Eur J Nutr 38:51–75 (1999)

REVIEW

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The concept of iron bioavailability and its assessment

Received: 26 March 1997
Accepted: 9 October 1998

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Summary In this review a broad overview of historical and current methods for the assessment of iron bioavailability was given. These methods can be divided into iron solubility studies, iron absorption studies, endpoint measures, and arithmetic models.The pros and cons of all methods were discussed. First, studies on in vitro and in vivo iron solubility have been described. The disadvantages of iron solubility include the impossibility of measuring absorption or incorporation of iron. Furthermore, only the solubility of nonheme iron, and not heme iron, can be studied. Second, we focused on iron absorption studies (either with the use of native iron, radioiron or stable iron isotopes), in which balance techniques, whole-body counting or postabsorption plasma iron measurements can be applied. In vitro determination of iron absorption using intestinal loops or cell lines, was also discussed in this part. As far as absorption studies using animals, duodenal loops, gut sacs or Caco-2 cells were concerned, the difficulty of extrapolating the results to the human situation seemed to be the major drawback. Chemical balance in man has been a good, but laborious and expensive, way to study iron absorption. Whole-body counting has the disadvantage of causing radiation exposure and it is based on a single meal. The measurement of plasma iron response did not seem to be of great value in determining nutritional iron bioavailability. The next part dealt with endpoint measures. According to the definition of iron bioavailability, these methods gave the best figure for it. In animals, the hemoglobin-repletion bioassay was most often used, whereas most studies in humans monitored the fate of radioisotopes or stable isotopes of iron in blood. Repletion bioassays using rats or other animals were of limited use because the accuracy of extrapolation to man is unknown. The use of the rat as a model for iron bioavailability seemed to be empirically based, and there were many reasons to consider the rat as an obsolete model in this respect. The double-isotope technique was probably the best predictor of iron bioavailability in humans. Disadvantages of this method are the single meal basis and the exposure to radiation (as far as radioisotopes were used). Finally, some arithmetic models were described. These models were based on data from iron bioavailability studies and could predict the bioavailability of iron from a meal.

Key words Iron – bioavailability

Introduction

The term bioavailability is surrounded by confusion. Many attempts have been made to define an adequate working definition, but still different points of view exist. These may depend partly on the scientific background of the investigator, as has been illustrated by Schümann et al. (233). They stated that in human nutritional sciences the concept of bioavailability was regarded as the efficiency with which nutrients are utilized. In animal nutrition, a similar view exists, although here bioavailability is usually expressed as the nutritive value of the feed to support growth and maintenance of the animals. In pharmacology, bioavailability is considered to be the fraction of a dose that reaches the systemic circulation after oral administration, or the "area under the curve".

When we limit ourselves to the nutritional sciences, it is obvious that bioavailability is a function of at least the digestibility, the absorbability, and the ability to use a nutrient for metabolic functions. Furthermore, bioavailability should be quantifiable. These aspects emerge in the definition of iron bioavailability that is given by Fairweather-Tait (59), according to whom iron bioavailability is a measure of the proportion of the total in a food or diet that is digested, absorbed, and metabolized by normal pathways. An overview of proposed definitions for (iron) bioavailability is given in Table 1.

There is some controversy about whether or not to include storage iron as bioavailable iron. According to some definitions, utilization must be demonstrated, whereas in other definitions terms like metabolizable are used, implicating that the possibility to use a nutrient for metabolism is sufficient proof for its bioavailability. In the case of iron, liver storage iron does not fulfill any metabolic function, but if necessary, it can be used in the future to some extent.

In practice, the working definition of iron bioavailability will be partly determined by the methods available to measure its use or potential use in metabolism. Therefore, it may be more pragmatic to choose one or more parameters that could be used to quantify iron bioavailability. As pointed out by Schlemmer (230), the measurement at some endpoint of the pathway can be regarded as a biomarker for bioavailability.

It is obvious that ultimate iron bioavailability is the result of many preceding steps. These can be divided in three parts. First, the digestibility and more specific, the solubility of iron in digesta is a determinant for its subsequent bioavailability. A second determinant is iron absorption and its delivery to the circulation. The third determinant of iron bioavailability is the processing of iron once it has entered the circulation, and its incorporation into a functional entity. Iron bioavailability is always assessed during one or more of these three steps. In this overview of iron bioavailability assessments, we will deal first with the determination of soluble iron in the gastrointestinal tract or in simulated digesta systems. Then, an overview is given of the methods that are applicable to measure iron absorption in man, animals, and in vitro. We will end this review with a discussion regarding the measurement at different endpoints, with special emphasis on measurements of iron in hemoglobin.

Iron bioavailability should be quantifiable. The best method can be chosen when all advantages and limitations of individual methods are clear. The aim of this review is to describe the development and implementation of methods that have been used and/or are still in use to assess iron bioavailability and to discuss the advantages and drawbacks of them.

Iron solubility studies

It has to be kept in mind that all studies regarding iron solubility deal with nonheme iron. Heme iron is hardly influenced by gastrointestinal conditions because the heme molecule is absorbed as such and the porphyrin ring is split off within the mucosal cells (214, 256, 259).

It is generally accepted that only soluble iron can be absorbed; thus, only a fraction of the soluble iron is bioavailable. Soluble iron can be either in the ferric or in the ferrous form. Ferric iron is rapidly hydrolyzed at a pH >1, whereas ferrous iron does not hydrolyze at a pH below 7. However, ferrous iron is rapidly oxidized to ferric

Table 1 Proposed definitions for bioavailability; references in parentheses

That portion of the total [iron] which is metabolizable (156).
The measure of the proportion of the total in a food or diet that is digested, absorbed, and metabolized by normal pathways (59).
The proportion of the total [mineral] in a food, meal or diet that is utilized for normal body functions (61).
The percentage of ingested [iron] that becomes available for metabolic action (250).
The measure of the ability of man and animals, or the effectivity, by which nutrients, in a given chemical form, are liberated from food in the presence of certain food components. It moreover includes intestinal absorption and transport of nutrients to organs and cells, where they finally fulfill their physiological function (230).

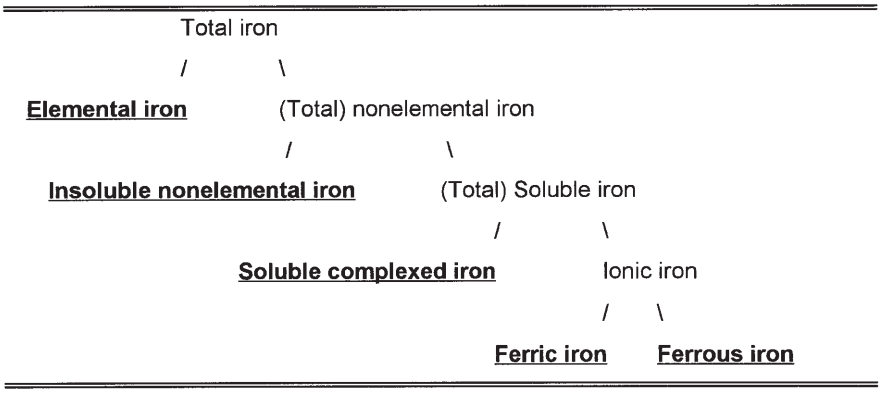

Fig. 1 Chemical iron profile in foods according to Lee & Clydesdale (143).

iron. The hydrolysis of iron, i.e., the formation of iron hydroxides, usually renders the iron insoluble. This may not always happen, because the presence of ligands may lead to the formation of soluble iron-ligand complexes. Thus, two factors are important: iron must be kept in the ferrous form, or a sufficient amount of ligands must be present in order to keep iron in the soluble phase.

Iron solubility can be measured in vitro, usually in systems that simulate the situation in the gastrointestinal tract to some extent. It can also be measured in vivo, by collecting digesta samples from humans or ex vivo in animals.

In vitro studies

Food iron solubility

Early studies on this topic dealt with the determination of available iron in aqueous food extracts. The results were consistently expressed as ionizable iron. Because iron was analyzed with the use of the ferrous chromogen α-α' dipyridyl (235), these methods, in fact, measured the amount of ferrous iron. No attempts were made to simulate the gastrointestinal environment at that time. A more physiological approach was reported by Sanford (226), who introduced a pepsin-HCl incubation and subsequent analysis of ionizable iron in the supernatant. Most studies of this kind used 0.5% pepsin, 0.1 N hydrochloric acid, and an incubation period of 90 min (105, 216). Jacobs & Greenman (123) made another contribution by discriminating between ionizable and total soluble iron in the pepsin-HCl extract. They defined total soluble iron as ionizable iron + heme iron + ferritin + iron bound to other soluble complexes.

The proximal part of the small intestine is the principal site of iron absorption in man (258). At that site, the digesta is neutralized by bicarbonate and pancreatic secretions. Thus, before iron is absorbed it has also faced this environment. The method of Jacobs & Greenman was extended concurrently by adjusting the pH to 7.5 with NaOH and the addition of pancreatin (192).

Many in vitro solubility studies that have been described in the last two decades include an acid digestion with HCl or HCl-pepsin, subsequent neutralization with a base (usually NaOH or $NaHCO_3$) together with pancreatin-bile extracts, and appropriate incubation and centrifugation steps (47, 52, 137, 218). Iron analysis is performed by chromogens, atomic absorption spectrometry, mass spectrometry or, in the case of ^{59}Fe, by γ-counting.

There are many ways to classify the different chemical profiles of nonheme iron. Lee & Clydesdale (143) classified five chemically different forms of nonheme iron that can be present in food (Fig. 1) and described methods for their determination. Chemical iron profiles were determined in a variety of foods like bread (271), milk with cereals (36), and meat samples (212). However, iron solubility characteristics that were found in individual food components could not be extrapolated to plain foods (211).

Methods for the determination of nonheme iron, heme iron, and total iron in foods have been also described and validated (33).

Iron dialysability

A new approach towards in vitro estimation of iron solubility was introduced by Miller et al. (171). They digested a food sample with pepsin and HCl, after which a dialysis bag containing $NaHCO_3$ was added. After incubation, pancreatin-bile addition to the digest, and another incubation period, the iron in the dialysate was analyzed. An important methodological difference with previous systems is the introduction of equilibrium dialysis. Instead of the iron fraction present in the supernatant after centrifugation, dialyzable iron is measured, i.e., soluble iron with a molecular weight less than the molecular weight cutoff of the dialysis tube. Iron availability assessments using this method were highly correlated with human iron availability trials when similar foods were tested (67, 231), and this method has been used frequently (106, 119, 232).

Several adaptations of this method have been reported, an important one being the determination of iron with ferrozine instead of bathophenanthroline disulphonate (32, 35). It was shown that the latter analysis could lead to systematic errors at low pH and in the presence of competing ligands (90). The introduction of continuous sampling of the digesta is another recent extension (268). After digestion, the dialysate and digesta were pooled at the time of pancreatin-bile addition and continuous dialysis was performed by means of a hollow-fibre system. This system more closely mimics in vivo conditions.

Measures using human gastric juice

Bezwoda et al. (16) collected gastric juice from 48 subjects in order to determine iron solubilization from bread in vitro. Iron absorption was measured in vivo in 24 of the subjects, and it was shown that iron absorption from bread in vivo correlated highly ($r = 0.834$) with in vitro solubilization of bread iron when gastric juice from the same subject was used. Lock & Bender (146) measured iron solubility from 20 food samples after incubation with human gastric juice. They found a positive correlation between increasing acidity and the amount of iron solubilized for bran, cocoa powder, and curry powder, but a negative correlation for soybean flour, lentils, egg, and peas. An explanation for this phenomenon was not given. A positive correlation was found between in vitro iron solubility from potatoes versus ascorbic acid contents of these potatoes (58). Interindividual comparisons were not possible, because the gastric juice used was sampled from one subject.

Recently, an in vitro model has been developed which simulates the complete gastrointestinal tract (178). It consists of four compartments, serving as a model for the stomach, duodenum, jejunum, and ileum, respectively. Peristaltic movement, pH, and gastrointestinal secretion can be controlled. The applicability for in vitro iron availability studies has not yet been reported.

Fortification iron solubility

So far, in vitro methods have been described in which the conditions in the stomach and duodenum were simulated. Studies that dealt with the assessment of fortification iron availability were quite different, as the iron compounds under study were usually dissolved in diluted hydrochloric acid and the percentage soluble or insoluble iron was determined subsequently (190, 206, 210, 237). Effects of food and physiological conditions were not taken into account. Unfortunately, these studies are hardly comparable because they all differed in HCl concentration, pH, incubation temperature, incubation time, and method of iron analysis.

In vivo studies on iron solubility

Iron in gastric and/or intestinal contents can be analyzed directly. This type of study has been performed mostly in rats. Usually, the diets under study were fed and after 2 to 3 h the gastrointestinal tract was removed, and the contents centrifuged in order to obtain a soluble and an insoluble phase. The relative amount of iron in the soluble phase is considered a determinant for iron bioavailability. The effects of egg yolk protein (227), carbohydrates (27, 251), and calcium carbonate (263) on intestinal iron solubility have been reported.

Another approach is to classify the soluble-phase iron either by molecular weight or by its chemical nature. The former can be achieved chromatographically for protein-bound iron complexes in rats (128) or by ultrafiltration, as has been done in chick studies (236). Separation of iron on a chemical basis can be achieved by treating the soluble phase of the digesta with ferrozine, in order to measure the proportion of ferrozine-available iron, as has been described in mice (241). These authors recently introduced a sequential extraction procedure, which was applied to rodent diets and rat digesta (203, 242). The extracted iron was analyzed and the pellet subjected to the next extraction step. After five extractions, exchangeable, carbonate-bound, oxide-bound, organic-bound, and residual iron fractions were obtained, respectively.

Pros and cons of iron solubility studies

The most important limitation of every iron solubility study that is used to predict iron bioavailability is its unability to pronounce upon subsequent absorption and incorporation of iron. Moreover, no methods are available to study heme iron bioavailability by in vitro solubility.

The vast majority of iron solubility studies are based on in vitro models. The usefulness of in vitro solubility methods for the prediction of iron bioavailability has been reviewed by Miller & Berner (172). Generally, in vitro models offer the possibility to optimally control the conditions of the experiment, which may lead to a high accuracy. An additional advantage is the absence of possible disturbing effects, like interindividual differences in iron status, resulting in a lower variability of in vitro methods as compared to in vivo methods. Other advantages comprise its speed, the low cost, and the replacement of animal experiments.

On the other hand, in vitro models are not physiological. The development of these models during this century can be characterized as a continuous effort to come as close to the physiological process as possible. In vitro models for iron solubility evolved from measurements in aqueous extracts, pepsin digestion, pancreatin digestion, and the introduction of dialyzability, to systems that closely simulate in vivo conditions. However, several fac-

tors like in vivo effects of transit time, enzymes, pH, and diffusion barriers can not be accounted for sufficiently (172).

Surprisingly few studies have been published that use human gastric juice for the determination of iron solubility in vitro. These can be considered intermediate between in vitro methods using pepsin and pancreatin and methods that study in vivo solubility in digesta. These studies are less standardized but come closer to in vivo conditions.

Digesta obtained from an in vivo experiment give the best approach to in vivo conditions, but are most susceptible to variation. As they are performed in rats or other animals, a proper extrapolation to humans may be difficult.

It can be concluded that in vitro methods based on iron solubility or dialyzability are useful tools in studying factors that may affect subsequent iron absorption. The absence of interfering factors offers a chance to study determinants of iron solubility in detail. This approach may obtain a better understanding of the processes that take place in digesta. Since iron solubility methods cover only the first phase of the iron bioavailability process, they can be considered a qualitative measure at the most.

Iron absorption studies

Several methods can be used for the determination of iron absorption. These can be divided on the basis of the physical type of iron that is used. First, we will discuss methods that use native iron, that is, without enrichment of one of its isotopes. Then, the application of two radioisotopes of iron, ^{55}Fe and ^{59}Fe, will be discussed. Finally, we will describe methods in which preparations are used that are enriched in stable iron isotopes (^{54}Fe, ^{57}Fe, and ^{58}Fe).

Absorption measurements using native iron

Chemical balance

In chemical balance studies, the amount of iron that is absorbed or retained by the body from different preparations or foods is measured indirectly. This results in a figure for the apparent iron absorption which can be calculated from the following equation:

apparent Fe absorption (mg/d) = Fe intake (mg/d) – fecal Fe (mg/d)

Theoretically, some of the iron that is absorbed will be lost by urinary excretion. As this fraction of iron is absorbed but not retained, a figure for iron retention can also be calculated:

apparent Fe retention = Fe intake – fecal Fe – urinary Fe (all expressed in mg/d)

Urinary iron excretion is very low. Thus, apparent iron absorption will practically equal apparent iron retention.

Apparent iron absorption measurements by way of a chemical balance are easy to perform in small animals, as they can be housed in metabolic cages, which enables a quantitative and separate collection of excreta. Iron balance studies in larger animals are also possible, but care has to be taken in order to collect the excreta quantitatively.

Early studies on iron bioavailability in humans depended almost solely on chemical iron balances. At present, iron balances are mainly used because many nutrients can be measured in the same samples, a reflection of iron absorption from the whole diet during a prolonged period can be obtained, and the use of radioactivity can be avoided (118, 122, 221). Complete fecal collections can be ensured by using quantitative fecal markers like chromic oxide, polyethylene glycol or radio-opaque pellets.

Postabsorption plasma iron measurements

In pharmacology, drug bioavailability is usually measured in plasma by determining either the area under the curve or the maximal concentration of the drug after administration. This method has been used in humans to study the increase in plasma iron after oral iron administration. Early studies originated from clinical interests in the treatment of anemia (108). Soon thereafter, studies appeared in which the primary goal was to compare the increase in plasma iron after administration of different iron compounds (185, 186). Most studies that use postabsorption plasma iron curves as a measure are performed in order to answer pharmacological questions, like determination of the bioavailability of iron supplements as such (50, 234) or to study the effect of drugs like antacids (51, 200) or pancrelipases (272) on iron bioavailability. Only a few human studies exist in which changes in plasma iron were induced by dietary iron or meal components given together with iron (167, 208). Nutritional studies that used plasma iron increase as a response criterion have been performed in rats. Kim & Atallah (131) compared the effects of different pectins on plasma iron levels in portal blood samples and found that these were highly correlated with calculated absorbed iron. A rat study in which the effect of different protein sources was compared yielded high correlations between plasma iron in portal blood versus both intestinal iron solubility and apparent iron absorption (132).

Postabsorption plasma iron measurements are also applicable when radioiron or stable isotopes of iron are used. Results thereof are discussed below.

Absorption measurements using radioiron

Radioiron in feces and urine

During the first 20 years after the discovery of radioiron, two methods were available to assess its absorption. One measured the amount of radioiron that was incorporated in blood. It will be discussed later because it is considered as an endpoint measurement of iron bioavailability. The other method was based on radioactivity measurements in excreta. Feces and urine were collected after oral administration of radioiron, and from the difference between the ingested and the excreted dose of radioiron the apparent absorption could be calculated. Thus, this technique is nearly similar to the chemical balance technique, except that possible endogenous iron losses are not taken into consideration. Feces and urine were analyzed for radioiron for the first time in a study using rats (10). Its use for studies in humans (179) and rats (58, 244) continued. As long as feces can be collected quantitatively, the calculation of ^{59}Fe absorption as the difference between ingested and excreted radioiron will provide a reliable figure. In rats, iron absorption calculated by this difference versus iron absorption calculated from ^{59}Fe in blood showed a close correlation (115). In non-anemic rats, however, correlations may be weakened because a variable amount of the absorbed ^{59}Fe will be stored and ^{59}Fe measurements in blood will then not necessarily reflect the amount that is absorbed.

Radioiron in digesta

Occasionally, ^{59}Fe absorption is calculated after oral supply of a known amount of ^{59}Fe to rats that are subsequently killed before any fecal ^{59}Fe emerges. The gastrointestinal tract and its contents are removed and counted for iron. Apparent iron absorption is calculated by subtracting the activity in gut and gut contents from the applied dose (137, 239). The resulting figure for iron absorption was reported to be comparable to iron absorption as calculated from 7-day fecal excretions (23). However, the diet fed may affect the gastrointestinal transit time, which in turn may determine the outcome. Iron absorption will be underestimated when collection takes place too early. It has been reported that gastric clearance of ^{59}Fe containing diets in rats was affected by diet composition and iron status (28).

Whole-body counting

A new approach towards the study of radioiron absorption was introduced by the use of whole-body counting. This was reported in rats (63), and the method was also applied in man (252, 213). In general, ^{59}Fe in an iron preparation or food is orally administered and after a short time (usually 1 h) a whole-body count is made. The resulting count rate is regarded as the 100% value. The amount of ^{59}Fe in the body will subsequently decrease as a result of fecal excretion. After sufficient time (5 to 7 d in rats, and 10 to 14 d in humans) a steady state is reached. The accompanying count rate, divided by the 100% value and corrected for radioactive decay is a direct measure for retained ^{59}Fe. Only ^{59}Fe can be used for whole-body counting, as ^{55}Fe does not emit γ rays.

In humans, a simultaneous comparison of ^{59}Fe absorption by means of fecal ^{59}Fe recovery versus ^{59}Fe retention as measured by whole-body counting was made by Lunn et al. (148). From their results, it can be calculated that both assessments of iron absorption were closely correlated ($r = +0.969$; $n = 16$). However, when iron absorption was low, a discrepancy between both methods emerged as absorption according to fecal recovery was always higher than whole-body counting. The authors believed this difference to be the result of incomplete feces collection, but it is more likely that the use of different counting methods and equipment was a major source of error. In a study using iron-deficient rats, an excellent correlation ($r = -0.995$) was found between whole-body counting and counting of feces for ^{59}Fe (12).

In man, whole-body counting is now preferred to counting of fecal collections, because the former provides a direct measurement of the amount of ^{59}Fe in the body, and it does not demand the careful collection of feces. Numerous studies using food iron (109, 9) or iron preparations (195, 166) have been performed using this approach. In small animals, whole-body counting is easily performed. Rats are most widely used (255, 263), but studies in mice (135), guinea pigs (14), chicks (31), and rhesus monkeys (49) have also been reported.

Mucosal uptake and mucosal transfer of iron

With the use of radioiron it is possible to distinguish between two steps of iron absorption, viz. mucosal uptake and mucosal transfer. Wheby & Crosby (257) showed in rats that the intestine initially takes up more iron than is ultimately transferred to the plasma. When the epithelial cells exfoliate, this iron is defecated, yet with some delay. Boender & Verloop (22) developed a method using ^{59}Fe and a second, non-absorbable isotope, i.e., ^{131}Ba, in order to quantify in a non-invasive way the amount of ^{59}Fe that was initially taken up by the mucosa, but not ultimately absorbed. In man, the amount of ^{59}Fe that was initially absorbed was calculated from the ratio ^{59}Fe:^{131}Ba in both the test dose and the feces of the first days. This method has been adapted in order to measure by whole-body counting (163, 164). The method has also been used for veal calves (177).

True iron absorption

Apparent ^{59}Fe absorption does not account for any endogenous iron losses and may underestimate true iron absorption. For some minerals, like zinc and manganese, en-

dogenous fecal losses may be considerable, because the homeostatic regulation of these minerals is partly dealt with by excretion via the feces. A method for the determination of true absorption was published by Heth & Hoekstra (113). They used ^{65}Zn, but the procedure can be used for ^{59}Fe as well. Two groups of rats are needed. In one group the isotope is administered orally, while in the other group the isotope is injected intravenously. The retention in both groups is measured by whole-body counting. After 2 to 4 d both measures will follow a simple exponential curve of time versus isotope retention. The retention curve of the injected isotope is a measure for endogenous isotope loss. Extrapolation of both curves to zero time gives two intercept values, whose ratio is a measure for the true absorption (113). This method, which has been referred to as the comparative balance technique, has been applied to study the true iron absorption in rats and guinea pigs between 1 and 200 d of age (193). The whole-body retention of the radioiron injected was 84% in rats 1 d of age, and 90% or more when the animals were 10 d and older. In the guinea pig, injected radioiron retention always exceeded 92%. Thus, endogenous iron excretion in these species is low.

Another approach for the determination of true iron absorption is the so-called isotope-dilution technique. True absorption is estimated indirectly by assessing the endogenous fecal excretion after ^{59}Fe injection linked to a chemical balance in the same animals. The key assumption is that the specific activity in fecal ^{59}Fe is equal to the specific activity of body ^{59}Fe during the period of investigation. The latter can be estimated in an organ or a blood sample. This technique has been applied in rats that were fed different amounts of iron, and it was shown that iron homeostasis was not controlled by endogenous iron excretion (136). Apparent iron absorption will, therefore, match true iron absorption in most cases.

Postabsorption plasma radioiron measurements

Radioiron has been used just like native iron to measure postabsorption plasma iron responses. By using radioiron instead of native iron, large diurnal variations in plasma iron can be corrected.

Crofton et al. (48) studied in humans the effects of zinc on plasma iron response and found close correlations between ^{59}Fe whole-body retention versus the amount of ^{59}Fe that was transferrred to the plasma in 6 h ($r = 0.77$), or the total iron area under the curve at 3 h ($r = 0.64$). The latter correlation became non-significant after 6 h ($r = 0.54$). Ekenved et al. (56) compared directly the increase of serum iron versus ^{59}Fe retention as measured by whole-body counting after administration of ferrous sulphate solutions. A close correlation ($r = 0.86$) was found between both methods. It was conluded that serum iron determinations were satisfactory for semiquantitative comparisons between iron preparations, but could not be used to determine the absolute amount of iron absorbed from an oral dose (55).

Absorption measurements using stable iron isotopes

Native iron is a mixture of four isotopes with atomic masses of 54, 56, 57, and 58. Their natural abundance is 5.82, 91.66, 2.19, and 0.33%, respectively. It is possible to increase the fractions of ^{54}Fe, ^{57}Fe and/or ^{58}Fe. The resulting preparation is called a stable isotope of iron. Stable isotopes do not emit radiation and can only be measured on the basis of their mass. Therefore, whole-body counting is impossible and the only way to study iron absorption from stable isotopes is by fecal balance. Stable isotopes can also be measured in blood samples, which will be discussed in the section on endpoint measurements. Samples can be analyzed either after transmutating them to radioisotopes by neutron-activation analysis (NAA) and measure these accordingly, or directly by mass spectrometry. Corrections for the natural abundance of the stable isotope always have to be made.

One of the first studies was reported by King et al. (133). Several studies that investigated the usefulness of stable isotopes of iron in human absorption studies have been published (124, 60). They all used the fecal balance technique and the samples were analyzed by NAA. Mass spectrometric methods for the determination of ^{54}Fe (126) and ^{58}Fe (54) have been published. Currently, TIMS (thermal-ionization mass spectrometry) and ICP-MS (inductively coupled plasma mass spectrometry) are used frequently. Instruments available for mass spectrometrical analysis have been reviewed recently (270).

The main advantage of the use of stable isotopes for iron absorption measurements is the absence of radiation. Therefore, they are especially useful in studies in infants and pregnant women.

In situ and in vitro assessment of iron absorption

Several techniques have been described that study iron absorption in isolated parts of the small intestine. These may be classified according to the level of structural integrity of the intestine that is maintained after isolation. Iron absorption can be studied in situ with the use of an isolated loop of intestine, so that blood circulation in the loop is maintained. The loop can also be dissected and connected to a circulation unit. Another method is to close a dissected loop either right side out or everted, or to use a longitudinally opened dissected loop as a separating sheet of tissue in a two-compartment model. Also, brush border membrane vesicles can be prepared to study iron absorption. For a review of iron absorption studies using intestinal segments see Forth & Rummel (71).

A completely different in vitro approach is the use of cell lines to study iron accumulation and transport.

Intestinal loops in situ

It is possible to inject a known amount of radioiron into a tied-off intestinal loop with intact blood supply. By measuring the gut loop, its contents, and the remaining carcass for radioactivity after incubation, iron absorption can be estimated (159).

Instead of creating an intestinal loop by way of ligating a segment and measuring its contents after incubation, perfusion methods of an isolated intestinal segment have also been described (121). This appears to be a refinement in terms of extrapolation, as in vivo conditions are simulated more closely with continuous perfusion as compared to the more stagnant condition in a non-perfused ligated loop. A system in dogs has also been described that made use of a duodenal and jejunal cannula, thus, creating a loop of about 1 m (62). Test solutions could be perfused in the former cannula, and the effluent could be collected in the latter.

Studies using intestinal loops are mainly performed in rats, but studies in mice (240), chicks (224), and dogs (53) have also been reported. By using intestinal loops, possible sources of variation like gastrointestinal secretions can be eliminated, and other determining factors like luminal pH can be controlled.

Isolated intestinal loops, sheets or sacs, and brush border membrane vesicles

The use of adequately oxygenated, isolated intestinal loops was first described by Fisher & Parsons (64). A rat intestinal loop was perfused with a buffer solution by means of a circulation unit and subsequently isolated. Fluids were circulated both through and around the intestinal segment. The viability of isolated segments is limited, therefore, absorption can not be studied longer than a few hours. The feasibility of this method for iron absorption studies was shown by Forth & Rummel (68).

An isolated intestinal loop can be excised longitudinally so that an intestinal sheet is obtained. By mounting this sheet in a so-called Ussing chamber a two-compartment model is obtained. In studies concerning iron absorption, it has been used occasionally (110).

When a gut sac is prepared from an isolated intestinal segment, this can be done either with the right side out, or everted, that is, with the serosal side positioned inside the sac. The method was introduced by Wilson & Wiseham (266), who used rats and hamsters. With respect to this method, effects of age (223), iron dose (69), and phenobarbital pretreatment (247) on iron absorption have been reported. In all cases, only a small percentage of iron can be found at the serosal side after incubation, which makes it difficult to draw conclusions in a quantitative way.

The mucosal brush border plays a part in iron absorption, but its exact role is still not known. Studies have been performed in which the brush borders were isolated and studied separately in an attempt to gain insight in the accompanying mechanisms. Brush border membrane vesicles can be used to study possible iron uptake mechanisms and competition with other ions (165), but their use in iron bioavailability assessment is limited.

Iron accumulation and transport in intestinal cell lines

Cell culture models are increasingly being used for the study of uptake and transport of iron, and the Caco-2 cell line is without any doubt the most popular tool in this regard (99, 225). This cell line originates from a human colon adenocarcinoma, it differentiates spontaneously and exhibits many features of small intestinal cells, including polarization, the formation of brush border microvilli, and the presence of brush border associated enzymes (204). The cells can be cultured on microporous membranes, so that a two-compartment model could be created (114, 98). Since then, Caco-2 cells have proved their usefulness in intestinal iron uptake and transport studies. Caco-2 cells mimic the effects of in vivo food iron absorption in many ways. The cells show a higher uptake and transport from ferrous as compared to ferric iron (1). It was shown that ferric iron had to be reduced before it could be taken up by the cells. This could be achieved both by a postulated ferrireductase at the apical side of the cells, and/or by reducing agents like ascorbate (197, 104). Iron uptake and transport were inhibited by inositol phosphates (102), and this inhibition could be overcome by ascorbate (103). Calcium addition also inhibited iron accumulation and transport in Caco-2 cells (265).

Recently, some studies have been reported that combined in vitro solubility or dialyzability studies with the use of Caco-2 cells. Garcia et al. (83) added supernatants from homogenates of different foods on top of Caco-2 monolayers and measured cell iron uptake. Qualitatively, the results were in good agreement with human studies, as supernatants from meat homogenates resulted in significant higher uptake as compared to soybean protein, egg albumin, and bovine serum albumin. A combination of in vitro dialyzability and subsequent measurement of iron uptake by Caco-2 monolayers was reported by Gangloff et al. (82) and adapted by Glahn et al. (86). The Caco-2 cells were cultured in six-well plates. For uptake experiments, an upper chamber was created by using a dialysis membrane as the bottom of an insert. A peptic-pancreatic digest was put on top of this membrane. The soluble ^{59}Fe in the digest was able to diffuse through the membrane into the lower compartment and could subsequently be taken up by the cells. The dialysis membrane prevented damage to the cells from the digestive enzymes.

HT-29 is another colonic cell line that is occasionally used for the study of iron metabolism. In particular, clone 19A of this cell line has been used for the study of transepithelial transport, including transport of lactoferrin (169).

Among the nontransformed small intestinal cell lines, IEC-18 may be a useful model for transport studies. These cells are derived from native rat ileal crypt cells and have a transepithelial electrical resistance that unlike Caco-2 cells resembles that of the small intestine. It is possible to culture these cells on microporous membranes (150), but transport or uptake of iron by these cells has not been reported yet.

Finally, IEC-6 cells have been shown to take up iron (194). IEC-6 cells have a finite life-span and were originally isolated from rat small intestine. They can also be cultured on microporous membranes.

Pros and cons of iron absorption studies

The terms iron absorption and iron bioavailability are frequently exchanged. Both terms usually match closely, but they are not totally similar, as iron bioavailability takes the potential use in the body into account, whereas iron absorption does not. The classical way to determine iron absorption in humans is the chemical balance with the use of native iron. Advantages of this method include: (1) study of the whole diet (although this can be considered a drawback when the effect of a single food item is to be assessed); (2) study for a prolonged time; (3) no exposure to radiation; (4) possibility for analysis of several nutrients simultaneously. The first two points may be important, as studies with radioisotopes or stable isotopes are usually based on a single test meal. They deviate from the normal situation in that a single meal is applied after an overnight fast, and subsequently, the subjects have to fast again. In addition, single meal studies give only an indication of the iron absorption from a particular diet at one moment. It has been suggested that single meal studies may overestimate iron absorption as compared to absorption from a daily diet (45), and this has been corroborated recently in man (248). Disadvantages of chemical balance studies include: (1) a limited number of participants, because the studies are expensive and very laborious (collection and analysis of duplicate meals, quantitative excreta collection); (2) large margin of error, because net retention is only a small fraction of iron intake and excretion figures; (3) no correction for endogenous iron excretion, although this factor is often of minor importance as endogenous losses are low. It is crucial that excreta be collected quantitatively. If not, iron absorption will be underestimated to a significant extent. As an example, let's assume a study in which a dietary iron intake of 15 mg/d is measured, with a fecal excretion of 14 mg/d, and a balance period of 2 weeks. When only one day's feces is missing, a net iron absorption of + 2 mg/d will be calculated, which is double the actual iron absorption.

With the use of radioiron or stable isotopes, the analysis of a balance in humans is easier and more accurate. However, fecal collections are still imperative. Whole-body counting does not have the drawbacks of cumbersome and possibly incomplete collection of excreta, but radioiron is needed and the limitations of single meal studies still exist when using this type of study.

Chemical balance studies in animals are easy to perform. Another advantage as compared to the use of human subjects is the uniformity in iron status that can be obtained. The most important drawback is the questionable accuracy of extrapolation from the animal model to humans.

The use of postabsorption plasma iron curves has important limitations. The kinetics of iron absorption may differ between iron preparations, which may lead to a misinterpretation of the results. An iron preparation that is absorbed slowly will result in a lower maximal plasma iron increase and a smaller area under the curve when the time course of the measurement is limited. This was suggested to occur in rats for ferric hydroxide-carbohydrate complexes (84). When native iron is used, no correction for the large diurnal variation in plasma iron can be made. The method can only be used within a narrow dose range, because a high iron dose may saturate plasma transferrin so that the surplus of iron will be deposited in the liver during first passage. In that case, total iron binding capacity will be measured. On the other hand, if the dose is too low, the plasma iron response will not emerge from the basal variations in plasma iron. In sum, plasma iron responses may be useful in a clinical setting when comparing the pharmacological bioavailability of different iron preparations, provided that the pharmacokinetic characteristics of these preparations are known. For determination of the nutritional bioavailability of iron, the method does not seem to be of great value.

Intestinal loops, gut sacs, and other derivatives have been useful to study mechanisms at the site of absorption. Interaction effects of iron and other elements can be demonstrated with the use of these models. However, they do not seem to have special advantages for the study of iron bioavailability, and as these models use animals, extrapolation to humans is a problem. Caco-2 cells may have an advantage in this respect, as they are from human origin. The results obtained until now show a remarkable similarity to human studies. The method is relatively new and more research will be needed to confirm the similarities for a broad range of substances. With the use of Caco-2 cells, it may be possible to pick up intramucosal effects of dietary factors that interfere with iron transport, which can not be done with the use of in vitro solubility studies. The Caco-2 cell system may also become a tool in the study of heme iron metabolism. Caco-2 cells contain heme oxygenase and its activity can be increased after exposure to heme (29). However, it is not known if the intestinal heme-iron receptor is present on these cells. Disadvantages of Caco-2 cells include: (1) the transformed nature of the cells, as they are derived from a colon carcinoma; it remains questionable to what extent normal metabolic processes are maintained in these cells. The following disadvantages relate to this: (2) the absence of

a mucin layer, which may play a significant role in intestinal iron absorption (37); (3) the transepithelial resistance is much higher than in human small intestine and resembles that of human colon; (4) low carrier expression, resulting in very low transport rates, so that a scaling factor may be required (144). This last point may apply also for iron transport, since it has been repeatedly shown that iron transport through Caco-2 monolayers is low.

In summary, a chemical balance study using native iron resembles best the normal situation, but is very difficult to perform accurately in humans. Stable isotopes are an improvement in terms of accuracy of determination, but they are based on a single meal and require fecal recovery. Radioisotopes also lead to an accurate measure of fecal recovery, but they cause radiation exposure. Besides, complete collection still can not be assured by these methods. Use of animals avoids some of the drawbacks, but introduces the problem of species differences. Whole-body counting is a direct and possibly the most reliable measure for iron retention, but radioactive ^{59}Fe is needed. The use of plasma iron response as a measure for iron bioavailability is limited, as well as in vitro models based on intestinal loops or gut sacs. Caco-2 cells are a promising tool, in that they might combine in vitro solubility methods with interactions at or in intestinal cells.

Endpoint measurements

The hemoglobin-repletion bioassay

The rat hemoglobin (Hb)-repletion bioassay is the recommended method according to the Association of Official Analytical Chemists (8). Young, male rats are depleted of iron by feeding an iron-deficient diet, so that anemia develops. After at least 4 weeks, they are divided in comparable groups and receive diets containing the iron compound under study in three different concentrations. The Hb repletion is measured relative to the reference source of iron, i.e., ferrous sulphate, which is fed also to three groups of rats. The level of Hb increase will be the result of both the dietary iron concentration and the dietary iron source. Hb values are measured after two weeks of repletion. By plotting these values against the dietary iron concentrations for each iron source, two lines are obtained. The ratio of the slopes gives a quantitative measure for the bioavailability of the iron compound under study. The relative biological value (RBV) of the iron source is expressed relative to the effect of ferrous sulphate.

The method was originally developed for the study of iron sources for fortification purposes. Several variations on this assay have been used. Moreover, many suggestions have been made as to improve the quality of the measurements. Some of these will be discussed in this section.

Effect of strain, sex and species

The AOAC method does not specify the rat strain that should be used. This may lead to variation between rat bioassays. Rao & Jagadeesan (217) compared the dietary induction of anemia between Fischer 344, Wistar, and Sprague-Dawley rats and found that the Sprague Dawley strain was less susceptible to iron deficiency anemia. After feeding an iron-deficient diet for 6 weeks, the mean Hb values for both Sprague-Dawley and Wistar rats were still higher than the maximum permitted Hb value for entering the bioassay. No information is available as to the relative speed of Hb repletion between rat strains.

Female rats showed a higher Hb gain than male rats, which implies that direct comparisons should be limited within the same sex (243). However, when food intake was equal, no sex difference was observed (249). Chapman & Campbell (34) found a significant effect of sex on liver iron contents in rats that were recovering from iron-deficiency-anemia.

The rat is the recommended species, but many bioassays have been performed with other animals. In early proposals of the AOAC method, the use of either chicks or rats as test animals was suggested (206, 207). As comparable responses in two different species would increase the reliability of the method, both species were used alternately (73). The agreement between the responses in chicks and rats was generally good (2). After close examination of the data, however, it was found that chicks were more able than rats in utilizing reduced iron with a large particle size (209, 189), possibly as a result of the grinding action in the gizzard of the chick. Tests with reduced iron preparations in human volunteers showed no plasma iron response when large-particle reduced iron was given. Thus, rats resembled humans closer than chicks in this respect, and rats were regarded the preferred test animal (209). The effects of iron salts (sodium iron pyrophosphate and ferric ortho-phosphate) were compared for rats, chicks, and humans, and in this case it was found that the results for chicks were in closer agreement with the results for humans (202). It seemed that rats were more comparable to humans when reduced iron preparations were used, whereas chicks responded like humans in the case of iron salts. In these studies, the RBV for humans was obtained by measuring the increase in plasma iron 2 h after ingestion of the iron preparation under study. In a collaborative study, the bioavailability of electrolytic iron and ferric ortho-phosphate were compared both in humans and in rats (67). The Hb repletion rat bioassay served as a good predictor for both compounds, when compared to the results for humans. In this case, the RBV in humans was measured with a double-isotope, extrinsic tag technique. Possible advantages when using chicks are the ease of rendering chicks anemic, which can be achieved in one week, as well as the absence of coprophagy, which may be a disturbing factor

in rats, dependent on the iron source that is used (215, 274). Pigs have been used in Hb repletion assays as well, with promising results (78, 116). The similarity of gastrointestinal anatomy and physiology between piglets and humans makes it an attractive model.

Hb repletion studies in human subjects have been reported in a study involving ten healthy men. Iron deficiency was induced by way of phlebotomy and dietary iron absorption was studied for 5 months by Hb increase (196, 199). The use of Hb repletion made it possible to study the bioavailability of dietary iron for a prolonged period. However, the method is not commonly used because of its invasive nature, the small number of subjects that can be studied, and the large individual variation.

Effects of dietary background

Iron bioavailability can be affected by the diet in which it is incorporated, as enhancing and inhibiting dietary factors will influence it to a huge extent (for reviews see 94, 188). Because of this, the diet to be mixed with the iron source under investigation has been prescribed (8). Differences in diet composition will induce changes in reponse (4, 202), although the qualitative effects of treatments may be maintained (73, 74). It has been shown that purified versus natural-ingredient diets resulted in a higher increase in several iron status parameters in rats (264).

Length of the depletion period. Curative and prophylactic assays

According to the AOAC method, rats have to be depleted by feeding iron-deficient diets for at least 28 d, but shorter depletion periods have been reported also (174, 176, 15).

Some studies reported the combined use of an iron-deficient diet and bleeding at regular intervals (70). Flanagan et al. (65) compared in mice the effects on metal absorption of iron deficiency induced either by bleeding or by a low-iron diet. It was found that a low-iron diet induced the intestinal metal transport system to a higher extent, and for a longer period.

The main objective of depletion is to standardize the iron status of all rats to ensure a uniform response to iron. Therefore, the depletion period can be shorter as long as iron status is comparable. The ultimate reduction of the depletion period would be its elimination. In that case, the rats are fed the experimental diets immediately after weaning. This test has been called the prophylactic assay, opposed to the “curative” AOAC method, in which rats are first rendered anemic.

The response to different iron sources by using a prophylactic assay is studied similar to the AOAC method. Comparisons between both methods have been made (3, 77, 176, 189, 201). Amine & Hegsted (3) studied iron sources and food products containing iron in different concentrations. Although they did not make direct comparisons, i.e., there were no compounds tested using both assays, some of the dose-response curves that were obtained in the prophylactic assay were significantly different when comparing the expected with the actual intersection of the lines, that is, without any iron addition. According to the authors, this could have been caused by additional effects of the compound under study. For instance, when the compound studied contains not only iron, but ascorbic acid as well, there will be an additional effect of the latter, resulting in a deviation in the interception point. For the study of simple iron salts or concentrated iron sources, both assays were expected to be appropriate, as no significant differences between both assay types were found in rat studies (189, 201). Fritz et al. (77) reported that the prophylactic test agreed with the Hb repletion bioassay, but that the length of the prophylactic test may be critical. Results for a 4-week assay agreed with the results of the repletion test, whereas those for a 3-week assay did not. Miller (176) compared the RBV of egg yolk iron in rats by using both assays. An 11-d curative assay resulted in higher RBV’s as compared to a 21-d prophylactic assay. When ascorbic acid was added to the diets, this difference disappeared. Thus, the effect of ascorbic acid addition on RBV was larger in the prophylactic than in the curative assay. It was concluded that the repletion assay was more sensitive because the rats used dietary iron more efficiently, whereas the prophylactic assay was more discriminatory.

As mentioned earlier, the main objective of the introduction of a depletion period is standardization of iron status. This implicates that a depletion period is not necessary, if the iron status of the rats at the start of the study can be considered similar. The prophylactic assay has two advantages: studies can be performed in less time, even in the case of a 4-week assay, whereas the absence of a depletion period will cause less discomfort to the animals.

Length of the repletion period

The outcome of iron bioavailability assessments depends on the length of the repletion period. The curve that describes the increase in Hb as a function of dietary iron intake appears to be sigmoid-shaped and, therefore, becomes flat with time. Comparisons must be made from the linear part of the graph. Thus, total dietary iron intake, or more specifically, the total amount of iron that is available for the animal, determines the outcome of the measure. Miller (174) obtained close dose-response correlations when animals were repleted for 7 or 11 d, corresponding with an iron intake of 1.5 to 4 mg, whereas a 3-d repletion period proved unsatisfactory. In an evaluation using data from four studies, this author found

that the response of Hb was linear up to an intake of 7 mg of iron as ferrous sulphate (175). This corroborated other studies, in which Hb gain was linear for up to 4 weeks, at which time 7.04 mg of ferrous sulphate was consumed by the highest dose group (46). Ranhotra et al. (216) found that ferrous sulphate had more a pronounced effect on Hb regeneration after 15 d than after 30 d, during which periods 4.4 and 9.4 mg of iron were consumed, respectively. Wienk & Beynen (264) also found more pronounced differences between treatments after 7 versus 15 d of repletion (during which periods 7.3 versus 20.5 mg iron was consumed).

Criteria of response in repletion bioassays

Hb concentration after repletion and Hb concentration gain

According to the AOAC method, the Hb value after two weeks of repletion is the parameter of response for iron bioavailability. The use of this parameter dates from the 1920s and 1930s (180, 57). The Hb gain during the repletion period can also be used instead of the final Hb value (76).

Several other response criteria have been compared to Hb concentrations and the most important will be discussed hereafter.

Gain in total Hb and gain in total Hb iron

Hb concentration and Hb gain do not take into account any difference in body weight gain. However, different body weights will coincide with different blood volumes. Response criteria that include not only Hb concentration, but also body weight, by using the product of both, have been used (238, 89). Total Hb gain, expressed in g, can be calculated when blood volume is estimated as a percentage of body weight, and the initial and final body weights are known. Blood volume in growing rats was about 7.5% of body weight, both in animals that were anemic or recovering from iron-deficiency anemia (260), but other values, ranging from 5.5 to 8.1%, have been used, too.

Iron bioavailability can be quantified specifically as the amount of Hb iron that is gained during the repletion period (7, 153). For this purpose, assumptions for both the blood volume of rats and the amount of iron in Hb have to be made. The whole-body amount of Hb iron can then be calculated (6) using:

whole-body Hb-Fe (mg) =

Hb (mg/l) × body wt (kg) × Fe content of Hb (%) × blood volume (% of body wt)

Hb contains 0.335% (w/w) iron. Both the initial and the final amounts of Hb iron can be calculated using this formula.

Hb regeneration efficiency (HRE)

When an iron supplement is studied by the use of a repletion bioassay, the dietary background should be similar. However, when iron bioavailability from different food sources is to be assessed, the diet can not be similar. Despite equal iron concentrations, dietary iron intake will differ between groups if dietary intake is different. To minimize potential interference of this factor a correction for different iron intakes can be made (153) by dividing the whole-body Hb-iron gain by the amount of iron that is ingested. The resulting figure is called HRE (156):

$$HRE_{day\ x} = \frac{\textit{whole-body Hb-Fe}_{day\ x}\ \textit{(mg)} - \textit{whole-body Hb-Fe}_{day\ 0}\ \textit{(mg)}}{\textit{Fe intake}_{day\ 0\ to\ x}\ \textit{(mg)}}$$

The calculation of the amount of whole-body Hb-iron has been given in the preceding paragraph. A linear response between Hb-iron gain versus daily iron intakes of 0.2 up to 0.6 mg was found, indicating that the efficiency of conversion of dietary iron into Hb was not affected by dietary iron level (154). However, it is important to choose the length of the repletion period such that a total dietary iron intake of about 7 mg is not exceeded in order to guarantee a linear response. Occasionally, dietary iron concentration instead of iron intake was used as denominator (176), which will diminish the accuracy of the assessment if diet consumption differs between rats.

HRE was unaffected by differences in growth response as induced by adding increasing amounts of fat to the diet (155). Results regarding the effect of a restricted feed intake on HRE are controversial. Mahoney et al. (158) found no effects after feeding 60% of the ad libitum amount, whereas Miller (173) found an increased HRE after feeding 83% of the ad libitum amount of diet.

Hematocrit (Ht)

In early studies concerning the Hb repletion assay in chicks it was reported that the Hb response to graded levels of ferrous sulphate was more consistent than the Ht response (206), although the differences between both measures were small. Hb and Ht are not always completely interchangeable (7, 130) but usually give similar results (2, 127, 15).

Free erythrocyte porphyrin (FEP)/Hb ratio

The ratio of FEP to Hb is occasionally used as an indicator for anemia. FEP is high in iron deficiency (26). FEP/Hb shows a decrease with increasing Hb values during the repletion period (81), but this ratio does not seem to give any additional information.

Iron in liver and other organs

The amount or the concentration of liver iron as affected by different dietary regimens has often been determined (134, 107, 100) for assessing iron bioavailability. Iron in other organs has been used for the same purpose. They all give an indication of the animal's iron status but it makes no sense to use tissue iron contents instead of Hb for iron bioavailability assessment. Furthermore, iron storage in liver or any other organ does not always point at an increased iron bioavailability of the compound that was fed. Rather, the diet under investigation may eventually cause disturbances in iron metabolism, leading to a redistribution of body iron, which has been shown in vitamin A-deficient rats (220). Finally, it depends on the definition of iron bioavailability whether or not liver iron is to be considered bioavailable, because it is storage iron, and therefore non-functional.

In chicks, close correlations between dietary iron intake versus iron concentrations in liver and kidney were found when conventional diets supplemented with iron up to 800 mg/kg were fed (30). It seems that this method can be used instead of Hb repletion, but it is unknown if this holds true for other animals as well.

Carcass iron

This is the most absolute way to determine the amount of iron in the body. Carcass iron content shows good agreement with the gain in Hb (72), and it was strongly correlated (r = 0.97) with ferrous sulphate intake up to about 9 mg of iron (175), but that held true also for Hb-iron gain (r = 0.96). A limitation of the method is the presence of diet in the gastrointestinal tract. This will lead to an overestimation of total body iron, which will even increase in groups that were fed diets with higher iron concentrations. On the other hand, removal of the entire gastrointestinal tract will cause a consistent underestimation of total body iron. Another disadvantage of measuring total body iron is that only final values can be obtained. The method is seldom used.

Body weight gain

Iron-depleted rats have a reduced growth rate. On entering the repletion period, the intake of iron and the increase of Hb will be positively correlated with body weight gain for some time. Up to a total iron intake of about 4 mg, the response of body weight gain is highly correlated with the amount of iron that is consumed, but response criteria based on body iron gain correlate better (175). Therefore, the use of this response criterion is limited. Another disadvantage of this criterion is that it can be used only in growing animals.

Interpretation of the results

For the statistical evaluation of bioavailability assays, the reader is referred to a recent review (145). We will briefly discuss three types of assay that are used for the determination of iron bioavailability: graphic assays and procedures using standard curves, the slope-ratio assay, and the parallel-lines assay.

In early bioassays the RBV of iron was calculated using a graphic assay (207). From a plot of furnished dietary iron versus Hb gain, it could be determined which Hb gain was obtained by feeding a diet with 20 mg/kg sample iron. From the same plot it was determined which concentration of ferrous sulphate led to an equal increase in Hb. The RBV of a sample was assigned on the basis of the quantity of iron supplied by ferrous sulphate that produced an equal Hb response:

$$RBV\ (\%) = \frac{mg\ Fe/kg\ from\ ferrous\ sulphate}{mg\ Fe/kg\ from\ sample},$$

that gives equal Hb response.

Similar results can be obtained by using a standard curve that is based on the response to different levels of ferrous sulphate. The response to the iron compound under study can be expressed relative to the corresponding ferrous sulphate response with the use of this curve. Theoretically, the use of only one concentration of the iron source under study, i.e., one group of animals, is sufficient to obtain a value for the RBV, provided that the response to the iron sample is in the linear range of the curve.

With the slope-ratio technique, the slope of the response to the iron sample relative to the slope of the response to ferrous sulphate is expressed. Two basic assumptions are linearity of the response and equal response in the absence of iron, that is, intersection at the Y-axis. For this method at least three groups of animals are necessary for each iron source studied.

In the parallel-lines assay, which was adopted by the AOAC (75, 8), the amount of dietary iron is expressed logarithmically versus the response criterion. This transformation may be useful in the case of a non-linear response. The parallel-lines assay was compared to several other methods of data analysis. All methods led to similar conclusions regarding the RBV of supplemental iron (76).

With the use of the HRE method, the RBV of an iron compound can be obtained by dividing its HRE with the HRE of ferrous sulphate (156, 246). However, with severe iron depletion the HRE from ferrous sulphate became higher, whereas the HRE from iron-containing foods did not change (273). In that case, the RBV of the compound under study may appear to be lower, although still an equal amount is bioavailable.

Studies in blood using one radioisotope

The first study using radioiron was published by Hahn et al. (91). They monitored the fate of ^{59}Fe in anemic dogs. Radioactivity measurements were made on ashed viscera and blood, after oral administration of ^{59}Fe(III) sulfate. For food iron absorption studies in humans, mustard greens and spinach were grown in the presence of ^{59}Fe, while laying hens and rabbits were injected with ^{59}Fe to label intrinsically eggs, liver, and muscle. The amount of ^{59}Fe that appeared in the blood during the two weeks after feeding a labeled foodstuff was determined (187). This method went out of use for several reasons. As the interindividual variation in iron bioavailability is very high, subjects had to be their own control if comparisons between treatments were to be made. With the introduction of techniques that made it possible to measure accurately a second iron radioisotope, ^{55}Fe, intraindividual comparisons could be made simultaneously (see below). For reasons of convenience, ^{59}Fe determinations in blood are still performed in animals (168, 80, 101).

Studies in blood using two radioisotopes

Oral and intravenous administration

Absorbed iron is primarily incorporated into Hb. However, the amount incorporated is less than 100%. A small and variable amount is distributed in other tissues. Hence, although the amount of radioiron in the red cell mass reflects the amount of iron that is absorbed, the actual percentage of absorption can not be calculated from this value. This can be corrected for by a technique that was first described in rats by Saylor & Finch (229). A known amount of ^{55}Fe was given orally and a known amount of ^{59}Fe was injected intravenously. A blood sample was taken after 9 d. The ratio of ^{55}Fe to ^{59}Fe in blood is a measure for the true absorption of ^{55}Fe, provided that the internal distributions of the oral and intravenous administered isotopes are similar. It can be calculated by:

$$^{55}Fe\ absorption\ (\%) = \frac{^{55}Fe\ activity\ in\ blood\ sample \times {}^{59}Fe\ dose\ injected}{^{55}Fe\ dose\ orally\ administered \times {}^{59}Fe\ activity\ in\ blood\ sample} \times 100$$

This procedure was also applicable in man (25, 205).

Oral administration of both isotopes: The double-isotope method

A variation on this method was employed by supplying both radioisotopes orally on consecutive days in different meals, after which their ratio in blood was determined. This made it possible to estimate iron absorption comparatively within the same subject (25, 205). This oral administration of ^{55}Fe and ^{59}Fe to humans has become a frequently used technique for the estimation of iron absorption from different foods. It will be referred to here as the double-isotope method.

Briefly, experiments in which the double-isotope method is applied are designed as follows. The foods or food components under investigation are tagged: one with ^{59}Fe and the other with ^{55}Fe. The subjects are served one tagged food on day 0, and the other one on day 1. A blood sample may be taken in advance for determination of baseline hematological values. Blood is drawn on day 15. Eventually, a second pair of tagged foods may be administered similarly and a final blood sample has to be drawn then on day 30. The isotope ratios in the blood samples of day 15 (and day 30) are measured. In practice, a maximum of four different foods can be studied in one subject.

When Layrisse et al. (138) estimated iron absorption from different intrinsically labeled foods, they observed mutual interactions. For example, when labeled corn and veal muscle were given together, the incorporation of corn iron increased whereas the incorporation of veal iron decreased when compared to the resulting iron incorporation from the single foods. These findings were corroborated by subsequent experiments generally showing that iron incorporation from vegetable foods increased when they were given with fish (160) or meat (161). This could be explained by assuming that all the nonheme iron in a meal is able to enter a common pool from which absorption takes place. Evidence for the existence of this common pool was provided by experiments that measured incorporation from a food that was intrinsically labeled with ^{55}Fe and extrinsically labeled with inorganic ^{59}Fe (39, 17). Incorporation from intrinsic and extrinsic radioiron was highly correlated and independent of iron valence, iron dose, and efficiency of incorporation (39). Soon thereafter, it was shown that there was a common pool for heme iron too, and that the addition of tagged hemoglobin to a meal provided a measure for heme-iron absorption from the total meal (139). The validity of extrinsic tagging was corroborated by subsequent studies for a number of single foods and meals (18, 228). However, there may be some pitfalls using the double-isotope method because the isotopic exchange is not always complete. Iron in unpolished rice (18), hemosiderin and ferritin iron (141, 162) and contamination iron (95) did not seem to exchange sufficiently with the extrinsic iron tag. For human milk, it takes up to 72 h after extrinsic tagging before a similar distribution as compared to the intrinsic milk iron is reached (49, 147). Finally, some types of fortification iron may not enter the common nonheme-iron pool sufficiently, which may result in either a decreased absorption, as for carbonyl iron (96), or an increased absorption, as for ferrous fumarate (120).

Quantification of the double-isotope method using estimates

The use of the double-isotope method results in a ratio of both radioiron isotopes in blood. In order to quantify the outcome in terms of absorption it may be adapted. Several approaches have been used for this, which comprise methods that estimate both the percentage radioiron incorporation in erythrocytes and blood volume, with additional use of a reference dose of iron, and/or normalization of the results to a fixed iron status.

The percentage of absorbed radioiron that is incorporated into the erythrocytes and the total blood volume of the subject are both unknown. In healthy subjects having a normal iron status, about 80% of the radioiron absorbed from an oral dose will be utilized for incorporation in the red cell mass, whereas in subjects suffering from iron-deficiency anemia about 96% of the absorbed radioiron is incorporated. In various anemias of different origin, this red cell iron utilization may vary from 18% in thalassemia up to 83% in anemias caused by infection (26). Estimates for blood volume can be obtained experimentally, but are usually estimated either as a fixed percentage of body weight or derived from tables that are based on a combination of sex, weight, and height.

It is not reliable to compare iron absorption between different subjects without any additional correction, because interindividual variation in iron absorption will surely interfere with the results. This can be corrected for by using a reference dose of iron that is administered to all subjects. The calculated iron absorption levels can then be standardized relative to this reference dose. This method was applied in many studies (40, 140, 67). A standard meal can also be used as the reference. The effect on iron absorption of various additions to the meal is then expressed relative to that standard meal. In this way, the effect of different proteins (42, 119), the effect of deleting or doubling the amount of carbohydrates, fat or protein in a semisynthetic meal (183), and the effect of different types of fiber (43) have been reported. When a standard meal is used as the reference source of iron, even dose-response curves can be obtained by adding increasing quantities of a compound to it (19, 41, 142, 151).

Iron absorption is determined by the iron content and composition of the food studied, and the iron status of the subject. In order to ease comparisons between different studies, the absorption percentage of the reference dose is sometimes “normalized” to subjects without iron stores that have not yet developed anemia. It was established that the absorption in these so-called borderline iron-deficient subjects of a 3 mg reference dose of ferrous iron is about 40% (152). Thus, in order to obtain a normalized figure for iron absorption from a food sample, the ratio of iron absorption from the sample versus absorption of 3mg of ferrous ascorbate was simply multiplied by 40, resulting in so-called A_{40} values (11, 97).

Instead of a reference dose, serum ferritin has also been used as a predictor for iron status. Taylor et al. (245) compared in a meta-analysis comprising 2018 subjects iron absorption from four sources with three indicators of iron status: serum ferritin, plasma transferrin saturation, and iron absorption from a reference dose of iron. They found that transferrin saturation was a poor predictor for iron absorption when it was higher than 25%. However, serum ferritin was practically as efficient as the reference dose absorption in predicting food iron absorption from meat and/or vegetable meals. Heme iron absorption formed an exception in that serum ferritin levels correlated poorly. On the other hand, Olivares et al. (198) found a good correlation between heme iron absorption and serum ferritin levels in a study that comprised 39 subjects.

Quantification of the double-isotope method using whole-body counting

An accurate way of quantification of the double-isotope method is to extend it with a whole-body count. When this is done few hours and 15 d after ingestion of the test meal a figure for the total ^{59}Fe retention is obtained. With this figure and the isotope ratio in blood the retention from ^{55}Fe can then be calculated (21, 222, 87). Hence, no estimates for blood volume and red cell iron incorporation are necessary.

The joined use of the double-isotope method and whole-body counting is particularly useful for studying total iron retention from a meal containing heme and non-heme iron. Both common pools can be tagged extrinsically and the comparative absorption from both pools within a meal can be studied. The retention from the whole diet can then be quantified with the use of whole-body counting (92, 20).

Application of the double-isotope method in animals

Most iron bioavailability studies are performed in order to predict the contribution of a particular food to iron bioavailability in man. As the double-isotope method is usually applied in man, there is no need for extrapolation. Nevertheless, the double-isotope method has also been used in animals, mainly for comparing the results of this technique for humans and animals, in order to determine the feasibility of animal models for iron bioavailability. In an attempt to validate the method of extrinsic tagging in iron-depleted and normal rats, studies with extrinsically and intrinsically labeled food components have been reported (181, 255).

The relative absorption of ferrous and ferric compounds was compared using the double-isotope technique in guinea pigs and rhesus monkeys. It was shown that the latter used ferrous iron preferentially. In a subsequent experiment, rhesus monkeys absorbed iron from rice-based

and wheat-based meals to the same extent as humans, indicating the feasibility of rhesus monkeys as an animal model in this respect (191).

Animals have also been used as a model for man in order to investigate extrinsic iron exchangeability in milk. As human milk can not be intrinsically tagged, the appropriateness of extrinsic labeling had to be studied in an animal model. In milk of sows, intrinsic and extrinsic iron were found to be distributed differently, suggesting that extrinsic labeling of human milk may also yield invalid results (85).

Reddy & Cook (219) used the double-isotope method in rats and humans in order to study the relative sensitivity of rats to dietary factors (meat, ascorbic acid, tea, bran, and soy protein) known to influence iron absorption in humans. It was found that rats were relatively insensitive to these factors, although rats and humans responded similar in a qualitative way.

Studies in blood using stable isotopes

Early studies using stable iron isotopes made use of one stable isotope without correction for interindividual differences (170, 125). Whittaker et al. (261) measured in four healthy women fractional iron absorption by using two enriched isotopes, of which ^{54}Fe was given orally, while ^{57}Fe was injected intravenously. This method was subsequently used to study iron absorption from a ferrous sulphate solution in healthy women during pregnancy and post delivery (262) and to study food iron absorption from a breakfast extrinsically labeled with ^{54}Fe in the same target group (13). In these studies, fractional absorption of the orally administered isotope was calculated by measuring the area under the curve for both isotopes:

$$\textit{oral absorption (\%)} = (AUC_{oral}/AUC_{iv}) \times (dose_{iv}/dose_{oral}) \times 100$$

As these authors argue, the level of erythrocyte incorporation from orally administered iron is not exactly known in pregnancy. Therefore, the appearance in plasma, instead of erythrocyte incorporation was used as the measure.

A promising method to assess iron bioavailability comparatively in humans is to apply the double-isotope method with the use of stable isotopes of iron (129). Infant formula was labeled either with ^{57}Fe or ^{58}Fe. The isotopic enrichment in erythrocytes was measured subsequently. Assumptions for blood volume and fraction of iron that is incorporated in erythrocytes have to be made if absorption is to be expressed as a percentage of iron uptake. Theoretically, as these authors pointed out, it would even be possible to use a triple-isotope method, by using ^{54}Fe as well. One stable isotope could then be added to a reference dose of iron for standardization purposes, while the other two stable iron isotopes would still be available for comparative studies within the same subject.

Stable isotopes are particularly useful in the assessment of iron bioavailability in vulnerable groups. For this reason, many stable isotope studies have been performed in infants and children (269, 66), and in pregnancy (262, 13).

Iron status parameters

All endpoint measurements that have been discussed so far are based on the incorporation of absorbed radioiron or stable isotopes into the red cell mass. Hematological parameters as such have also been used for the assessment of iron bioavailability, including Hb, Ht, serum ferritin, serum iron, TIBC, transferrin saturation, MCV, and free erythrocyte protoporphyrin. These have been applied in studies regarding the effectiveness of either iron supplementation or iron fortification of food, and to study the effects of different iron regimens.

Common characteristics for all iron supplementation studies are the low iron status of the group under investigation at the start of the study and the long duration of the study, ranging from 3 weeks up to 1 year depending on the level of iron supplementation (88, 24).

Food iron fortification can be measured in a clinical setting in order to test its effectiveness. Next, it should be assessed in field studies, in which iron status before and after the implementation of the fortification program should be determined. Fortification programs may be aimed at special groups or comprise the whole population. Hertrampf et al. (112) tested the effects of iron-fortified cereal on iron status in infants. The effects of supplying iron-fortified cookies in a school lunch program has been studied in 1000 boys and girls in Chile (254). Recently, the results of an iron fortification program on community scale were reported (253).

Information regarding food iron effects on iron status is scanty. Hertrampf et al. (111) compared infant iron status after feeding soy formula, fortified cow's milk or breast feeding. Fuchs et al. (79) compared the effects of formula versus cow's milk with iron-fortified cereal. The effect on iron status of additional meat consumption as compared to iron supplementation was studied in women (149). The effects of dietary manipulation on iron status are hardly demonstrable in subjects with normal body iron stores as shown by Cook et al. (44), who found no increase in serum ferritin levels in healthy subjects when 2 g ascorbic acid was supplemented daily for 16 weeks.

Pros and cons of endpoint measures

When it is agreed that solubility, absorption, and incorporation in the functional compartment are all involved in ultimate bioavailability (230), then an endpoint measure

in a representative part of the functional compartment gives the best figure for iron bioavailability. For this purpose, Hb seems to be the best candidate. In general, two methods can be applied: repletion bioassays or (double-) isotope studies.

Hb repletion in rats serves as the standard method according to the AOAC. For Hb repletion, we have to rely on animal models, as repletion studies were only seldom performed in humans. The rat model offers some clear advantages: it is relatively simple to perform, large numbers of samples can be screened, and responses to treatment are uniform. When the anemic rat model is to be used, calculation of HRE instead of the use of Hb repletion seems to be favorable. HRE determination only demands body weigths and food intake to be measured in addition to Hb, in order to correct for both factors. When a HRE assay is performed, only one group of rats is needed for each iron source, including the reference source, provided that the response is in the linear range. This approach may decrease the number of rats that have to be used to about 30%.

The Hb-repletion bioassay was originally designed to determine iron bioavailability of fortification iron. For that purpose, addition in different concentrations of the iron compound under study was sufficient. However, when the bioavailability of food iron is to be compared according to the guidelines of the bioassay, different amounts of the food under study must be added to the basal diet at the expense of one or more of the other dietary components, in order to reach a range of different dietary iron concentrations. It is obvious that this approach leads to variations in the amounts of other food components. This error is inherent to the repletion bioassay. Another limitation of the bioassay is the difficulty to study foods or food components which enhance iron bioavailability. As iron absorption in iron-deficient rats is already highly induced, any enhancing effect of a dietary component may not emerge from the results. A major cause for concern is the suitability of the rat to act as a model for man. Rats respond qualitatively similar to different dietary factors as compared to man (157). For that reason, these authors feel that the rat model is potentially useful to estimate iron bioavailability for humans. Others come to a different conclusion (219). There is no obvious reason for the rat as an animal model in this case. It is, of course, simple and inexpensive, but the use of the model seems to be empirically based. Scientific evidence is accumulating that the rat does not fit well as a model for man when iron bioavailability is to be studied. Rats are able to absorb ferrous and ferric iron to an equal extent, while man preferentially absorbs ferrous iron; the bioavailability to rats of heme and nonheme iron is approximately similar, where man preferentially absorbs heme iron; man is able to raise iron absorption from a nonheme iron reference dose to approximately 40% in case of a low iron status, where rats are able to absorb double the amount. Rats are able to synthesize ascorbic acid, and they possess intestinal phytase activity, where man does not. If quantitative results are to be obtained, these factors advocate against the use of the rat as a model for iron bioavailability in man. Other animal models may prove to resemble man in a more quantitative way. The pig has been considered useful as an animal model, as its gastrointestinal tract resembles that of humans. However, results are scanty as the vast majority of studies on iron nutrition has been performed in rats. Moreover, the usefulness of every animal model is questionable as long as the accuracy of extrapolation to man is unknown.

Double-isotope studies are an important source of current knowledge of iron nutrition and iron bioavailability in man. The advantages of this method include the use of human subjects, hence no extrapolation problem, as well as the relative simplicity of the method. Disadvantages of the method include its single meal basis, the exposure to radiation, and the impracticability of extrinsic tagging for some compounds. Single meal studies may not give a similar result as compared to whole-diet studies. This drawback can be overcome by assessing iron bioavailability from the whole diet for a few days, but this demands the tagging of each meal. Only a few studies have been performed in this way (93, 87, 117). In some cases, extrinsic tagging of food is not feasible, because it results in a different distribution of extrinsic vs. intrinsic iron. It has to be kept in mind, however, that by far most foodstuffs do not cause any difficulties in this respect. Whenever the iron bioavailability of a foodstuff or iron preparation is to be tested with the use of the double-isotope method without knowing the feasibility of extrinsic tagging, this should be investigated. The use and limitations of extrinsic tagging in iron bioavailability have been reviewed by Consaul & Lee (38).

The same advantages and disadvantages hold true when stable isotopes are used in the double-isotope method, except for the exposure to radiation. The presence of three stable isotopes against two radioisotopes of iron leads to a higher potential in designing experiments. The use and determination of stable isotopes still has many practical limitations. As stable isotopes do not emit radiation, whole-body counting can not be performed. Therefore, it is impossible to link the double-isotope method to whole-body counting in order to obtain a direct measure for iron retention, as has been done for radioiron. An additional limitation is the cost both of the isotopes and the detection equipment. Finally, in order to reach sufficient enrichment of the foodstuff under study, relatively large quantities of stable isotopes are needed. Thus, the iron content of the foodstuff is increased significantly, which may influence the results, for the percentage iron absorbed drops with increasing dosage, whereas the amount absorbed rises (26).

Other hematological parameters for studying iron bioavailability may be useful in a clinical setting for the treat-

ment of iron-deficiency anemia, and in field studies to monitor the effectiveness of iron fortification programs. Hb and serum ferritin are useful parameters in this respect. In Third World countries, however, the use of serum ferritin as the sole iron status parameter may mask the incidence of anemia, because it may be increased during infections, regardless of iron status.

Prediction of iron bioavailability by means of arithmetic models

Some attempts have been made to predict iron bioavailability from a whole diet by means of calculation. Two approaches can be distinguished. The first is a method to calculate iron bioavailability from a whole meal, based on both the concentration of iron and enhancing factors in the meal. The second approach departs from mathematical models that are derived from in vitro data in order to predict the impact of an enhancing or inhibiting factor on the in vitro availability of iron in food products.

Two arithmetic models

Monsen et al. (182) reported for the first time a model to predict iron bioavailability for a given diet. Five parameters are needed, i.e., the amounts in the meal of total iron, heme iron, nonheme iron, ascorbic acid, and meat + poultry + fish (i.e., animal tissue). If known, body storage iron may be used as a sixth, person-bound parameter. The assumptions are: (1) relative heme iron absorption depends on body iron stores, and does not depend on meal composition; (2) relative nonheme iron absorption depends on both body iron stores and meal composition; the latter is determined by the amounts of ascorbic acid and/or animal tissue (i.e., enhancing factors); (3) animal tissue iron consists of 40% heme and 60% nonheme iron. For each meal, the amounts of total iron, nonheme iron and heme iron, ascorbic acid, and animal tissue can be calculated from food tables. Subsequently, the amount of absorbable iron can be calculated for each meal. In the original method, depending on the amount of enhancing factor, the availability of nonheme iron in a meal was considered either low, medium or high, with three accompanying absorption percentages. In a refinement on the method (184), the amount of enhancing factors was substituted in a logarithmic equation in order to obtain a better estimate for the percentage of nonheme iron absorption. This so-called "Monsen method" generally gives a good indication with respect to the amount of absorbable iron from a meal. However, some factors have not been taken into account, and these must be considered carefully when applying the model. First, no correction was made for any inhibiting factors in the meal, although these may affect ultimate iron bioavailability to a considerable extent. Second, no adjustments were made for an increase in total available iron, although higher iron doses induce a decrease in absolute iron absorption. Third, the assumption that 40% of animal tissue iron consists of heme iron is questionable. It was noticed that heme iron contents of animal tissues were not invariably 40% of total iron, but the figure was used for convenience (182). However, a recent study indicates that heme iron contents in different meats are not only variable (from 22 up to 80% in cooked meat samples) but usually also higher than 40% (33). Fourth, no adjustments could be made for nonheme iron sources that do not enter the common pool and as a result are not absorbed. For these reasons, variations on the model have been used. As an example, Cook et al. (45) adjusted for inhibiting factors in the diet.

Mathematical models for the study of iron bioavailability can be derived from in vitro solubility data in order to predict the impact of an enhancing or inhibiting factor on the in vitro availability of iron in food products. Wolters et al. (267) developed a model that was based on theoretical chemical considerations. With the use of this model it was possible to describe the effect on iron bioavailability of a single enhancing or inhibiting factor, as well as on combinations of these factors. Iron availability of cereal products, fruits, vegetables, and nuts was best described by taking ascorbic acid into account as an enhancer, and both arabinose and phytic acid as inhibitors, each with its own weight factor. Anand & Seshadri (5) used a linear regression model in order to predict iron bioavailability from Indian vegetarian meals. Enhancing (ascorbic acid, citric acid) and inhibiting (tannic acid, calcium phosphate) parameters were incorporated in the equation. Both mathematical models may give insight in possible interactions of enhancing and inhibiting factors with iron, but also have important limitations. For both methods, data were used that were derived from in vitro dialyzability or solubility studies. Thus, physiological conditions which partly determine iron bioavailability are not taken into account. Some other potentially important factors, like competitive trace element interactions have not been incorporated into the model.

Pros and cons of arithmetic models

Both the Monsen method and mathematical modeling are based on experimental data in order to quantify the inhibiting and enhancing factors. The model of Monsen et al. (182) is primarily based on data from iron bioavailability studies in humans. It provides an easy tool to calculate iron bioavailability from a meal, and heme iron is included in the model. However, as the effect of absorptive inhibitors was not included in the original model, an updated version (45) will give better results. Mathematical models may be useful to predict the relative impact of an enhancing or inhibiting factor on iron bioavailability for a wide range of foods. As they are based on in vitro data, all limitations of in vitro experiments are applicable to them.

Concluding remarks

As has been stated in the introduction, iron bioavailability should ideally be quantifiable. Several methods are used to assess iron bioavailability and they all quantify it in their own way. If a consensus is to be reached about a general method for the quantification of bioavailability, this might result in some kind of an absolute figure for iron bioavailability. But do we have to strive for such a measure? At this moment, there is no method available combining all advantages without any limitations. Therefore, the best approach should be to study iron bioavailability in an integrated matter. With a thorough knowledge of the possibilities and limitations of individual methods, the most feasible method or set of methods can be chosen.

For large-scale screening in vitro solubility and dialyzability studies are suitable. Cell lines may even give a better picture, as effects on the intestinal level may be demonstrable. However, much work needs to be done before the suitability of cell lines, like Caco-2, can be judged properly. Animals may be useful in that they respond qualitatively equal to several modulators of iron bioavailability, but it seems that this can also be achieved with in vitro models. For the study of fortification iron, in vitro solubility methods seem to give results that are comparable to the Hb-repletion bioassay. The mechanistic effects of minor additions (like micronutrients) may be studied using the Hb-repletion rat bioassay, but Caco-2 cells may become equally suitable. For assessment of iron bioavailability in whole food or major components of food the experimental design of the Hb-repletion bioassay is unsuitable.

Ultimately, human subjects have to be studied in order to corroborate results from in vitro and/or animal studies, for the latter can only predict iron bioavailability, whereas balance and isotope methods in man give a more direct figure by getting around the extrapolation of the results. Although radioisotopes have added much to our knowledge of iron bioavailability, exposure to radiation just for scientific purposes remains a serious drawback of the method. Stable isotopes are promising in this respect and may take over the role of radioiron, but much work has to be done in order to develop this method further.

References

1. Alvarez-Hernandez X, Nichols GM, Glass J (1991) Caco-2 cell line: a system for studying intestinal iron transport across epithelial cell monolayers. Biochim Biophys Acta 1070:205–208
2. Amine EK, Neff R, Hegsted DM (1972) Biological estimation of available iron using chicks or rats. J Agr Food Chem 20:246–251
3. Amine EK, Hegsted DM (1974) Biological assessment of available iron in food products. J Agr Food Chem 22:470–476
4. Amine EK, Hegsted DM (1975) Effect of dietary carbohydrates and fats on inorganic iron absorption. J Agr Food Chem 23:204–208
5. Anand AN, Seshadri S (1995) A quantitative model for prediction of iron bioavailability from Indian meals: an experimental study. Int J Food Sci Nutr 46:335–342
6. Anderson HD, McDonough KB, Elvehjem CA (1940) Relation of the dietary calcium-phosphorus ratio to iron assimilation. J Lab Clin Med 25:464–471
7. Anderson TA, Kim I, Fomon SJ (1972) Iron status of anemic rats fed iron-fortified cereal-milk diets. Nutr Metabol 14:355–361
8. AOAC (1990) Bioavailability of iron. Rat Hemoglobin Repletion Bioassay. In: Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed.; Helrich K, (ed) Association of Official Analytical Chemists Inc., Arlington, VA, pp 1098–1099
9. Ashworth A, Milner PF, Waterlow JC, Walker RB (1973) Absorption of iron from maize (*Zea mays* L.) and soya beans (*Glycine hispida* Max.) in Jamaican infants. Br J Nutr 29:269–278
10. Austoni ME, Greenberg DM (1940) Studies in iron metabolism with the aid of its artificial radioactive isotope. The absorption, excretion, and distribution of iron in the rat on normal and iron-deficient diets. J Biol Chem 134:27–41
11. Ballot D, Baynes RD, Bothwell TH, Gillooly M, Macfarlane BJ, MacPhail AP, Lyons G, Derman DP, Bezwoda WR, Torrance JD, Bothwell JE, Mayet F (1987) The effects of fruit juices and fruits on the absorption of iron from a rice meal. Br J Nutr 57:331–343
12. Bannerman RM, O'Brien JRP, Witts LJ (1962) Studies in iron metabolism. IV. Iron absorption in experimental iron deficiency. Blood 20:532–546
13. Barrett JFR, Whittaker PG, Williams JG, Lind T (1994) Absorption of non-haem iron from food during normal pregnancy. BMJ 309:79–82
14. Bates GW, Boyer J, Hegenauer JC, Saltman P (1972) Facilitation of iron absorption by ferric fructose. Am J Clin Nutr 25:983–986
15. Beard JL, Burton JW, Theil EC (1996) Purified ferritin and soybean meal can be sources of iron for treating iron deficiency in rats. J Nutr 126:154–160
16. Bezwoda W, Charlton R, Bothwell TH, Torrance J, Mayet F (1978) The importance of gastric hydrochloric acid in the absorption of nonheme food iron. J Lab Clin Med 92:108–116
17. Björn-Rasmussen E, Hallberg L, Walker RB (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 25:317–323
18. Björn-Rasmussen E, Hallberg L, Walker RB (1973) Food iron absorption in man. II. Isotopic exchange of iron between labeled foods and between a food and an iron salt. Am J Clin Nutr 26:1311–1319
19. Björn-Rasmussen E, Hallberg L (1974) Iron absorption from maize. Effect of ascorbic acid on iron absorption from maize supplemented with ferrous sulphate. Nutr Metab 16:94–100
20. Björn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B (1974) Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. J Clin Invest 53:247–255
21. Björn-Rasmussen E, Hallberg L, Magnusson B, Rossander L, Svanberg B, Arvidsson B (1976) Measurement of iron absorption from composite meals. Am J Clin Nutr 29:772–778
22. Boender CA, Verloop MC (1969) Iron absorption, iron loss and iron retention

in man: studies after oral administration of a tracer dose of $^{59}FeSO_4$ and $^{131}BaSO_4$. Br J Haematol 17:45–58
23. Bogunjoko FE, Neale RJ, Ledward DA (1983) Availability of iron from chicken meat and liver given to rats. Br J Nutr 50:511–520
24. Borch-Iohnsen B, Meltzer HM, Stenberg V, Reinskou T, Trygg K (1990) Bioavailability of daily low dose iron supplements in menstruating women with low iron stores. Eur J Clin Nutr 44:29–34
25. Bothwell TH, Pirzio-Biroli G, Finch CA (1958) Iron absorption. I. Factors influencing absorption. J Lab Clin Med 51:24–36
26. Bothwell TH, Charlton RW, Cook JD, Finch CA (1979) Iron metabolism in man. Blackwell Scientific Publications, Oxford
27. Brouwer IA, Lemmens AG, Beynen AC (1993) Dietary fructose v. glucose lowers ferrous-iron absorption in rats. Br J Nutr 70:171–178
28. Buchowski MS, Mahoney AW (1992) Intestinal ^{59}Fe distribution and absorption in rats with different iron status and given wheat bran or ferrous sulfate as dietary iron sources. Nutr Res 12:1479–1490
29. Cable JW, Cable EE, Bonkovsky HL (1993) Induction of heme oxygenase in intestinal epithelial cells: studies in Caco-2 cell cultures. Molec Cell Biochem 129:93–98
30. Cao J, Luo XG, Henry PR, Ammerman CB, Littell RC, Miles RD (1996) Effect of dietary iron concentration, age, and length of iron feeding on feed intake and tissue iron concentration of broiler chicks for use as a bioassay of supplemental iron sources. Poultry Sci 75:495–504
31. Carmichael D, Christopher J, Hegenauer J, Saltman P (1975) Effect of milk and casein on the absorption of supplemental iron in the mouse and chick. Am J Clin Nutr 28:487–493
32. Carpenter CE, Mahoney AW (1989) Proteolytic digestion of meat is not necessary for iron solubilization. J Nutr 119:1418–1422
33. Carpenter CE, Clark E (1995) Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. J Agric Food Chem 43:1824–1827
34. Chapman DG, Campbell JA (1957) Effect of bone meal on the utilization of iron by anaemic rats. Br J Nutr 11:117–126
35. Chidambaram MV, Reddy MB, Thompson JL, Bates GW (1989) In vitro studies of iron bioavailability. Probing the concentration and oxidation-reduction reactivity of pinto bean iron with ferrous chromogens. Biol Trace Elem Res 19:25–40
36. Clydesdale FM, Nadeau DB (1984) Solubilization of iron in cereals by milk and milk fractions. Cereal Chem 61:330–335
37. Conrad ME, Umbreit JN, Moore EG (1991) A role for mucin in the absorption of inorganic iron and other metal cations. Gastroenterol 100:129–136
38. Consaul JR, Lee K (1983) Extrinsic tagging in iron bioavailability research: a critical review. J Agric Food Chem 31:684–689
39. Cook JD, Layrisse M, Martínez-Torres C, Walker R, Monsen E, Finch CA (1972) Food iron absorption measured by an extrinsic tag. J Clin Invest 51:805–815
40. Cook JD, Minnich V, Moore CV, Rasmussen A, Bradley WB, Finch CA (1973) Absorption of fortification iron in bread. Am J Clin Nutr 26:861–872
41. Cook, JD, Monsen ER (1975) Food iron absorption. I. Use of a semisynthetic diet to study absorption of nonheme iron. Am J Clin Nutr 28:1289–1295
42. Cook JD, Monsen ER (1976) Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am J Clin Nutr 29:859–867
43. Cook JD, Noble NL, Morck TA, Lynch SR, Petersburg SJ (1983) Effect of fiber on nonheme iron absorption. Gastroenterol 85:1354–1358
44. Cook JD, Watson SS, Simpson KM, Lipschitz DA, Skikne BS (1984) The effect of high ascorbic acid supplementation on body iron stores. Blood 64:721–726
45. Cook JD, Dassenko SA, Lynch SR (1991) Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr 54:717–722
46. Cowan JW, Esfahani M, Salji JP, Azzam SA (1966) Effect of phytate on iron absorption in the rat. J Nutr 90:423–427
47. Crews HM, Burrell JA, McWeeny DJ (1985) Trace element solubility from food following enzymolysis. Z Lebensm Unters Forsch 180:221–226
48. Crofton RW, Gvozdanovic D, Gvozdanovic S, Khin CC, Brunt PW, Ashley N, Mowat G, Aggett PJ (1989) Inorganic zinc and the intestinal absorption of ferrous iron. Am J Clin Nutr 50:141–144
49. Davidson LA, Litov RE, Lönnerdal B (1990) Iron retention from lactoferrin-supplemented formulas in infant rhesus monkeys. Pediatr Res 27:176–180
50. Dietzfelbinger H (1987) Bioavailability of bi- and trivalent oral iron preparations. Investigations of iron absorption by postabsorption serum iron concentrations curves. Arzneim-Forsch/Drug Res 37:107–112
51. Disch G, Classen H-G, Spätling L, Leifert U, Schumacher E (1996) Therapeutic availability of iron administered orally as the ferrous gluconate together with magnesium-L-aspartate hydrochloride. Arzneim-Forsch/Drug Res 46:302–306
52. Dundar MS, Haswell SJ (1995) Use of a model gut system to study the effects of dietary fiber and multivitamins on the speciation of copper, zinc and iron. Analyst 120:2085–2088
53. Duthie HL, Code CF, Owen CA (1962) Absorption of iron from the small bowel of dogs. Gastroenterol 42:599–611
54. Eagles JE, Fairweather-Tait SJ, Self R (1985) Stable isotope ratio mass spectrometry for iron bioavailability studies. Anal Chem 57:469–471
55. Ekenved G (1976) Absorption from different types of iron tablets – correlation between serum iron increase and total absorption of iron. Scand J Haematol Suppl 28:51–63
56. Ekenved G, Norrby A, Sölvell L (1976) Serum iron increase as a measure of iron absorption – studies on the correlation with total absorption. Scand J Haematol Suppl 28:31–49
57. Elvehjem CA, Hart EB, Sherman WC (1933) The availability of iron from different sources for hemoglobin formation. J Biol Chem 103:61–70
58. Fairweather-Tait SJ (1983) Studies on the availability of iron in potatoes. Br J Nutr 50:15–23
59. Fairweather-Tait SJ (1987) The concept of bioavailability as it relates to iron nutrition. Nutr Res 7:319–325
60. Fairweather-Tait SJ, Portwood DE, Symss LL, Eagles JE, Minski MJ (1989) Iron and zinc absorption in human subjects from a mixed meal of extruded and nonextruded wheat bran and flour. Am J Clin Nutr 49:151–155
61. Fairweather-Tait SJ (1992) Bioavailability of trace elements. Food Chem 43:213–217
62. Fernandez R, Phillips SF (1982) Components of fiber impair iron absorption in the dog. Am J Clin Nutr 35:107–112
63. Field M, Seki M, Mitchell ML, Chalmers TC (1960) Studies in iron absorption. I. Determination in rats by measurement of total body radioactivity. J Lab Clin Med 55:929–935
64. Fisher RB, Parsons DS (1949) A preparation of surviving rat small intestine for the study of iron absorption. J Physiol 110:36–46
65. Flanagan PR, Haist J, Valberg LS (1980) Comparative effects of iron deficiency induced by bleeding and a low- iron diet on the intestinal absorptive interactions of iron, cobalt, mangan- ese, zinc, lead and cadmium. J Nutr 110:1754–1763

66. Fomon SJ, Ziegler EE, Nelson SE (1993) Erythrocyte incorporation of ingested ^{58}Fe by 56-day-old breast-fed and formula-fed infants. Pediatr Res 33:573–576
67. Forbes AL, Arnaud MJ, Chicester CO, Cook JD, Harrison BN, Hurrell RF, Kahn SG, Morris ER, Tanner JT, Whittaker P, Adams CE (1989) Comparison of in vitro, animal, and clinical determinations of iron bioavailability: International Nutritional Anemia Consultative Group Task Force report on iron bioavailability. Am J Clin Nutr 49:225–238
68. Forth W, Rummel W (1965) Eisen-Resorption an isolierten Dünndarmpräparaten von normalen und anämischen Ratten. Naunyn-Schmiedebergs Arch Exp Path Pharmakol 252:205–223
69. Forth W, Rummel W (1966) Beziehungen zwischen Eisen-Konzentration und -Resorption am isolierten Dünndarm normaler und anämischer Ratten. Med Pharmacol Exp 14:289–296
70. Forth W, Andres H (1969) Methode zur Erzeugung eines Eisenmangels bei Ratten. Arzneim-Forsch/Drug Res 19:363–364
71. Forth W, Rummel W (1973) Iron absorption. Physiol Rev 53:724–792
72. Freeman S, Ivy AC (1942) The influence of antacids upon iron retention by the anemic rat. Am J Physiol 137:706–709
73. Fritz JC, Pla GW, Roberts T, Boehne JW, Hove EL (1970) Biological availability in animals of iron from common dietary sources. J Agr Food Chem 18:647–651
74. Fritz JC, Pla GW (1972) Application of the animal hemoglobin repletion test to measurement of iron availability in foods. J Assoc Off Anal Chem 55:1128–1132
75. Fritz JC, Pla GW, Harrison BN, Clark GA (1974) Collaborative study of the rat hemoglobin repletion test for bioavailability of iron. J Assoc Off Anal Chem 57:513–517
76. Fritz JC, Pla GW, Harrison BN, Clark GA (1975) Estimation of the bioavailability of iron. J Assoc Off Anal Chem 58:902–905
77. Fritz JC, Pla GW, Harrison BN, Clark GA, Smith EA (1978) Measurement of the bioavailability of iron, using the rat hemoglobin repletion test. J Assoc Off Anal Chem 61:709–714
78. Frølich W, Lysø A (1983) Bioavailability of iron from wheat bran in pigs. Am J Clin Nutr 37:31–36
79. Fuchs GJ, Farris RP, DeWier M, Hutchinson SW, Warrier R, Doucet H, Suskind RM (1993) Iron status and intake of older infants fed formula vs cow milk with cereal. Am J Clin Nutr 58:343–348
80. Fuchs V, Kühnert M, Golbs S, Dedek W (1990) Untersuchungen zur enteralen Resorption von Eisen (II) aus Huminsäure-Eisen-Komplexen beim Saugferkel mit Hilfe radiomarkierten Eisens (^{59}Fe). Dtsch Tierärztl Wochenschr 97:208–209
81. Galdi M, Bassi A, Barrio Rendo ME, Valencia ME (1988) Ferric glycinate iron bioavailability determined by hemoglobin regeneration method. Nutr Rep Int 37:591–598
82. Gangloff MB, Glahn RP, Miller DD, Van Campen D (1996) Assessment of iron availability using combined in vitro digestion and Caco-2 cell culture. Nutr Res 16:479–487
83. Garcia MN, Flowers C, Cook JD (1996) The Caco-2 cell culture system can be used as a model to study food iron availability. J Nutr 126:251–258
84. Geisser P, Müller A (1987) Pharmacokinetics of iron salts and ferric hydroxide-carbohydrate complexes. Arzneim-Forsch/Drug Res 37:100–104
85. Gislason J, Jones B, Lönnerdal B, Hambraeus L (1992) Iron absorption differs in piglets fed extrinsically and intrinsically ^{59}Fe-labeled sow's milk. J Nutr 122:1287–1292
86. Glahn RP, Wien EM, Van Campen D, Miller DD (1996) Caco-2 cell iron uptake from meat and casein digests parallels in vivo studies: use of a novel in vitro method for rapid estimation of iron bioavailability. J Nutr 126:332–339
87. Gleerup A, Rossander-Hulthén L, Gramatkovski E, Hallberg L (1995) Iron absorption from the whole diet: comparison of the effect of two different distributions of daily calcium intake. Am J Clin Nutr 61:97–104
88. Gordeuk VR, Brittenham GM, Hughes M, Keating LJ, Opplt JJ (1987) High-dose carbonyl iron for iron deficiency anemia: a randomized double-blind trial. Am J Clin Nutr 46:1029–1034
89. Gordon DT, Godber JS (1989) The enhancement of nonheme iron bioavailability by beef protein in the rat. J Nutr 119:446–452
90. Gorman JE, Clydesdale FM (1983) The behavior and stability of iron-ascorbate complexes in solution. J Food Sci 48:1217–1220
91. Hahn PF, Bale WF, Lawrence EO, Whipple GH (1939) Radioactive iron and its metabolism in anemia. Its absorption, transportation, and utilization. J Exp Med 69:739–753
92. Hallberg L, Björn-Rasmussen E (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 9:193–197
93. Hallberg L, Björn-Rasmussen E, Rossander L, Suwanik R (1979) The measurement of food iron absorption in man. A methodological study on the measurement of dietary non-haem-Fe absorption when the subjects have a free choice of food items. Br J Nutr 41:283–289
94. Hallberg L (1981) Bioavailability of dietary iron in man. Ann Rev Nutr 1:123–147
95. Hallberg L, Björn-Rasmussen E, Rossander L, Suwanik R, Pleehachinda R, Tuntawiroon M (1983) Iron absorption from some Asian meals containing contamination iron. Am J Clin Nutr 37:272–277
96. Hallberg L, Brune M, Rossander L (1986) Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour. Am J Clin Nutr 43: 59–67
97. Hallberg L, Rossander-Hultén L, Brune M, Gleerup A (1992) Bioavailability in man of iron in human milk and cow's milk in relation to their calcium contents. Pediatr Res 31:524–527
98. Halleux C, Schneider Y-J (1991) Iron absorption by intestinal epithelial cells: 1. CaCo2 cells cultivated in serum-free medium, on polyethyleneterephthalate microporous membranes, as an in vitro model. In Vitro Cell Dev Biol 27A:293–302
99. Halleux C, Schneider Y-J (1994) Iron absorption by CaCo2 cells cultivated in serum-free medium as in vitro model of the human intestinal epithelial barrier. J Cell Physiol 158:17–28
100. Hämäläinen MM, Mäkinen KK (1985) Duodenal xanthine oxidase (*EC* 1.2.3.2.) and ferroxidase activities in the rat in relation to the increased iron absorption caused by peroral xylitol. Br J Nutr 54:493–498
101. Hamdaoui M, Doghri T, Tritar B (1995) Effect of different levels of an ascorbic acid and tea mixture on nonheme iron absorption from a typical Tunisian meal fed to healthy rats. Ann Nutr Metab 39:310–316
102. Han O, Failla ML, Hill AD, Morris ER, Smith JC Jr (1994) Inositol phosphates inhibit uptake and transport of iron and zinc by a human intestinal cell line. J Nutr 124:580–587
103. Han O, Failla ML, Hill AD, Morris ER, Smith JC Jr (1995) Ascorbate offsets the inhibitory effect of inositol phosphates on iron uptake and transport by Caco-2 cells. PSEBM 210:50–56
104. Han O, Failla ML, Hill AD, Morris ER, Smith JC Jr (1995) Reduction of Fe(III) is required for uptake of nonheme iron by Caco-2 cells. J Nutr 125:1291–1299
105. Hart HV (1971) Comparison of the availability of iron in white bread, fortified with iron powder, with that of iron naturally present in wholemeal bread. J Sci Food Agric 22:354–357

106. Hazell T, Johnson IT (1987) In vitro estimation of iron availability from a range of plant foods: influence of phytate, ascorbate and citrate. Br J Nutr 57:223–233
107. Hegsted DM, Finch CA, Kinney TD (1949) The influence of diet on iron absorption. II. The interrelation of iron and phosphorus. J Exp Med 90:147–156
108. Heilmeyer L, Plötner H (1936) Eisenmangelzustände und ihre Behandlung. Klin Wochenschr 15:1669–1673
109. Heinrich HC, Gabbe EE, Kugler G, Pfau AA (1971) Nahrungs-Eisenresorption aus Schweine-Fleisch, -Leber, und -Hämoglobin bei Menschen mit normalen und erschöpften Eisenreserven. Untersuchungen zur diätetischen Eisen-Prophylaxe und Therapie. Klin Wochenschr 49:819–825
110. Helbock HJ, Saltman P (1967) The transport of iron by rat intestine. Biochim Biophys Acta 135:979–990
111. Hertrampf E, Cayazzo M, Pizarro F, Stekel A (1986) Bioavailability of iron in soy-based formula and its effect on iron nutriture in infancy. Pediatrics 78:640–645
112. Hertrampf E, Olivares M, Pizarro F, Walter T, Cayazzo M, Heresi G, Llaguno S, Chadud P, Stekel A (1990) Haemoglobin fortified cereal: a source of available iron to breast-fed infants. Eur J Clin Nutr 44:793–798
113. Heth DA, Hoekstra WG (1965) Zinc-65 absorption and turnover in rats. I. A procedure to determine zinc-65 absorption and the antagonistic effect of calcium in a practical diet. J Nutr 85:367–374
114. Hidalgo IJ, Raub TJ, Borchardt RT (1989) Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterol 96:736–749
115. Hisayasu S, Orimo H, Migita S, Ikeda Y, Satoh K, Shinjo S, Hirai Y, Yoshino Y (1992) Soybean protein isolate and soybean lectin inhibit iron absorption in rats. J Nutr 122:1190–1196
116. Howard L, Buchowski M, Wang B-J, Miller DD (1993) Bioavailability of electrolytic iron in fortified infant cereal determined by hemoglobin repletion in piglets. Nutr Res 13:287–295
117. Hultén L, Gramatkovski E, Gleerup A, Hallberg L (1995) Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. Eur J Clin Nutr 49:794-808
118. Hunt JR, Gallagher SK, Johnson LK, Lykken GI (1995) High- versus low-meat diets: effects on zinc absorption, iron status, and calcium, copper, iron, magnesium, manganese, nitrogen, phosphorus, and zinc balance in postmenopausal women. Am J Clin Nutr 62:621–632
119. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD (1989) Iron absorption in humans as influenced by bovine milk proteins. Am J Clin Nutr 49:546–552
120. Hurrell RF, Reddy MB, Dassenko SA, Cook JD, Shepherd D (1991) Ferrous fumarate fortification of a chocolate drink powder. Br J Nutr 65:271–283
121. Hyde AS (1957) Absorption of radioiron perfused through the duodenum of the rat. Am J Physiol 191:265–270
122. Ivaturi R, Kies C (1992) Mineral balances in humans as affected by fructose, high fructose corn syrup and sucrose. Plant Foods Hum Nutr 42:143–151
123. Jacobs A, Greenman DA (1969) Availability of food iron. Br Med J 1:673–676
124. Janghorbani M, Ting BTG, Young VR (1980) Absorption of iron in young men studied by monitoring excretion of a stable iron isotope (^{58}Fe) in feces. J Nutr 110:2190–2197
125. Janghorbani M, Ting BTG, Fomon SJ (1986) Erythrocyte incorporation of ingested stable isotope of iron (^{58}Fe). Am J Hematol 21:277–288
126. Johnson PE (1982) A mass spectrometric method for use of stable isotopes as tracers in studies of iron, zinc, and copper absorption in human subjects. J Nutr 112:1414–1424
127. Johnson PE, Lukaski HC, Korynta ED (1992) Effects of stearic acid and beef tallow on iron utilization by the rat. PSEBM 200:480–486
128. Kapsokefalou M, Miller DD (1995) Iron speciation in intestinal contents of rats fed meals composed of meat and nonmeat sources of protein and fat. Food Chem 52:47–56
129. Kastenmayer P, Davidsson L, Galan P, Cherouvrier F, Hercberg S, Hurrell RF (1994) A double stable isotope technique for measuring iron absorption in infants. Br J Nutr 71:411–424
130. Kim M, Atallah MT (1992) Structure of dietary pectin, iron bioavailability and hemoglobin repletion in anemic rats. J Nutr 122:2298–2305
131. Kim M, Atallah MT (1993) Intestinal solubility and absorption of ferrous iron in growing rats are affected by different dietary pectins. J Nutr 123:117–124
132. Kim M, Lee D-T, Lee Y-S (1995) Iron absorption and intestinal solubility in rats are influenced by dietary proteins. Nutr Res 15:1705–1716
133. King JC, Raynolds WL, Margen S (1978) Absorption of stable isotopes of iron, copper, and zinc during oral contraceptive use. Am J Clin Nutr 31:1198–1203
134. Kletzien SW (1940) Iron metabolism. 1. The role of calcium in iron assimilation. J Nutr 19:187–197
135. Kontoghiorghes GJ (1990) Chelators affecting iron absorption in mice. Arzneim-Forsch/Drug Res 40:1332–1335
136. Kreuzer M, Kirchgessner M (1991) Endogenous iron excretion. A quantitative means to control iron metabolism? Biol Trace Elem Res 29:77–92
137. Latunde-Dada GO, Neale RJ (1986) Pigeon (*Columba* L.) meat iron solubility and availability for absorption in rats. Br J Nutr 55:409–418
138. Layrisse M, Martínez-Torres C, Roche M (1968) Effect of interaction of various foods on iron absorption. Am J Clin Nutr 21:1175–1183
139. Layrisse M, Martínez-Torres C (1972) Model for measuring dietary absorption of heme iron: test with a complete meal. Am J Clin Nutr 25:401–411
140. Layrisse M, Martínez-Torres C, González M (1974) Measurement of the total daily dietary iron absorption by the extrinsic tag model. Am J Clin Nutr 27:152–162
141. Layrisse M, Martínez-Torres C, Renzy M, Leets I (1975) Ferritin iron absorption in man. Blood 45:689–698
142. Layrisse M, Martínez-Torres C, Leets I, Taylor P, Ramírez J (1984) Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. J Nutr 114:217–223
143. Lee K, Clydesdale FM (1979) Quantitative determination of the elemental, ferrous, ferric, soluble, and complexed iron in foods. J Food Sci 44:549–554
144. Lennernäs H, Palm K, Fagerholm U, Artursson P (1996) Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in vivo. Int J Pharm 127:103–107
145. Littell RC, Lewis AJ, Henry PR (1995) Statistical evaluation of bioavailability assays. In: Bioavailability of nutrients for animals: amino acids, minerals, vitamins; Ammerman CB, Baker DH, Lewis AJ (eds) Academic Press, San Diego, CA, pp 5-33
146. Lock S, Bender AE (1980) Measurement of chemically-available iron in foods by incubation with human gastric juice in vitro. Br J Nutr 43: 413–420
147. Lönnerdal B, Glazier C (1989) An approach to assessing trace element bioavailability from milk in vitro. Biol Trace Elem Res 19:57–69
148. Lunn JA, Richmond J, Simpson JD, Leask JD, Tothill P (1967) Comparison between three radioisotope methods for measuring iron absorption. Br Med J 3:331–333
149. Lyle RM, Weaver CM, Sedlock DA, Rajaram S, Martin B, Melby CL (1992) Iron status in exercising women: the effect of oral iron therapy vs

increased consumption of muscle foods. Am J Clin Nutr 56:1049–1055
150. Ma TY, Hollander D, Bhalla D, Nguyen H, Krugliak P (1992) IEC-18, a non-transformed small intestinal cell line for studying epithelial permeability. J Lab Clin Med 120:329–341
151. MacPhail AP, Patel RC, Bothwell TH, Lamparelli RD (1994) EDTA and the absorption of iron from food. Am J Clin Nutr 59:644–648
152. Magnusson B, Björn-Rasmussen E, Hallberg L, Rossander L (1981) Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. Scand J Haematol 27:201–208
153. Mahoney AW, VanOrden CC, Hendricks DG (1974) Efficiency of converting food iron into hemoglobin by the anemic rat. Nutr Metab 17:223–230
154. Mahoney AW, Hendricks DG (1976) Effect of dietary iron level on efficiency of converting food iron into hemoglobin by the anemic rat. Nutr Metab 20:222–227
155. Mahoney AW, Farmer BR, Hendricks DG (1980) Effects of level and source of dietary fat on the bioavailability of iron from turkey meat for the anemic rat. J Nutr 110:1703–1708
156. Mahoney AW, Hendricks DG (1982) Efficiency of hemoglobin regeneration as a method of assessing iron bioavailability in food products. In: Nutritional bioavailability of iron. ACS Symposium series 203; Kies C (ed) American Chemical Society, Washington DC, pp 1–10
157. Mahoney AW, Hendricks DG (1984) Potential of the rat as a model for predicting iron bioavailability for humans. Nutr Res 4:913–922
158. Mahoney AW, Whittaker P, Farmer BR, Hendricks DG (1985) Iron bioavailability in an anemic rat model: effect of food restriction. Nutr Rep Int 31:457–462
159. Manis JG, Schachter D (1962) Active transport of iron by intestine: features of the two-step mechanism. Am J Physiol 203:73–80
160. Martínez-Torres C, Layrisse M (1970) Effect of amino acids on iron absorption from a staple vegetable food. Blood 35:669–682
161. Martínez–Torres C, Layrisse M (1971) Iron absorption from veal muscle. Am J Clin Nutr 24:531–540
162. Martínez-Torres C, Renzi M, Layrisse M (1976) Iron absorption by humans from hemosiderin and ferritin, further studies. J Nutr 106:128–135
163. Marx JJM (1979) Normal iron absorption and decreased red cell iron uptake in the aged. Blood 53:204–211
164. Marx JJM (1979) Mucosal uptake, mucosal transfer and retention of iron, measured by whole-body counting. Scand J Haematol 23:293–302
165. Marx JJM, Aisen P (1981) Iron uptake by rabbit intestinal mucosal membrane vesicles. Biochim Biophys Acta 649:297–304
166. Maxton DG, Thompson RPH, Hider RC (1994) Absorption of iron from ferric hydroxypyranone complexes. Br J Nutr 71:203–207
167. McCance RA, Edgecombe CN, Widdowson EM (1943) Phytic acid and iron absorption. Lancet ii:126–128
168. McGuire SO, Miller WJ, Gentry RP, Neathery MW, Ho SY, Blackmon DM (1985) Influence of high dietary iron as ferrous carbonate and ferrous sulfate on iron metabolism in young calves. J Dairy Sci 68:2621–2628
169. Mikogami T, Heyman M, Spik G, Desjeux J-F (1994) Apical-to-basolateral transepithelial transport of human lactoferrin in the intestinal cell line HT-29cl.19A. Am J Physiol 267:G308–315
170. Miller DD, Van Campen DR (1979) A method for the detection and assay of iron stable isotope tracers in blood serum. Am J Clin Nutr 32:2354–2361
171. Miller DD, Schricker BR, Rasmussen RR, Van Campen DR (1981) An in vitro method for estimation of iron availability from meals. Am J Clin Nutr 34:2248–2256
172. Miller DD, Berner LA (1989) Is solubility in vitro a reliable predictor of iron bioavailability? Biol Trace Elem Res 19:11–24
173. Miller J (1977) Effect of feed restriction on rate of hemoglobin regeneration in anemic rats. Nutr Rep Int 15:305–309
174. Miller J (1977) Study of experimental conditions for most reliable estimates of relative biological value of iron in bread. J Agric Food Chem 25:154–158
175. Miller J (1982) Assessment of dietary iron availability by rat hemoglobin repletion assay. Nutr Rep Int 26:993–1005
176. Miller J (1982) Factors affecting evaluation of relative biological value of dietary iron for rats. J Nutr 112: 2381–2385
177. Miltenburg GAJ, Wensing Th, Breukink HJ, Marx JJM (1993) Mucosal uptake, mucosal transfer and retention of iron in veal calves. Vet Res Comm 17:209–217
178. Minekus M, Marteau P, Havenaar R, Huis in 't Veld JHJ (1995) A multi-compartmental dynamic computer-controlled model simulating the stomach and small intestine. ATLA 23:197–209
179. Minnich V, Okçuoğlu A, Tarcon Y, Arcasoy A, Cin Ş, Yörükoğlu O, Renda F, Demirağ B (1968) Pica in Turkey. II. Effect of clay upon iron absorption. Am J Clin Nutr 21:78–86
180. Mitchell HS, Schmidt L (1926) The relation of iron from various sources to nutritional anemia. J Biol Chem 70:471–486
181. Monsen ER (1974) Validation of an extrinsic iron label in monitoring absorption of nonheme food iron in normal and iron-deficient rats. J Nutr 104:1490–1495
182. Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W, Finch CA (1978) Estimation of available dietary iron. Am J Clin Nutr 31:134–141
183. Monsen ER, Cook JD (1979) Food iron absorption in human subjects. V. Effects of the major dietary constituents of a semisynthetic meal. Am J Clin Nutr 32:804–808
184. Monsen ER, Balintfy JL (1982) Calculating dietary iron bioavailability: refinement and computerization. J Am Diet Assoc 80:307–311
185. Moore CV, Arrowsmith WR, Welch J, Minnich V (1939) Studies in iron transportation and metabolism. IV. Observations on the absorption of iron from the gastro-intestinal tract. J Clin Invest 18:553–580
186. Moore CV, Minnich V, Dubach R (1943) Absorption and therapeutic efficacy of iron phytate. J Am Diet Assoc 19:841–844
187. Moore CV, Dubach R (1951) Observations on the absorption of iron from foods tagged with radioiron. Trans Assoc Am Physicians 64:245–256
188. Morris ER (1983) An overview of current information on bioavailability of dietary iron to humans. Fed Proc 42:1716–1720
189. Motzok I, Pennell MD, Davies M, Ross HU (1975) Effect of particle size on the biological availability of reduced iron. J Assoc Off Anal Chem 58:99–103
190. Motzok I, Verma RS, Chen S-S, Rasper J, Hancock RGV, Ross HU (1978) Bioavailability, in vitro solubility, and physical and chemical properties of elemental iron powders. J Assoc Off Anal Chem 61:887–893
191. Narasinga Rao BS, Siva Prasad J, Vijaya Sarathy C (1977) An animal model to study iron availability from human diets. Br J Nutr 37:451–456
192. Narasinga Rao BS, Prabhavathi T (1978) An in vitro method for predicting the bioavailability of iron from foods. Am J Clin Nutr 31:169–175
193. Naylor GPL, Harrison JD (1995) Gastrointestinal iron and cobalt absorption and iron status in young rats and guinea pigs. Human Exp Toxicol 14:949–954
194. Nichols GM, Pearce AR, Alverez X, Bibb NK, Nichols KY, Alfred CB, Glass J (1992) The mechanisms of nonheme iron uptake determined in IEC-6 rat intestinal cells. J Nutr 122:945–952

195. Nielsen P, Dresow B, Fischer R, Heinrich HC (1990) Bioavailability of iron and cyanide from oral potassium ferric hexacyanoferrate(II) in humans. Arch Toxicol 64:420–422
196. Norrby A, Sölvell L (1974) The absorption of food iron measured by haemoglobin regeneration and phlebotomy. Scand J Haematol Suppl 20:15–32
197. Núñez MT, Alvarez X, Smith M, Tapia V, Glass J (1994) Role of redox systems on Fe^{3+} uptake by transformed human intestinal epithelial (Caco-2) cells. Am J Physiol 267:C1582–C1588
198. Olivares M, Hertrampf E, Pizarro F (1993) Effect of iron stores on heme iron absorption. Nutr Res 13:633–638
199. Olszon E, Isaksson B, Norrby A, Sölvell L (1978) Food iron absorption in iron deficiency. Am J Clin Nutr 31:106–111
200. O'Neil-Cutting MA, Crosby WH (1986) The effect of antacids on the absorption of simultaneously ingested iron. JAMA 255:1468–1470
201. Pennell MD, Wiens W-D, Rasper J, Motzok I (1975) Factors affecting the relative biological value of food grade elemental iron powders for rats and humans. J Food Sci 40:879–883
202. Pennell MD, Davies MI, Rasper J, Motzok I (1976) Biological availability of iron supplements for rats chicks and humans. J Nutr 106:265–274
203. Peters TJ, Raja KB, Simpson RJ (1992) Speciation of trace metals, with special reference to intestinal iron absorption. Food Chem 43:315–320
204. Pinto M, Robine-Leon S, Appay M-D, Kedinger M, Triadou N, Dussaulx E, Lacroix B, Simon-Assmann P, Haffen K, Fogh J, Zweibaum A (1983) Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. Biol Cell 47:323–330
205. Pirzio-Biroli G, Bothwell TH, Finch CA (1958) Iron absorption. II. The absorption of radioiron administered with a standard meal in man. J Lab Clin Med 51:37–48
206. Pla GW, Fritz JC (1970) Availability of iron. J Assoc Off Anal Chem 53:791–800
207. Pla GW, Fritz JC (1971) Collaborative study of the hemoglobin repletion test in chicks and rats for measuring availability of iron. J Assoc Off Anal Chem 54:13–17
208. Pla GW, Fritz JC (1972) Human plasma iron responses following test doses of iron from known sources. J Assoc Off Anal Chem 55:197–199
209. Pla GW, Harrison BN, Fritz JC (1973) Comparison of chicks and rats as test animals for studying bioavailability of iron, with special reference to use of reduced iron in enriched bread. J Assoc Off Anal Chem 56:1369–1373
210. Pla GW, Fritz JC, Rollinson CL (1976) Relationship between the biological availability and solubility rate of reduced iron. J Assoc Off Anal Chem 59:582–583
211. Platt SR, Clydesdale FM (1984) Binding of iron by cellulose, lignin, sodium phytate and beta-glucan, alone and in combination, under simulated gastrointestinal pH conditions. J Food Sci 49:531–535
212. Politz ML, Clydesdale FM (1988) Effect of enzymatic digestion, pH and molecular weight on the iron solubilizing properties of chicken muscle. J Food Sci 53:1081–1085
213. Price DC, Cohn SH, Wasserman LR, Reizenstein PG, Cronkite EP (1962) The determination of iron absorption and loss by whole body counting. Blood 20:517–531
214. Raffin SB, Woo CH, Roost KT, Price DC, Schmid R (1974) Intestinal absorption of hemoglobin iron-heme cleavage by mucosal heme oxygenase. J Clin Invest 54:1344–1352
215. Ranger CR, Neale RJ (1984) Iron availability from soy, meat and soy/meat samples in anemic rats with and without prevention of coprophagy. J Food Sci 49:585–589
216. Ranhotra GS, Hepburn FN, Bradley WB (1971) Availability of iron in enriched bread. Cereal Chem 48:377–384
217. Rao J, Jagadeesan V (1995) Development of a rat model for iron deficiency and toxicological studies: comparison among Fischer 344, Wistar, and Sprague Dawley strains. Lab Anim Sci 45:393–397
218. Reddy MB, Chidambaram MV, Fonseca J, Bates GW (1986) Potential role of in vitro iron bioavailability studies in combatting iron deficiency: a study of the effects of phosvitin on iron mobilization from pinto beans. Clin Physiol Biochem 4:78–86
219. Reddy MB, Cook JD (1991) Assessment of dietary determinants of nonheme-iron absorption in humans and rats. Am J Clin Nutr 54:723–728
220. Roodenburg AJC, West CE, Yu S, Beynen AC (1994) Comparison between time-dependent changes in iron metabolism of rats as induced by marginal deficiency of either vitamin A or iron. Br J Nutr 71:687–699
221. Rosado JL, Lopez P, Morales M, Munoz E, Allen LH (1992) Bioavailability of energy, nitrogen, fat, zinc, iron and calcium from rural and urban Mexican diets. Br J Nutr 68:45–58
222. Rossander L Hallberg L, Björn-Rasmussen E (1979) Absorption of iron from breakfast meals. Am J Clin Nutr 32:2484–2489
223. Ruliffson WS, Hopping JM (1963) Maturation, iron deficiency, and ligands in enteric radioiron transport in vitro. Am J Physiol 204:171–175
224. Sáiz MP, Martí MT, Mitjavila MT, Planas J (1993) Iron absorption by small intestine of chickens. Biol Trace Elem Res 36:7–14
225. Sánchez L, Ismail M, Liew FY, Brock JH (1996) Iron transport across Caco-2 cell monolayers. Effect of transferrin, lactoferrin and nitric oxide. Biochim Biophys Acta 1289:291–297
226. Sanford R (1960) Release of iron from conjugates in foods. Nature 185:533–534
227. Sato R, Noguchi T, Naito H (1987) The effect of feeding demineralized egg yolk protein on the solubility of intra-intestinal iron. Nutr Rep Int 36:593–602
228. Sayers MH, Lynch SR, Jacobs P, Charlton RW, Bothwell TH, Walker RB, 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
229. Saylor L, Finch CA (1953) Determination of iron absorption using two isotopes of iron. Am J Physiol 172: 372–376
230. Schlemmer U (1995) Bioavailability of nutrients. Conceptual aspects of definition and problems of determination. Br J Nutr 73:150–151
231. Schricker BR, Miller DD, Rasmussen RR, Van Campen D (1981) A comparison of in vivo and in vitro methods for determining availability of iron from meals. Am J Clin Nutr 34:2257–2263
232. Schricker BR, Miller DD, Van Campen DR (1982) In vitro estimation of iron availability in meals containing soy products. J Nutr 112:1696–1705
233. Schümann K, Elsenhans B, Gordon DT, Schlemmer U (1994) Bioavailability '93: different aspects of 'bioavailability' in perspective. Br J Nutr 72:950–952
234. Seligman PA, Caskey JH, Frazier JL, Zucker RM, Podell ER, Allen RH (1983) Measurements of iron absorption from prenatal multivitamin-mineral supplements. Obstet Gynecol 61: 356–362
235. Shackleton L, McCance RA (1936) The ionisable iron in foods. Biochem J 30:582–591
236. Shafey TM, McDonald MW, Dingle JG (1991) Effect of dietary calcium and available phosphorus concentration on digesta pH and on the availability of calcium, iron, magnesium and zinc from the intestinal contents of meat chickens. Br Poultry Sci 32: 185–194
237. Shah BG, Giroux A, Belonje B (1977) Specifications for reduced iron as a food additive. J Agric Food Chem 25:592–594
238. Shah BG, Belonje B (1985) Rat bioassay of hydrogen-reduced iron powder in flour and bread. Nutr Rep Int 32:1171–1178

239. Shears GE, Neale RJ, Ledward DA (1989) Effect of dietary iron deficiency and tungsten supplementation on ^{59}Fe absorption and gastric retention from ^{59}Fe compounds in rats. Br J Nutr 61:573–581
240. Simpson RJ, Peters TJ (1986) Mouse intestinal Fe^{3+} uptake kinetics in vivo. The significance of brush-border membrane vesicle transport in the mechanism of mucosal Fe^{3+} uptake. Biochim Biophys Acta 856:115–122
241. Simpson RJ, Peters TJ (1990) Forms of soluble iron in mouse stomach and duodenal lumen: significance for mucosal uptake. Br J Nutr 63:79–89
242. Simpson RJ, Sidhar S, Peters TJ (1992) Application of selective extraction to the study of iron species present in diet and rat gastrointestinal tract contents. Br J Nutr 67:437–444
243. Smith MC, Otis L (1937) Hemoglobin regeneration in anemic rats in relation to iron intake. With suggestions for improvement of the bioassay technic for measuring available iron. J Nutr 13:573–582
244. Storey ML, Greger JL (1987) Iron, zinc and copper interactions: chronic versus acute responses of rats. J Nutr 117:1434–1442
245. Taylor P, Martínez-Torres C, Leets I, Ramírez J, Nieves García-Casal M, Layrisse M. (1988) Relationships among iron absorption, percent saturation of plasma transferrin and serum ferritin concentration in humans. J Nutr 118:1110–1115
246. Thannoun AM, Mahoney AW, Hendricks DG, Zhang D (1987) Effect of meat-bread mixtures on bioavailability of total dietary iron for anemic rats. Cereal Chem 64:399–403
247. Thomas FB, McCullough FS, Greenberger NJ (1972) Effect of phenobarbital on the absorption of inorganic and hemoglobin iron in the rat. Gastroenterol 62:590–599
248. Tidehag P, Hallmans G, Wing K, Sjöström R, Ågren G, Lundin E, Zhang J-X (1996) A comparison of iron absorption from single meals and daily diets using radioFe (^{55}Fe, ^{59}Fe). Br J Nutr 75:281–289
249. Underwood EJ (1938) A comparison of ferrous and ferric iron in the nutrition of the rat. J Nutr 16:299–308
250. Van Dokkum W (1992) Significance of iron bioavailability for iron recommendations. Biol Trace Elem Res 35:1–11
251. Van Gils APM, Lemmens AG, Beynen AC (1994) Fructose versus glucose in the diet of rats lowers the apparent absorption of dietary iron from both ferrous and ferric sulphate. J Anim Physiol a Anim Nutr 71:22–29
252. Van Hoek R, Conrad ME Jr (1961) Iron absorption. Measurement of ingested iron^{59} by a human whole-body liquid scintillation counter. J Clin Invest 40:1153–1159
253. Viteri FE, Alvarez E, Batres R, Torún B, Pineda O, Mejía LA, Sylvi J (1995) Fortification of sugar with iron sodium ethylenediaminotetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations. Am J Clin Nutr 61:1153–1163
254. Walter T, Hertrampf E, Pizarro F, Olivares M, Llaguno S, Letelier A, Vega V, Stekel A (1993) Effect of bovine-hemoglobin-fortified cookies on iron status of schoolchildren: a nationwide program in Chile. Am J Clin Nutr 57:190–194
255. Weaver CM, Schmitt HA, Stuart MA, Mason AC, Meyer NR, Elliott JG (1984) Radiolabeled iron in soybeans: intrinsic labeling and bioavailability of iron to rats from defatted flour. J Nutr 114:1035–1041
256. Weintraub LR, Weinstein MB, Huser H-J, Rafal S (1968) Absorption of hemoglobin iron: the role of a heme-splitting substance in the intestinal mucosa. J Clin Invest 47:531–539
257. Wheby MS, Crosby WH (1963) The gastrointestinal tract and iron absorption. Blood 22:416–428
258. Wheby MS (1970) Site of iron absorption in man. Scand J Haematol 7:56–62
259. Wheby MS, Suttle GE, Ford III KT (1970) Intestinal absorption of hemoglobin iron. Gastroenterol 58:647–654
260. Whittaker P, Mahoney AW, Hendricks DG (1984) Effect of iron-deficiency anemia on percent blood volume in growing rats. J Nutr 114:1137–1142
261. Whittaker PG, Lind T, Williams JG, Gray AL (1989) Inductively coupled mass spectrometric determination of the absorption of iron in normal women. Analyst 114:675–678
262. Whittaker PG, Lind T, Williams JG (1991) Iron absorption during normal human pregnancy: a study using stable isotopes. Br J Nutr 65:457–463
263. Wienk KJH, Marx JJM, Lemmens AG, Brink EJ, Van der Meer R, Beynen AC (1996) Mechanism underlying the inhibitory effect of high calcium carbonate intake on iron bioavailability from ferrous sulphate in anaemic rats. Br J Nutr 75:109–120
264. Wienk KJH, Beynen AC (1996) Efficacy of iron repletion in anemic rats after the feeding of a purified or natural-ingredient diet with ferrous sulphate or carbonyl iron. Nutr Res 16:615–626
265. Wienk KJH, Marx JJM, Van der Meer R, Lemmens AG, Brink EJ, Beynen AC (to be submitted) Calcium inhibits uptake but not subsequent fractional transfer of nonheme iron by Caco-2 cells
266. Wilson TH, Wiseham G (1954) The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J Physiol 123:116–125
267. Wolters MGE, Diepenmaat HB, Hermus RJJ, Voragen AGJ (1993) Relation between in vitro availability of minerals and food composition: a mathematical model. J Food Sci 58:1349–1355
268. Wolters MGE, Schreuder HAW, Van den Heuvel G, Van Lonkhuijsen HJ, Hermus RJJ, Voragen AGJ (1993) A continuous in vitro method for estimation of the bioavailability of minerals and trace elements in foods: application to breads varying in phytic acid content. Br J Nutr 69:849–861
269. Woodhead JC, Drulis JM, Nelson SE, Janghorbani M, Fomon SJ (1991) Gender-related differences in iron absorption by preadolescent children. Pediatr Res 29:435–439
270. Yergey AL (1996) Analytical instruments for stable isotopic tracers in mineral metabolism. J Nutr 126:355S–361S
271. Zemel MB, Shelef LA (1982) Phytic acid hydrolysis and soluble zinc and iron in whole wheat bread as affected by calcium containing additives. J Food Sci 47:535–537
272. Zempsky WT, Rosenstein BJ, Carroll JA, Oski FA (1989) Effect of pancreatic enzyme supplements on iron absorption. Am J Dis Child 143:969–972
273. Zhang D, Hendricks DG, Mahoney AW (1989) Bioavailability of total iron from meat, spinach (*Spinacea oleracea* L.) and meat-spinach mixtures by anaemic and non-anaemic rats. Br J Nutr 61:331–343
274. Zhang D, Hendricks DG, Mahoney AW (1992) Effect of coprophagy on bioavailability of iron from plant foods fed to anemic rats. Plant Foods Hum Nutr 42:97–108