Bioavailability of iron measurement in two nutrients multiple solutions by *in vitro* and *in vivo*; a comparative methodology between methods

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Abstract

**Objectives:** The bioavailability of dietary iron present in a nutritional formulation may be evaluated by *in vitro* and *in vivo* methods since they provide for a cohesive line study and provided in the literature. The aim of this study was to evaluate the bioavailability of iron targeting a comparative analysis of two nutritional supplement formulations (A and B).

**Methods:** For this study were using *in vitro* and *in vivo* methods, both described in the literature for availability of iron in an enteral feeding after ingestion supplement nutrition with much nutrients.

**Results:** The results obtained by *in vitro* simulation of the human gastrointestinal tract were 0.70 ± 0.02 and 0.80 ± 0.01 % iron availability by formulations A and B. *In vivo* studies, as measured by the curves of serum iron in humans after ingestion of formulations allowed the calculation of coefficient of variation $\Delta < 0$, indicating that there was a low absorption of iron. The bioavailability of iron as two multi-nutrients solutions obtained by *in vitro* and *in vivo* showed that there were comparisons of those methodologies used in this study.


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Key words: Bioavailability. Iron. *In vitro*. *In vivo*.

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Introduction

The bioavailability of iron in many nutrient solutions can be studied by in vitro and in vivo methods for estimated on iron absorption. 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.\(^1\)

Luten et al.\(^1\) in a collaborative study to compare the methods using in vitro and in vivo to assess the absorption of nonheme iron, found a statistically significant correlation indicating that the results obtained using the method in vitro can be extrapolated to humans. Chiplonkar et al.\(^4\) and Narasinga Rao\(^5\) studying different types of diets and food for the purpose of measuring the iron dialysability components at different concentrations in order to test methods in vitro and in vivo and have shown a correlation of \(r = 0.94\), suggesting the data was reflected nonheme iron absorption in humans.

Conway et al.\(^6\) proposed the serum iron curves obtained after ingestion of food or multiple formulations containing nutrients to be used to assess dietary iron absorption in humans. Conway et al.\(^6\) and Hoppe et al.\(^7\) showed good correlation between the method of the study the area of the curves of iron and serum stable isotopes technique for iron to check on iron absorption at food and it is possible to analyze the absorption and circulation of iron in humans.

In previous studies in our group whose experimental designs were similar to that used in this study showed response of serum iron levels after administration of iron sodium EDTA (NaFeEDTA)\(^8\) and iron bis-glycine chelate\(^9\), in healthy volunteers. Rosa\(^10\) was observed the iron absorption in obese patients after ingesting 15 mg of elemental iron by ferrous sulfate before and after bariatric surgery.

The objective was to evaluate the bioavailability of iron as two multi-nutrient solutions by in vitro simulation of the human gastrointestinal tract and in vivo through the response curve of serum iron level by means of a delta (\(\Delta\)) of variation serum levels of mineral obtained after intake of the formulations A and B in healthy volunteers and obeses, in order to compare the methods in vitro and in vivo to evaluate the availability of iron absorption in nutritional formulations.

Methodology

Materials and methods

Preparation and Composition of the Nutritional Supplement Formulations

We prepared two multiples nutrient formulation that would reproduce the nutrient composition of products used for oral supplementation or polymeric enteral diet (table I). All components (protein soy isolate, malt dextrin, 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.

We prepared two multiples nutrients formulations A and B described by Bueno\(^11\) that would reproduce the nutrient composition of products used for oral supplementation or polymeric enteral diet (table II). All components (protein soy isolate, malt dextrin, canola and corn oils, medium-chain-triglycerides (MCTs), soy lecithin, partially hydrolyzed guar gum, and a mixture of minerals and vitamins) used to prepare those formulations were purchased. Regardless of the nutritional composition of the formulations, the iron concentration was maintained constant in all the formulations tested.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Formulation A</th>
<th>Formulation B</th>
<th>Bueno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>3.10</td>
<td>3.10</td>
<td>13.34</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>63.10</td>
<td>64.10</td>
<td>59.12</td>
</tr>
<tr>
<td>Lipid (g)</td>
<td>4.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.00</td>
<td>7.31</td>
<td></td>
</tr>
<tr>
<td>Canola oil</td>
<td>3.50</td>
<td>7.74</td>
<td></td>
</tr>
<tr>
<td>Soy lecithin</td>
<td>3.00</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4.00</td>
<td>2.15</td>
<td></td>
</tr>
<tr>
<td>Fe (mg)</td>
<td>25.00</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>Ca (mg)</td>
<td>1000.00</td>
<td>800.00</td>
<td>80.00</td>
</tr>
<tr>
<td>Vitamin (g)</td>
<td>1.00</td>
<td>4.30</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fiber (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partially hydrolyzed guar gum</td>
<td>25.00</td>
<td>4.30</td>
<td></td>
</tr>
<tr>
<td>Total (g)</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Salt mixture (4/3g): Mg (15 mg), P (75 mg), K (90 mg), Zn (0.50 mg, 1.09 mg), Mn (0.11 mg), Cu (0.08 mg), Na (60 mg). Vitamin mixture (1 g): Vitamin A (500 µgRE), Vitamin D (4µg), Vitamin E (8 µg TE), Vitamin K (40 µg), Vitamin B\(_2\) (1 mg), Vitamin B\(_3\) (1 mg), Niacin (10 mg), Ácido Pantoténico (5 mg), Vitamin B\(_6\) (1,5 mg), Ácido Fólico (150 µg), Vitamin B\(_7\) (0,5 µg), Biotina (120 µg), Vitamin C (50 mg)
Bioavailability of iron measurement by in vitro and in vivo

The quantities of nutrients were changed to demarcate the possible effects of calcium, fiber and MCTs on iron availability. A pot of each formulation was selected to be applied in vitro in the same manner as formulations A and B were prepared to be given to the study in vivo.

Patients

The study was conducted on twenty two volunteers of both genders aged 18 to 50 years and eutrophic (n = 7) and obeses (n = 15) the Center for the Treatment of Bariatric Surgery of the Discipline of Nutrology, University Hospital, Faculty of Medicine of Ribeirão Preto (HCFMRP). The study was approved by the Research Ethics Committee of the Hospital, and the data were collected from November 2007 to December 2008.

Inclusion Criteria: Adult subjects aged 18 to 50 years with no diseases potentially interfering with absorptive capacity and giving informed consent to participate.

Exclusion Criteria: Adult with anemia (hemoglobin level of less than 10.0 mg/dL), with chronic renal insufficiency, alcoholism, intestinal parasitic diseases, diabetes, and chronic diarrhea were excluded from the study.

Experimental design

The experimental assays involving the research subjects were started in the morning. The formulation of the nutritional supplement provided 25.0 mg elemental iron from heptahydrated ferrous sulfate, and its interaction with 800 mg calcium and 25 g fiber was determined. Those formulations contained 38 additional nutrients for simulation a normal meal in nutrients, whose quality and quantity are listed in table I.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Partially hydrolyzed guar gum (g)</th>
<th>Salt mixture (g)</th>
<th>Vitamin mixture (mg)</th>
<th>Calcium (mg)</th>
<th>Vitamin C (mg)</th>
<th>MCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous iron solution</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aqueous iron solution + guar goma</td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Solução aquosa de ferro + mistura salina</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Aqueous iron solution + salt mixture</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Aqueous iron solution + calcium carbonate (A/B)</td>
<td>1000/800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous iron solution + vitamin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formulation A</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>1000</td>
<td>50</td>
<td>4.5</td>
</tr>
<tr>
<td>Formulation B</td>
<td>25</td>
<td>3</td>
<td>1</td>
<td>800</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Digestion and dialysability of the samples

The in vitro bioavailability of iron in the samples was determined by the method of Miller et al, modified by Luten et al. For the simulation of the digestive process, a 250mL sample of the multiple nutrient formulations 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
ed water, transferred to a pot with a lid and stored in the refrigerator for 12 hours. The experimental assays involving the research subjects were started in the morning. The formulation of the nutritional supplement provided 25.0 mg elemental iron from heptahydrated ferrous sulfate, and its interaction with 800 mg calcium and 25 g fiber was determined. Those formulations contained 38 additional nutrients for simulation a normal meal in nutrients, whose quality and quantity are listed in table I.
period of equilibrium through the dialysis membrane.

The process was finalized by removing the dialysis tubes from the solutions and the content of the beakers was transferred to a 25 mL 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 dialysability, 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 NaHCO₃ 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.

**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 (HNO₃) and hydrogen peroxide (H₂O₂) at a 5:1 proportion at 100 °C in a block digestor (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.

**Determination of Serum Iron Levels after the Ingestion of the Nutritional Supplements Formulations**

Samples collected at time 0, 1, 2, 3 and 4 hours were spun to separate serum and red blood cells were immediately discarded. Serum samples were placed in demineralized Eppendorf tubes and stored frozen at -20 °C until the time for analysis. Iron concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) in the DRC mode according to the method of Palmer et al. with the samples being diluted 1:20 with 0.5 % HNO₃ diluent (v/v) + 0.005 % TRITON X-100 (v/v).

Readings were then obtained with a Perkin Elmer ELAN DRC PLUS instrument equipped with a cyclonic chamber and coupled to a Meinhard nebulizer under conditions of optimization of gas flow of 0.60 mL/min, lens voltage of 6.00 A and radiofrequency power of 1100.00 W.

**Determining of delta variations (Δ) to the levels of serum iron obtained in the volunteers**

Variations in the time intervals between serum iron levels were made to measure of the iron absorption in volunteers. All intervals of time were found in a number of variations measured 10, called delta (Δ) or coefficient of variation between serum iron and the time that were taken these values. Were made \[ \Delta \sum_{\Delta i=1}^{X} \Delta x_i /N \] and the classification was \( \Delta < 0 \) without absorption and \( \Delta > 0 \) with absorption.

**Statistical analysis**

Descriptive analysis of experimental data in vitro and in vivo was made with means and standard deviations. For in vivo testing was considered the ratio of the sum of serum levels iron in volunteers. Those spreadsheets were tabulated in EXCEL program.

**Results**

Studies of these formulations analyzed by in vitro, the scope of the methodology is to show the solubility of the chemical binding molecules according to their affinity for electrons, resulting in 0.70 ± 0.02 and 0.80 ± 0.01 % of iron dialysability respectively, for the formulations A and B. In an aqueous solution on 25 mg of iron was showed 70 ± 6 %. In the same solution in which iron has been added ascorbic acid had increased to 90 ± 3 % of iron availability, confirming the positive effect of iron absorption by the method to describe in simulation of the human gut condition. Fibers in an aqueous solution of iron the value of dialysability of iron was showed 1.00 ± 0.01 %. This showed that fiber has a binding affinity for hydrogen atoms and due to is low activation energy and fibers are not capable of forming organ metallic complexes.

Different calcium concentration 800 and 1000 mg/L the low iron dialysability for 0.80 ± 0.01% and 1.30 ± 0.02% showing interaction between calcium and iron in an availability of iron (table III).
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The results obtained by in vivo assays shown by the
sum of the variances between the experimental period
and level of serum iron measured in the volunteers
noted that there was poor absorption of iron by the
ingestion of the formulation (table IV). Comparing
with the results observed in vitro and in vivo inhibitory
effects of nutrients influencing the bioavailability of
iron were potentiated in humans especially because the
quantity of fiber and calcium in the formulation.

Discussion

Van Dyck et al14. studied the influence of the nutri-
tional components of multiple supplement nutrition
formulations by iron dialysability and concluded that
the fibers are interfering negative because the presence
of phytate and calcium in the fibers components.
Azevedo15 showed that the proportion of calcium and
iron ranging from 50:1 to 60:1 and the components of
the fibers negative strongly influence of iron
dialysability in many formulations of enteral nutrition.
The percentages in these formulations of iron dialyzed
was 2.34 to 9.67%. These parameters of iron
dialysability were classified by low iron availability to
< 5%, mean availability 5-8% and good availability >
8%. Bueno11 showed the solution enteral formulation
should contain 10 g / L of fiber, 0 (zero) of MCT and
320 mg / L of calcium and keeping the amount of 10
mg / L iron from ferrous sulfate, to provide a
dialysability of 7% iron bioavailability was estimated
by mathematical modeling of the ingested amount cor-
responding to 0.7 mg / L.

Fibers have been added to the formulations of nutri-
tional supplements because of their functional charac-
teristics and benefits for the human organism16,17. On
the other hand, studies of the action of fibers on the
bioavailability of minerals have demonstrated that
these components interfere with the absorption of iron,
zinc, copper, calcium and magnesium5,17,18 . Gupta et
al19. to assess the bioavailability of calcium and iron in
leafy vegetables, by in vitro dialysis concluded that the
components present in the chemical structure such as
food fibers, oxalate, phytic acid and tannins are the pri-
mary interfering bioavailability of iron.

Minerals bioavailability was measured by the habit-
ual consumption of foods such as wheat, rice, corn and
soy and in a study of the Chinese population showed
that the amounts of phytate and fiber in these foods
enabled the formation of insoluble compounds that
decreased the iron bioavailability20. In cereals, fortified
or not, the interaction of iron absorption was reduced in
the presence of fibers and other types of foods such as
coffee and milk, probability of presence that caffeine
and calcium21. Kapsokefalou and Miller 22 to compare
the solubility and dialysability of iron sources
(pyrophosphate, 2-glycinate, glutamate, lactate and
ferrous sulfate) in samples of milk prior to addition of
ascorbic acid they authors can observed that infants
products with lower amounts of calcium and total pro-
tein in their composition showed an availability of iron

<table>
<thead>
<tr>
<th>Variations of Δ</th>
<th>n</th>
<th>Sum of Serum Iron Levels (µg/dL)</th>
<th>Means of Serum Iron Levels (µg/dL)</th>
<th>Standard Deviation (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ (J-1H)</td>
<td>22</td>
<td>-824.60</td>
<td>-37.50</td>
<td>89.80</td>
</tr>
<tr>
<td>Δ (J-2H)</td>
<td>22</td>
<td>-775.10</td>
<td>-35.20</td>
<td>124.90</td>
</tr>
<tr>
<td>Δ (J-3H)</td>
<td>22</td>
<td>-831.35</td>
<td>-38.00</td>
<td>122.80</td>
</tr>
<tr>
<td>Δ (J-4H)</td>
<td>22</td>
<td>-861.70</td>
<td>-39.20</td>
<td>116.20</td>
</tr>
<tr>
<td>Δ (2H-1H)</td>
<td>22</td>
<td>-49.50</td>
<td>2.20</td>
<td>63.60</td>
</tr>
<tr>
<td>Δ (3H-1H)</td>
<td>22</td>
<td>-11.50</td>
<td>-0.52</td>
<td>58.80</td>
</tr>
<tr>
<td>Δ (4H-1H)</td>
<td>22</td>
<td>-37.00</td>
<td>-1.70</td>
<td>66.00</td>
</tr>
<tr>
<td>Δ (3H-2H)</td>
<td>22</td>
<td>-61.00</td>
<td>-2.80</td>
<td>60.30</td>
</tr>
<tr>
<td>Δ (4H-2H)</td>
<td>22</td>
<td>-86.60</td>
<td>-4.00</td>
<td>77.80</td>
</tr>
<tr>
<td>Δ (4H-3H)</td>
<td>22</td>
<td>-25.50</td>
<td>-1.20</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Table III
Means and Standard Deviation of percent iron in a purê
daqueous solution or in the same solution after addition of
individual components and the supplement nutrition
formulations A and B

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Dialysability of iron (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous iron solution</td>
<td>70.00 ± 6.00</td>
</tr>
<tr>
<td>Aqueous iron solution + guar goma</td>
<td>1.00 ± 0.01</td>
</tr>
<tr>
<td>Aqueous iron solution + salt mixture</td>
<td>2.00 ± 0.06</td>
</tr>
<tr>
<td>Aqueous iron solution + vitamin mixture</td>
<td>25.00 ± 0.12</td>
</tr>
<tr>
<td>Aqueous iron solution + calcium (A)</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>Aqueous iron solution + calcium (B)</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>Aqueous iron solution + vitamin C</td>
<td>90.00 ± 3.00</td>
</tr>
<tr>
<td>Formulation A</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>Formulation B</td>
<td>0.80 ± 0.01</td>
</tr>
<tr>
<td>Formulation was described by Bueno11</td>
<td>7.00 ± 0.40</td>
</tr>
</tbody>
</table>

Table IV
Variation of Δs and somatory by levels serum iron were obtained by in vitro and in vivo methods in the volunteers
after ingestion of A and B supplement nutrition formulations

Bioavailability of iron measurement by
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around 62% higher than the milk suitable for older children.

Velasco-Reynold et al. have shown the mean dialysability of magnesium found in duplicate in hospital meals (daily, lunch, dinner) was 13.2% per meal. The dark green vegetables and vegetables in general are primary sources of bioavailable magnesium in daily diet. The magnesium dialysabilities were significantly influenced only by dialysable calcium, magnesium, zinc, chromium, and iron fractions. Consequently, important similarities in the magnesium and calcium in foods and behaviors as well as meals in their absorptive processes exist. The fiber content of duplicate meals did not influence the dialysable calcium fraction and calcium dialysabilities. Dietary fat positively affects perhaps the calcium absorption by the chelating action of fatty acids. Only total magnesium and dialysable magnesium levels and magnesium dialysabilities significantly influenced on dialysable calcium fractions.

The partially hydrolyzed guar gum are important for the production of short chain fatty acids in human gut and to provide supply energy to the body and were selected to be included in the nutritional formulation for maintain their characteristics without altering the viscosity, the solution solubility and can be used in drinks and nutritional enteral and supplement nutrition formulations.

Yoon et al. 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.

By studying the interactions of Fe++, Ca++, and Fe+++ in the formulation of enteral nutrition by in vitro methods in different concentrations of soluble fiber, insoluble fiber and different pHs, simulating physiological different conditions, observed that high amounts of fiber and physical-chemical unsuitable can lead to poor availability of iron. Cook, Dassenko and Whittaker 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 and Cook observed that different iron/calcium proportions (above 1:40) and the types of salt sources of the minerals interfere with the bioavailability of iron.

Those MCTs were the nutrient present in the formulation A and absent in the formulation B in agreement with the results showed by Bueno. They are not stored in liver and adipose tissue and were used quickly, in conjunction with glucose as energy source for the organism. No need of action with plasma albumin, in cellular metabolism or transport by carnitine when activated in the mitochondria for oxidation and were showed not interference by an iron availability or 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 increate 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).

The bioavailability of iron by in vitro and in vivo methods by multiple supplement nutrition formulations showed that comparison between these methodologies and by the low iron availability and absorption in humans. Studies aimed at the optimization of iron in nutritional formulations should include in vitro methods followed by an assessment of iron absorption in vivo in order to better investigate their metabolic behavior.

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