IRON ABSORPTION FROM BIOFORTIFIED BEANS EVALUETED BY STABLE ISOTOPES

ABSORÇÃO DE FERRO DO FEIJÃO BIOFORTIFICADO AVALIADA POR ISÓTOPOS ESTÁVEIS

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RESUMO - A introdução de alimentos bioforticados como o feijão com maior teor de ferro na alimentação é uma ferramenta útil na prevenção de doenças como a anemia. A biofortificação visa atingir a raiz do problema da desnutrição, tem como alvo a população mais necessitada, utiliza mecanismos de distribuição incorporados, é cientificamente viável e efetiva em termos de custos, além de complementar outras intervenções em andamento para o controle da deficiência em micronutrientes. Desta forma a medida da absorção de minerais é fundamental para viabilizar o uso de alimentos biofortificados. O presente estudo avaliou a biodisponibilidade do ferro de variedades de feijão comum BRS Estilo e biofortificado BRS Pontal no homem por meio de técnicas fidedignas e ainda não utilizadas no Brasil. O feijão utilizado foi produzido pela EMBRAPA/Agroindústria de Alimentos. **Indivíduos e métodos**: Participaram do estudo 29 voluntários adultos jovens divididos em 2 grupos Grupo CB (13 indivíduos) receberam 100g de feijão comum (BRSEstilo) cozido e o Grupo BB (16 indivíduos) receberam 100g de feijão biofortificado BRS Pontal, cozido, após o consumo foi conduzida a avaliação isotópica de ferro, para a medida da incorporação de ferro no eritrócito. Resultados: A absorção de ferro foi medida pela razão isotópica e não foi diferente entre os dois grupos estudados.

Palavras-chave: Isótopos, biodisponibilidade, ferro, feijão.

ABSTRACT - The introduction of biofortified crops like beans with higher iron content is a useful tool in preventing diseases such as anemia. The biofortification aims to reach the root of the problem of malnutrition, targets the neediest population, uses embedded distribution mechanisms, is scientifically feasible and effective in terms of cost, and complements other ongoing interventions to control micronutrient deficiency. Thus the measurement of mineral absorption is essential to enable the use of biofortified crops. This study evaluated the iron bioavailability of common bean varieties BRS Estilo and iron biofortified BRS Pontal in man through reliable techniques and have not yet used in Brazil. The beans used was produced by EMBRAPA / Agro Food. Subjects and Methods: The study included 29 young adult volunteers divided into 2 groups: Group CB (13 subjects) received 100g of common beans (BRS Estilo) cooked labeled with iron58 (^{58}Fe) and Group BB (16 patients) received 100g biofortified beans (BRS Pontal), cooked and labeled with iron58 (^{58}Fe). The next day they receive the reference dose of ferrous sulfate enriched iron-57 (^{57}Fe). After consumption, an isotopic evaluation of iron was conducted for measurement of iron incorporation into erythrocyte. Results: The iron absorption was evaluated by isotopic ratio and did not differ between the groups.

Keywords: isotopes, bioavailability, iron, beans.

INTRODUCTION

Brazil is a major bean producer and consumer, a crop that is part of the basic diet of the population. Beans are rich in iron, but low when compared to iron availability of animal foods. The biofortified bean was developed by EMBRAPA to be introduced in basic nutrition to prevent anemia. This study aims to evaluate the bioavailability of iron from carioca beans through the application of isotope techniques to measure the true absorption of this mineral from the beans separately, yet unprecedented in Brazil technique for biological studies. In Brazil to evaluate the bioavailability of iron are performed only *in vitro* studies, repletion / depletion of hemoglobin in animals and increase in hemoglobin after long term food consumption in human study. With the use of stable isotopes after consumption of fortified food and increased isotope of iron (Fe57 or Fe58) in just one day can be measured within 14 days its incorporation in red cells and in very small amounts that are not detected by traditional evaluation methods of serum. Although the number of studies in fortified biofortified foods is growing, the evaluation of the bioavailability of these minerals in biological studies is still inefficient in Brazil. The objective of this study was to evalueted the iron absorption from biofortified beans with the use of stable isotopes to provide essential information needed to determine the efficacy and feasibility of biofortification.

METHOD

Study population:

Twenty-nine healthy adults, 20-45 years of age (13 male and 16 female), were recruited from the Medicine School of Ribeirão Preto - São Paulo University, Brazil. Volunteers were considered eligible if they are healthy, had no underlying medical problems, took no medications or vitamin supplements. The Ethical Committee of Clinical Hospital of Ribeirão Preto approved the protocol, and informed written consent was obtained from the volunteers before enrollment.

Study design

On the morning of the study, fasted subjects were admitted to the Metabolic Research Unit of the Clinical Hospital of Ribeirão Preto of University. Height and weight were measured by standard clinical methods and divided in two groups: Group CB (conventional beans – BRS Estilo) and Group BB (biofortified beans – BRS Pontal). They were given 100mg of cooked beans to which 2 mg of aqueous ferrous sulfate enriched iron-58 (^{58}Fe). Once this portion was consumed, a further 60 mL of water was used to rinse the glass, and the volunteers were encouraged to consume as much of this as possible. The volunteers were then discharged home and required to fast for an additional 2 hours. The following day, they return and received 5mg of reference dose ferrous sulfate enriched iron-57 (^{57}Fe) with 30 mL of natural orange juice and um capsule of ascorbic acid 60 mg. The volunteers returned to the Metabolic Research Unit 14 days later, when 5 mL of blood was drawn for isotope ratios, a complete blood cell count, and measurement of ferritin and Unsaturated iron binding capacity (UIBC) levels. Iron absorption was measured by a well-described and validated method that used stable isotopes (Chen et al., 2005). Ferrous sulphate enriched iron-57 (^{57}Fe) (95.82 atom percent) and ^{58}Fe (93.13 atom percent) were purchased from Trace Sciences International Corp, Richmond Hill, Ontario. Common beans (BRS Estilo) and biofortified beans (BRS Pontal), were produced by EMBRAPA (Table1).

Sample preparation and iron analysis

Blood samples were collected by venipuncture into an EDTA anticoagulated tube and a plain tube (no anticoagulant). A portion of the EDTA-anticoagulated sample was used to measure a complete blood cell count. Serum was separated from the plain tube by centrifugation and stored at −80°C pending analysis. To calculate iron absorption, iron isotope ratios were measured at the Children's Nutrition Research Center Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA, using high-resolution double-focusing inductively coupled plasma mass spectrometry Thermo Element 2 ICP-MS (Thermo). Red blood cell (RBC) iron incorporation of ^{57}Fe and ^{58}Fe was measured 14 days after isotope administration and calculated using a mean blood volume of 65 mL/kg, measured hemoglobin concentration, and isotope enrichment. Estimated iron absorption was calculated assuming that 90% of absorbed iron was incorporated into RBC. Iron isotope ratios were measured by Thermo Element 2 ICP-MS (Thermo) with software v2.354. Serum ferritin and UIBC concentration was measured with a Colorimetric Method, Cobas Integra 700® Roche Diagnostic Systems.

RESULTS

The difference between iron absorption from the meal containing common bean and that from the meal containing biofortified bean examined by means of a paired *t* test. Results (Table

2) were considered significant at *P*<0.05. The groups CB and BB did not differ in hemoglobin or serum ferritin concentrations. Serum ferritin ranged from 41 to 298 umol/L; all of the subjects in the sample were not anemic. Iron absorption was not statistically different. Mean isotopic ratio of iron absorption from the meal with common beans was 0.409% (±0.040%) and mean iron incorporation from the meal with biofortified beans was 0.407% (±0.038%).

Table 1. Iron concentration (mg/Kg) on beans from EMBRAPA.

Beans	Iron Concentration (mg/kg)
BRS ESTILO (conventional bean)	56.554
BRS Pontal (biofortified bean)	72.966

Table 2. Isotopic ratio absorption of ^{58}Fe (added to beans) and ^{57}Fe (reference dose); hemoglobin and ferritin of Groups Common Beans and Biofortified Beans.

Groups (n)	Hemoglobin (g/dL) (mean ±sd)	Ferritin (ng/mL) (mean ±sd)	%58abs/%57abs (mean±sd)
CB (13)	14.39 (±1.05)	107.58 (±72.44)	0.409 (±0.04)
BB (16)	14.14 (±1.09)	108.80 (±117.70)	0.407 (±0.038)

DISCUSSION

Despite considerable efforts, iron deficiency remains a major global health problem. International research efforts, including those funded by HarvestPlus, a Challenge Program of the Consultative Group on International Agricultural Research (CGIAR), are focusing on conventional plant breeding to biofortify staple crops such as maize, rice, cassava, beans, wheat, sweet potatoes, and pearl millet to increase the concentrations of micronutrients that are commonly deficient in specific population groups of developing countries (La Frano, 2014). Biofortification aims to increase nutrient levels in crops during plant growth rather than during processing of the crops into foods. Common beans are a staple food and the major source of iron for populations in Eastern Africa and Latin America. A major constraint to bean iron biofortification is low iron absorption, attributed to inhibitory compounds such as phytic acid (PA) and polyphenol(s) (PP).

In the present study iron absorption from the whole bean meal was similar in participants with normal iron status and non anemic. In this study, in which the meals contained only beans and were fed on a single day, the inhibitory effect of the PP was additional to the inhibitory effect of phytate and legume proteins. Finding no additional inhibition of bean PP in the composite meals is perhaps not surprising, as other food components in a meal have been reported to decrease the inhibitory effect of polyphenols. It is possible that other food components in potatoes or rice such as ascorbic acid or proteins facilitated iron absorption from the bean meals due to their reducing or chelating effects or simply diluted the effect of the inhibitors (PETRY, 2012). Multiple meal studies are preferred when stable isotopes are used to study the influence of food components on iron absorption. This is because the isotope dose can be added over many meals and thus has only a modest influence on the molar ratio of food component iron (PETRY et al., 2015) and can be taken to indicate long-term potential. On the other hand, due to the low bioavailability of bean iron shown in isotope studies, exclusively breeding for high iron concentration may not provide enough additional absorbable iron to impact iron status.

CONCLUSION

In the present study the iron absorption from the conventional beans and bifortified beans was not significantly different. This study tested a single food; if its results are considered in making programmatic decisions, consideration must be given to the balance between iron absorption enhancers and inhibitors in the whole meal. Additional implementation research will be needed to ensure maximization of the beneficial impact of this intervention and a smooth scaling up to make biofortification a sustainable intervention in public health. The multiple meal study is necessary to evaluate the real iron absorption. The challenge for the global health community remains how to take this efficacious intervention and implement at large scale in the real world.

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