# Food Iron Absorption in Man

## APPLICATIONS OF THE TWO-POOL EXTRINSIC TAG METHOD TO MEASURE HEME AND NONHEME IRON ABSORPTION FROM THE WHOLE DIET

ERIK BJÖRN-RASMUSSEN, LEIF HALLBERG, BJÖRN ISAKSSON, and BERTIL ARVIDSSON

*From the Department of Medicine II, Institute of Clinical Nutrition, and Department of Radiation Physics, University of Göteborg, Sahlgrenska Sjukhuset, Göteborg, Sweden*

ABSTRACT A new radioisotope method to measure iron absorption from the whole diet was used in this study. The method is based on the concept that food iron is absorbed from two pools, the heme iron pool and the nonheme iron pool, which can be especially labeled with two radioiron isotopes given as hemoglobin and as an iron salt. The purpose of this study was to test the accuracy of this two-pool extrinsic tag method.

The meals served were composed as an average of 6 wk consumption in the present material of 32 young enlisted men. The mean and total heme and nonheme iron absorption in all the 32 young men was 1.01±0.11. This figure agrees well with the mean daily losses expected for this group of subjects (1.0 mg). The conclusion can therefore be made that there are no major systematic errors of the present method to measure the total iron absorption from a mixed diet.

In one series a comparison was made of the absorption of heme and nonheme iron from the meals. A significant correlation between the absorption of the two kinds of iron was found. However, a much greater fraction of the heme iron was absorbed (37%) than of the nonheme iron (5%).

The absorption both from breakfast and lunch was in two series found to give a good prediction of the total daily nonheme iron absorption. One series was designed to compare the effect of two levels of iron fortification. There was a significant increase in iron absorption when the level of iron fortification of the meals was increased.

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## INTRODUCTION

Different methods have been used to get information about iron absorption from the whole diet. The earliest studies were made with chemical balance technic. This method is theoretically correct but is laborious and has serious inherent errors (1). Very long balance studies are needed to reduce errors in fecal demarcation. As the difference between oral intake and fecal loss is small, it is difficult to estimate with precision. Earlier balance studies have been reviewed by Josephs (2) and by Moore (3).

In another type of method, a tracer dose of inorganic radioiron was administered in a drink taken together with the meal (4). It is known that this way of administration of the radioiron leads to an overestimation of the real absorption (5, 6). Moreover, in most earlier studies the meals studied contained unknown amounts of heme iron which was not labeled by an extrinsic inorganic tracer (7–9).

The absorption from biologically labeled foods was first studied by Moore and Dubach (10). A great number of such studies have been published. For a review see Moore (3). Usually only one food was studied at a time. The absorption of iron from the whole diet is determined not only by its content of iron but also to a large extent by the content of known and unknown components favoring or inhibiting the iron absorption. It is therefore impossible to use the absorption figures of single foods to estimate total iron absorption from a given diet.

Recently a new radioisotope method to measure the absorption of both heme and nonheme iron from com-

TABLE I
*Experimental Design*

| Study | Purpose of study | Number of subjects | Method of labeling | | | | |
|---|---|---|---|---|---|---|---|
| | | | Labeled iron pool | Radioiron isotope added to | | | Specific radioactivity |
| | | | | Breakfast | Lunch | Dinner | |
| I | Measurement of total daily heme and nonheme iron absorption (1-day study) | 8 | Nonheme | $^{59}$Fe | $^{59}$Fe | $^{59}$Fe | Identical in all meals |
| | | | Heme | — | $^{55}$Fe | $^{55}$Fe | Identical in lunch and dinner |
| II | Nonheme iron absorption from breakfast in relation to total daily nonheme iron absorption (1-day study) | 7 | Nonheme | $^{59}$Fe | $^{55}$Fe | $^{55}$Fe | Identical in lunch and dinner |
| III | Nonheme iron absorption from lunch in relation to total daily nonheme iron absorption (1-day study) | 10 | Nonheme | $^{55}$Fe | $^{59}$Fe | $^{55}$Fe | Identical in breakfast and dinner |
| IV | Effect of additional iron fortification on total daily absorption of nonheme iron (2-day study) | 7 | Nonheme (normal fortification) | $^{59}$Fe | $^{59}$Fe | $^{59}$Fe | Identical in all meals |
| | | | Nonheme (additional fortification) | $^{55}$Fe | $^{55}$Fe | $^{55}$Fe | Identical in all meals |

posite meals has been proposed (11). The method is based on the new concept that iron absorption can be considered to take place from two pools, one heme iron pool and one nonheme iron pool. The absorption from these two pools can be independently measured with two radioiron isotopes as tracers of the two pools. The heme iron pool is labeled with biologically radioiron-labeled hemoglobin and the nonheme iron pool with an inorganic radioiron tracer.

The validity of this two-pool extrinsic tracer method is based on a number of recent studies. Two studies have shown that labeled hemoglobin is a good tracer for heme iron in food (11, 12). Different groups of investigators have shown that an extrinsic inorganic iron tag can be validly used as a measure of nonheme iron absorption from a great variety of foods (5, 6, 13–17). The reason for this is the rapid and complete isotopic exchange between the added inorganic radioiron tracer and the nonheme iron compounds in the food when a good mixing is ensured before the final preparation of the food.

The main purposes of the present investigation were to study the accuracy and precision of the method to measure iron absorption from the whole diet, to study the iron absorption from separate meals, to measure the increase in iron absorption obtained by iron fortification, and to study the practical applicability of the new method of measuring iron absorption from the whole diet.

## METHODS

*Experimental design.* The present investigation is composed of four studies (Table I). The subjects were served three radioiron-labeled meals (breakfast, lunch, and dinner), prepared and labeled as described below. In study I the total daily absorption of both heme and nonheme iron was measured. The two radioiron isotopes $^{55}$Fe and $^{59}$Fe were used to label the two types of dietary iron. In studies II, III, and IV only the nonheme iron absorption was measured. In studies II and III the two radioiron isotopes were used to relate the nonheme iron absorption from one meal (breakfast or lunch) to the absorption from the other two meals. Study IV was designed to compare the absorption of nonheme iron at two levels of iron fortification: the level presently used in Sweden, and a level about three times higher. The two levels of fortification were compared on two consecutive days with two different radioiron isotopes.

Before the absorption study blood samples were drawn to determine hemoglobin concentration, serum iron (18), and total iron-binding capacity (19).

*Subjects.* 32 young men doing their military service volunteered for the study.[1] The subjects were in the 7th mo of their duty and were all in good physical condition. They had no history of gastrointestinal disease or anemia.

*Preparation and labeling of meals.* All provisions used in the study were obtained locally, except for the wheat flour products.[2] The special wheat flour was fortified with the same amount of iron as is usual in Sweden (6.5 mg iron/100 g flour). However, the commonly used reduced

[1] The study was performed at Kungliga Älvsborgs Kustartilleriregementet in Göteborg and was approved by the Medical Board of the Armed Forces in Sweden.

[2] Supplied by Swedish Cooperative Union.

TABLE II
*Composition of the Meals**

| | Food products | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Without heme iron | | | | | | | | With heme iron | | | |
| | Greens and legumes | Fruits | Potatoes and roots | Milk products | Egg | Cereals | Fats | Sugar | Meats | Fish | Sausages | Total |
| **Breakfast:** | | | | | | | | | | | | |
| Number of items | | | | 6 | | 5 | 1 | 3 | | 1 | 3 | 19 |
| Amount, *g* | | | | 177 | | 70 | 15 | 58 | | 5 | 10 | 335 |
| Iron, *mg* | | | | 0.5 | | 2.0 | — | — | | 0.1 | 0.4 | 3.1‡ |
| Energy, *kcal* | | | | 170 | | 210 | 120 | 160 | | 10 | 37 | 700 |
| **Lunch-dinner:** | | | | | | | | | | | | |
| Number of items | 11 | 6 | 4 | 4 | 1 | 5 | 3 | 5 | 8 | 3 | 2 | 52 |
| Amount, *g* | 36.7 | 14.3 | 74.1 | 189 | 2.3 | 41.2 | 9.8 | 17.5 | 39.2 | 12.4 | 9.5 | 446 |
| Iron, *mg* | 0.6 | 0.1 | 0.6 | 0.3 | 0.1 | 1.6 | — | — | 1.4 | 0.1 | 0.1 | 4.9 |
| Energy, *kcal* | 30 | 10 | 59 | 150 | 4 | 130 | 76 | 54 | 110 | 13 | 30 | 670 |

* Iron and energy estimated from food tables (34).
‡ Total non heme iron in breakfast estimated to be less than 0.01 mg.

iron was exchanged for ferrous sulphate to ensure a complete exchange with the added nonheme iron tag. In study IV the amount of fortification iron was increased to a total of 12.2 mg in the three meals served on the 2nd day, as compared with 4.1 mg in all other studies.

Each meal consisted of a pudding in which the relative amounts of provisions were close to the average amounts calculated from a 6-wk master menu and from detailed supply records for the corresponding meals served at the military unit. As only slight differences were found in the composition of the average lunch and dinner meals, identical meals were prepared for lunch and dinner (in the following called lunch-dinner).

The amounts of food served were considered sufficient as determined by pilot studies in which the subjects were served three unlabeled puddings in free amounts during a day. The final composition of the meals is given in Tables II and III.

The food used to prepare the meals was thoroughly minced and mixed. The radioiron was added during the final mixing. The lunch-dinner meals were prepared in two steps. Two batches were first prepared, one with all heme iron-containing foods (meat and fish), and one with the remaining foodstuffs. The final lunch-dinner mix was then prepared by mixing these two batches. The reason for this two-step procedure was to increase the accuracy in determining the amount of heme iron in the lunch-dinner meal. Heme iron formed only about 6% of the total iron content of the diet whereas it formed about 40% of the iron content in the batch containing meat and fish. From the breakfast mix and the final lunch-dinner mix, weighed amounts (290 and 425 g, respectively) were put into aluminum forms. The forms were placed in a water bath and boiled for 2 h in an oven set at 200°C. The puddings were kept frozen at −20°C until used. Before serving, the puddings were warmed in a water bath. Aliquots were taken from the batches before baking as standards for determination of $^{55}Fe$ and $^{59}Fe$.

*Radioiron labeling of the meals.* To measure heme iron absorption in study I, $^{59}Fe$-labeled rabbit hemoglobin (sp act about 30 $\mu$Ci/g hemoglobin) was added to the lunch-dinner mix. The heme iron in breakfast was not labeled as the amount was negligible (< 0.01 mg).

The nonheme iron was labeled with $^{55}FeCl_3$ or $^{59}FeCl_3$

TABLE III
*Food Analysis*

| Meal | Energy* | Protein | Fat | Carbohydrates* | Calcium | Phosphorus: In phytic acid | Phosphorus: Total | Iron‡: Nonheme | Iron‡: Heme |
|---|---|---|---|---|---|---|---|---|---|
| | *Kcal* | *g* | *g* | *g* | *mg* | *mg P* | *mg P* | *mg Fe* | *mg Fe* |
| Breakfast | 700 | 19 | 22 | 100 | 400 | 26 | 400 | 4.2 | <0.01 |
| Lunch | 670 | 24 | 25 | 70 | 260 | 17 | 400 | 6.1 | 0.5 |
| Dinner | 670 | 24 | 25 | 70 | 260 | 17 | 400 | 6.1 | 0.5 |
| Total | 2040 | 67 | 72 | 240 | 920 | 60 | 1200 | 16.4 | 1.0 |

* Estimated from food tables (34).
‡ Additionally fortified meals of study IV not analyzed.

(sp act about 10 $\mu$Ci/$\mu$g Fe) in amounts calculated to obtain equal specific radioactivity of the nonheme iron in the meals labeled with the same radioiron tracer (Table I). The total amounts of $^{55}$Fe and $^{59}$Fe administered to the subjects were 6 $\mu$Ci and 2 $\mu$Ci, respectively.

*Serving of meals.* After an overnight fast, breakfast was served at 7 a.m., lunch at noon, and dinner at 5 p.m. The meals were served with water and coffee. Salt and pepper were allowed to improve the taste of the meals. No other food or drink was allowed until 9 p.m. the day of the study. The subjects were cooperative and no major complaints with respect to the taste of the meals were raised. The food returns were small as determined by weighing.

*Absorption measurement.* The absorption of $^{59}$Fe was measured in a whole-body counter.[3] The relative absorption of $^{55}$Fe and $^{59}$Fe was calculated from radioactivities in the food consumed and in blood samples drawn 2 wk after serving the labeled meals. The total absorption of $^{55}$Fe was calculated from the relative absorption of $^{55}$Fe and $^{59}$Fe, and the absorption of $^{59}$Fe measured in the whole body counter.

Determinations of $^{55}$Fe and $^{59}$Fe in the blood and food samples were performed according to a modification of the method of Eakins and Brown (20). Before the study the baseline activities of $^{55}$Fe and $^{59}$Fe in blood samples were determined, and the subjects were measured in the whole-body counter. The measurements of whole body retention of $^{59}$Fe were performed in two different ways. In studies II and III, where only one meal was labeled with $^{59}$Fe, the subjects were measured 30 min after this meal and then 2 wk later. In studies I and IV, where all three meals were labeled with $^{59}$Fe, a measurement was made 30 min after breakfast and then 2 wk later. The total intake of $^{59}$Fe was then calculated from the content of $^{59}$Fe in breakfast and the other meals.

[3] A large sensitive $3\pi$ plastic scintillation whole-body counter.

*Other methods.* Total iron in food was determined in aliquots ashed at +600°C overnight, dissolved in boiling iron-free 6 M HCl, and diluted to 100 ml with iron-free water. After reduction with thioglycolic acid, the iron was determined at pH 5.2 as a ferrobathophenanthroline sulfonate complex at 546 nm in a spectrophotometer (18).

Nonheme iron was determined in freeze-dried food samples extracted with 5 M HCl according to Hallgren (23). After reduction with thioglycolic acid, the nonheme iron present in the extract was taken up into a solution of bathophenanthroline in pentanol and determined spectrophotometrically (24). Due to the difficulties in determining heme directly in mixtures containing other chromogens, heme iron in the lunch-dinner meals was calculated as the difference between total iron and nonheme iron in the batch containing meat and fish.

The figures given for total iron and nonheme iron content are mean values based on 10 measurements. The SEM values were 1.9 and 2.5%, respectively. The accuracy of the method for measurement of nonheme iron in food was studied in two ways. In foods containing no heme iron, a comparison was made of the methods for total and nonheme iron. No systematic difference was observed. In another series of studies using radioactive hemoglobin, it was found that only 2% of iron from freeze-dried labeled hemoglobin was determined as nonheme iron.

Phytic acid phosphorus and total phosphorus was determined by the methods proposed by the Nordic Committee on Food Analysis (25, 26). Potassium, sodium, and calcium were determined in freeze-dried samples by means of an Eppendorf flame photometer (Gerätebau Netheler & Hinz GmbH, Hamburg) as described earlier (27). Nitrogen was determined in a Technicon Auto Analyser (Technicon Instruments Corp., Tarrytown, N. Y.). The results of the food analysis are shown in Table III.

To calculate the significance of difference between means, the Wilcoxon signed rank test was used (28). The best fit for the linear regression lines was calculated according to the least squares method.

TABLE IV

*Absorption of Nonheme Iron and Heme Iron from Breakfast, Lunch, and Dinner, Study I*

| Subject | Hemoglobin concn | Serum iron concn | Total iron-binding capacity | Body wt | Iron absorption from breakfast, lunch, and dinner: nonheme iron labeled with $^{59}$Fe, 16.4 mg | | heme iron labeled with $^{55}$Fe, 1.0 mg | | Total, 17.4 mg |
|---|---|---|---|---|---|---|---|---|---|
| | *g/100 ml* | *µg/100 ml* | *µg/100 ml* | *kg* | % | *mg* | % | *mg* | *mg* |
| 1 | 14.1 | 110 | 370 | 88 | 0.6 | 0.10 | 23.2 | 0.23 | 0.33 |
| 2 | 14.0 | 127 | 348 | 79.5 | 1.6 | 0.26 | 34.5 | 0.35 | 0.61 |
| 3 | 13.7 | 81 | 312 | 60.5 | 2.0 | 0.33 | 41.6 | 0.42 | 0.74 |
| 4 | 15.2 | 140 | 386 | 66.5 | 2.8 | 0.46 | 37.2 | 0.37 | 0.83 |
| 5 | 15.5 | 108 | 332 | 64 | 4.6 | 0.75 | 39.3 | 0.39 | 1.15 |
| 6 | 14.9 | 79 | 348 | 78.5 | 7.2 | 1.18 | 34.2 | 0.34 | 1.52 |
| 7 | 14.9 | 141 | 405 | 70 | 7.3 | 1.20 | 36.6 | 0.37 | 1.56 |
| 8 | 14.1 | 144 | 389 | 57 | 16.6 | 2.72 | 51.8 | 0.52 | 3.24 |
| Mean | | | | | 5.3 | 0.88 | 37.3 | 0.37 | 1.25 |
| SEM | | | | | 1.8 | 0.30 | 2.8 | 0.03 | 0.32 |

Three radioiron-labeled meals, breakfast, lunch, and dinner, were served to the subjects during 1 day. The total amount of nonheme iron labeled with $^{59}$Fe was 16.4 mg and of heme iron labeled with $^{55}$Fe 1.0 mg. The correlation between absorbed amounts of nonheme iron and heme iron was significant ($r = 0.748$, $P < 0.05$).

TABLE V

*Absorption of Nonheme Iron from Breakfast and from Lunch and Dinner, Study II*

| Subject | Hemoglobin concn | Serum iron concn | Total iron-binding capacity | Body wt | Nonheme iron absorption: Breakfast labeled with $^{59}$Fe, 4.2 mg | | Lunch and dinner labeled with $^{55}$Fe, 12.2 mg | | All meals, 16.4 mg |
|---|---|---|---|---|---|---|---|---|---|
| | *g/100 ml* | *μg/100 ml* | *μg/100 ml* | *kg* | % | *mg* | % | *mg* | *mg* |
| 9 | 15.8 | 76 | 317 | 65.5 | 0.5 | 0.02 | 0.9 | 0.11 | 0.13 |
| 10 | 14.9 | 75 | 333 | 61 | 0.5 | 0.02 | 1.2 | 0.15 | 0.17 |
| 11 | 15.2 | 122 | 346 | 79.5 | 1.0 | 0.04 | 1.7 | 0.21 | 0.25 |
| 12 | 15.1 | 98 | 283 | 64.5 | 1.1 | 0.05 | 2.7 | 0.33 | 0.38 |
| 13 | 14.9 | 121 | 350 | 78 | 2.4 | 0.10 | 3.3 | 0.40 | 0.50 |
| 14 | 14.2 | 130 | 320 | 68.5 | 2.4 | 0.10 | 4.5 | 0.55 | 0.65 |
| 15 | 14.0 | 85 | 307 | 81 | 7.0 | 0.29 | 7.2 | 0.88 | 1.17 |
| Mean | | | | | 2.1 | 0.09 | 3.1 | 0.38 | 0.46 |
| SEM | | | | | 0.9 | 0.04 | 0.8 | 0.10 | 0.14 |

The correlation between nonheme iron absorption from breakfast and the two meals, lunch and dinner, was highly significant ($r = 0.956$, $P < 0.001$). The difference between the percentual iron absorption from breakfast and from lunch and dinner was statistically significant ($P < 0.05$).

## RESULTS

To facilitate interpretation of the results from the four separate studies, the subjects of each study are listed in Tables IV–VII in order of increasing iron absorption.

*In study I* the total daily absorption of nonheme and heme iron was separately measured in eight subjects. The results are given in Table IV. Of the total daily nonheme iron intake (16.4 mg) 5.3±1.8% was absorbed, and of the heme iron intake (1 mg) 37.3±3.8% was absorbed. There was a significant correlation between the absorption of the two kinds of iron ($r = 0.748$; $P < 0.05$).

*In study II* the absorption of nonheme iron from breakfast was compared with that from lunch and dinner. The results in the seven subjects studied are given in Table V. The mean percentual absorption of nonheme iron was 2.1±0.9 from breakfast and 3.1±0.8 from lunch and dinner. The difference between these two means was statistically significant ($P < 0.05$). The correlation between iron absorption from breakfast and from the lunch and dinner meals was highly significant ($r = 0.956$; $P < 0.001$).

*In study III* the absorption of nonheme iron from lunch was compared with that from breakfast and dinner,

TABLE VI

*Absorption of Nonheme Iron Absorption from Lunch and from Breakfast and Dinner Meals, Study III*

| Subject | Hemoglobin concn | Serum iron concn | Total iron-binding capacity | Body wt | Nonheme iron absorption: Lunch labeled with $^{59}$Fe, 6.1 mg | | Breakfast and dinner labeled with $^{55}$Fe, 10.3 mg | | All meals, 16.4 mg |
|---|---|---|---|---|---|---|---|---|---|
| | *g/100 ml* | *μg/100 ml* | *μg/100 ml* | *kg* | % | *mg* | % | *mg* | *mg* |
| 16 | 15.8 | 146 | 348 | 61.5 | 0.6 | 0.04 | 1.0 | 0.10 | 0.14 |
| 17 | 14.4 | 84 | 302 | 72 | 2.3 | 0.14 | 2.3 | 0.24 | 0.38 |
| 18 | 15.4 | 137 | 382 | 76.5 | 2.3 | 0.14 | 3.0 | 0.31 | 0.45 |
| 19 | 14.1 | 114 | 381 | 57 | 2.4 | 0.15 | 2.1 | 0.22 | 0.36 |
| 20 | 16.5 | 72 | 284 | 72 | 2.5 | 0.15 | 2.4 | 0.25 | 0.40 |
| 21 | 15.8 | 172 | 306 | 51 | 2.7 | 0.17 | 2.9 | 0.30 | 0.46 |
| 22 | 16.2 | 99 | 302 | 72 | 3.6 | 0.22 | 3.1 | 0.32 | 0.54 |
| 23 | 16.0 | 72 | 291 | 65.5 | 4.2 | 0.26 | 3.4 | 0.35 | 0.61 |
| 24 | 13.9 | 98 | 309 | 66.5 | 8.0 | 0.49 | 8.6 | 0.89 | 1.37 |
| 25 | 15.7 | 110 | 318 | 58 | 11.8 | 0.72 | 11.2 | 1.15 | 1.87 |
| Mean | | | | | 4.0 | 0.25 | 4.0 | 0.41 | 0.66 |
| SEM | | | | | 1.1 | 0.07 | 1.0 | 0.10 | 0.17 |

The correlation between the nonheme iron absorption from lunch and the two meals breakfast and dinner was highly significant ($r = 0.988$, $P < 0.001$).

TABLE VII

*Effect of Additional Iron Fortification on the Total Daily Absorption of Nonheme Iron, Study IV*

| Subject | Hemoglobin concn | Serum iron concn | Total iron-binding capacity | Body wt | Nonheme iron absorption from the meals: Ordinarily fortified served the 1st day, 16.4 mg | | Additionally fortified served the 2nd day, 25.5 mg | |
|---|---|---|---|---|---|---|---|---|
| | *g/100 ml* | *μg/100 ml* | *μg/100 ml* | *kg* | % | *mg* | % | *mg* |
| 26 | 15.2 | 104 | 376 | 65.5 | 1.0 | 0.16 | 6.1 | 1.56 |
| 27 | 15.7 | 121 | 365 | 69 | 2.4 | 0.39 | 3.2 | 0.82 |
| 28 | 14.1 | 106 | 315 | 77.5 | 2.7 | 0.44 | 5.6 | 1.43 |
| 29 | 13.9 | 100 | 278 | 64 | 3.7 | 0.61 | 2.1 | 0.54 |
| 30 | 14.2 | 33 | 354 | 62 | 4.7 | 0.77 | 3.1 | 0.79 |
| 31 | 14.9 | 157 | 338 | 73.5 | 4.9 | 0.80 | 1.5 | 0.38 |
| 32 | 15.3 | 118 | 292 | 89 | 5.1 | 0.84 | 6.3 | 1.60 |
| Mean | | | | | 3.5 | 0.57 | 4.0 | 1.02 |
| SEM | | | | | 0.6 | 0.10 | 0.8 | 0.19 |

No correlation was found between the nonheme iron absorption from the meals ordinarily fortified and the meals additionally fortified ($r = -0.38$, $P < 0.1$).

in 10 subjects (Table VI). The percentual absorption from lunch was 4.0±1.1, and from breakfast and dinner 4.0±1.0. The correlation between the iron absorption from lunch and from the breakfast and dinner was found to be highly signficant ($r = 0.988$; $P < 0.001$).

***In study IV*** the nonheme iron absorption was measured at two levels of iron fortification on two consecutive days. On the first day, when the meals were fortified at the level presently used in Sweden, 3.5±0.6% of the daily intake of 16.4 mg nonheme iron was absorbed. On the second day, when the daily intake of nonheme iron was increased to 25.5 mg, the mean absorption was 4.0±0.8%. On an average, 0.45 mg more iron was absorbed at the higher level of iron fortification of the flour. This difference was statistically significant ($P < 0.05$). The correlation between the absorption from the meals ordinarily fortified and the meals additionally fortified was not statistically significant (Table VII).

***The total daily iron absorption*** (heme and nonheme iron) was measured only in the eight subjects in study I. In the 24 subjects of studies II–IV, only, the nonheme iron absorption was measured. In these subjects the total absorption was estimated simply by adding the mean heme iron absorption found in study I (0.37 mg) to the total nonheme iron absorption figures observed. In study IV the absorption from the meals ordinarily fortified was used to estimate the total iron absorption. The mean total heme and nonheme iron absorption of the subjects calculated in this way was 1.02 mg. The distribution of the individual absorption values is shown in Fig. 1.

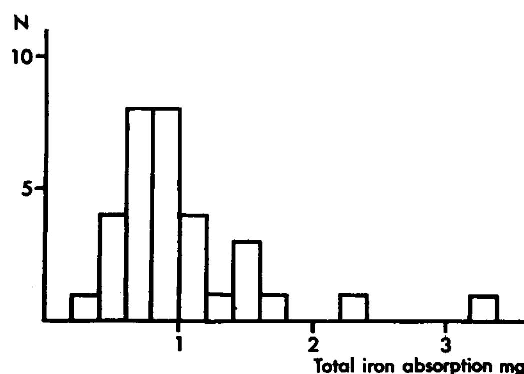


FIGURE 1 Distribution of total daily (heme and nonheme) iron absorption in 32 subjects. The absorption of heme and nonheme iron was measured in eight subjects in study I. In the 26 subjects in studies II–IV only nonheme iron absorption was measured and the total iron absorption was estimated by adding the mean heme iron absorption (0.37 mg) obtained in study I.

## DISCUSSION

Information about the iron absorption from the whole diet is needed both in nutritional and clinical studies. To study the possible role of the diet in explaining a high prevalence of iron deficiency, it is necessary to measure iron absorption from whole typical diets in a representative sample of the population. Similar studies are needed to evaluate the effect of different measures to improve iron nutrition. Studies on clinical materials may be needed, for instance, to evaluate the effect of different methods of gastric surgery on iron balance. The chemical balance technique is too complicated and laborious to be used other than to study very special problems. There is thus a need for a much simpler but

still accurate and precise method to measure iron absorption from the whole diet.

The present studies were designed to allow estimations of the accuracy and precision of the new extrinsic tag two-pool method. It is difficult to study the accuracy of the present method directly, e.g. by comparison with simultaneously made balance studies. An estimation of the systematic error was therefore made by comparing the average daily absorption measured and the average losses of iron expected, assuming that the subjects were in iron balance. The subjects and experimental conditions were chosen to increase the validity of this comparison. The subjects were very homogenous with respect to age, calorie expenditure, and dietary habits, and the meals were composed as averages of a 6 wk food intake. The assumption that absorption should equal losses in the present subjects, i.e. that the total iron content of the body was constant, was also based on the facts that all subjects had passed the age of growth in body height and that their physical activity was moderate and had been constant for a long period of time.

The results from studies on total body iron losses indicate that on an average the daily loss is about 14 $\mu$g/kg body wt/day (29, 30). The average body weight was 70 kg in the present group, which gives an estimate of average total iron losses of about 1 mg. The average iron absorption calculated as a mean from all four series was 1.02 mg. The two figures are very close and may give a false impression of very high precision in determining total body iron losses and total dietary iron absorption. The conclusion seems to be valid, however, that there are no major systematic errors in determining iron absorption from the whole diet with the present method.

It should be pointed out that nonheme iron absorption was measured in all subjects whereas heme iron absorption was only measured in study I. In the present diet the heme iron formed only 6% of the total intake of iron and the variation in absorption of heme iron was small. The estimation of the total absorption in studies II–IV was therefore simply made by adding the average absorption of heme iron observed in study I (0.37 mg) to the nonheme iron absorption figures. The error of using this procedure in the present very homogeneous material was considered not to be greater than an estimation from the correlation between the absorption of heme and nonheme iron obtained in study I.

The accuracy of the extrinsic tag method is mainly based on the validity of the assumption that a complete isotopic exchange occurs between the extrinsic tag and the main part of the nonheme iron compounds in the diet. Although a complete isotopic exchange is shown to take place in a great number of food stuffs (5, 6, 13–17), it cannot be excluded that there may be foods in which the isotopic exchange is incomplete or slow or both. However, the magnitude of this source of error must be small, at least in the present type of diet. In a previous study it was shown that the isotopic exchange is not affected by variations in iron status of the subjects. It was also shown that a complete rapid exchange occurs even in the presence of gastric disorders such as anacidity or rapid gastric emptying caused by partial gastric resection (14). It is therefore probable that individual variations have little influence on the accuracy of the present method.

Each meal was composed of a great number of food items, which were carefully minced and mixed. Therefore the taste and the consistency of the food served differed from ordinary meals. Generally, effects of such differences on digestion and absorption are to be considered. In the present studies, however, the results indicate that there were no major effects on the absorption of iron.

It is well known that there is a marked variation in absorption of iron not only between different, apparently normal, individuals, but also in the same individual at different days. This fact makes it difficult to study the precision or reproducibility of methods to measure iron absorption. In the present series of studies the reproducibility cannot be calculated, as duplicate studies were not made. In studies II and III comparisons were made of the absorption from different meals and in study IV from 2 days at two levels of iron fortification. It is possible, however, to make rough estimates of the precision of the method to measure nonheme iron absorption. In studies II and III there are two sources of variation in the absorption within subjects, the real variation between meals, and the experimental error. The total variation in absorption, which is not explained by the correlation in absorption between meals $(1 - r^2)$, was 8.6 and 2.4% in these two studies, respectively. It is therefore reasonable to assume that the experimental error of the method does not exceed this magnitude.

In study IV the absorption on two consecutive days was compared. Two levels of iron fortification were studied, which must increase the variation between days. When comparing the results of studies II and III with those of study IV, one is tempted to conclude that the real variation in absorption between consecutive days is greater than between different meals the same day. Further studies, however, are needed to clarify this point, which may be of importance in the future design of comparative studies on food iron absorption.

The variation in absorption between subjects was great, as shown in Fig. 1. The magnitude of this variation is probably not greater than that observed in other studies on iron absorption, i.e. from iron salts and la-

beled single foods (31). Part of the variation observed is probably related to a variation in iron status of the subjects, even if this is not reflected in the individual hematological data available. Another and perhaps the main part of the variation in absorption in this homogeneous material is related to the day-to-day variation in absorption. The magnitude of this variation makes it difficult to make a valid assessment of the average iron absorption in the single individual. The main use of the present method will therefore be in studies on groups of subjects.

Iron in the breakfast was found to be less well-absorbed than iron in the lunch and dinner meals (study II). There are two main differences between these meals. Breakfast contained only insignificant amounts of fish and meat products, which have been shown to enhance iron absorption (12, 32, 33), and the content of phytic acid phosphorous was much higher than in the other meals. The ratio of phytic acid phosphorous to nonheme iron was more than six in breakfast and less than three in the lunch-dinner. It should be noted that the total amounts of phosphorous in the meals were equal.

One of the objectives of the present investigation was to study problems related to the practical applicability of the new method. The diet served was composed of a great number of food items. These were all carefully minced and mixed according to procedures earlier shown to ensure a complete exchange of iron between the nonheme iron compounds in the foods and the inorganic tracer added. The mincing and mixing procedure used was fairly time-consuming. It is possible that a much simpler procedure and especially a less thorough mixing might give as accurate results. The taste and consistency of the very finely ground food is different from that of an ordinary meal. It might be advantageous even from this point of view if a simpler procedure could be used. Studies are in progress to develop a simpler method more suitable for field studies than the present one.

The concept that all dietary iron except heme iron compounds is absorbed from one single pool has to be carefully considered in iron nutrition. The absorption from this pool is determined by the net effect of factors inhibiting or promoting iron absorption. An increased nutritive value of diets with respect to iron can therefore be obtained not only by iron fortification but also by changing the relative amounts of factors in the diet that influence the iron absorption. Very little is known about the relative importance of such factors. The results of the present study indicate that the new two-pool extrinsic tag method will be of value in studies on the nutritive value of different diets to clarify the causes of iron deficiency in different populations. The method will also greatly facilitate systematic studies on factors influencing iron absorption from the diet in order to find effective and realistic ways to improve iron nutrition in different populations.

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## REFERENCES

1. Isaksson, B., and B. Sjögren. 1967. A critical evaluation of the mineral and nitrogen balances in man. *Proc. Nutr. Soc.* **26**: 106.
2. Josephs, H. W. 1958. Absorption of iron as a problem in human physiology. A critical review. *Blood J. Hematol.* **13**: 1.
3. Moore, C. V. 1968. The absorption of iron from foods. *In* Occurrence, Causes and Prevention of Nutritional Anemias (Symp. VI, Swedish Nutrition Foundation). G. Blix, editor. Almqvist & Wiksell, Publishers, Upsala. 92.
4. Sharpe, L. M., W. C. Peacock, R. Cooke, and R. S. Harris. 1950. The effect of phytate and other food factors on iron absorption. *J. Nutr.* **41**: 433.
5. Björn-Rasmussen, E., L. Hallberg, and R. B. Walker. 1972. Food iron absorption in man. I. Isotopic exchange between food iron and inorganic iron salt added to food: studies on maize, wheat, and eggs. *Am. J. Clin. Nutr.* **25**: 317.
6. Cook, J. D., M. Layrisse, C. Martinez-Torres, R. Walker, E. Monsen, and C. A. Finch. 1972. Food iron absorption measured by an extrinsic tag. *J. Clin. Invest.* **51**: 805.
7. Hallberg, L., and L. Sölvell. 1964. Absorption of hemoglobin iron in man. *Am. J. Digest. Dis.* **9**: 787.
8. Hallberg, L., and L. Sölvell. 1967. Absorption of hemoglobin iron in man. *Acta Med. Scand.* **181**: 335.
9. Turnbull, A., F. Cleton, and C. A. Finch. 1962. Iron absorption. IV. The absorption of hemoglobin iron. *J. Clin. Invest.* **41**: 1897.
10. Moore, C. V., and R. Dubach. 1951. Observations on the absorption of iron from foods tagged with radioiron. *Trans. Assoc. Am. Physicians Phila.* **64**: 245.
11. Hallberg, L., and E. Björn-Rasmussen. 1972. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as haem and non-haem iron. *Scand. J. Haematol.* **9**: 193.
12. Martinez-Torres, C., and M. Layrisse. 1971. Iron absorption from veal muscle. *Am. J. Clin. Nutr.* **24**: 531.
13. Björn-Rasmussen, E. 1973. Food iron absorption in man. Effect of iron salt, ascorbic acid and desferrioxamine on the isotopic exchange between native food iron and an extrinsic inorganic iron tracer. *Scand. J. Haematol.* **5**: in press.
14. Björn-Rasmussen, E. 1973. Food iron absorption in man. Validity of the extrinsic tag two-pool method for

measurement of dietary nonheme iron absorption in patients with various clinical disorders. *Scand. J. Gastroenterol.* In press.
15. Sayers, M. H., S. R. Lynch, P. Jacobs, R. W. Charlton, R. B. Walker, F. Mayet, and T. H. Bothwell. 1973. The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat, and soya. *Br. J. Haematol.* **24**: 209.
16. Layrisse, M., and C. Martinez-Torres. 1972. Model for measuring dietary absorption of heme iron: test with a complete meal. *Am. J. Clin. Nutr.* **25**: 401.
17. Layrisse, M., C. Martinez-Torres, J. D. Cook, R. Walker, and C. A. Finch. 1973. Iron fortification of food. Its measurement by the extrinsic tag method. *Blood J. Hematol.* **41**: 333.
18. International Committee for Standardization in Hematology. 1971. Proposed recommendations for measurement of serum iron in human blood. *Br. J. Haematol.* **20**: 451.
19. Herbert, V., C. W. Gottlieb, K-S. Lau, M. Fisher, N. R. Gevirtz, and L. R. Wasserman. 1966. Coated charcoal assay of unsaturated iron-binding capacity. *J. Lab. Clin. Med.* **67**: 855.
20. Eakins, J. D., and D. A. Brown. 1966. An improved method for the simultaneous determination of iron -55 and iron -59 in blood by liquid scintillation counting. *Int. J. Appl. Radiat. Isot.* **17**: 391.
21. Arvidsson, B., H. Sköldborn, and B. Isaksson. 1972. Clinical application of a high-sensitivity whole-body counter. The Third International Conference on Medical Physics, including Medical Engineering. Chalmers University of Technology, Göteborg, Sweden. **41**: 2.
22. Sköldborn, H., B. Arvidsson, and M. Andersson. 1972. A new whole body monitoring laboratory. *Acta Radiol. Suppl.* **313**: 233.
23. Hallgren, B. 1953–54. Determination of non-haemin iron in the tissues. *Acta Soc. Med. Ups.* **59**: 84.
24. Schilt, A. A. 1969. Analytical applications of 1.10-Phenantroline and related compounds. *Int. Ser. Monogr. Anal. Chem.* **32**: 59.
25. Nordic Committee on Food Analysis. 1965. Method number 57. Teknisk Forlag, Copenhagen.
26. Nordic Committee on Food Analysis. 1966. Method number 17:2. Teknisk Forlag, Copenhagen.
27. Isaksson, B., and B. Sjögren. 1965. On the concept "constant diet" in metabolic balance studies. *Nutr. Dieta.* **7**: 175.
28. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th edition. Iowa State University Press, Ames, Iowa. 128.
29. Green, R., R. Charlton, H. Seftel, T. Bothwell, F. Mayet, B. Adams, C. Finch, and M. Layrisse. 1968. Body iron excretion in man. A collaborative study. *Am. J. Med.* **45**: 336.
30. Bothwell, T. H., and C. A. Finch. 1968. Iron losses in man. *In* Occurrence, Causes, and Prevention of Nutritional Anemias (Symp. VI, Swedish Nutrition Foundation). G. Blix, editor. Almqvist & Wiksell, Publishers, Uppsala. 104.
31. Cook, J. D., M. Layrisse, and C. A. Finch. 1969. The measurement of iron absorption. *Blood J. Hematol.* **33**: 421.
32. Layrisse, M., C. Martinez-Torres, and M. Roche. 1968. Effect of interaction of various foods on iron absorption. *Am. J. Clin. Nutr.* **21**: 1175.
33. Martinez-Torres, C., and M. Layrisse. 1970. Effect of amino acids on iron absorption from a staple vegetable food. *Blood J. Hematol.* **35**: 669.
34. Abrahamson, E. 1971. Kosttabell 5:e uppl. Läromedelsförlagen. Teknik och Ekonomi, Stockholm.