Bioavailability of Nutrients and Other Bioactive Components from Dietary Supplements

Iron[1]

Susan J. Fairweather-Tait[2]

Institute of Food Research, Norwich Research Park, Colney, Norwich, United Kingdom

ABSTRACT The most useful and appropriate methods for assessing the bioavailability of (nonheme) iron supplements are described. When the supplement can be labeled isotopically, the best method for measuring bioavailability is hemoglobin incorporation, followed by fecal monitoring. Caco-2 cell in vitro systems can be used for rapid screening to predict potential availability for absorption. If the compound cannot be labeled, then the plasma appearance/disappearance of oral iron given together with an intravenous dose of iron isotope can be used to quantify absorption. With oral doses in excess of 25 mg, the 4- to 6-h plasma concentration can provide a qualitative assessment of bioavailability. Approaches for normalizing results to minimize intraindividual and interindividual variability in efficiency of iron absorption are discussed. J. Nutr. 131: 1383S–1386S, 2001.

KEY WORDS: • *bioavailability* • *supplements* • *iron*

There are a number of reviews in the literature describing methods that can be used to study iron bioavailability (Hallberg 1981, van Dokkum et al. 1996, Wienk et al. 1999). The aim of the present article is to focus on methods that can be used to assess the bioavailability of iron supplements, not dietary iron. However, the bioavailability of any iron supplement taken with food(s) or beverage(s) will be modified by the presence of a wide range of dietary inhibitors and enhancers. Similarly, physiological factors that determine the efficiency of dietary iron absorption will also influence the absorption of supplemental iron (Fairweather-Tait 1998).

Dietary iron is found in two predominant forms, heme and nonheme, and these are absorbed by separate pathways, heme as the intact moiety and nonheme iron from the common iron pool within the gut (Hallberg 1981). Because heme iron is rarely, if ever, used as an iron supplement per se, the methods discussed in this article relate specifically to nonheme iron supplements.

Available standardized and validated methods

According to the generally accepted definition, bioavailability is a measure of fractional utilization of orally ingested nutrient. Currently, iron is the only micronutrient for which there is a direct measure of bioavailability. Because ~80–90% of absorbed iron is used for the synthesis of hemoglobin in reticulocytes, bioavailability can be measured as hemoglobin incorporation using isotopes. However, because there are no major excretory pathways for iron, various measures of absorption are also used to predict bioavailability.

Early studies made use of animal models, particularly the rat, for assessing the bioavailability of different forms of iron. However, there are some major discrepancies between results from studies in rodents and man, and it is now generally accepted that rats cannot be used to assess the quantitative importance of dietary factors in human iron nutrition (Reddy and Cook 1991). Therefore, animal models are not recommended for studying the bioavailability of iron supplements.

Techniques currently used to assess iron bioavailability are summarized in **Table 1**. Most involve radio- or stable isotope labels to monitor the absorption and/or subsequent metabolism of different forms of iron, but it is also possible to assess bioavailability by measuring the rate of hemoglobin repletion in anemic individuals. However, this requires recruitment of volunteers who are already anemic or who are made anemic by venesection, which may create logistical and/or ethical problems.

Isotopic labeling of iron supplements

The methods of choice for measuring bioavailability of iron supplements involve the use of isotope labels to follow the metabolism of the test compound. One of the major difficulties with measuring the bioavailability of iron supplements is preparing suitable test material. Examples of iron compounds marketed as supplements are shown in **Table 2**. Some are slow release preparations, designed to reduce unpleasant gastrointestinal side effects. Efficacy is clearly improved when bioavailability is maximized, but for people who are not iron deficient and, hence, will not absorb iron efficiently, the advisability of

[1] Presented at the conference "Bioavailability of Nutrients and Other Bioactive Components from Dietary Supplements" held January 5–6, 2000 in Bethesda, Maryland. This conference was sponsored by the Office of Dietary Supplements, National Institutes of Health and the Life Science Research Office, American Society for Nutritional Sciences. Conference proceedings are published as a supplement to *The Journal of Nutrition*. Guest editors for the supplement publications were Mary Frances Picciano, Pennsylvania State University, University Park, PA and Daniel J. Raiten, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

[2] To whom correspondence should be addressed.
E-mail: sue.fairweather-tait@bbsrc.ac.uk

TABLE 1

Methods for assessing iron bioavailability

Hemoglobin incorporation (radio- and stable isotopes)
Fecal monitoring (radio- and stable isotopes)
Caco-2 cell systems (in vitro)
Plasma appearance/disappearance
Whole-body counting (^{59}Fe)
Hemoglobin repletion

ingesting high doses of oral iron has been questioned, particularly in relation to increased risk of bowel cancer (Wurzemann et al. 1996). The mechanism is not understood but it may relate to the generation of free radicals in feces, as demonstrated with oral ferrous sulfate supplements in iron-replete individuals (Lund et al. 1999). Thus, bioavailability is a key determinant of both efficacy and safety.

To label an iron supplement, the added isotope must either be in exactly the same physicochemical form or must be fully exchangeable with the supplemental iron. With iron salts that are completely soluble in water or dilute hydrochloric acid (pH 2), as found under typical gastric conditions, then it is easy to label them with a solution of iron isotope. If the supplement is insoluble or only partially soluble, the isotopic label must be prepared in a similar form before mixing with the supplement. The costs may be prohibitive when the yields from the chemical synthesis are low (e.g., ferrous gluconate) and expensive stable isotopes are used for labeling. In some cases, it may be impossible to synthesize isotopically enriched forms of iron, e.g., polysaccharide iron complexes containing oxides of iron, e.g., hematite (α-Fe_2-O_3) and magnetite (Fe_3O_4) and metallic iron.

There are two radioisotopes of iron, ^{59}Fe and ^{55}Fe, both of which have been used extensively for human iron studies (Hallberg 1981). These are generally supplied as high specific activity ferric chloride solution. More recently, attention has focused on developing stable isotope techniques for studying iron bioavailability because of ethical concerns about exposure to ionizing radiation. Iron has four stable isotopes, three of which (^{54}Fe, ^{57}Fe and ^{58}Fe) have a low enough natural abundance for iron bioavailability studies (5.8, 2.2 and 0.28%, respectively).

Hemoglobin incorporation

The method of choice for assessing the bioavailability of iron supplements is hemoglobin incorporation, where the test compound is labeled with a radioisotope, fed to fasting individuals on one or more occasions and percentage incorporation into red blood cells measured 14 d after the last oral dose (Brise and Hallberg 1962). A comparison can be made between two different sources of iron, labeled with ^{55}Fe or ^{59}Fe, or between the test compound and a well-absorbed reference dose comprised of 3 mg of iron as ferrous sulfate plus 30 mg of ascorbic acid (Hallberg et al. 1997). Sensitive sample preparation and detection methods have been developed that require the administration of levels of radioactivity as low as 37 kBq of ^{59}Fe or 111 kBq of ^{55}Fe. The percentage of iron absorption is calculated from estimates of blood volume (based on a height and weight nomogram) and an assumed red cell incorporation of absorbed iron of 80 or 100% [with serum ferritin concentration $>$ 15 or $<$ 15 μg/L, respectively (Cook et al. 1991)].

An alternative, but rarely used, dual isotope method for obtaining absolute instead of relative values for iron absorption can be used (Saylor and Finch 1953) that avoids the need for making assumptions about percent incorporation of absorbed iron into red blood cells. In this method, ^{59}Fe is given orally at the same time that ^{55}Fe, bound to plasma, is injected intravenously. Absorption is calculated by relating the ratio of the two isotopes in the red cells to the ratio of administered isotopes, and whole-body ^{59}Fe counting can be used for verification. Stable isotopes have also been used to measure absorption in women from an oral dose of 5 mg of ^{57}Fe and an intravenous dose of 250 μg of ^{58}Fe (Barrett et al. 1994). In principle, the method could be used for studying more than one iron compound if mixtures of radio- and stable isotopes are used.

When radioisotope administration is not permitted, hemoglobin incorporation of stable isotopes can be measured to assess iron bioavailability (van Dokkum et al. 1996). Unlike the situation with radioisotopes, where background activity is not a problem, relatively large doses of stable isotopes have to be given to produce measurable enrichment of red blood cells. This creates labeling problems for food iron, where intakes of iron are relatively low, and necessitates multiple dosing protocols. Thus, early applications of stable isotope methodology were in infants (Fairweather-Tait et al. 1995) whose blood volume is smaller than that of adults. However, the constraints concerning bioavailability measurements of iron supplements relate to the cost of isotope and labeling of the iron, not to dose per se. Increased sensitivity may be achieved by separating the young erythroid cells from blood for the determination of isotopic enrichment, and this will reduce the oral dose of isotope by up to one third (van den Heuvel et al. 1998).

Fecal monitoring

Because there is no appreciable loss of absorbed iron through feces and very little iron excreted in the urine, fecal monitoring can be used to measure true iron absorption. This method applies mainly to stable isotopes, where doses are dependent on the detection system but are generally less than those needed for the hemoglobin incorporation technique.

The main problem with fecal monitoring is incomplete fecal collection, particularly in individuals with long transit times. Short collection periods may lead to an overestimate of absorption due to incomplete recovery of unabsorbed isotope from luminal contents and/or incomplete collection of mucosal cells that contain isotope that was not transferred to the systemic circulation. A number of markers have been used to test for completeness of fecal collection, the most recent being the rare earth elements (Fairweather-Tait et al. 1997).

Whole-body counting

Whole-body counting used to be considered to be the gold standard technique for measuring iron absorption but it is less

TABLE 2

Examples of forms of iron found in supplements

Ferrous sulfate
Ferrous gluconate
Ferrous fumarate
Ferrous succinate
Ferrous glycine sulfate
Ferrous carbonate
Iron glycine
Ferritin
Polysaccharide iron complexes

commonly used today because it needs access to a whole-body counter and associated specialist techniques (Bothwell et al. 1979). The supplement must be labeled with the gamma-isotope, ^{59}Fe. An initial baseline count is made to measure background activity (from ^{40}K and any residual radioactivity from previous studies involving radioisotopes) and the volunteer is given ^{59}Fe-labeled iron compound and recounted 1–5 h and 10–14 d later. The difference is calculated as absorbed isotope, once corrections have been made for isotope decay and geometry.

Plasma appearance/disappearance

The wide diurnal variations in plasma iron restricts the use of plasma iron tolerance as a method for measuring iron absorption from unlabelled iron supplements, except for large doses (see below). Alternative approaches using stable isotope-enriched iron, validated against radioisotope methods, have been introduced (Barrett et al. 1994). To quantify iron absorption using plasma appearance data, information on the rate of removal of iron from the plasma is required; this can be obtained by giving an intravenous dose of a second iron isotope at the same time as the oral dose and monitoring its concentration in plasma. Plasma concentrations of the isotopes are calculated using deconvolution analysis to obtain the area under the curve. In practice, it is often not possible to take blood samples until all of the isotopes have disappeared from the plasma; therefore, a simulation has to be performed from the last sample point. This is more straightforward for the intravenous dose (which follows an exponential decay) than for the oral dose. To simulate beyond the last point of the oral data, sampling must be taken beyond the time required for absorption to be completed and the subsequent appearance of oral isotope is assumed to follow the same form of exponential decay as the intravenous isotope.

A simpler method for assessing bioavailability that does not require isotopic labeling can be used for large (25–100 mg) doses of iron. The serum iron increase 4–6 h postingestion is significantly correlated with absorption of ^{59}Fe measured by whole-body counting (Ekenved et al. 1976). It is possible to use this technique to make a qualitative assessment of the bioavailability of different iron supplements.

Caco-2 cell systems

A new in vitro Caco-2 cell system has been developed for predicting iron bioavailability from food (Garcia et al. 1996) and meal digests (Glahn et al. 1996). Caco-2 TC7 cells have recently been shown to express Nramp2 (a divalent cation transporter) in the apical membrane, with a substrate preference for iron and transport occurring by a proton-dependent mechanism (Tandy et al. 2000). These results indicate that this in vitro system could provide a rapid screening technique to measure the maximum potential bioavailability of iron supplements, provided the iron can be labeled isotopically.

Normalization of results

One of the difficulties encountered with in vivo studies measuring iron bioavailability is the wide intersubject and intrasubject variability in iron absorption (Kuhn et al. 1968). Multiple dosing protocols can reduce day-to-day fluctuations in efficiency of absorption, but this approach is not always possible. Interindividual variation is primarily due to differences in body iron stores and, hence, physiological requirements for iron (Hallberg et al. 1998). Two main approaches have been proposed to normalize the differences.

1. Comparison with a reference dose. Individuals are given 3 mg of iron (as ferrous sulfate plus 30 mg of ascorbic acid) and absorption from the test compound is compared with the well-absorbed reference dose. The bioavailability of the iron supplement is either expressed as a ratio (ferrous sulfate:iron supplement) or corrected to a mean reference value of 40% by multiplying absorption by 40/R, where R is the reference dose absorption (Magnusson et al. 1981).
2. Correction for body iron stores. The absorption data are corrected to a value corresponding to a serum ferritin of 40 μg/L (Cook et al. 1991) using the equation:

$$\text{Log } A_c = \text{Log } A_o + \text{Log } F_o - \text{Log } 40,$$

where A_c is the corrected absorption from the iron supplement, A_o is the observed absorption and F_o is the observed serum ferritin.

Recently, Hallberg et al. (1998) examined the relationship between iron stores and iron absorption in relation to dietary requirements for iron. They have developed a new equation relating serum ferritin and iron stores, which may affect the normalization procedure. They also emphasize the inertia in changes of iron stores by presenting a series of graphs illustrating the rate of increase of iron stores in individuals with different iron requirements. The latter clearly demonstrates that it takes years, not months, for iron stores to reach a plateau, regardless of dietary iron bioavailability. Thus, long-term endpoints are not appropriate for studying the bioavailability of iron supplements.

The best method for assessing bioavailability of supplemental iron is hemoglobin incorporation, followed by fecal monitoring, using radioisotope or stable isotope labeling. Caco-2 cell systems can be used for rapid screening of labeled supplements to predict availability for absorption. If the compound cannot be labeled, then plasma appearance/disappearance of oral iron given together with an intravenous dose of iron isotope can be used to quantify absorption. With oral doses in excess of 25 mg, the 4- to 6-h plasma iron concentration can be taken as a qualitative assessment of iron bioavailability. For all methods, it is important to design a protocol that enables the results from individual studies to be normalized, for example, by comparing the supplement with an iron source of known bioavailability, e.g., 3 mg of ferrous sulfate plus 30 mg of ascorbic acid, or to estimate maximum potential bioavailability.

LITERATURE CITED

Barrett, J.F.R., Whittaker, P. G., Fenwick, J. D., Williams, J. G. & Lind, T. (1994) Comparison of stable isotopes and radioisotopes in the measurement of iron absorption in healthy women. Clin. Sci. 87: 91–95.

Brise, H. & Hallberg, L. (1962) A method for comparative studies on iron absorption in man using two radioiron isotopes. Acta Med. Scand. Suppl. 59: 7–22.

Bothwell, T. H., Charlton, R. W., Cook, J. D. & Finch, C. A. (1979) Iron Metabolism in Man. Blackwell Scientific Publications, Oxford, U.K.

Cook, J. D., Dassenko, S. A. & Lynch, S. R. (1991) Assessment of the role of nonheme-iron availability in iron balance. Am. J. Clin. Nutr. 54: 717–722.

Ekenved, G., Norrby, A. & Solvell, L. (1976) Serum iron increase as a measure of iron absorption: studies on the correlation with total absorption. Scand. J. Haematol. Suppl. 28: 51–63.

Fairweather-Tait, S. J. (1998) Trace element bioavailability. In: Role of Trace Elements for Health Promotion and Disease Prevention (Sandstrom, B. & Walter, eds.), pp. 29–39. Karger, Basel.

Fairweather-Tait, S., Fox, T., Wharf, S. G. & Eagles, J. (1995) The bioavailability of iron in different weaning foods and the enhancing effect of a fruit drink containing ascorbic acid. Pediatr. Res. 37: 389–394.

Fairweather-Tait, S. J., Minihane, A. M., Eagles, J., Owen, L. & Crews,

H. (1997) Rare earth elements as nonabsorbable fecal markers in studies of iron absorption. Am. J. Clin. Nutr. 65: 970–976.
Garcia, M. N., Flowers, C. & Cook, J. D. (1996) The Caco-2 cell culture system can be used as a model to study food iron availability. J. Nutr. 126: 251–258.
Glahn, R. P., Wien, E. M., Van Campen, D. R. & Miller, D. D. (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.
Hallberg, L. (1981) Bioavailability of dietary iron in man. Annu. Rev. Nutr. 1: 123–147.
Hallberg, L., Hulthen, L. & Garby, L. (1998) Iron stores in man in relation to diet and iron requirements. Eur. J. Clin. Nutr. 52: 623–631.
Hallberg, L., Hulten, L. & Gramatkovski, E. (1997) Iron absorption from the whole diet in men: how effective is the regulation of iron absorption? Am. J. Clin. Nutr. 66: 347–356.
Kuhn, I. N., Monsen, E. R., Cook, J. D. & Finch, C. A. (1968) Iron absorption in man. J. Lab. Clin. Med. 71: 715–721.
Lund, E. K., Wharf, S. G., Fairweather-Tait, S. J. & Johnson, I. T. (1999) Oral ferrous sulfate supplements increase the free radical-generating capacity of feces from healthy volunteers. Am. J. Clin. Nutr. 69: 250–255.
Magnusson, B., Bjorn-Rasmussen, E., Hallberg, L. & Rossander, L. (1981) Iron absorption in relation to iron status: model proposed to express results to food iron absorption measurements. Scand. J. Haematol. 27: 210–208.
Reddy, M. B. & Cook, J. D. (1991) Assessment of dietary determinants of nonheme-iron absorption in humans and rats. Am. J. Clin. Nutr. 54: 723–728.
Saylor, L. & Finch, C. A. (1953) Determination of iron absorption using two isotopes of iron. Am. J. Physiol. 172: 372–376.
Tandy, S., Williams, M., Leggett, A., Lopez-Jimenez, M., Dedes, M., Ramesh, B., Srai, S. K. & Sharp, P. (2000) Nramp2 expression is associated with pH-dependent iron uptake across the apical membrane of human intestinal Caco-2 cells. J. Biol. Chem. 275: 1023–1029.
van den Heuvel, E., Muys, T., Pellegrom, H., Bruyntjes, J. P., van Dokkum, W., Spanhaak, S. & Schaafsma, G. (1998) A new method to measure iron absorption from the enrichment of ^{57}Fe and ^{58}Fe in young erythroid cells. Clin. Chem. 44: 649–654.
van Dokkum, W., Fairweather-Tait, S. J., Hurrell, R. & Sandstrom, B. (1996) Study techniques. In: Stable Isotopes in Human Nutrition (Mellon, F. A. & Sandstrom, B., eds.), pp.23–42. Academic Press, London, U.K.
Wienk, K.J.H., Marx, J.J.M. & Beynen, A. C. (1999) The concept of iron bioavailability and its assessment. Eur. J. Nutr. 38: 51–75.
Wurzemann, J. l., Silver, A., Schreinemachers, D. M., Sandler, R. S. & Everson, R. B. (1996) Iron intake and risk of colon cancer. Cancer Epidemiol. Biomarkers Prev. 5: 503–507.