Iron Biofortification and Revisiting the Accuracy of Extrinsic Labeling in Studies of Iron Absorption

Dear Editor:

Extrinsic isotopic labeling of the iron in a food or meal is a method that has been used many times over the past few decades to assess iron bioavailability. The primary assumption of this methodology is that the extrinsic label exchanges or equilibrates fully with the intrinsic iron of the food or meal. In 1983, Consaul and Lee (1) published a critical review of this methodology. They concluded that “...this method cannot be considered proven with regards to all types of foods. ...At present it is not known how completely the different nonheme forms of iron are labeled by an extrinsic tag.”

Since 1983, only 2 studies (2, 3) have revisited the issue of validity and accuracy of extrinsic labeling of iron in foods, with both studies comparing varieties of beans. In one study (2), comparison of absorption of iron from the intrinsic iron and the extrinsic iron isotope was used as the measure of iron exchange. In this study, iron absorption was very low (i.e., 1–1.8%), and no statistical difference in absorption was observed. The authors broadly concluded that “…extrinsic labeling may be used to screen various varieties of beans for iron bioavailability in humans.” In the other study (3), in vitro digestion was used and extrinsic and intrinsic iron was directly measured in supernatant and pellet fractions of the digests. These authors found that extrinsic iron does not always equilibrate well with the intrinsic iron of beans. Taken together with evidence summarized by Consaul and Lee (1), there are clearly times when the primary assumption of extrinsic iron labeling is not met, yet since 1983, a multitude of studies have used extrinsic labeling of food iron, and there are no published results in these studies that demonstrate testing of the degree of isotopic exchange. As a scientist working in the field of iron nutrition, I am concerned by what appears to be broad acceptance of methodology based on an unreliable and improperly tested assumption.

The recent publication by Petry et al. (4) is an example in which this methodology has again been used without evaluating the exchange of the extrinsic label with the intrinsic iron of the food (i.e., bean) and/or the complete meal. Petry et al. cite several references (2, 5–8) as validation of extrinsic labeling methodology. I encourage readers to review those references carefully and consider the different bean and staple crop varieties, the different meal components, and the various instances that indicate miscalibration of extrinsic and intrinsic iron. For example, in Cook et al. (5; Tables III, IV, and V), there are many results in which the extrinsic-to-intrinsic iron absorption ratios are different from a value of 1. Moreover, let us keep in mind that in these studies the measure of iron exchange or equilibration was based solely on absorption. Absorption is an indirect measure of equilibration and clearly it is possible that similar absorption values can be obtained from intrinsic and extrinsic iron without full equilibration of the 2 sources of iron. For example, in a bean meal, the intrinsic iron could be primarily in the iron phytate form or complexed to cotyledon proteins, whereas the extrinsic iron could be bound to polyphenols emanating from the seed coat, with the end result being a similar percentage absorption of each isotope. To my knowledge, no published studies exist to demonstrate this possibility, but clearly the possibility exists. This scenario would be even more likely when iron absorption values are very low, such as <5%, which is common with human iron absorption. In a thorough search of the literature, I can find no studies other than the one by Jin et al. (3) that test equilibration directly. All published results on this subject tested iron exchange via absorption. I therefore question how extrinsic labeling can be considered valid for a broad range of foods when the primary assumption of isotopic exchange has seldom if ever been directly tested. Moreover, because equilibration has seldom been tested directly, and given the many examples of miscalibration, one could argue that there is no available literature to indicate where extrinsic labeling is a valid and accurate technique.

The important evolution of the biofortification strategy for alleviating iron deficiency over the past decade warrants careful and accurate assessment of iron bioavailability from staple food crops. Numerous varieties of iron-biofortified staple food crops are being developed that can differ dramatically in phytate and polyphenolic profiles, and these lines need to be accurately evaluated and monitored in the diets of the regions for which they are targeted. Given the evidence mentioned above, it seems careless to assume that because extrinsic labeling of iron may have worked well under one set of conditions, it can be considered valid for all food combinations. Extrinsic labeling of iron can have a role in studies of iron absorption from staple food crops because it has certain practical advantages; however, this method must be validated for each application, perhaps via an in vitro digestion method (3), because the method appears to bring with it a major assumption that is not always met, and it can therefore result in misleading information.

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References


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**Reply to RP Glahn**

Dear Editor:

We appreciate the interest of Glahn (1) in our work and thank him for his remarks. We do, however, disagree with his suggestion that the extrinsic tag technique to estimate nonheme iron absorption in humans has not been sufficiently validated and may give misleading information. On the contrary, we would argue that this method has been instrumental in estimating fractional iron absorption from a wide variety of foods, meals, and diets (2), has helped identify the relative potency of the inhibitors and enhancers of iron absorption (3), and has facilitated the setting of dietary reference values for iron with respect to the nature of the diet (4). The method is not perfect, however, because single meal studies will overestimate the potency of inhibitors and inhibitors compared with multiple meal studies (5), and there are a small number of identified situations in which the native meal iron does not readily enter the common iron pool, and in which the extrinsic tag technique does not then give a reliable estimate of iron absorption. These specific situations were summarized by Consaul and Lee (6) and include iron from insoluble or chelated iron fortification compounds, contamination iron from soil or equipment, when the tag is given separately with a drink, and native iron from whole grains that are not completely digested and do not release their native iron as readily as other foodstuffs.

For the extrinsic tag technique to give an accurate estimation of iron absorption, the isotopically labeled tag added to the meal must equilibrate with the native food iron during the digestive process and be absorbed and used to the same extent. Studies to validate the extrinsic tag technique were carried out by several different research groups in the 1970s and have been reviewed by Consaul and Lee (6). The review describes almost 50 separate human iron absorption studies with multiple adult subjects. The studies compared iron absorption estimated based on erythrocyte incorporation of intrinsically labeled food iron with iron absorption estimated based on the erythrocyte incorporation of extrinsic radioiron labels added to the same meals. The meals included intrinsically radioiron-labeled wheat, rice, maize, eggs, common beans, or soybeans, cooked or processed in different ways, and fed alone or in combination. With the exception of the specific situations described above, good agreement was observed in absorption estimated with the use of the extrinsic or intrinsic tags. The ratio of extrinsic to intrinsic tag absorption in 45 studies varied from 0.90–1.22 with a mean of 1.06. Such variability is not uncommon in human studies; these finding were interpreted as indicating that the intrinsically labeled native food iron was released during digestion into a common pool containing the extrinsic tag and that the 2 tags were similarly influenced by all the food components and subject factors that affect iron absorption and utilization. The main exception was rice grains, for which the extrinsic to intrinsic tag ratio was 1.62 from unpolished whole grains, decreasing to 1.17 with polished grains and 1.02 with rice flours, indicating a slow release of native iron from rice grains during digestion (7).

In relation to the known inhibitors and enhancers of iron absorption, these early studies showed that iron absorption as measured by the intrinsic and extrinsic iron tags was similarly enhanced by ascorbic acid, but no specific studies were made with phytic acid and polyphenols, which are the major inhibitors of iron absorption. Most of the meals, however, contained high concentrations of phytic acid from wheat, maize, and/or soybean, and 7 studies fed meals containing polyphenols from black beans. The good agreement in these studies between iron absorption as measured by extrinsic or intrinsic tags indicates that the tags behave similarly in the presence of phytic acid and polyphenols from black beans. Since these early studies, only one further study has tested the ability of the extrinsic tag technique to predict native iron absorption from common bean meals (8). In this study, with 23 women fed meals of red or white beans, there was almost perfect agreement in absorption of the extrinsic and intrinsic tags for fractional iron absorption values ranging from <1% to ~8%.

In the current letter, Glahn questions the validity of the extrinsic tag studies to measure iron absorption in humans based on the results of one series of experiments investigating the equilibration of native bean iron and an extrinsic iron tag added to an in vitro simulated digestion of white and red beans (9). The native iron was not intrinsically labeled for this comparison. The simulated gastric digestion used in these studies included a 1 h digestion at 37˚C with pepsin at pH 2 and on a rocking platform, before increasing the pH to 5.5–6, adding pancreatin bile, increasing the pH to 7, and centrifuging. The ratios of extrinsic tag iron and the native iron were then compared in the supernatant and pellet after centrifugation. With white beans, the ratios of extrinsic to native iron were slightly higher in the supernatant than the pellet, which is comparable to the results of the human studies. With the red beans, however, the ratio of extrinsic tag to native iron was much lower, indicating a poor equilibration of the extrinsic tag with native iron and suggesting that a greater proportion of the tag had been removed from the supernatant into the pellet. Glahn’s explanation for these results is that native bean iron is mostly bound to phytic acid in the bean cotyledon and is released into the supernatant during the in vitro digestion more slowly than the phenolic compounds in the hull of the red bean. The phenolic compounds in the supernatant then bind preferentially to the extrinsic tag and the insoluble extrinsic tag polyphenol complex is removed in the pellet.

We agree that this is a logical explanation for the results of the in vitro digestion study with red beans but suggest that these results