Improving the iron delivery from staple food crops and food products

Dr Raymond Glahn, US Department of Agriculture and Cornell University, has pioneered several techniques to assess how much iron (Fe) is bioavailable from foods, meals and the overall diet. Such techniques are essential to identify and optimise dietary sources of Fe and alleviate iron deficiency, one of the leading global nutritional deficiencies. Staple food crops are a focus of this research, particularly beans as they are relatively high in Fe and a major staple in regions where Fe deficiency is prevalent. Measurement of Fe delivery from foods is challenging as greater Fe content does not always equate to more absorbable iron; hence the need for efficient tools to assess the nutritional quality of this essential micronutrient.

A balanced diet must include adequate sources of all macro- and micronutrients. However, deficiencies in micronutrients, such as vitamins and minerals are common. Perhaps the most common nutritional deficiency worldwide is iron deficiency.

Iron has many roles in human health, but it is arguably best known for its role in the molecule haemoglobin, a protein in red blood cells which transports oxygen around the body. The consequences of mild iron deficiency can be fatigue and lethargy, whereas more severe deficiencies can lead to health issues such as heart problems, increased risk of infections, pregnancy complications, and in children, cognitive deficiencies and poor neurological development.

There are many good dietary sources of iron, including animal and plant sources. Animal sources of iron, such as red meat, are more easily absorbed by the body compared to plant-based sources, such as maize, wheat, beans, chickpeas, peas and lentils.

However, it is often difficult and expensive to assess iron bioavailability from foods. The term 'bioavailable' refers to the iron that is accessible to the body for absorption, compared to iron which may be bound to other components of food thus rendering it unavailable to the body.

Early work carried out by Dr Raymond Glahn of the US Department of Agriculture produced methods that could be used for assessing bioavailable iron from foods. Glahn's method provided a way by which food companies, crop breeders and nutrition professionals can measure iron bioavailability and therefore identify and develop food products and processing practices that can deliver more bioavailable Fe in the food supply.

A BIOASSAY TO ASSESS IRON BIOAVAILABILITY

Dr Glahn developed a model known as the Caco-2 cell bioassay for iron bioavailability. The Caco-2 cell line is a human intestinal cell line widely used for studies of nutrient uptake. Dr Glahn coupled simulated stomach and intestinal digestion with Caco-2 cell cultures to produce a model of the upper human intestinal tract. These experimental conditions enabled Dr Glahn to accurately assess the most critical step that determines iron bioavailability, uptake by the intestinal epithelial cell. Iron uptake is assessed via measurement of the iron storage protein, ferritin, which has been shown to form in direct proportion to Fe uptake.

Measurement of intestinal cell ferritin enables high throughput of the model system, makes it extremely cost efficient, and negates challenges and flaws of other methods that have been used to assess iron bioavailability. Since the model's inception in 1998, it has been thoroughly validated via direct comparison to numerous animal and human studies, predicting the correct effect on Fe bioavailability observed in the corresponding *in vivo* trial. This thorough validation of the model now means that it can be applied with confidence in applications such as plant breeding for improved Fe bioavailability from staple food crops, or for food science applications to optimise Fe nutrition in food products.

It is important to note that the Caco-2 cell bioassay predicts the amount of Fe that can be delivered from a food. This distinction is a key feature of the bioassay as the chemical nature of Fe and its interaction with food components means that Fe concentration in a food or meal does not always correlate with Fe absorption. The bioassay is therefore a practical and data-driven approach to Fe nutrition.

A FOCUS ON BEANS

Over the past 20 years there have been numerous applications of the Caco-2 cell bioassay. Perhaps the most notable applications have been the ongoing studies of Fe delivery from the common bean. Dr Glahn's research was the first to demonstrate that the colour of the seed coat can have profound effects on Fe delivery from beans. Studies have shown that beans in market classes such as red, red-mottled, dark red, purple, cranberry and black generally have low Fe bioavailability; whereas most white and some yellow bean varieties have higher Fe bioavailability. Seed coat colour is due to the presence or absence of compounds known as polyphenols.

Further studies by the Glahn research team identified compounds in a class of polyphenols known as flavonols, specifically designating the flavonols quercetin and myricetin as inhibitors of Fe uptake. Additional inhibitors of Fe uptake were identified in the polyphenol class of cyanidins; however, most importantly this research identified polyphenolic compounds in the flavonol class that *promote* Fe uptake. Specifically, the compounds known as kaempferol, kaempferol

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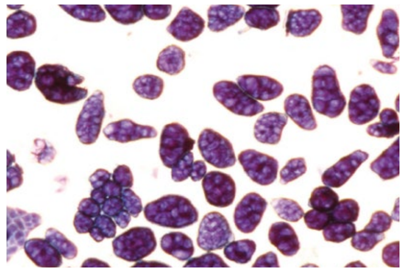
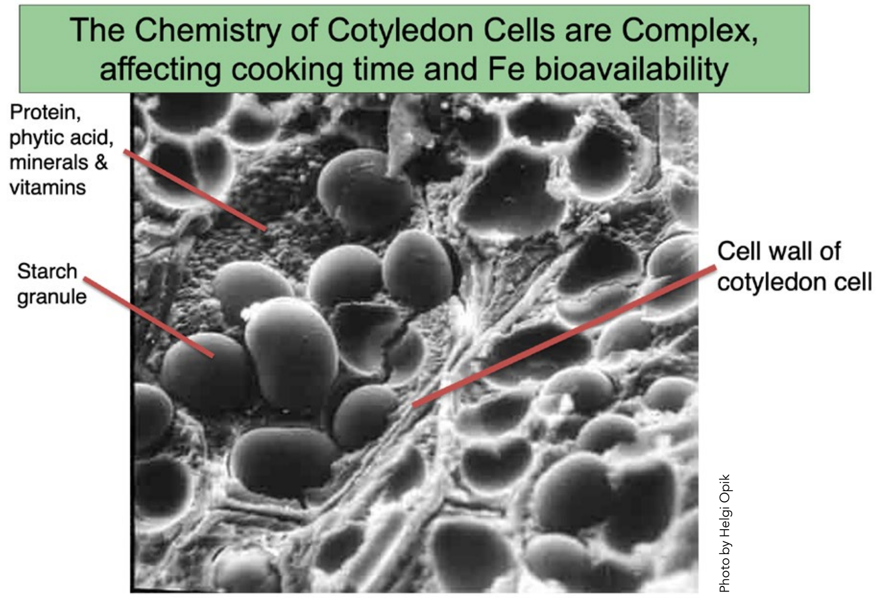
Yellow beans have unique qualities and nutritional traits that help improve the delivery of iron from a plant-based diet.

Caco-2 cells provide breeders a cost-effective tool for evaluating the iron bioavailability of pulse crops.



A microscopic image of cooked Trypan blue stained cotyledon cells from white (navy) bean seeds following *in vitro* gastric and intestinal digestion (magnification 80Å~). This image illustrates how the low pH and enzymes of the gastrointestinal tract have little effect on the intact cell wall structure. Glahn *et al.*, *Food Funct*. 2016. doi:10.1039/c6fo00490c

Storage cells in a bean seed (*Phaseolus vulgaris*), imaged with scanning electron microscopy.



Dr Glahn's work has advanced the field of nutritional iron studies by providing methods to assess iron bioavailability in staple food crops, food products and the overall diet.

3-glucoside, catechin and epicatechin have been shown to promote Fe uptake. Moreover, certain varieties in the yellow bean market class have been identified that are high in these compounds and have little or no inhibitory polyphenols and thus exhibit high Fe bioavailability. Recently, the slow-darkening trait observed in some varieties of pinto beans also results in high levels of these Fe uptake promoting polyphenols, and low levels of the inhibitory compounds thus delivering more Fe for absorption.

Above: Yellow bean breeding lines with fast cooking times and improved iron bioavailability being tested at the Montcalm Research Farm, Michigan. Left: Lentil crop. Dr Glahn's research has identified that the dehulling process improves Fe delivery in lentils.

Additional studies of beans by the Glahn lab have looked at the components of the bean seed in regards to Fe concentration and bioavailability. These studies found that within the cotyledon, i.e. the centre white part of the bean, the individual cotyledon cell walls do not break down due to cooking, chewing or gastrointestinal digestion. Given that 70-95% of the Fe in a bean is located in the cotyledon, within the cotyledon cells, this observation represents a new factor affecting Fe delivery from beans. This same factor applies to other legumes. Identifying this factor is significant as it identifies an obstacle that can be overcome to unlock additional Fe nutrition from beans and other legumes.

Finally, the Caco-2 cell bioassay has found that the fast-cooking trait, highly desirable to many bean consumers, has been linked to improved Fe delivery. Fast cooking is believed to be associated with the same polyphenolic compounds that promote Fe uptake, as well as genes that may control thickness of cotyledon cell walls. In summary, the above observations are all good news for bean growers, food producers and consumers who seek improved Fe nutrition from this staple food crop. This research gives new targets for bean breeders, and ultimately provides enhanced nutritional quality for consumers and value-added traits for producers.

OTHER APPLICATIONS OF THE CACO-2 CELL BIOASSAY FOR FE DELIVERY

Maize: The Caco-2 cell has identified the germ fraction (ie. embryo) of the maize kernel as the major component that inhibits Fe uptake from maize. Thus, degermed maize flour may represent an opportunity to provide improved Fe nutrition. In addition, these studies suggested that identifying and/or developing maize varieties with higher Fe density in the endosperm of the kernel should also provide more bioavailable Fe.

Lentils: Red lentils are commonly dehulled post-harvest. The Caco-2 cell bioassay has identified the dehulling process as improving Fe delivery from lentils.

Plant breeders require high throughput systems to identify and develop traits that can improve Fe delivery from staple food crops.

Peas: Overall, peas have been identified as an excellent legume to deliver more bioavailable Fe.

Complementary Foods for Infants: As infants are particularly prone to Fe deficiency, complementary foods represent an excellent application of the Caco-2 cell bioassay to identify specific foods and food combinations to improve Fe nutrition for this vulnerable age group.

Food Products: Major food companies routinely make use of the Caco-2 cell bioassay to identify optimal formulations to deliver bioavailable Fe while balancing sensory and other quality concerns.

POULTRY MODEL FOR IRON BIOAVAILABILITY

Human studies of Fe bioavailability are expensive and time-consuming; thus, there is a critical need for the combination of the Caco-2 cell bioassay and an animal model to refine objectives for definitive human studies, and if possible reduce the number of human trials. Thus, to complement the bioassay, Dr Glahn and his research team also developed a poultry model for iron bioavailability studies. In combination with the bioassay, the poultry model has been validated in direct parallel comparison to human trials. In studies of crops such as beans and pearl millet, the bioassay and the poultry model correctly predicted the human efficacy studies. As a result, the combination of these research tools now represent a popular approach to studies of Fe nutrition.

Behind the Research

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Dr Glahn is a Research Leader with the United States Department of Agriculture, Agricultural Research Service. He has over 32 years of experience in nutritional physiology and over 170 peer-reviewed publications. His major research focus is on factors that improve iron nutrition from staple foods, food products and food systems.

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Personal Response

What can people do at home to minimise their risk of iron deficiency and to maximise the bioavailability of iron in their diet?

" Humans require a diversity of foods for optimal Fe nutrition, which also promotes other beneficial nutrition outcomes. Therefore, consumption of diverse diet of nutrient dense foods containing a variety of "protein foods", such as legumes (beans and peas), lean meat (poultry and beef), seafood, eggs, and nuts. In addition, a variety of vegetables and whole fruits are highly recommended. Whole grains should make up at least half of all grain intake. Recommended amounts of dairy products should also be followed. Adherence to these dietary guidelines generates a healthy intestinal tract that facilitates Fe absorption and overall health. "

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