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CLINICAL STUDIES OF HIP: AN ORAL HEME-IRON PRODUCT

Paul A. Seligman, M.D.[✉,1], Gary M. Moore, B.S[2], and Rhoda B. Schleicher, B.S.[1]

School of Medicine, Department of Medicine, Division of Hematology[1], University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Campus Box B-170, Denver, Colorado 80220, and Colorado BioLabs[2], Aurora, Colorado, e-mail address: Paul.Seligman@UCHSC.edu, telephone: 303\315-8474, facsimile: 303\315-8477.

ABSTRACT

Iron bound to heme appears to be more bioavailable than iron salts, but in the United States no heme iron supplement presently exists. We performed iron absorption studies to evaluate a heme iron product made from digested hemoglobin (HIP) and compared iron absorption from this preparation to iron salts and placebo. In the study 14 subjects were given a standard breakfast and tested with three different preparations: 20 mg of iron as HIP; 20 mg of iron as ferrous fumarate and placebo. Change in serum iron was measured at 3 hour and 6 hours post ingestion. Statistical analysis was done using paired T tests. HIP allowed for significantly increased iron absorption taken with a meal when compared to iron salts or placebo ($p<0.03$ and $p<0.02$, respectively). There was a correlation between iron stores, as estimated by serum ferritin, and iron absorption from HIP with iron absorption from HIP significantly increased compared to placebo for those with lower ferritin values. Thus, a new heme iron product is bioavailable when taken with a meal and would have potential advantages over iron salts as a supplement.

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Key Words: Heme, Iron, and Ferritin

✉ To whom correspondence and reprint requests should be addressed.

INTRODUCTION

Iron in the diet is typically described as belonging to one of two distinct pools: heme-iron and inorganic iron (1). Heme-iron is a common dietary component contributed from animal tissue including red meat, poultry, and fish. Inorganic iron, also called iron salts, is contributed primarily by vegetables and cereals. Several prior studies suggest that iron from heme (as hemoglobin) is more bioavailable (2,3). One reason inorganic iron is thought to be less bioavailable is due to the inhibition of absorption caused by food (4,5), particularly dietary constituents such as polyphenolic tannins, phytates, soy, and dairy products (6,7,8,9).

The use of hemoglobin as an iron supplement is impractical since about 6 grams of purified hemoglobin (0.33% iron) would need to be ingested to deliver 20 mg of iron (10). In the United States no heme iron supplement is presently marketed, possibly because pure heme extracted from hemoglobin would polymerize into insoluble complexes at the low pH of the stomach (11).

We have studied a product used in Japan called heme iron polypeptide (HIP) derived from porcine hemoglobin digested with proteolytic enzymes which results in a highly soluble heme moiety with short polypeptide chains containing more than 1% iron (10,12,13). To evaluate the product for use in the US we compared iron absorption from HIP to inorganic iron and placebo in the presence of a meal.

METHODS & MATERIALS

Heme iron polypeptide was obtained from Asahi Chemical Industry Company, Ltd. (Tokyo, Japan), ferrous fumarate from Generichem Corp. (Totaway, NJ) and glucose (placebo) from Ashland Nutritionals (Irvine, CA). All preparations were put into opaque "00" sized gelatin capsules.

After institutional review board approval, subjects were included based on study criteria: over 18 years of age, healthy, no inflammatory conditions, non-anemic by both hemoglobin and hematocrit values using our laboratory reference range. Subjects included ranged between 22 and 52 years of age. After reading and signing informed consent, subjects fasted from 8 p.m. the evening prior to testing and upon arrival in the morning had 10 ml of blood drawn. They immediately thereafter consumed the test-dose with the standard meal consisting of 8 oz of coffee with 0.5 oz of cream, 1 C corn flakes with 4 oz of skim milk, and 2 slices of toasted wheat bread with 0.5 oz of margarine. The basal iron content of the meal was not determined. After eating the meal, the subjects continued fasting and returned at 3-hour and 6-hour intervals for blood draws. Each subject received, in a double blinded randomized fashion, 3 capsules containing either 20 mg of HIP iron, 20 mg of iron as ferrous fumarate, or placebo.

For assessing iron absorption, we used a change in serum iron technique (14,15). Detailed studies by Ekenved and coworkers have shown that absorption of radioactive iron as measured with the whole body counter is highly correlated with the increase in serum iron concentration 2-6 hours after ingestion (16,17). These studies have demonstrated that the increase in the concentration of serum iron can be used for comparative studies of the absorption of iron when iron is given in the form of tablets and in the presence of various substances such as food and antacids which inhibit iron absorption (4-9,18,19).

Blood samples drawn above were allowed to clot and in siliconized tubes, centrifuged, and the serum was decanted and serum iron measured at 0, 3, and 6 hours post ingestion using a standard assay (Sigma Chemical, St. Louis, MO). Subjects also had iron stores assessed by serum ferritin determinations (clinical kit).

RESULTS

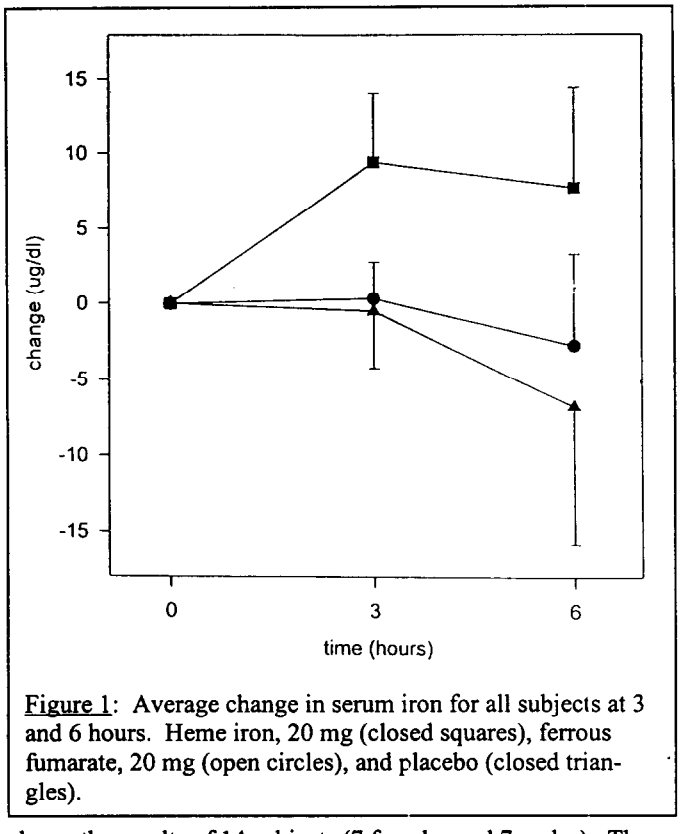

Figure 1: Average change in serum iron for all subjects at 3 and 6 hours. Heme iron, 20 mg (closed squares), ferrous fumarate, 20 mg (open circles), and placebo (closed triangles).

Figure 1 shows the results of 14 subjects (7 females and 7 males). The average change in serum iron with the HIP dose was higher as compared to the average change seen with ferrous fumarate or placebo at 3 hours. After 6 hours, all of the values including placebo were lower reflecting decreased iron absorption and possibly diurnal variation of serum iron (20). Using paired T analysis, the change at 3 hours for HIP was significantly higher as compared to placebo ($p<0.02$) as well as when compared to ferrous fumarate ($p<0.03$).

When subjects were separated according to starting ferritin values, two groups were easily identified using a 50 ng/ml "cut-off", a value used in previous studies to distinguish decreased iron stores in anemic subjects (21). As shown in Figure 2 (shown on page 3), at 3 hours the low ferritin group had statistically higher serum iron levels after HIP when compared to placebo (means 16.98 and –1.38, respectively) using paired T analysis ($p<0.01$), and ferrous fumarate (iron salt) ($p<0.02$). The high ferritin group change with HIP compared to placebo (means 3.84 and 0.26, respectively) was not statistically significant ($p=0.4$).

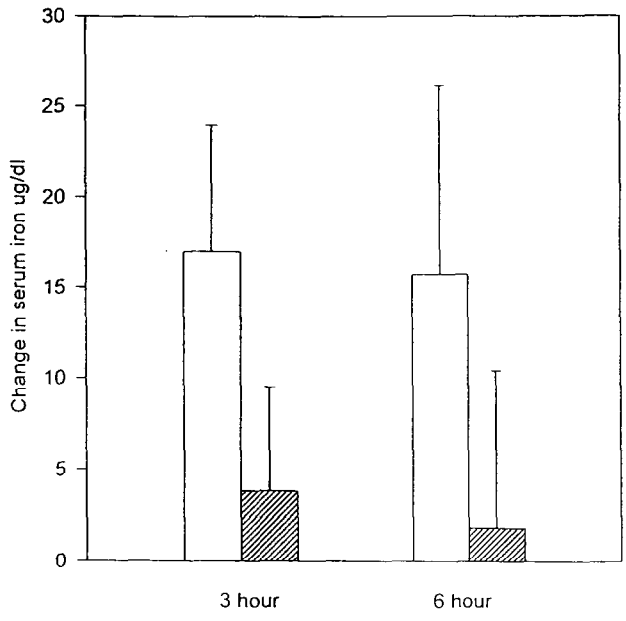

Figure 2: Response to heme iron (20 mg) as measured by changes in serum iron at 3 and 6 hours. Subjects were divided into two groups according to starting ferritin levels. Clear bars represent six subjects with ferritins <50 ng/ml. Filled bars represent 8 subjects with ferritins >50 ng/ml. Error bars represent standard error of the mean.

Side effect data was obtained by questionnaire in a double-blind fashion following the 6-hour blood draw. Besides complaints of hunger due to the 6-hour fast, only two subjects complained of gastrointestinal side-effect described as "heart burn". Both subjects had taken the ferrous fumarate preparation.

In an additional study, we selected 10 female subjects who were nonanemic and had ferritins below 50 ng/ml to determine if HIP given as 60 mg dose of elemental iron would result in markedly increased absorption as compared to the 20 mg dose. This study required a large number of gelatin capsules to be given in order to maintain the double-blind nature of the experiment with all subjects receiving nine gel caps. Although the 60 mg HIP dose was higher at 3 hours and 6 hours post ingestion as compared to the 20 mg dose, neither difference was statistically significant (change at 3 hours for 60 mg (17.90 $\pm$ 5.45 SEM) vrs 20 mg (12.20 $\pm$ 5.14SEM). Change at 6 hours for 60 mg (16.60 $\pm$ 10.55 SEM) vrs 20 mg (4.50 $\pm$ 8.40 SEM))

DISCUSSION

Previous studies suggest that a specific receptor for heme in the duodenum and jejunum is different from the newly characterized reductase system that allows for absorption of inorganic

iron (22,23,24). Iron salts are relatively inexpensive but many subjects complain of gastrointestinal side-effects (25), some patients have inadequate gastrointestinal absorption (26) and for all subjects the absorption of iron salts with a meal decreases iron bioavailability based on numerous food components (6-9). Previous studies using radio-labeled hemoglobin have suggested that iron absorption from these preparations is also controlled by iron stores but not inhibited when taken with a meal (27).

In our studies of bioavailability of iron from HIP, we chose a product available in Japan that has been shown to increase iron parameters including ferritin values when given in doses of 6-7 mg of iron per day for those at risk for iron deficiency (28,29,30).

For our study, we did additional bioavailability comparing iron absorption of HIP to the same dose of iron as ferrous fumarate and placebo when taken with a meal. So as to avoid the use of radioactivity in order to use the unaltered HIP preparation presently used in Japan, we used a technique that has been shown by Ekenved to correlate with radioactive iron absorption and allow for comparison of preparations (16,17). Using our formula obtained from Ekenved's data to calculate iron absorption (19), the amount of iron absorbed for those taking 20 mg of iron as HIP was at least 2.0 mg whereas <1 mg was absorbed from 20 mg iron as ferrous fumarate.

Thus our studies indicate that iron present in HIP, similar to hemoglobin, when taken with a meal, allowed for significantly increased iron absorption using 20 mg of iron which, based on US RDA and RDI recommendations, is recommended for those at risk for iron deficiency (31). This finding, taken together with the increased absorption by those with lower serum ferritin values, indicates that regulation of iron absorption from HIP does occur.

Continual supplementation would not be expected to lead to iron overload due to our additional study, where we found that 60 mg of iron as HIP did not show proportional increase in bioavailability, as compared to the 20 mg dose even for those with relatively low ferritin values. Although an increased number of time points may have made the difference significant, the results with higher iron dosing from HIP suggest that iron transfer across the lining of the intestine may be a saturable process. However, with this product, the nine capsules necessary to contain the 60 mg dose would also appear to be impractical and might actually interfere with iron absorption. Our data does suggest, however, that two 20 mg doses given with breakfast and dinner might provide sufficient iron to treat iron deficiency anemia. HIP iron is bioavailable even when taken with a meal and compliance might be improved due to anticipated lack of gastrointestinal side-effects. Further studies, therefore, are now warranted to assess the efficacy of this preparation in both the prevention and treatment of iron deficiency.

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