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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 enhancers 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 flour, 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 studies, 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...
indicate that the in vitro methodology used by Jin et al. (9) was too short and not vigorous enough to be a good predictor of the human digestion process. Gastric digestion of a solid meal in humans is variable but is often much longer than 1 h. The median half gastric emptying time for a solid meal was recently reported to be 127 min (10). A longer, more vigorous in vitro simulated digestion procedure as used by other researchers (11, 12) might improve the equilibration between native iron and extrinsic tags. The in vitro technique as used by Jin et al. (9) has only been used to evaluate the extrinsic tag technique in beans, and we would suggest that further studies should be made with a greater variety of meals, including meals used in the human validation experiments, in which we are sure that the extrinsic tag predicts the absorption of native food iron. Evidence of poor equilibration of extrinsic and native iron in the in vitro technique of Jin et al. (9) would indicate that this method does not adequately simulate gastric digestion in humans. Evidence of good equilibration would indicate that the problem could be specifically with colored beans.

We consider the results of Jin et al. (9) to be a particularity of the simulated digestion method used. The reason is that human iron absorption studies with red and black beans have reported good agreement between the absorption of intrinsically labeled native iron and the added extrinsic tag. In the early validation, a study with intrinsically labeled black beans, boiled and mashed, reported a ratio of extrinsic to intrinsic tag of 0.99 (13); in 6 human absorption studies in which black beans were part of composite meals with maize or wheat, the mean extrinsic to intrinsic tag ratio was 1.15 (6). Thus in the human studies, slightly more of the extrinsic tag was absorbed than the intrinsically labeled native iron. This is opposite to what was predicted by the in vitro digestion method of Jin et al. (9), who reported much lower concentrations of extrinsic tag in the supernatant, suggesting that the extrinsic tag would be less well absorbed that native iron. Lastly, in the more recent study with included red and white beans (8), the agreement between the absorption of extrinsic and intrinsic tags was excellent (1.0).

Glahn stated that close agreement in humans between absorption of intrinsically labeled native iron and an extrinsically labeled iron tag is not an indication of close equilibration. We would disagree with this. We believe that good agreement in absorption and utilization between extrinsic and intrinsic tags indicates that the tags have behaved in a similar way at all stages of the digestion and absorption process, including similar equilibration in a common iron pool. We thus conclude that, apart from the few exceptions discussed above, there is strong evidence that the extrinsic tag technique performs as it was designed to do, and usefully estimates the absorption of native food iron from individual foods and composite meals.

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References


Communication of Randomized Controlled Trial Results Must Match the Study Focus

Dear Editor:

We read with interest the recent article by Hernández-Cordero et al. (1) that adds another data point to a growing body of randomized, controlled evidence demonstrating that sugar-sweetened–beverage-reduction initiatives attempted, to date, do not have large or reliable effects on obesity (2). We express concern about how this article may be interpreted, however, because the article’s title and focus revolves around findings related to a secondary analysis of the primary outcome and a tertiary analysis of a tertiary outcome. By focusing the title and abstract on secondary and tertiary analyses, the article may distract readers from the statistically nonsignificant primary findings in favor of statistically significant exploratory findings.

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