

# Iron Dialyzability in a Multiple Nutrient Formulation and Effect of the Addition of Separate Nutrients

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**Abstract** Introduction: Different nutrients added to nutritional formulations can facilitate, reduce or block the iron absorption. Objective: The aims of this study was to assess the modulation of iron bioavailability by other minerals and vitamins in an multiple nutrient formulation and in an aqueous iron solution. Material and Methods: We analyzed the iron dialyzability in the multiple nutrients formulation developed to simulate an enteral diet. Furthermore, the iron dialyzability was determined in aqueous solutions containing 25 mg of iron, which were added separately usual amounts of soluble fiber, salt mixture, vitamin mixture, calcium and vitamin C. The dialysed iron was measured by atomic absorption spectrometry. Results: In the multiple nutrients formulation, we documented low bioavailability of iron ( $0.80 \pm 0.01\%$ ). Compared with the aqueous iron solution ( $70.0 \pm 6.0\%$ ), the addition of 135 mg of vitamin C increased the iron dialyzability ( $90.0 \pm 3.0\%$ ). There was reduction of iron dialysis after addition of soluble fiber ( $1.00 \pm 0.01\%$ ), the vitamin mixture ( $25.00 \pm 0.12\%$ ), salt mixture ( $2.00 \pm 0.06\%$ ) and calcium ( $0.80 \pm 0.02\%$ ). Conclusion: The low iron bioavailability in the multiple nutrients formulation can be attributed to the protein source and supply of fiber and calcium, thereby affecting the absorption of iron.

**Keywords** Bioavailability, Iron, Soy Protein, Soluble Fiber, Calcium

## 1. Introduction

Iron absorption is affected by body iron stores, by the chemical structure of the salt, and by dietary factors (1) which have positive or negative effects on iron bioavailability (2,3). The solubility, dialyses, and entry/transport of iron by Caco-2 cells are *in vitro* methods used to assess the addition of nutrients on bioavailability of iron. *In vitro* dialyzability analysis the iron dialyzes through a semi-permeable membrane after simulation of gastric and duodenal digestion (4) represents a methodological option for the assessment of iron bioavailability after the addition of different nutrients. This methodology may provide useful information on iron bioavailability in foods and dietary products (5), like oral supplement and enteral diet.

Patients unable to consume sufficient quantities of food to satisfy their nutrient requirements need oral supplement or enteral nutrition (6). The possible interactions between the components of the formula may alter the bioavailability of specific nutrients and interfere with the clinical course of sick individuals. The aims of this study was to assess the modulation of iron bioavailability by other minerals and vitamins in an multiple nutrient formulation and in an iron

aqueous solution.

## 2. Materials and Methods

### 2.1. Preparation of The Experimental Formula and of the Aqueous Solutions

We prepared a multiple nutrient formulation that would reproduce the nutrient composition of products used for oral supplementation or polymeric enteral diet (Table 1). All components (protein soy isolate, maltodextrin, canola and corn oils, soy lecithin, partially hydrolyzed guar gum, and a mixture of minerals and vitamins) used to prepare the formulation were purchased. In parallel, we prepared an aqueous ferrous sulphate solution containing 25 mg elemental iron to which the following nutrients were later added: partially hydrolyzed guar gum (25 g); salt mixture (3 g); vitamin mixture (10 g); calcium (800 mg); and vitamin C (135 mg). A total volume of 250 mL and an iron concentration of 25 mg were kept constant regardless of the nutritional composition of the enteral formula or aqueous iron solution.

### 2.2. Digestion and Dialyzability of the Samples

The *in vitro* bioavailability of iron in the samples was determined by the method of Miller and others (7) and modified by Luten and others (8). For the simulation of the digestive process, a 250mL sample of the multiple nutrient

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formulation was homogenized and 6 N HCl was added until a pH value of 2 was reached. Five 20g aliquots were separated and pepsin was added at the proportion of 0.125 g/g protein. The solution was incubated at 37 °C in a water bath with shaking for 2 h. Finally, titration with 0.5 N KOH was performed up to pH 7. A sodium bicarbonate solution was prepared and added to the dialysis tube until pH 5 was reached after 30 min under constant shaking. The pancreatin-bile solution was then prepared at the proportion of 25 mg pancreatin/g protein in the sample and of 0.4 g pancreatin/2.5 g bile extract. The pancreatin-bile solution (4 mL) was added to 3 beakers containing 20 g of the digest and the mixture was incubated in a water bath with shaking for 2 h.

The process was finalized by removing the dialysis tubes from the solutions and the content of the beakers was transferred to a 25mL volumetric round-bottom flask and reconstituted to its final volume with deionized water. For the samples of aqueous solutions containing 25.0 mg iron, only pH control was performed by acidification and neutralization with the reagents used in the method, without the addition of digestive enzymes.

For the evaluation of iron dialyzability, 20 g of the digest or of the aqueous solutions was placed in a beaker together with the dialysis tube previously hydrated in deionized water for 10 min and filled with 25 mL of an NaHCO<sub>3</sub> solution. The flasks were covered and kept in a water bath at 37 °C with shaking for 30 min. Four mL of the bile-pancreatin suspension was added to each flask and incubation was continued for 2 additional hours. At the end of the incubation period the dialyzed content was transferred to volumetric balloons and deionized water was added to complete the volume to 25 mL, followed by storage in a freezer at -20 °C until the time for reading.

### 2.3. Determination of Total and Dialyzed iron

For the determination of total iron in the aqueous solutions and in the various formulations tested, 2 g samples were obtained and digested with nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at a 5:1 proportion at 100 °C in a block digester (Pyrotec®). The material was diluted with deionized water in a 50 mL round-bottom flask. The analyses were performed using a Shimadzu® atomic absorption spectrophotometer model AA 6200 (Shimadzu Corporation,

Tokio, Japan) with an air/acetylene oxidant under the following conditions: hollow cathode lamp, 248.3 wavelength for iron and a 0.2 nm slit. The solutions for the standard iron curve were prepared with Tritisol ferric chloride (Merck® 9972) at concentrations of 0.5, 2.0, and 4.0 µgFe/mL. All determinations were carried out in triplicate and data are reported as means + SD.

Iron dialyzability was estimated as the proportion of dialyzed iron in relation to iron concentration at the beginning of the *in vitro* digestion process after a period of equilibrium through the dialysis membrane.

## 3. Results

Table 2 shows that percent dialyzed iron was very low in the multiple nutrient formulation ( $0.80 \pm 0.01\%$ ), indicating a possible impairment of iron bioavailability in products used in enteral formula, like this multiple nutrient formulation. In aqueous solutions, iron dialyzability was facilitated by the addition of ascorbic acid ( $90.00 \pm 3.00\%$ ), and was reduced by the addition of guar gum ( $1.00 \pm 0.01\%$ ), and calcium carbonate ( $0.80 \pm 0.02\%$ ).

## 4. Discussion

In the present study, iron dialyzability in a multiple nutrient formulation was very low ( $0.80 \pm 0.01\%$ ). This study's results confirm the inhibitory effect of calcium and of soluble fiber on iron bioavailability assessed by the method of *in vitro* dialyzability.

Ascorbic acid has long been known to enhance absorption of iron from test meals (9). Despite the presence of vitamin C, the iron dialyzability was low from multiple nutrient supplements, indicating that other factors were harming the bioavailability of iron. Calcium carbonate was used in the present study, that possibly determined the formation of insoluble complexes with iron, explaining the inhibitory effect of the addition of calcium on the iron dialyzability (10,11). A negative effect on iron absorption has been documented with calcium phosphate (12) and calcium carbonate (13) supplementation, although this effect did not occur in a radioisotope study on humans (14) or in experiments of iron dialyzability (15).

**Table 1.** Nutritional composition of 250 mL of a multiple nutrient solution

Nutrient	Sources and composition
Protein	Soy protein isolate (3.1 g)
Carbohydrate	Maltodextrin (63.1 g)
Lipid	Com oil (1.0 g), canola oil (3.5 g), soy lecithin (0.3 g)
Fiber	Partially hydrolyzed guar gum (25.00 g)
Salt mixture	FeSO <sub>4</sub> ·7H <sub>2</sub> O (25 mg Fe <sup>++</sup> ), MgCO <sub>3</sub> (15 mg Mg <sup>++</sup> ), KH <sub>2</sub> PO <sub>4</sub> (75 mg P <sup>++</sup> ; 90 mg K <sup>+</sup> ), ZnSO <sub>4</sub> ·7H <sub>2</sub> O (0.5 mg Zn <sup>++</sup> ), KIO <sub>3</sub> (0.09 mg I <sup>-</sup> ), MnSO <sub>4</sub> ·H <sub>2</sub> O (0.11 mg Mn <sup>++</sup> ), CuSO <sub>4</sub> ·5H <sub>2</sub> O (0.08 mg Cu <sup>++</sup> ), NaCl (60 mg Na <sup>+</sup> ), CaCO <sub>3</sub> (800 mg Ca <sup>++</sup> )
Vitamin mixture	Vitamin A (50 µgRE), vitamin D (0.4 µg), vitamin E (0.8 mg TE), vitamin K (4.0 µg), vitamin B <sub>1</sub> (0.1 mg), vitamin B <sub>2</sub> (0.1 mg), niacin (1.0 mg), pantothenic acid (0.5 mg), vitamin B <sub>6</sub> (0.15 mg), folic acid (15.0 µg), vitamin B <sub>12</sub> (0.5 µg), biotin (12.0 µg), vitamin C (5.0 mg)

**Table 2.** Mean and standard deviation of percent iron in a pure aqueous solution (A) or in the same solution after the addition of individual components (B, C, D, E and F) and in the nutritionally complete formulation (G)

	Formulation	Dialyzed iron (%)
A	Aqueous iron solution	70.00 ± 6.00
B	Aqueous iron solution + guar goma	1.00 ± 0.01
C	Aqueous iron solution + salt mixture	2.00 ± 0.06
D	Aqueous iron solution + vitamin mixture	25.00 ± 0.12
E	Aqueous iron solution + calcium carbonate	0.80 ± 0.02
F	Aqueous iron solution + vitamin C	90.00 ± 3.00
G	Multiple nutrient formulation	0.80 ± 0.01

*In vitro* methods are relatively simple, rapid and inexpensive and can simulating the digestion gastric and duodenal, followed by dialysis. The proportion of the element diffused through the semi permeable membrane during the process, is the dialysability element after an equilibration period, being used as an estimate of nutrient bioavailability (7-8).

In a collaborative study to compare the methods using *in vitro* and *in vivo* to assess the absorption of non-heme iron, found a statistically significant correlation indicating that the results obtained using the method *in vitro* can be extrapolated to humans (8).

*In vitro* studies have shown that iron absorption from breakfast cereals was reduced after the addition of fibers (16) and also in foods such wheat, rice, corn and soy (17). In contrast to these findings, rats fed with partially hydrolyzed guar gum presented greater intestinal iron absorption than rats receiving a diet without the addition of fiber (18). In a study on healthy humans, the daily addition of 40 g soluble fiber to a mixed diet did not alter the apparent absorption of iron (17). The discordance in the literature regarding the effect of fibers on iron bioavailability may be attributed to differences in the potential of soluble or insoluble fibers to form precipitate that limit iron absorption. There is also the hypothesis that the consumption of soluble fiber will increase the bacterial flora, resulting in a stimulus of iron absorption by the colonic mucosa (18,19) and explaining the difference between *in vitro* and *in vivo* studies. Thus, when the content of soluble fiber is modified in formulations, it is possible that *in vitro* methods will not be feasible for the assessment of iron bioavailability.

This multiple nutritional formulation was studied because is similar to enteral nutrition who composited by multiple nutrients and offered to people in the same time.

In various commercial enteral diet products, percent dialyzed iron ranged from 2.34 to 9.67 % according to the presence of fiber and to the calcium-iron proportion (20). The study of optimization of dialyzed iron in a multiple nutrient solution showed that the ideal formulation for a maximum of 7 % dialyzability required 10 mg/L iron, 10 g/L fiber, 320 mg/L calcium and no addition of medium-chain triacylglycerides (21).

In the present study, the highest concentration of nutrients of the solution can justify poor results of both the iron dialyzabilidade formula with multiple nutrients and in aqueous solution of ferrous sulfate. The enteral formula

used in this study contains soy protein extract, which is known to decrease iron absorption (22-24). The inhibitory effect of soybean products is thought to be due to the protein component (7) than phytic acid (25).

Cook et al. (14) assessed the effect of calcium salts commonly used as supplements on iron absorption when administered during the interval between meals and observed that calcium carbonate at the dose of 600 mg did not inhibit the absorption of ferrous sulfate (18 mg), at an iron/calcium proportion of 1:33). When the same assays were repeated using citrate and phosphate salts as a source of calcium at the same concentrations, iron absorption was reduced to 44 % and 62 %, respectively, showing that the type of salts used can also affect the bioavailability of minerals. Reddy & Cook (26) observed that different iron/calcium proportions (above 1:40) and the types of salt sources of the minerals interfere with the bioavailability of iron.

Yoon et al. (27) discussed the possibility of fiber acting on the human gastrointestinal tract by causing changes in the utilization of nutrients and showed that greater amounts of fiber (> 20 g/day) can affect the bioavailability of minerals. The supplements studied here contained 25 g fiber that may have represented a factor capable of reducing iron absorption.

Numerous interactions exist between the different trace elements affecting absorption via the gastrointestinal tract. Factors affecting bioavailability of trace elements include the actual chemical form of the nutrient (eg, organic form of iron is better absorbed than the ionic form), antagonistic ligands (eg, zinc absorption is decreased by phytate and fiber; iron absorption is decreased by fiber), facilitatory ligands (eg, zinc absorption is aided by citric acid or iron absorption is increase by amino acids or fermented products), and competitive interactions (eg, iron depresses the absorption of copper, and zinc; zinc depresses copper absorption and vice versa) (28).

Studies evaluating the effect of different protein, fiber and calcium sources on iron dialyzability in multiple nutrition formulation are needed in order to provide an adequate nutrition supply without a reduction in iron bioavailability. The iron bioavailability in a multiple nutrient solution proved to be low, a fact that may harm the health of individuals who use enteral nutrition.

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