

1)  Turnland, J. (1989): The use of stable iron isotopes in mineral nutrition research. *J. Nutr.*, 120, 7–14.
2)  Janghorbani, M., Ting, B. T. G., and Young, V. R. (1980): Absorption of iron in young men studied by monitoring excretion of a stable iron isotope ($^{56}$Fe) in feces. *J. Nutr.*, 110, 2190–2197.
3)  Lowman, J. T., and Krivit, W. (1963): New in vivo tracer method with the use of nonradioactive isotopes and activation analysis. *J. Lab. Clin. Med.*, 61, 1042–1048.
4)  Disler, P. B., Lynch, S. R., Charlton, R. W., Torrance, J. D., Bothwell, T. H., Walker, R. B., and Mayet, F. (1975): The effect of tea on iron absorption. *Gut*, 16, 193–200.
5)  Fairweather-tait, S. J., Minski, M. J., and Richardson, D. P. (1983): Iron absorption from a malted cocoa drink fortified with ferric orthophosphate using the stable isotope $^{56}$Fe as an extrinsic label. *Br. J. Nutr.*, 50, 51–60.
6)  Kaneko, K., Nishida, K., Koike, G., Hirai, Y., and Yoshino, Y. (1986): Absorption of iron studied by using stable isotope $^{56}$Fe in women. *J. Jpn. Food. Sci.*, 39, 165–168.
7)  Fairweather-tait, S. J., and Minski, M. J. (1986): Studies on iron availability in man, using stable isotope techniques. *Br. J. Nutr.*, 55, 279–285.
8)  Sayers, M. H., Lynch, S. R., Jacobs, P., Charlton, R. W., Bothwell, T. H., Walker, R. B., and Mayet, F. (1973): The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya. *Br. J. Haematol.*, 24, 209–218.
9)  Gilllooly, M., Torrance, J. D., Bothwell, T. H., MacPhail, A. P., Derman, D., Mills, W., and Mayet, F. (1984): The relative effect of ascorbic acid on iron absorption from soy-based and milk based infant formulas. *Am. J. Clin. Nutr.*, 40, 522–527.
10) Sayers, M. H., Lynch, S. R., Charlton, R. W., Bothwell, T. H., Walker, R. B., and Mayet, F. (1974): The fortification of common salt with ascorbic acid and iron. *Br. J. Haematol.*, 28, 483–495.
11) Hallberg, L., Rossander, L., Persson, H., and Svahn, E. (1982): Deleterious effects of prolonged warming of meals on ascorbic acid content and iron absorption. *Am. J. Clin. Nutr.*, 36, 846–850.
12) Hallberg, L., Björn-rasmussen, E., Howard, L., and Rossander, L. (1979): Dietary heme iron absorption. *Scand. J. Gastroenterol.*, 14, 769–779.
13) Cook, J. D., and Monsen, E. R. (1977): Vitamin C, the common cold, and iron absorption. *Am. J. Clin. Nutr.*, 30, 235–241.
14) Taniguchi, M., Imamura, H., Shirota, T., Okamatsu, H., Fujii, Y., Toba, M., and Hashimoto, F. (1991): Improvement of iron deficiency anemia through therapy with ferric ammonium citrate and vitamin C and the effects of aerobic exercise. *J. Nutr. Sci. Vitaminol.*, 37, 161–171.

15) Japan Science and Technology Agency (1989): Tables of Food Composition, 5th ed., pp. 247, Daiichi pub.

16) Yoshino, Y., Hirai, Y., Aoki, T., Morita, T., Eguchi, Y., Yajima, T., and Kagawa, Y. (1980): Serum ferritin concentration and isoferritin patterns detected by immunoradiometric assay. *J. Nutr. Sci. Vitaminol.*, 26, 87–97.

17) Monsen, E. R., Hallberg, L., Layrisse, M., Hegsted, D. M., Cook, J. D., Mertz, W., and Finch, C. A. (1978): Estimation of available dietary iron. *Am. J. Clin. Nutr.*, 31, 134–141.

18) Heubers, H., and Rummel, W. (1984): Pharmacology of intestinal permeation I. Csaky ed., 513–541.

19) Hallberg, L., and Björn-rasmussen, E. (1972): Determination of iron absorption from whole diet. *Scand. J. Haematol.*, 9, 193–197.

20) Björn-rasmussen, E., and Hallberg, L. (1974): Iron absorption from maize. *Nutr. Metabol.*, 16, 94–100.

21) Cook, J. D., Layrisse, M., Martinez-torres, C., Walker, R., Monsen, E., and Finch, C. A. (1972): Food iron absorption measured by an extrinsic tag. *J. Clin. Invest.*, 51, 805–815.

22) Linder, M. C., and Munro, H. N. (1977): The mechanism of iron absorption and its regulation. *Fed. Proc.*, 36, 2017–2023.

23) Huebers, H. A. (1986): Iron absorption: molecular aspects and its regulation. *Acta Haematol. Jpn.*, 49, 1528–1535.

24) Baker, S. J. (1978): Nutritional anemia: A major controllable health problem. *Bull. WHO*, 56, 659–675.

25) Finch, C. A., and Cook, J. D. (1984): Iron deficiency. *Am. J. Clin. Nutr.*, 44, 471–477.

26) Dallman, P. R., Yip, R., and Johnson, C. (1984): Prevalence and causes of anemia in the United States, 1976 to 1980. *Am. J. Clin. Nutr.*, 39, 437–455.

27) McCane, R. A., and Widdowson, E. M. (1937): Absorption and excretion of iron. *Lancet*, ii, 680–684.

28) Moore, C. V., and Dubach, R. (1951): Observations on the absorption of iron from foods tagged with radioiron. *Trans. Assoc. Am. Physicians*, 64, 245–256.

29) Dubach, R., Moore, C. V., and Callender, S. (1955): Studies in iron transportation and metabolism. IX. The excretion of iron as measured by the isotope technique. *J. Lab. Clin. Med.*, 45, 599–615.