Clinical Nutrition ESPEN xxx (2018) e1–e5

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

Original article

Iron absorption from beans with different contents of iron, evaluated by stable isotopes

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ARTICLE INFO

Article history:
Received 4 December 2017
Accepted 17 March 2018

Keywords:
Iron absorption
Bioavailability
Beans
Biofortification
Stable isotopes
Iron deficiency
Anemia

SUMMARY

Background: The introduction of biofortified foods such as beans with higher iron content may be a useful tool in preventing iron deficiency. 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. However, to ensure effectiveness, measurement of the absorption of minerals is essential.
Objective: The objective of this study was to evaluate the iron bioavailability of common bean BRS Pontal (PO), targeted for biofortification, compared with common bean BRS Estilo in man through reliable techniques that have not been previously used in Brazil.
Methods: The study included 29 young adult volunteers divided into 2 groups: Group CB (13 subjects) received 100 g of common beans (BRS-Estilo) cooked labeled with iron-58 (^{58}Fe) and Group TBB (16 patients) received 100 g common bean target for iron biofortification (BRS-Pontal), cooked and labeled with iron58 (^{58}Fe). The next day they received the reference dose of ferrous sulfate enriched iron-57 (^{57}Fe). Isotopic evaluation of iron for measurement of iron incorporation into erythrocytes was performed 14 days after consumption. The beans used, were produced, through conventional breeding program, by EMBRAPA/Rice and Beans.
Results: The iron absorption was evaluated by assessing the isotopic enrichment of the stable isotope. Mean iron absorption from the meal with common beans was 0.409% (±0.040%) and mean iron incorporation from the meal with target beans for biofortification 0.407% (±0.038%) and did not differ between the groups.
Conclusions: This study tested the iron absorption from a single bean meal in healthy volunteers or non anemics, In the present study the iron absorption ratio from common bean Pontal (PO), targeted for biofortification and compared with common bean BRS Estilo was not significantly different. The iron concentration of the TBB, probably was not sufficient to improve higher bioavailability. It is recommended to evaluate the not only the absorption of iron in cultivars that present a higher difference in iron concentration but also in association with other components of the Brazilian diet, such as rice and beans.
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1. Introduction

Dietary strategies to prevent iron deficiency and consequently anemia, include iron supplementation, food fortification, or dietary modification. Food fortification is often propagated as the most

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https://doi.org/10.1016/j.clnesp.2018.03.120
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realistic way to increase iron intake on a widespread and sustainable basis and is currently implemented in the United States, Britain, and most of Latin America, among other locations [1,2]. Currently, biofortification of crops is also being intensively evaluated as a more sustainable and complementary alternative to food fortification [3]. Biofortification seeks to improve the nutrient density of staple food crops through conventional plant breeding, agronomic management, or genetic engineering [3–5]. For biofortification, the most common micronutrients targeted are iron, zinc, and provitamin A carotenoids, due to the high prevalence of deficiencies of these micronutrients among children under the age of 5 years and women of childbearing age in developing areas of Africa, Asia, and Latin America [3]. Since a key issue in these areas is the low concentration of these micronutrients in the most commonly consumed foods, biofortification of staple food crops has been suggested as a way to help alleviate these deficiencies [3,6].

Biofortification is particularly relevant in the current economy, when price increases for nonstaple foods further curtail dietary diversity and food and nutrition security among the poor [6]. In principle, this strategy allows the population to grow and consume the same foods they are accustomed to eating while improving their micronutrient intake. The development and implementation of biofortification food programs is still in the initial stages of growth. HarvestPlus, part of the Consultative Group on International Agriculture Research (CGIAR) Research Program on Agriculture for Nutrition and Health (A4NH), is currently supporting the research and dissemination of staple crops biofortified through conventional plant breeding [7,8]. Brazil is a major beans producer and consumer, food which is part of the basic diet of the population and EMBRAPA coordinates the Biofortification Program that development crops like beans with high level of iron, to be used to anemia prevention [9]. Although concentrations of micronutrients in many of these biofortified crops will remain relatively low, staple foods are eaten in such large quantities in many at-risk populations that, over time, the micronutrients consumed in this manner can enhance micronutrient status and prevent deficiency. Furthermore, the increase in iron concentration in food crops may not result in proportional increment of absorbed iron, as crop varieties with high iron content can also have increased or decreased concentrations of inhibitors or enhancers of iron absorption. Therefore, there is a need to analyze the concentration of iron and its bioavailability in new cultures with high concentrations of minerals and carotenoids [10]. In addition, the body's need for iron is the most important factor that guides iron absorption and this homeostasis is regulated by transcriptional mechanisms, through regulation of gene expression of proteins involved in iron metabolism [11,12].

To assess which of the bean cultivars would provide most of the bioavailable iron, we compared iron bioavailability from a commonly consumed control bean (CB) and a common bean targeted for biofortification, (TBB), in Brazilians healthy. Beans are a major component of this populations diet. This study evaluated 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 [13,14].

The objective of this study was to evaluate the iron absorption of common bean Pontal (PO), targeted for biofortification, compared with common bean BRS Estilo with the use of stable isotopes to provide essential information needed to determine the efficacy and feasibility of biofortification.

2. Subjects and methods

2.1. Study population

On the morning of the study (day 0), fasted subjects were admitted to the Metabolic Research Unit of the Clinical Hospital of Ribeirão Preto of University. They were recruited from the Medicine School of Ribeirão Preto - São Paulo University, Brazil. During this screening (day 0), body weight and height were measured, and the first blood sample was taken for hemoglobin and ferritin measurements. The selected subjects, twenty-nine healthy adults, 20–45 years of age (13 male and 16 female), were randomly assigned to start. Volunteers were considered eligible if they are healthy, had no underlying medical problems, took no medications or vitamin supplements. They were divided aleatory in two groups: Group CB (common beans – BRS Estilo) and Group TBB (common beans target for biofortification BRS Pontal).

2.2. Staple food crop preparation

The material used were *Phaseolus vulgaris*, or common bean, “carioca” (cream-and brown-striped) type, developed by Embrapa Conventional Breeding Program. The cultivars used were a traditionally consumed BRS-Estilo, and BRS-Pontal, targeted for biofortification . Briefly, the biofortification crop improvement was divided into three phases [1]: Early-stage product development and parent building [2]; Intermediate product development [3]; Final product development. BRS-Pontal is a promising cultivar for biofortification, since it does not present the 100% of target for biofortification, but it has the minimum amount of iron required to be considered as an intermediate product development (75 μg Fe/g). BRS-Pontal has 50% of the target (50 ppm + 25 ppm iron) [13]. The beans were cooked in three replicates in a conventional pressure cooker for 40 min using a bean/water ratio of 1:2.2 (w/v). They were given 100 mg of cooked beans and labeled with 2 mg of aqueous ferrous sulfate enriched iron-58 (^{58}Fe) after overnight fasting. 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 h. The following day, they return and received 5 mg of reference dose ferrous sulfate enriched iron-57 (^{57}Fe) with 30 mL of natural orange juice and one capsule of ascorbic acid 60 mg [15]. 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 levels.

2.3. Ethics approval

The Ethical Committee of Clinical Hospital of Ribeirão Preto approved the protocol, Informed consent was obtained individually from the subjects who participated in the study before enrollment.

2.4. Blood tests

Hemoglobin and Serum ferritin concentration was measured with a Colorimetric Method, Cobas Integra 700® Roche Diagnostic Systems.

2.5. Iron analysis

Blood samples were collected by venipuncture into an EDTA anticoagulated tube (metal free) and a plain tube (no anticoagulant). Serum was separated from the plain tube by centrifugation

Please cite this article in press as: Junqueira-Franco MVM, et al., Iron absorption from beans with different contents of iron, evaluated by stable isotopes, Clinical Nutrition ESPEN (2018), https://doi.org/10.1016/j.clnesp.2018.03.120

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