5-1997

# Relative Absorption of Iron and Magnesium from Sulfate Salts, Amino Acid Chelates Complexed and/or Mixed with Vegetables, and Taste-Free Supplements

Jennifer A. Bowden
*Utah State University*

# RELATIVE ABSORPTION OF IRON AND MAGNESIUM FROM SULFATE SALTS, AMINO ACID CHELATES COMPLEXED AND/OR MIXED WITH VEGETABLES, AND TASTE-FREE SUPPLEMENTS

by

Jennifer A. Bowden

A thesis submitted in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1997

# ABSTRACT

Relative Absorption of Iron and Magnesium from Sulfate Salts,
Amino Acid Chelates Complexed and/or Mixed with
Vegetables, and Taste-Free Supplements

by

Jennifer A. Bowden, Master of Science

Utah State University, 1997

Major Professor: Dr. Deloy G. Hendricks
Department: Nutrition and Food Science

Common methods for determining mineral absorption by the body are invasive and frequently utilize radioisotopes. In experiment one, rats were given a dose of radiolabeled ferrous sulfate. Relative absorption was estimated by changes in serum iron and by appearance of the radiotracer in the serum. There were no differences in relative absorption determined by the methods, although the low overall absorptions by the iron-replete rats may have decreased the sensitivity.

In experiments two and three, iron and magnesium supplements were given orally to 12 women age 19-25. Each subject received iron and magnesium supplements once each week for 5 weeks. Blood samples were taken via venous catheter every 30 minutes for 2 hours and 30 minutes following dosing, and a urine sample was taken following the collection time period. Samples were analyzed for serum iron, serum magnesium,

hematocrit, ferritin, urinary magnesium, and urinary creatinine. The increase in serum iron was evaluated from both the peak increase in serum iron and from the integrated increase in serum iron over the blood collection time. The data for each supplement were compared by analysis of variance. For the iron supplements, the taste-free iron supplement increased serum iron less than either the Ferrochel or the ferrous sulfate supplements. When the iron absorption was then compared to ferritin stores (low, medium, and high), the relative absorption of Ferrochel was higher in the low ferritin range (0-15 ng/ml) than in the upper ranges (P=.001 for peak and P=.0002 for area). Relative absorption from Ferrochel iron was also higher than the other supplements for subjects with low ferritin stores.

Neither serum nor urinary magnesium values differed significantly among the three compounds examined. Serum magnesium values are stable in healthy individuals, and the urinary magnesium data were not evaluated over 24 h as in typical magnesium load tests.

In summary, both the ferrous sulfate and Ferrochel supplements were absorbed more efficiently than the taste-free iron supplement. The Ferrochel was also absorbed more efficiently in individuals with low iron stores, demonstrating better regulation by the body than with the other supplements examined.

(79 pages)

# ACKNOWLEDGMENTS

I wish to express my appreciation to all those who assisted in this thesis project. First and foremost, I thank my dear husband, Anton, for all of his love, support, and help with data collection and analysis. All of his efforts made completing this project possible.

I also wish to thank Dr. Deloy Hendricks for all of his diligent work in helping me with this project, especially with the animal studies. He has been a wonderful major professor.

Special thanks goes to Jim Talty in radiation safety for his assistance and instruction, to Kent Udy in the LARC facility for accommodating and assisting with the animal study, and to Dr. Stan Allen for his instruction, assistance, and surgical skill.

Thank you to Roxanne Pfister for her assistance with statistical analysis. I also wish to thank Noreen Schvaneveldt, Janet Anderson, and Deloy Hendricks for their constant help and encouragement, and for allowing me to work as a teaching assistant for them. Thank you to Dr. Nedra Christensen and Dr. Charles Carpenter for their willingness to serve as committee members.

Finally, thank you to Albion Laboratories for funding this project, and to Steven Ashmead for his assistance in making this research possible.

Jennifer A. Bowden

# CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

Iron and magnesium are two essential minerals with important functions in the body. Iron is needed for oxygen transport and is a cofactor for several enzymes and immune factors. Magnesium is a necessary part of bone structure and plays a role in hundreds of biochemical reactions. Deficiencies in either of these minerals lead to biochemical imbalances and deficiency symptoms. Adequate dietary intake is the preferred method of preventing deficiency although dietary limitations of several groups of individuals prevent them from meeting nutrient needs.

Iron is a mineral frequently limited in the diet. As with all essential minerals, levels of appropriate intake for iron have been established by the Food and Nutrition Board of the National Research Council in the form of Recommended Dietary Allowances (1). Often these levels are not reached in the diet, particularly in women. Due to limited dietary intake, supplementation may be needed in some individuals who are at high risk of or suffering from poor iron status or anemia. There is a high incidence of iron deficiency anemia worldwide with a lack of information regarding how to most effectively increase iron absorption to treat anemia.

Magnesium is another mineral with established Recommended Dietary Allowances. In contrast to iron, people typically meet these recommendations since magnesium is a ubiquitous mineral in the diet. Deficiency occasionally occurs, for example, in association with gastrointestinal and renal diseases (2) and may occur in up to 65% of patients in intensive care settings, often going undiagnosed (3). Adequate magnesium in critical care settings is therefore important in deficient individuals, and

supplementation may be needed. As with iron, information on how to best treat magnesium deficiency is lacking.

The use of nutrient supplements must be evaluated, and the particular supplements must be studied to determine their most appropriate use. The American Dietetic Association has recently released the following position statement in regard to nutrient supplements:

> It is the position of the American Dietetic Association that the best nutritional strategy for promoting optimal health and reducing the risk of chronic disease is to obtain adequate nutrients from a wide variety of foods. Vitamin and mineral supplementation is appropriate when well-accepted, peer-reviewed, scientific evidence shows safety and effectiveness (4:73).

As recommended in this statement, this study examined the effectiveness of iron and magnesium absorption from six supplements. This information will help evaluate their potential use in treating deficiencies.

To evaluate the absorption of minerals, invasive techniques and radioactive assays are frequently used. Experiment one compared two methods for measuring iron absorption. The first method followed the increase in serum iron levels after gavage-feeding the mineral supplement. Evaluating change in serum levels has been utilized frequently to determine mineral absorption (5-10). Examining change in serum iron is minimally invasive and potentially less dangerous than utilizing radioactive substances. The second measurements were made using the more accepted technique of adding a small amount of radioisotope to the supplement and following isotope absorption. The results of the two methods were compared.

When utilizing a supplement of an essential mineral, the level of absorption or

uptake into the blood is important in evaluating the supplement's effectiveness. One factor affecting absorption of a mineral is the form of the mineral when consumed. From previous studies involving compounds similar to the Ferrochel supplement being examined in this study, minerals chelated to amino acids appear to be absorbed more effectively into intestinal mucosal cells than are inorganic minerals (11, 12). In addition, iron chelated to an amino acid helps increase hematocrit and hemoglobin levels in iron deficiency anemia with lower doses than with ferrous sulfate. For example, 30 mg of chelated iron is as effective as 120 mg of ferrous sulfate in increasing low hemoglobin levels (13).

In a process developed and patented by Albion Laboratories, minerals have been chelated to amino acids, primarily glycine, and complexed and/or mixed with dry vegetable concentrates in an attempt to increase the bioavailability of the nutrients in the supplement and increase the supplement's nutrient density by including vegetables, though the amount used does not make this increase significant. Albion added these vegetable concentrates for their known nutritional benefits as well as the hypothesized benefits of disease prevention, which are currently being researched (12). This study examined the uptake of iron and magnesium from these vegetable and mineral-amino acid chelate combinations to evaluate their benefit as nutrient supplements.

For the evaluation of magnesium absorption, the magnesium dose was followed by a 2.5-h urine collection, based on the principles of a magnesium load test. Urine was assayed for magnesium and creatinine, and absorption was determined based on magnesium excreted per milligram creatinine per hour. Serum magnesium was examined

simultaneously in the blood samples taken every 30 minutes. Serum magnesium levels measured in this study reflect the tight regulation of this element seen in the bodies of healthy individuals.

# REVIEW OF RELATED LITERATURE

## Iron deficiencies

Iron deficiency is often defined as the point at which iron stores are completely exhausted and functional deficiencies begin to occur. Risks associated with low iron stores are due to the potential for depleting iron stores completely (14). There is evidence that iron deficiency is associated with decreased attention span and learning, impaired growth and increased morbidity in children, decreased immunity, decreased work capacity, decreased athletic performance, increased premature uterine contractions, and possibly increased premature birth (14). Iron deficiency affects up to 6% of the United States population, particularly affecting individuals in periods of growth and women of child-bearing years (1). Worldwide, deficiency may affect as many as 500 to 600 million people (15).

In pregnancy, one of these periods of growth, physiological changes naturally occur. These normal changes in iron status and use have influenced the formation of different ideas regarding appropriate iron intake, absorption, and anemia. During pregnancy, red blood cell mass increases by approximately 18% and plasma volume increases by approximately 50%, reflecting the need for increased oxygen and the greater need for increased circulation and vasodilation (16). Indiscriminate iron supplementation can increase red blood cell mass, leading to hyperviscosity (16). Barrett et al. (17) suggested that in normal pregnancy absorption of non-heme iron increases to match increased iron demands when the food iron is bioavailable. This study also suggested that

iron deficiency anemia in women who had normal iron stores in early pregnancy and consumed a normal diet may develop a deficiency later in pregnancy due to a defect in metabolizing absorbed iron (17). In this deficiency, iron supplementation may be beneficial as has been demonstrated in other women with depleted iron status. Due to depleted iron status, severe iron deficiency anemia is common in developing countries. One contributing factor may be the dietary iron consumed by women in these developing nations. Diets tend to be low in heme iron and high in grain products, which frequently contain inhibitors of iron absorption such as phytates. Other considerations are the higher parity in women of developing countries as well as a less common use of iron supplements (18). Iron deficiency in pregnancy is a problem as it leads to poor growth and survival of infants.

Infants and children are also at risk of iron deficiency. Manifestation of deficiency in infants and children may include impaired psychomotor development and cognitive function, in part due to the infants being more fearful, tired, and tense. Iron supplementation is important in reversing the anemia although reversibility of these effects remains uncertain. Thus, preventing the deficiency is of primary importance (19). One subgroup of children at risk of iron deficiency anemia is children with phenylketonuria (PKU). These children are already at risk of neurological toxicity due to phenylalanine and may be at increased risk from low iron intake. Studies have shown that iron deficiency changes neurotransmissions involving dopamine, which affects behavior. This population typically has low iron stores, which may result from inadequate intake, decreased iron absorption, and low iron bioavailabilty (i.e., no heme iron in the diet) (20).

In adults, iron deficiency can lead to decreased work performance. This may result from decreased hemoglobin levels leading to decreased oxygen transport to tissues or from a decreased capacity of the muscle to consume oxygen (19). Women are most susceptible, especially those with heavy menstrual losses or high parity. Treatment with iron is effective in reversing this deficit in work or exercise capacity (19).

Iron deficiency anemia is also prevalent in the elderly population, likely due to factors including normal physiological changes in aging, relatively higher levels of malnutrition than in younger United States populations, and a higher rate of chronic disease. Iron deficiency occurs in the elderly despite the increase in liver iron stores associated with advancing age. In elderly individuals, gastrointestinal blood loss is the most frequent cause of iron deficiency (21). This blood loss may result from polyps, stomach or colon cancers, peptic ulcers, hiatal hernia, hemorrhoids, diverticulosis, nonsteroidal antiinflamatory drugs, anticoagulants, genitourinary diseases, frequent blood draws, and other disease conditions (21). Gastrointestinal disease may not manifest itself with obvious blood loss but may result in partial or complete iron malabsorption. Iron deficiency due to gastrointestinal disease responds well to oral iron supplementation although mechanisms are unclear (22). Iron deficiency appears more prevalent among elderly individuals seeking medical attention for other conditions. Some institutionalized elderly may be at risk of dietary iron deficiency as well. Iron intake typically decreases with advancing age, although a large proportion of the elderly population takes iron supplements (21).

Iron deficiency occurs in three stages: iron depletion where iron stores are

diminished, iron deficient erythropoiesis, and iron deficiency anemia (1, 23). The first stage of iron depletion presents with diminished iron stores in the liver, spleen, and bone marrow and is reflected by a decrease in serum or plasma ferritin concentration (23, 24). Iron deficiency erythropoisis, the second stage, is characterized by decreased serum iron, increased total iron binding capacity, decreased percent transferrin saturation, and increased erythrocyte protoporphyrin concentration due to a lack of iron for heme synthesis. In iron deficient erythropoisis, hematocrit and hemoglobin remain within normal limits. In the third stage of iron deficiency, a microcytic, hypochromic anemia exists, along with decreased hemoglobin and hematocrit levels, further decreased serum iron and ferritin, and further increased erythrocyte protoporphyrin concentration and total iron binding capacity (24).

Serum ferritin is a key indicator of iron stores. Its function is primarily to provide cells with iron (25). Serum ferritin is most informative when assessing iron reserves in iron-replete populations. Serum ferritin is also important in clinical assessment of anemic patients because in all types of anemia, except iron deficiency anemia, a decrease in hemoglobin is accompanied by an increase in storage iron. If iron deficiency is the sole cause of anemia, serum ferritin is almost always less than 12 $\mu$g/l (14). Ferritin is the single most concentrated source of physiological iron in the body (26, 27). Iron must be in the ferrous state to be incorporated into ferritin. It requires reduction and the presence of an iron chelator to release iron from ferritin (27). Deposition and release of ferritin iron must be tightly regulated, and therefore it is likely controlled by enzymes (26). Ferritin consists of an apoprotein shell enclosing a core of iron in the form of ferrichydroxy-

phosphate, which may contain up to 4500 iron atoms (25, 28).

**Iron requirements**

Established iron requirements consider the bioavailability of iron in the diet, the ability of the body to absorb iron, obligatory losses, and iron requirements for growth. Recommended Dietary Allowances for iron are based on an average American diet consumed by a person with average iron stores. This diet is assumed to include iron of medium bioavailability where about 10% of total dietary iron is absorbed. These requirements also take into account iron losses and needs. Obligatory losses in a 70-kg male are about 1 mg/day; for a 55-kg female losses are about 0.8 mg/day plus menstrual losses of about 0.6 mg/month in a skewed pattern. Losses for infants are approximately 0.3 mg/day for the first year, 0.4 mg/day for the second year, and from 0.5 to 0.8 mg/day for children. Adolescents lose about 1.6 mg of iron per day. Losses for expectant women vary throughout the pregnancy, the total loss being about 1000 mg of iron (about 20 mg Fe/kg mass of the woman). There is a significant increase in iron losses during the second and third trimester of pregnancy (about 5-6 mg/day) (29). There are also pathological iron losses that can result from bleeding and parasites such as hookworm. Hookworm affects about 450 million people, although its effects on iron status in most affected people are often minimal when compared to other factors, such as limited dietary iron, which contribute more to the poor iron status of these individuals (29).

Food iron availability is in two major pools, heme iron and nonheme iron. Heme iron is highly bioavailable; it is absorbed intact with the porphyrin ring and is not affected by dietary factors. Nonheme iron is affected by both inhibitors and promoters in

the diet. Most dietary iron is characterized by poor bioavailability, heme iron absorption ranging from 12-35% (29, 30) and nonheme ranging from 2-20% (30).

**Iron in the body**

Iron is a trace mineral found throughout the body. It is particularly associated with hemoglobin, myoglobin, transferrin, tissue enzymes such as the cytochromes, and enzymes needed for immune system function. These functions make iron essential for humans. Iron is important due to its redox reactivity in two stable, interchangeable forms (31). The body has the capacity to conserve iron, but a limited ability to absorb iron. The body of a healthy adult contains between 3 and 4 g of total iron (about 50 mg/kg) found primarily in iron porphyrin complexes, hemoglobin, myoglobin, and the enzymes. Thus, in iron-replete individuals, men have about 1000 mg storage iron while women have 300 mg storage iron (29).

Body iron content is regulated primarily through changes in the amount of iron absorbed by the intestinal mucosal cells, and usually varies between 10-15% of total iron intake. Absorption is influenced by body iron stores, by the amount and chemical form of iron in the ingested food, and by dietary enhancers and inhibitors (1). If body iron status is high, iron absorption is low; if body iron is low, absorption of dietary iron is high. Ferrous sulfate is used as the standard when comparing absorption from other sources and efficiency in converting the iron to hemoglobin (32). Bioavailability of iron from ferrous sulfate varies according to research in efficiency of converting iron to hemoglobin (33).

Iron metabolism is not strictly absorption and loss, but it is a cycle of storage, utilization, transportation, and reuse. There is no known regulated way to dispose of

excess iron (28). Iron is ingested, oxidized, combined with a carrier, passed through an apical membrane of the gut mucosa, passed through the cytosol of the gut mucosal cell, then stored within the cell cytoplasm or transported through the basolateral membrane of this cell (28). It is then carried in the serum and transported into a cell's cytoplasm for utilization in the synthesis of iron containing enzymes and hemoglobin, or incorporated into the storage protein, ferritin (28).

**Chelation of iron**

Iron transport and storage by cells is facilitated by its chelation to proteins such as transferrin and ferritin, respectively. These iron-binding proteins can withhold iron needed from bacterial growth, thus preventing infectious growth of microorganisms (34). It has also been shown that many cells can acquire a substantial amount of iron from low molecular weight iron chelates (34). The concentration of these chelates seems to increase under pathological conditions of iron overload. It has been suggested that human macrophages can acquire iron from these chelates, allowing the macrophages to clear acute conditions of iron overload, as in areas of cell injury and thus limit oxidant-mediated tissue injury (34). Dietary enhancers and inhibitors of iron absorption are examples of chelators. If an iron chelate maintains solubility and the chelate can release iron to the mucosal cell, iron absorption is enhanced. If the iron is strongly bound, the iron chelate is excreted and absorption is inhibited (30).

**Measuring iron absorption**

Absorption of minerals and mineral chelates can be accurately measured by

following changes in serum levels of minerals after supplementation. This method is less expensive, less invasive, and less dangerous than radioassay methods. Measuring changes in serum levels involves performing an initial blood draw, administering a dose of the nutrient to be examined, and repeating blood draws at intervals thereafter. The absorption of the nutrient may be determined by the peak rise in serum levels, or the area under the serum iron versus time curve. In a previous human study examining serum iron as a measure of iron absorption, the results obtained were very similar to results using methods involving radioactive iron (10).

**Magnesium deficiency**

Magnesium deficiency may be associated with impaired intake or with intestinal or renal tubular magnesium malabsorption. One type of depletion is a failure to ingest, absorb, or retain magnesium in otherwise well nourished individuals. Another deficiency is associated with concomitant nutrient deficiencies, catabolism, potassium depletion, acidosis, and/or drug use resulting in the loss of bone and muscle magnesium. Clinical deficiency symptoms can include muscle fasiculations, tremors, spasms, personality changes, anorexia, nausea and vomiting, and Trousseau and Chvostek signs (35), as well as growth failure, pallor, weakness, electromyogram changes, hypocalcemia, and hypokalemia (2). Decreased magnesium status is also associated with low levels of immunoparathyroid hormone (iPTH) (35). Magnesium deficiency may occur frequently in acutely or chronically ill patients. Deficiency can also be found in patients with alcoholism, diabetes mellitus (particularly with ketoacidosis), malabsorption, protein energy malnutrition, renal disease, post parathyroidectomy hypomagnesemia, congenital

disorders, and hypertension. Reported prevalence of hypomagnesemia in hospitalized patients varies, ranging from 4.6% to 47%, and in intensive care patients may be as high as 65% (3).

Low magnesium status is related to coronary artery disease. Any changes in intracellular magnesium concentration can profoundly affect cardiac physiology (35). Several studies suggest that magnesium deficiency affects lipid metabolism and is associated with tissue injury in the membrane bilayer lipids as well as intensifying cardiovascular lipid deposition and lesions. Magnesium deficiency also affects the susceptibility of the lipoproteins to peroxidation; thus magnesium supplementation may help in preventing atherosclerosis (36).

Magnesium may also play an important role in osteoporosis. High calcium intake can alter the calcium-to-magnesium ratio, decreasing the efficiency of magnesium absorption and further aggravating the consequences of low estrogen. These effects act in decreasing magnesium movement into the bone as well as increasing the demineralizing activity of parathyroid hormone (2).

Assessment of magnesium status to detect deficiency may take several forms. Although serum magnesium analysis is unreliable, measuring ionized serum magnesium may be more reliable, as may determining free intracellular magnesium, and blood mononuclear cell magnesium concentration measurements (35). Urinary magnesium is a good method of assessment, and may be performed along with a magnesium load test or a classic balance study. Magnesium load tests consist of determining urinary magnesium excretion over a determined time period following a dose of magnesium. Clinical

observations may also help in assessing magnesium status (35).

**Magnesium in the body**

Magnesium is the fourth most abundant cation in the body and the second most abundant intracellular cation. Magnesium can be found in the body in three states: ionized or free, complexed to anions, and bound to proteins. The primary site of action for magnesium is in protein and nucleic acid synthesis (37). Magnesium functions in over 300 biochemical reactions, including fatty acid synthesis, amino acid activation, protein synthesis, phosphorylation of glucose and glucose derivatives in the glycolytic pathway, oxidative decarboxylation of citrate, transketolase reactions, and the formation of cAMP. Over half of the magnesium in the body is found in the bone, with most of the rest located in soft tissues (35). Absorption of magnesium has been found to take place primarily in the ileum and to some extent in the colon (37, 38).

**Albion mineral chelation**

Several studies have demonstrated actual chelation, bioavailability, absorption, and gastrointestinal tolerance of amino acid chelated minerals developed by Albion Laboratories (39). Absorption of minerals in dietary supplements is important as an individual can receive no benefit from a supplement unless it can be delivered to the cells. Absorption of these chelated minerals has been determined by evaluating mineral and mineral chelate levels in intestinal tissues, particularly jejunal tissues, evaluating organ tissue levels (11), examining hemoglobin levels, and monitoring excretion rates (13, 40). When evaluating serum levels of the minerals and mineral chelates, radioisotopes have

been the primary measure used to determine the absorption of the minerals and chelates. The rationale for utilizing radioactive substances is to assure accuracy in measuring absorption (12).

# EXPERIMENT ONE--IRON

## STUDY IN RATS

### Methods

Two techniques, serum iron and radioisotope measurements, were used to determine amounts of iron absorbed from ferrous sulfate. This experiment utilized a group of 35 male Spauge-Dawley rats weighing approximately 150 g each. The rats were housed in pairs at the Laboratory Animal Research Center (LARC) at Utah State University. The rats were held in isolation for 3 days and fed the LARC's standard rat feed and water ad libidum. Hemoglobin and hematocrit levels of each rat were determined from blood collected in capillary tubes from the orbital cavity of each rat. Hemoglobin values were found using the Drabkin method (41). See Appendix A for the methodology and Appendix B (Table 4) for rat blood data.

The rats were divided into five groups, each group of rats having similar hemoglobin levels to avoid confounding the results. The rats received 0.8 mg of iron as ferrous sulfate. This dose is slightly above the estimated iron requirement of 0.7 mg/day based on a dietary recommendation of 35 mg/kg of diet assuming an average intake of 20 g of diet per day (42). This dose is within the range of doses provided in previous studies (43).

Prior to dosing the groups of rats, one rat from each group was anesthetized by carbon dioxide for blood collection via cardiac puncture. These five rats served as a baseline for serum iron levels for their groups. This method of blood collection via

cardiac puncture was utilized for all rats in the experiment and is explained below.

Each rat received the ferrous sulfate and 1 μCi of the $^{59}Fe$ isotope as an oral dose given in 0.5 mL water by gavage. Only one rat was dosed every minute to allow appropriate timing for the blood draws. Prior to blood collection, each rat was anesthetized in a carbon dioxide chamber. Cardiac puncture was performed by inserting a Vacutainer needle through the skin directly to the left of the xiphoid process at approximately a 15-degree angle above the abdomen and into the heart. The needle was then inserted into a Vacutainer tube for the blood collection. Following blood collection, the rats were euthanized by carbon dioxide. Carbon dioxide chambers are an appropriate method of euthanasia for rats approved by the American Veterinary Medical Association (44). All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (approval #762). Blood was collected from one rat in each group at 20-min intervals following iron dosing. Blood collection was repeated in each group every 20 min for 2 h, with five subjects being utilized for each time period.

Part of each blood sample (1 ml) was analyzed with a gamma counter for determination of $^{59}Fe$ absorbed into the blood. Total counts in the serum were calculated based on a blood volume equal to 7% of each rat's weight (45). Total serum iron was determined with the Ferrozine color reagent (46, 47). This method of determining serum iron compares absorbance measured by spectrophotometry in a set of standards with absorbance measured in the samples. See Appendix A for the complete methodology. The percents of dose absorbed calculated by change in serum iron and by appearance of the $^{59}Fe$ radiotracer were compared.

**Results**

Measuring change in serum iron and appearance of the radiotracer provided similar (P=0.668) estimates of iron absorption from ferrous sulfate. Data were analyzed using paired comparisons for each time period (Appendix E, Table 10). Both methods reflected extremely low absorption of the dose provided (Table 1).

**Discussion**

There were no significant differences in percent of dose appearing in the serum calculated at each time period over 120 min when measuring the serum iron increase and isotope appearance. However, the inability to compare data when only one sample could be taken from each rat caused difficulty in determining any significant changes in absorption over time. The need to take samples from different rats and average the data,

**TABLE 1**
Percent of dose in serum determined by change in serum iron and by appearance of $^{59}Fe$ isotope for each blood draw time following dosing

| Time after | Serum: | | isotope: | |
|---|---|---|---|---|
| dose (min) | % of dose absorbed | range (% dose) | % of dose absorbed | range (% dose) |
| 20 min | 0.61% | 1.44 to -0.15 | 0.39% | 0.73 to 0.21 |
| 40 min | 0.54% | 1.51 to -0.07 | 0.69% | 1.19 to 0.27 |
| 60 min | 0.30% | 0.68 to -0.35 | 0.83% | 1.65 to 0.16 |
| 80 min | 0.59% | 1.02 to 0.21 | 0.69% | 1.13 to 0.41 |
| 100 min | 0.99% | 1.91 to 0.42 | 0.99% | 1.63 to 0.62 |
| 120 min | 0.35% | 0.60 to 0.04 | 1.05% | 1.52 to 0.42 |

as well as the extremely low iron absorption in these rats due to high initial iron status, proved to be significant problems. Greater changes in serum iron may have occurred had the rats been iron depleted prior to the study. This was not done as the researchers wanted the method to be similar to experiment two, where the humans were neither depleted nor selected for low iron status.

# EXPERIMENT TWO--IRON

## STUDY IN WOMEN

### Methods

A group of 12 females age 19-25 years were recruited to participate in the study and consented to participate (Appendix C). Participants were of average body size, with a body mass index (BMI) ranging from 19.0 to 24.6. Most of the participants' BMI's ranged from 19 to 22, thus maintaining relatively constant blood volumes between the participants. However, adjustments for blood volume were made for each individual when calculating iron absorption. Participants were not selected by iron status, which allowed for a sample population with variable iron stores and status.

For the first week, the individuals were randomly provided with a dose of ferrous sulfate, the Albion chelated iron and vegetable concentrate supplement, or the taste-free iron supplement, with one-third of the participants receiving each supplement type. Doses were provided in capsules given by mouth with 8 oz of orange drink. Doses were given with the beverage primarily to provide kilocalories and fluid to the fasting subjects and prevent problems noted during the first week of the trial when several of the subjects fainted. Because the beverage contained vitamin C, there may have been an enhancement of the ferrous sulfate absorption. This effect was consistent throughout the study as the same beverage and serving was given each week. Each capsule contained 2 mg of iron (10). For composition of each capsule, see Appendix D.

Two registered nurses from Logan Regional Hospital took blood samples at zero, 30, 60, 90, 120, and 150 min following administration of the supplement dose (10). A

venous catheter was used at the site of the blood draw to reduce problems with venous access as well as the number of needle-sticks required. At 1-wk intervals sampling was repeated and each individual received a different supplement each week. The data from the first week were discarded due to errors created by dehydration of the subjects, severe hemolysis in several samples, and an inability to collect all blood samples over the study time due to fainting. Through the five remaining weeks, each subject received each supplement once and received two of the three supplements twice.

Blood samples were analyzed by evaluating the changes in serum iron levels using the initial blood sample as the control for each individual. Serum iron was determined by the hematology department at Logan Regional Hospital. The investigators determined hematocrit as well as serum ferritin, using an [$^{125}$I ] Ferritin RIA Kit (48).

Relative absorption of iron from the three supplements was determined by examining the peak increases in serum iron and by the integrated area under the increase in serum iron versus time curve. To estimate absorption from change in serum iron at peak levels, the following information was utilized:

body weight x .07% x (1 - hematocrit) = liters serum

Peak increase in serum iron was then determined by:

highest serum iron value obtained - initial serum iron value = gain in serum iron

As the dose given was 2 mg elemental iron, the percent of dose in the serum was then determined:

$$\frac{\text{gain x liters serum x 10 dl/l}}{\text{2 mg}} \times 100 = \text{peak serum iron increase}$$

The integrated increase in serum iron was examined using Simpson's Rule (49). The areas obtained by Simpson's Rule were then utilized to determine percent of the 2-mg iron dose absorbed, as with the peak serum iron increase values.

Serum iron levels were also evaluated in comparison to serum ferritin and hematocrit values. Statistical analysis of the data included analysis of variance and correlation of the factors under consideration.

**Results**

The results were examined by supplement for each of the subjects. Table 2 lists each subject, followed by pertinent data for each compound. See Appendix B (Table 5) for the complete data obtained. Weekly hematocrit and serum ferritin values are also given for each dosing because these values varied over time. We used both the peak increase in serum iron following dosing and the integrated increase in serum iron to determine relative absorption. Peak increase in serum iron appeared a more appropriate measure because there was a frequent drop from the initial serum iron level at the 30-min blood draw, which confounded the results when using the integrated increase in serum iron. Thus, our conclusions were made using the peak percent of dose absorbed rather than the area percent of dose absorbed.

The average increases in serum iron for each compound at the blood draw times are demonstrated in Figure 1. The increases in serum iron noted here differ from the results of experiment one. These differences will be discussed later. Note the negative value in the ferrous sulfate supplement at the 30-min blood draw. This demonstrates the drop from baseline seen in several subjects immediately following the dose. This drop

was seen with the other supplements as well, but was greater with the ferrous sulfate when the values were averaged. This drop may reflect a stimulation of iron clearance from the blood as the supplement dose was absorbed. The three different compounds were all absorbed by most of the subjects, although differences in absorption were noted. There was a slightly higher absorption from the Ferrochel supplement and a lower absorption from the taste-free iron compound than in the Ferrochel or the ferrous sulfate ($P = 0.0984$) (Appendix E, Table 12). When the averaged peak increase to determine serum absorption was used, the Ferrochel and ferrous sulfate supplements showed similar absorptions of 21.2% of dose and 20.7% of dose, respectively. The taste-free supplement demonstrated significantly less absorption at 10.2% of dose absorbed ($P = 0.0316$) (Appendix E, Table 12) when compared with the other two supplements (Figure 2).

When all of the subjects' data are averaged and the changes in serum iron at each time point are compared, there is a more pronounced difference between the compounds. This measurement is not the same as measurements used for comparing the compounds by subject or for the statistical analysis. By averaging all the data for each time point, one overall curve was created. By using the averaged area under this curve, the Ferrochel demonstrates the greatest level of absorption at 44.25% of dose absorbed. The ferrous sulfate also demonstrates a substantial absorption at 34.75% of dose absorbed while the taste-free iron supplement shows a much lower absorption at 1.8%. This low value is due in part to the frequent drops from baseline serum iron associated with this supplement over time (Figure 3).

**TABLE 2**

Summary of changes in serum iron for each supplement by subject

| Subject # | Supplement | Hematocrit (%) | Serum (ng/ml) | Peak % dose in | Area % dose in | Subject # | Supplement | Hematocrit (%) | Serum (ng/ml) | Peak % dose in | Area % dose in |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | FeSO4 | 43.00 | 30.00 | 0.00 | 0.00 | 7 | FeSO4 | 42.50 | 50.00 | 5.27 | neg |
| | Veg.Chelate | 45.00 | 21.00 | 9.01 | 10.51 | | Veg.Chelate | 42.50 | 90.00 | 13.18 | 12.74 |
| | | 43.50 | 24.00 | 12.96 | 21.90 | | | 42.00 | 46.00 | 26.59 | 55.40 |
| | Taste-free | 44.00 | 21.00 | 2.75 | neg | | Taste-free | 43.00 | 65.00 | 26.13 | 58.80 |
| | | 44.00 | 25.00 | 0.00 | neg | | | 43.50 | 39.00 | 12.95 | 12.95 |
| 2 | FeSO4 | 37.50 | 34.00 | 19.69 | 35.88 | 8 | FeSO4 | 36.50 | 6.50 | 50.89 | 138.05 |
| | | 39.00 | 26.00 | 0.00 | neg | | | 37.00 | <5 | 64.51 | 159.40 |
| | Veg.Chelate | 37.50 | 24.00 | 0.00 | neg | | Veg.Chelate | 36.00 | <5 | 49.86 | 67.91 |
| | | 39.00 | 24.00 | 1.28 | neg | | | 37.00 | <5 | 49.08 | 120.60 |
| | Taste-free | 40.50 | 27.00 | 3.75 | neg | | Taste-free | 35.50 | 5.50 | 1.44 | neg |
| | | 38.00 | 16.00 | 0.00 | neg | | | 35.00 | 5.20 | 15.90 | 27.40 |
| 3 | FeSO4 | 42.00 | 16.00 | 20.36 | 52.19 | 9 | FeSO4 | 41.50 | 5.60 | 43.71 | 127.20 |
| | | 43.00 | 12.00 | 12.51 | 20.85 | | Veg.Chelate | 44.00 | 13.00 | 39.59 | 85.58 |
| | Veg.Chelate | 41.00 | 46.00 | 11.66 | 21.58 | | | 41.50 | 7.00 | 31.90 | 86.64 |
| | | 40.00 | 14.00 | 52.67 | 144.84 | | Taste-free | 41.50 | 6.50 | 7.09 | 12.21 |
| | Taste-free | 43.00 | 23.00 | 21.26 | 38.40 | | | 40.50 | 8.70 | 6.01 | 6.81 |
| 4 | FeSO4 | 39.50 | 7.50 | 18.65 | 40.58 | 10 | FeSO4 | 39.00 | 15.00 | 2.52 | neg |
| | Veg.Chelate | 31.50 | 16.00 | 2.48 | neg | | Veg.Chelate | 40.00 | 65.00 | 0.00 | neg |
| | | 42.50 | 6.00 | 33.36 | 75.41 | | | 38.00 | 25.00 | 16.67 | 60.28 |
| | Taste-free | 44.00 | 21.00 | 0.00 | neg | | Taste-free | 39.50 | 38.00 | 20.02 | 19.61 |
| | | 39.00 | 16.00 | 12.17 | 26.54 | | | 39.50 | 16.00 | 18.77 | 57.98 |
| 5 | FeSO4 | 37.50 | 9.00 | 16.81 | 53.88 | 11 | FeSO4 | 43.50 | 80.00 | 25.71 | 37.39 |
| | | 40.50 | 12.00 | 9.85 | 33.64 | | | 42.00 | 26.00 | 0.00 | neg |
| | Veg.Chelate | 39.00 | 5.50 | 17.66 | 36.59 | | Veg.Chelate | 41.00 | 29.00 | 20.75 | 50.44 |
| | Taste-free | 30.50 | 8.50 | 7.19 | 17.73 | | | 43.00 | 32.00 | 10.61 | 15.72 |
| | | 39.00 | 7.50 | 0.00 | neg | | Taste-free | 41.50 | 32.00 | 16.94 | 37.95 |
| 6 | FeSO4 | 40.00 | 15.00 | 29.38 | 65.44 | 12 | FeSO4 | 31.00 | 18.00 | 24.58 | 54.78 |
| | Veg.Chelate | 42.00 | 24.00 | 20.66 | neg | | | 35.00 | 16.00 | 28.94 | 54.50 |
| | Taste-free | 40.00 | 28.00 | 30.72 | 88.14 | | Veg.Chelate | 36.00 | 10.00 | 25.64 | 46.06 |
| | | 40.00 | 16.00 | 12.02 | 23.59 | | Taste-free | 35.00 | 21.00 | 7.24 | neg |
| | | | | | | | | 37.50 | 8.50 | 1.39 | neg |

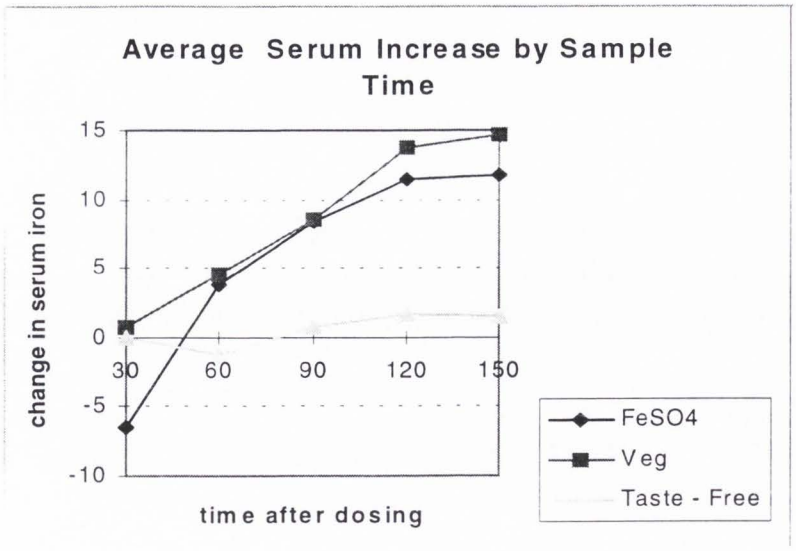


**FIGURE 1.** Changes in serum iron by sample time, averaged over all subjects for each supplement type.

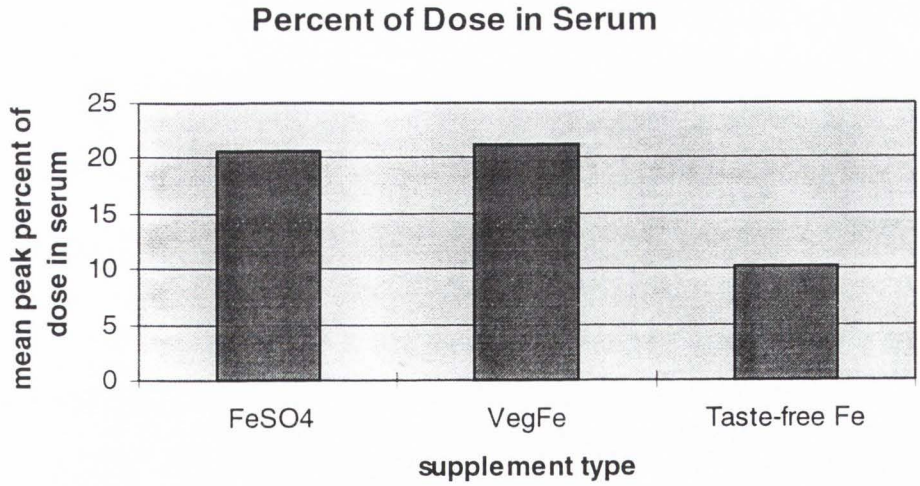


**FIGURE 2.** Mean relative absorption by supplement type for all subjects (P = 0.0984) (absorption ranges: $FeSO_4$ = 0 to 51, Ferrochel = 0 to 53, Taste-Free Fe = 0 to 31). When compared independently, the taste-free iron absorption was lower (P = 0.0316).

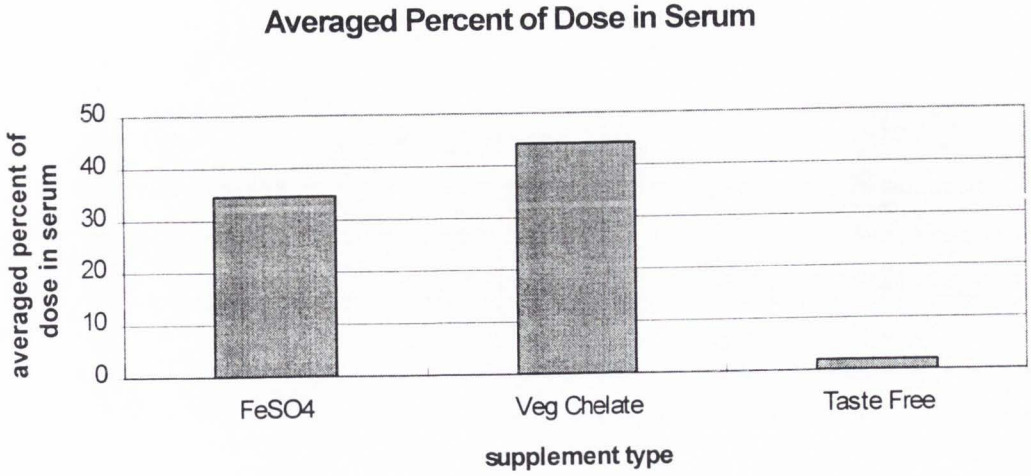


**FIGURE 3.** Integrated percent of dose in serum determined after data for all subjects were averaged for each blood draw time in each supplement. Significance could not be measured as the data were averaged.

To better examine the relationship between supplement absorption and serum ferritin status, subjects were categorized by their ferritin levels (high, 31 ng/mL or greater; medium, 16 through 30 ng/mL; and low, 0 through 15 ng/mL). Absorption was affected by ferritin level for the Ferrochel supplement but not for the ferrous sulfate or the taste-free iron supplement. For the Ferrochel supplement, there was a significantly higher absorption of iron for individuals in the low ferritin group than in the other two groups with higher ferritin status ($P = 0.0017$) (Appendix E, Table 15). Similarly, when the data were examined by ferritin level, there was a significantly lower absorption of the taste-free supplement than the Ferrochel ($P = 0.0185$) (Appendix E, Table 16) in subjects with low ferritin. There were no statistically significant differences between the absorption of the different supplement types in subjects with either moderate ($P = 0.3794$) (Appendix

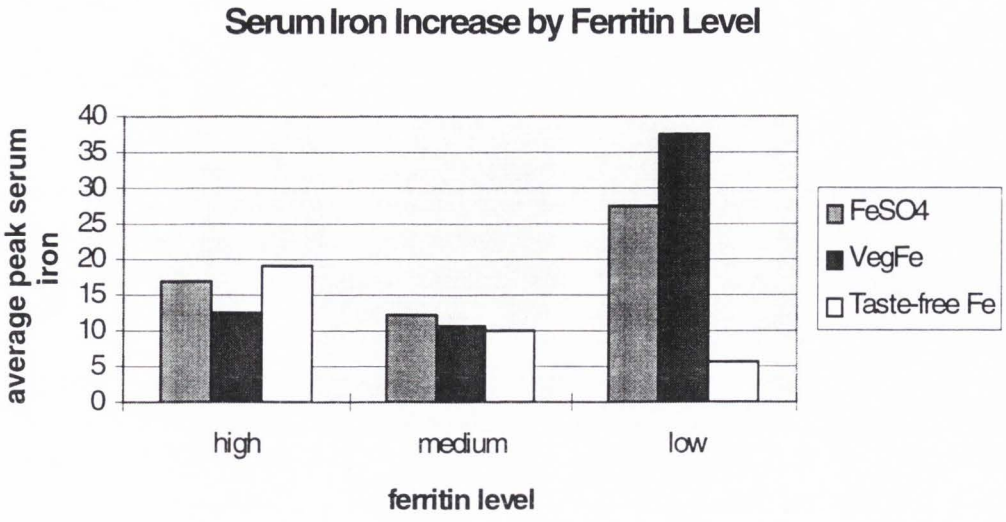


**FIGURE 4.** Average absorption by peak increase in serum iron for high ($\geq$ 31 ng/dl) (P = 0.7349), medium (16 - 30 ng/dl) (P = 0.3794), and low ($\leq$ 15 ng/dl) (P = 0.0017) ferritin status by supplement type. For subjects with low ferritin, absorption was higher in the Ferrochel (vegetable chelate) than the taste-free iron supplement (P = 0.0185).

E, Table 17) or high (P = 0.7349) (Appendix E, Table 18) ferritin levels (Figure 4).

The correlation between hematocrit, serum ferritin levels, and the absorption of the supplements was also a point of interest. Significant correlations ($P \leq 0.01$) were noted for ferritin in all supplements for both peak percent of dose absorbed (0.4492) and integrated percent of dose absorbed (0.5844) (Appendix E, Table 19).

**Discussion**

The human study in experiment two provides the most practical information, as the study's initial intent was to determine potential differences in absorption of the compounds for human use. Data obtained demonstrate the effectiveness of the Ferrochel when compared to ferrous sulfate. Measuring iron uptake by increases in serum iron is

minimally invasive when compared with other frequently utilized methods, particularly as no radioisotope is required.

Previous studies have demonstrated a correlation between the use of an $^{59}Fe$ tracer in examining iron absorption as compared with the changes in serum iron. Crosby and O'Neil-Cutting (50) utilized the increase in serum iron after 2 h as an effective indicator of mild iron deficiency. These data compared well to previous studies utilizing $^{59}Fe$ to determine absorption. The method was also confirmed by these researchers in examining the blocking effect of a dose of iron, as these data produced results similar to those noted in $^{59}Fe$ studies in animals (51). Wright (10) also noted that her studies with iron absorption from different sources produced absorption results similar to those seen in previous isotope studies.

Several other studies utilized changes in serum iron as a reflection of iron absorption following an oral iron load. Their methodologies were similar to the methods utilized in this study (6-9, 52, 53).

Measuring changes in serum iron underestimates iron absorption as only the rapid phase of uptake is considered and disappearance from serum is not included. One study by Costa et al. (5) considered disappearance in determining iron absorption from serum iron. Because the purpose of this study was to compare supplements with each subject receiving all three supplements, simply measuring the serum iron increase over a short period of time was appropriate to determine relative absorption. Because each subject had been provided with all three supplements, confounding factors such as iron status and clearance rates did not affect the results for each individual. All supplements were

measured in the same manner, and the study followed the methodology utilized in the literature by the majority of researchers. The drop in serum iron levels occurring after 30 min, which was seen most frequently with the ferrous sulfate, may indicate some differences in clearance. This effect should be considered in future studies.

Because ferrous sulfate is considered to be a well-absorbed form of nonheme iron, the lack of statistically significant differences between the peak increase in serum iron from ferrous sulfate and the Ferrochel suggests that the Ferrochel is also well absorbed. Similar iron chelate supplements are better absorbed than ferrous sulfate (54, 55). In these German studies, subjects given doses of the chelate supplement for 4 wk had improved blood iron values as compared to subjects given iron salts. Blood iron values examined were increases over time in serum iron, hemoglobin, and erythrocytes (54, 55). Kirchhoff examined hematocrit levels in addition to the stated biochemical indices. In addition to the duration of the study and doses given, the subjects in the two German studies suffered some degree of iron deficiency, but in this experiment the subjects were of varying iron status. In comparing this experiment to the previous experiments with chelated products, we found the short time span during which serum levels were measured and small doses that were administered may not have allowed a picture of the full effectiveness of the supplements. In the subjects with low ferritin status, the Ferrochel was much more effective than the other supplements, which reflects more closely the results of the German studies.

The presence of Vitamin C in the orange beverage in which each dose of supplement was given may have artificially increased the serum uptake of ferrous

sulfate. The beverage was needed to provide fluid and carbohydrate to the fasting subjects to maintain blood volume and glucose levels. This vitamin C would not be expected to affect the Ferrochel absorption, since Ferrochel mimics a heme iron molecule (13).

The taste-free iron supplement was absorbed less effectively than the other supplements in this study. These results differ from a study (56) done with ferric glycinate, a similar compound. These investigators found ferric glycinate to be absorped and retained at higher levels when compared with ferrous sulfate in infant formulas fed to weanling rats. Both iron compounds were extrinsically labeled with $^{59}FeCl_3$, and data were obtained by whole body counts taken daily for 2 wk (56).

Low serum ferritin is recognized as an effective predictor of high iron absorption due to decreased iron stores (10). The higher rate of absorption of the Ferrochel in subjects with low ferritin status demonstrates an advantage to this particular supplement. Individuals with low iron status, who are in greater need of supplementation to prevent or cure iron deficiency anemia, would utilize this supplement better than individuals with higher iron status. If iron status is currently high, the Ferrochel is not absorbed as effectively as if iron status is low. This characteristic could also be beneficial in fortified foods, as individuals with high iron stores or with iron overload disease may be less affected by this supplement than by the salts usually added to fortify food products. This issue would require further research with individuals diagnosed with iron overload disease. In previous studies, Albion mineral chelates have been shown to be well regulated supplements, with the LD-50 for the Ferrochel at a level 3.5 times higher than the soluble salt (57).

A change occurred in the hematocrit levels of some participants from week to week (Appendix B, Table 6). The initial hypothesis was that the drop may relate to blood loss from the menstrual cycle of the participants; however, no relationship was evident. Similarly, when ferritin levels were examined and compared with the hematocrit values, there were no consistencies in the hematocrit changes. The changes in hematocrit may have been due to the blood loss experienced through the experiment, or other currently undetermined factors.

Because of differences between the study participants, ranges of iron absorption and initial iron status were large. To attempt to identify any dietary or genetic factors affecting iron absorption, the subjects were asked to respond to a short questionnaire near the end of the study (Appendix C). The responses varied between participants in regards to dietary iron intake, family history, and the relationship between these factors and their iron status and absorption.

Subject #8 consistently demonstrated the highest iron absorption throughout the study and had the lowest ferritin status with essentially no ferritin stores as well as low hematocrit values (35-37%). Subject #7 had lower peak iron absorption, as might be expected as she had the highest serum ferritin values and higher hematocrit values (42-43.5%). Because the intake of highly bioavailable dietary iron sources was similar and adequate in these subjects, there may be genetic effects on iron status in these individuals. This information may also reflect data obtained by Gavin et al. (58) demonstrating a setpoint theory for iron absorption. An individual's iron stores may have a predetermined setpoint, with absorption regulation to maintain iron stores at this predetermined level.

Based on the data obtained, there was no clear relationship between the iron status of the study participants, their dietary iron intake, and family history of chronic disease (Appendix B, Table 7).

# EXPERIMENT THREE- MAGNESIUM STUDY IN WOMEN

## Methods

Concurrent with the iron dosing and blood draws, magnesium absorption was also studied using magnesium sulfate, Albion's magnesium amino acid chelate complexed and/or mixed with vegetables, and the taste-free magnesium supplement. Doses (100 mg) were given as two capsules, each containing 50 mg of elemental magnesium (Appendix D). Serum magnesium values were examined along with serum iron values. Because of the close physiological control of serum magnesium, these levels were expected to remain relatively constant.

Urinary magnesium was also measured. Subjects were instructed to void just prior to receiving the magnesium dose, and a total void of urine was collected at the end of the 2.5-h blood collection period. Magnesium was determined by atomic absorption spectrophotometry and compared to urinary creatinine levels, a value used as a constant for healthy individuals (59). For a description of the methods used in determining urinary magnesium and creatinine levels (60), see Appendix A.

Data from the magnesium absorption study were analyzed by analysis of variance. Serum magnesium absorption was evaluated based on net change, which was expected to be negligible. Urinary magnesium was examined in terms of creatinine excretion on a per hour basis.

**Results**

Magnesium absorption was examined by rise in serum magnesium and by urinary magnesium losses. Serum magnesium levels remained fairly constant, as would be expected (see Appendix B, Table 8 for complete data and Appendix E, Table 21 for statistical analysis). Urinary magnesium losses were measured over 2.5 h. Magnesium excretion for each subject was examined with respect to total volume of urine excreted and creatinine level (60). Data are presented for each subject in Table 3. See Appendix B (Table 9) for complete data obtained.

There was no difference between the excretion of the three different magnesium compounds when expressed in terms of creatinine excretion ($P = 0.333$) (Appendix E, Table 22). There was, however, a trend toward a slightly higher overall level of excretion from the chelated supplement and a lower excretion rate from the taste-free magnesium supplement (Figure 5).

**Discussion**

The magnesium study did not demonstrate statistically significant differences; however, the trend in the data ($P = 0.333$) showed a slightly higher absorption from the vegetable chelate, with the lowest absorption from the taste-free supplement as was seen in experiment two utilizing iron. The modified magnesium load test utilized may have limited the effectiveness of the doses as a full 24 h was not utilized for urine collection (61).

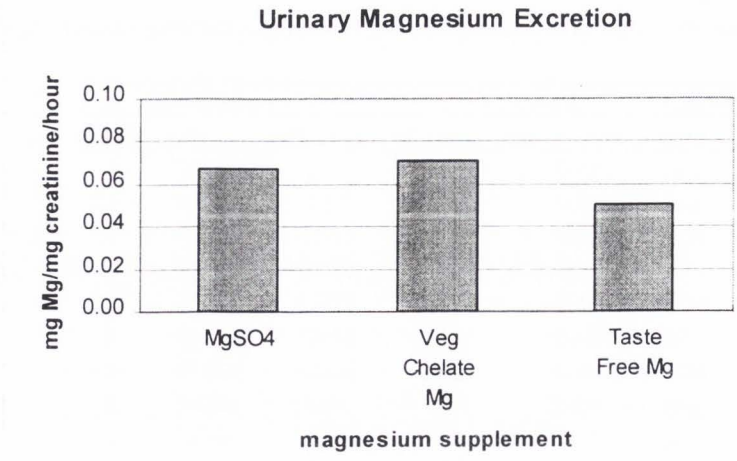


**FIGURE 5.** Absorption of each supplement dose as estimated by magnesium excretion corrected for creatinine and time demonstrating no significant difference between supplements (P = 0.333) (variability: MgSO4 = .03 to.13, Chelate = .02 to .13, taste-free = .02 to .16).

**TABLE 3**

Urinary magnesium excretion adjusted for creatinine and time for each subject.

| Subj # | creatinine (mg) | Mg (mg) | minutes | mg Mg/ mg creat/hr | supplement type |
|---|---|---|---|---|---|
| 1 | 111.36 | 5.77 | 157 | 0.02 | veg |
| 1 | 97.28 | 8.82 | 156 | 0.03 | veg |
| 1 | 129.60 | 11.11 | 157 | 0.03 | SO4 |
| 1 | 244.00 | 13.00 | 155 | 0.02 | TF |
| 2 | 32.64 | 11.34 | 155 | 0.13 | SO4 |
| 2 | 121.60 | 15.43 | 152 | 0.05 | TF |
| 2 | 121.60 | 12.28 | 155 | 0.04 | SO4 |
| 2 | 54.60 | 9.01 | 155 | 0.06 | veg |
| 3 | 30.96 | 6.12 | 158 | 0.08 | SO4 |
| 3 | 89.76 | 17.54 | 153 | 0.08 | SO4 |
| 3 | 131.12 | 12.90 | 160 | 0.04 | veg |
| 3 | 176.00 | 7.57 | 153 | 0.02 | TF |
| 4 | 60.80 | 10.26 | 129 | 0.08 | SO4 |
| 4 | 69.36 | 11.47 | 156 | 0.06 | SO4 |
| 4 | 126.48 | 18.49 | 158 | 0.06 | veg |
| 4 | 106.56 | 14.67 | 157 | 0.05 | TF |
| 5 | 39.78 | 4.68 | 163 | 0.04 | TF |
| 5 | 86.40 | 5.85 | 72 | 0.06 | veg |
| 5 | 62.40 | 11.97 | 156 | 0.07 | SO4 |
| 5 | 104.00 | 11.90 | 157 | 0.04 | veg |
| 6 | 220.80 | 22.69 | 138 | 0.04 | TF |
| 6 | 140.00 | 14.35 | 100 | 0.06 | SO4 |
| 6 | 19.36 | 4.45 | 155 | 0.09 | veg |
| 7 | 180.00 | 8.85 | 158 | 0.02 | TF |
| 7 | 188.00 | 15.12 | 157 | 0.03 | SO4 |
| 7 | 64.60 | 21.76 | 158 | 0.13 | veg |
| 7 | 34.00 | 13.82 | 156 | 0.16 | TF |
| 8 | 67.76 | 15.22 | 158 | 0.09 | veg |
| 8 | 168.96 | 23.94 | 156 | 0.05 | TF |
| 8 | 125.44 | 19.91 | 157 | 0.06 | veg |
| 8 | 120.00 | 12.30 | 157 | 0.04 | SO4 |
| 9 | 24.64 | 4.04 | 155 | 0.06 | veg |
| 9 | 61.20 | 14.59 | 155 | 0.09 | SO4 |
| 9 | 89.44 | 17.58 | 157 | 0.08 | TF |
| 9 | 79.68 | 19.12 | 156 | 0.09 | veg |
| 10 | 53.56 | 11.89 | 160 | 0.08 | veg |
| 10 | 86.36 | 23.62 | 150 | 0.11 | veg |
| 10 | 112.80 | 15.30 | 152 | 0.05 | TF |
| 10 | 60.76 | 13.63 | 154 | 0.09 | SO4 |
| 12 | 50.75 | 9.86 | 122 | 0.10 | SO4 |
| 12 | 42.70 | 6.91 | 120 | 0.08 | veg |
| 12 | 117.60 | 9.58 | 160 | 0.03 | SO4 |
| 12 | 118.40 | 9.93 | 126 | 0.04 | TF |

# CONCLUSIONS

Three main conclusions were drawn from the data obtained from the three experiments. First, over a short period of time, serum iron techniques and $^{59}Fe$ techniques provided similar estimates of iron absorption in rats. This reflects conclusions in previous studies that isotopes are not needed to measure iron absorption, and less invasive techniques may be utilized effectively. In humans, Ferrochel (Iron-Amino Acid Chelate Complexed and/or Mixed with Vegetables) was absorbed at least as effectively as ferrous sulfate and was better absorbed in subjects with low ferritin status. Further examination of the magnesium supplements is needed to determine if the lack of difference between the supplements was due to actual similarities in uptake or to the study design.

In the future, when evaluating the different mineral supplements examined, the effect of the design must be considered. The doses provided were small, the subjects were not severely depleted, and the supplements were not taken consistently--factors that may affect the overall effectiveness of the supplements in treating anemia and magnesium deficiencies.

# REFERENCES

1. National Research Council. Recommended Dietary Allowances. 10th ed. Washington, DC: National Academy Press, 1989.

2. Dreosti IE. Magnesium status and health. Nut Rev 1995; 53:S23-7.

3. Al-Ghamdi SMG, Cameron EC, Sutton RAL. Magnesium deficiency: pathophysiologic and clinical overview. Am J Kidney Dis 1994; 24:737-52.

4. American Dietetic Association. Position of the American Dietetic Association: vitamin and mineral supplementation. JADA 1996; 96:73-7.

5. Costa A, Liberato LN, Palestra P, Barosi G. Small-dose iron tolerance test and body iron content in normal subjects. Eur J Haematol 1991 46(3):152-7.

6. De Vizia B, Poggi V, Conenna R, Fiorillo A, Scippa L. Iron absorption and iron deficiency in infants and children with gastrointestinal diseases. J Pediatr Gastroenterol Nut 1992; 14(1):21-6.

7. Gross SJ, Stuart MJ, Swender PT, Oski FA. Malabsorption of iron in children with iron deficiency. J Pediatr 1976; 88:795-9.

8. Gumruk F, Gurgey A, Duru F, Altay C. Reevaluation of iron absorption and serum ferritin in beta-thalassemia intermedia. Pediatr Hematol-Oncol 1992; 9(4):359-363.

9. Seligman PA, Caskey JH, Frazier JL, Zucker RM, Podell ER, Allen RH. Measurements of iron absorption from prenatal multivitamin-mineral supplements. Obstet-Gynecol 1983; 61(3):356-62.

10. Wright MB. Change in serum iron as a measure of bioavailability of dietary iron. MS thesis. Utah State University, 1989.

11. Jeppsen RB. Biochemistry and physiology of Albion metal amino acid chelates as proofs of chelation. Albion International Conference. Salt Lake City: Albion Laboratories, 1995.

12. Ashmead HH, Ashmead SD. Albion research: past present and future. Albion International Conference. Salt Lake City: Albion Laboratories, 1995.

13. Pineda O, Ashmead HD, Perez JM, Lemus CP. Effectiveness of iron amino acid chelates on the treatment of iron deficiency anemia in adolescents. J Appl Nutr. 1994; 46(1&2):2-13.

14. Cook JD, Skikne BS. Iron deficiency: definition and diagnosis. J Intern Med 1989; 226:349-55.

15. Bothwell TH. Overview and mechanisms of iron regulation. Nut Rev 1995; 53: 237-245.

16. Hollan S, Johansen KS. Adequate iron stores and the "nil nocere" principle. Hematologia 1993; 25:69-84.

17. Barrett JFR, Whittaker PG, Williams JG, Lind T. Absorption of non-haem iron from food during normal pregnancy. BMJ 1994; 309:79-82.

18. Brunvand L, Henriksen C, Larsson M, Sandberg AS. Iron deficiency among pregnant Pakistanis in Norway and the content of phytic acid in their diet. Acta Obstet Gynecol Scand 1995; 74:520-525.

19. Dallman PR. Iron deficiency: does it matter? J Intern Med 1989; 226:367-72.

20. Bodley JL, Austin VJ, Hanley WB, Clarke JTR, Zlotkin S. Low iron stores in infants and children with treated phenylketonuria: a population at risk for iron-deficiency anaemia and associated cognitive defects. Eur J Pediatr 1993; 152:140-3.

21. Johnson MA, Fischer JG, Bowman BA, Gunter EW. Iron nutriture in elderly individuals. FASEB J 1994; 8:609-21.

22. Haurani FI, Marcolina MJ. The mechanisms of gastrointestinal loss of iron. Nut Research 1994;14:1779-87.

23. Johnson MA. Iron: nutrition monitoring and nutrition status assessment. J Nutr 1990; 120:1486-91.

24. Gibson RS. Assessment of iron status. In: Nutritional assessment. New York: Oxford University Press, 1993: 349-76.

25. Worwood M. An overview of iron metabolism at a molecular level. J Intern Med 1989; 226:381-91.

26. Miller DM, Buetther GR, Aust SD. Transition metals as catalysts of "autoxidation" reactions. Free Rad Biol Med 1990; 8:95-108.

27. Samokyszyn VM, Reif DW, Miller DM, Aust SD. Effects of ceruloplasmin on superoxide-dependent iron release from ferritin and lipid peroxidation. Free Rad Res Comms 1991; 12-13:153-9.

28. Lash A, Saleem A. Iron metabolism and its regulation. Ann Clin Lab Sci 1995; 25: 20-9.

29. Bothwell TH, Baynes RD, MacFarlane BJ, MacPhail AP. Nutritional iron requirements and food iron absorption. J Intern Med 1989; 226:357-65.

30. Monson ER. Iron nutrition and absorption: dietary factors which impact iron bioavailability. JADA 1988; 88:786-90.

31. Carpenter CE, Mahoney AW. Contributions of heme and nonheme iron to human nutrition. Critical Reviews in Food Science and Nutrition 1992; 31(4):333-67.

32. Mahoney AW, VanOrden CC, Hendricks DG. Efficiency of converting food iron into hemoglobin by the anemic rat. Nutr Metabol 1974; 17:223-30.

33. Park YW, Mahoney AW, Hendricks DG. Bioavailability of different sources of ferrous sulfate iron fed to anemic rats. J Nutr 1983; 113:2223-8.

34. Olakanmi O, Stokes JB, Britigan BE. Acquisition of iron bound to low molecular weight chelates by human monocyte-derived macrophages. J Immunol 1994; 153(6): 2691-703.

35. Shils ME. Magnesium. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea & Febiger, 1994: 164-84.

36. Rayssiguier Y, Gueux E, Bussiere L, Durlach J, Mazur A. Dietary magnesium affects susceptibility of lipoproteins and tissues to peroxidation in rats. J Am Coll Nutr 1993; 12:133-7.

37. Elin RJ. Magnesium: the fifth but forgotten electrolyte. Clin Pathol 1994; 102:616-22.

38. Spencer H, Fuller H, Norris C, Williams D. Effect of magnesium on the intestinal absorption of calcium in man. J Am Coll Nutr 1994; 13(5):485-92.

39. Motyka MR. The advantages and applications of Albion's amino acid chelates. Albion International Conference. Salt Lake City: Albion Laboratories, 1995.

40. Mervyn L. Human therapeutic applications of amino acid chelates. Albion International Conference. Salt Lake City: Albion Laboratories, 1995.

41. Crosby WH, Munn SI, Furth FW. Standardized method for clinical hemoglobinometry. US Arm Forces Med J 1954; 5:693-703.

42. BARR (Board on Agriculture and Renewable Resources) Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition. Nutrient Requirements of Laboratory Animals. 3rd rev ed. Nutrition Requirements of Domestic Animals Series. Washington, DC: National Academy of Sciences, 1978.

43. Buchowski MS, Mahoney AW, Kalpalathika MVP. Nonheme iron absorption, apparent iron absorption and hemoglobin regeneration efficiency in anemic and normal rats fed with dietary heme and nonheme iron at various levels. Nutrition Research 1989; 9:773-8.

44. American Veterinary Medical Association. 1993 Report of the AVMA panel on euthanasia. JAVMA 1993; 202:229-49.

45. Altman PL, Dittmer DS. (eds). Biological Handbooks: blood and other fluids. Washington DC: Federation of American Societies for Experimental Biology, 1961.

46. Stookey LL. Ferrozine - a new spectrophotometric reagent for iron. Anal Chem 1970; 42(7):779-81.

47. Carter P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal Biochem 1971; 40(2):450-8.

48. [125 I] Ferritin RIA Kit. Los Angeles, CA: Diagnostic Products Corporation, 1996.

49. Ellis R, Gulick D. Calculus with Analytic Geometry. 3rd ed. Orlando: Harcourt Brace Jovanovich Publishers, 1986.

50. Crosby WH, O'Neil-Cutting MA. A small-dose iron tolerance test as an indicator of mild iron deficiency. JAMA 1984; 251:1986-1987.

51. O'Neil-Cutting MA, Crosby WH. Blocking of iron absorption by a preliminary oral dose of iron. Arch Intern Med 1987; 147:489-91.

52. De Vizia B, Poggi V, Vajro P, Cucchiara S, Acampora A. Iron malabsorption in giardiasis. J Pediatr 1985; 107:75-8.

53. Domoto DT, Martin KJ. Failure of CAPD patients to respond to an oral iron absorption test. Adv Perit Dial 1992; 8:102-4.

54. Kirchoff HW. Zur behandling der eisenmangelanemie mit eisen chelat-tasbletten. Therapiewoche 1983; 33/37:4833-42.

55. Schrueffer H, Stein W. Erhrungen mit eisen chelat tabletten bei eisenmangelanalmein. Therapiewhoche. 1983; 33:2121-3.

56. Langini S, Cadbone N, Galdi M, Barrio Rendo ME, Portela ML, Caro R, Valencia ME. Ferric Glycinate iron bioavailability for rats, as determined by extrinsic radioisotopic labeling of infant formulas. Nutr Rep Int. 1988; 38:729-35.

57. Larson AE. L.D. 50 studies with chelated minerals. In: Ashmead D. (ed). Chelated Mineral Nutrition in Plants, Animals and Man. Springfield: Charles C. Thomas, 1982: 163-9.

58. Gavin MW, McCarthy DM, Garry PJ. Evidence that iron stores regulate iron absorption--a setpoint theory. Am J Clin Nutr 1994; 59:1376-80.

59. Berkow R, Fletcher AJ. (eds). The Merck manual of diagnosis and therapy. 16th ed. Rahway, NJ: Merck Research Laboratories. 1992.

60. Faulkner WR, King JW. (ed). CRC manual of clinical laboratory procedures. Cleveland, OH: The Chemical Rubber Co, 1970.

61. Caddell JL. The magnesium load test: I. a design for infants. Clin Ped 1975; 14(5):449-59,518-9.

# APPENDICES

# APPENDIX A

## Methods for Experiment Procedures

Determination of Hemoglobin:

1. Prepare Drabkin reagent

    Potassium cyanide 0.52 gm
    Potassium ferricyanide 0.198 gm
    Sodium bicarbonate 1.0 gm
    Distilled water to 1000 mL

Store the solution in a dark brown glass container at room temperature

2. Prepare commercial hemoglobin standards

    Dilute purchased standard to 50 mL with Drabkin reagent.
    Dilute aliquots of the solution to form standards of 5, 10, 15, and 20 gm Hgb/dl.

3. Determine the Hemoglobin level of each subject

    Remove the blood from two tubes (20 µl) using a hemoglobin pipette and place the blood in test tubes containing 5 mL Drabkin's solution and mix well. Add the prepared standards to 5 mL Drabkin solution and mix well. Also prepare the blank. Let tubes stand for 20 minutes. Read the absorbance in a spectrophotometer at 540 nm.

Ferrozine Method for the Determination of Total Serum Iron
(Stookey, 1970 and Carter, 1971)

Prepare reagents:

1. Reducing agent: 0.02% ascorbic acid in 0.2 N hydrochloric acid
Dissolve 20 mg ascorbic acid in a small quantity of water in a 100 mL volumetric flask. Add 1.67 mL concentrated hydrochloric acid and dilute to volume. Store at 4° C for not more than 3 days.
2. Protein precipitant: 11.3% trichloroacetic acid
Add water to a 0.25 pound container of trichloroacetic acid. Transfer to a 1 liter flask and dilute to volume.
3. Buffer solution: 10% ammonium acetate.
Dissolve 50 gm ammonium acetate in 500 mL water.
4. Mixed color reagent:
Place 75 mg ferrozine and 75 mg neocuproin in a 25 mL volumetric flask. Dissolve in water containing 1 drop of concentrated hydrochloric acid to obtain solution and dilute to volume. Store at 4° C.
5. Standard iron: 100 µg/dl
Transfer 0.702 gm $Fe(NH_4)_2SO_4 \cdot 6H_2O$ to a 1 liter flask containing water and 0.50 mL concentrated sulfuric acid. dilute to volume. A working standard containing 100 µg/dl is prepared biweekly by diluting the stock 100-fold with water.

Procedure:

With a 1.0 mL disposable pipette tip transfer 0.50 mL serum into a disposable 12x75 mm test tube. Add 0.50 mL reducing agent, mix, and allow to stand at room temperature for 5 minutes. Add 0.50 mL protein precipitant, mix well and cap to prevent evaporation, and centrifuge. Transfer 1.0 mL clear centrifugate to a second tube and add 0.40 mL buffer solution followed by 0.10 mL color reagent. Mix well and measure absorbance of the magenta-colored complex, which develops within 5 minutes, at 562 nm against water. With each set of unknowns, prepare a blank and a standard with 0.50 mL deionized water and 0.50 mL working standard, respectively.

Calculation:

$(Au-Ab)/(As-Ab) \times 100\mu g \text{ iron/dl} = \mu g \text{ iron/dl serum}$

Au = absorbance of unknown
As = absorbance of standard
Ab = absorbance of blank

Determination of Urinary Creatinine:
Faulkner and King (ed), 1970

Reagents:
1. Picric acid solution (1.17% W/V)
2. Alkaline picrate solution of 1 volume 10% sodium hydroxide with 5 volumes picric acid solution. Prepare just before use.
3. Creatinine standards of concentration ranging from 0.1 mg/dl to 5.0 mg/dl.

Procedure:
1. Create a standard curve with concentrations equivalent to 0.1, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.25, 3.0, and 5.0, with volumes of 2.0 mL for each standard. Create a blank with 2 mL distilled water. Add one mL of the alkaline picrate solution to each cuvette. Mix the contents of each cuvette by agitation and allow to stand for 10 minutes. Measure the percent transmittance at a wavelength of 490 nm
against the blank.
2. Make a 1:20 dilution of each urine sample to be analyzed. For each determination, place 2 mL diluted urine and 1 mL alkaline picrate solution in a cuvette and mix. Allow to stand for 10 minutes, and read the percent transmittance at 490 nm
3. Plot the %T readings on the log scale against mg/dl for the standard curve. Read the valued for the urine samples from the chart to determine their creatinine concentrations.

Determination of Urinary Magnesium:

1. Prepare working standard solutions of magnesium to a volume of 100 mL with distilled water to create standards ranging from 5 to 50 mg/L. Dilute each working standard 1:50 with distilled water.

2. Dilute each urine sample 1:100 with distilled water. Some concentrated samples may need further dilution to fit within the standards.

3. Analyze the standards and samples by atomic absorption spectrophotometry. Analyze at a wavelength of 285.2 nm with a 0.7 nm slit and air-acetylene flame using a Ca-Mg hollow cathode lamp.

# APPENDIX B

Tables

**TABLE 4**
Rat hemoglobin and hematocrit values

| Rat # | Weight (grams) | Hemoglobin (g/dl) | Hematocrit (%) |
|---|---|---|---|
| 1 | 183 | 17.70 | 47.50 |
| 2 | 176 | 18.14 | 52.00 |
| 3 | 183 | 17.70 | 47.00 |
| 4 | 176 | 17.24 | 47.00 |
| 5 | 169 | 17.06 | 46.50 |
| 6 | 186 | 16.81 | 46.00 |
| 7 | 181 | 16.81 | 48.50 |
| 8 | 178 | 16.84 | 46.50 |
| 9 | 179 | 16.41 | 45.50 |
| 10 | 186 | 17.31 | 47.00 |
| 11 | 173 | 17.17 | 46.00 |
| 12 | 181 | 16.19 | 47.00 |
| 13 | 166 | 17.35 | 46.00 |
| 14 | 170 | 17.17 | 49.00 |
| 15 | 183 | 17.17 | 50.00 |
| 16 | 180 | 17.38 | 50.00 |
| 17 | 185 | 17.38 | 48.50 |
| 18 | 180 | 16.99 | 48.50 |
| 19 | 182 | 16.70 | 48.00 |
| 20 | 184 | 16.63 | 45.50 |
| 21 | 185 | 16.99 | 48.00 |
| 22 | 187 | 17.13 | 45.50 |
| 23 | 183 | 17.89 | 46.00 |
| 24 | 176 | 17.71 | 46.50 |
| 25 | 183 | 17.42 | 50.00 |
| 26 | 188 | 17.10 | 48.00 |
| 27 | 173 | 18.28 | 48.00 |
| 28 | 186 | 17.45 | 46.00 |
| 29 | 184 | 16.74 | 45.00 |
| 30 | 184 | 16.05 | 44.50 |
| 31 | 181 | 15.77 | 44.50 |
| 32 | 184 | 16.70 | 47.00 |
| 33 | 170 | 16.88 | 47.00 |
| 34 | 178 | 17.60 | 47.00 |
| 35 | 188 | 15.98 | 48.00 |

# TABLE 5
Complete iron data by subject

| | Sample # | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subj # | 1 | 2 | 3 | 4 | 5 | 6 | HCT (%) | ferritin | weight | serum vol (L) | peak incr | net incr | Area (mg/dL) | corrected area | area % inc | peak % inc | Fe Type |
| 1 | 130 | 130 | 127 | 129 | 140 | 139 | 45 | 21 | 46.8 | 1.80 | 10 | 18.02 | 16.33 | 294.33 | 14.72 | 9.01 | veg |
| 1 | 207 | 210 | 196 | 201 | 207 | 197 | 44 | 21 | 46.8 | 1.83 | 3 | 5.50 | 1.00 | 18.35 | 0.92 | 2.75 | TF |
| 1 | 140 | 137 | 144 | 154 | 146 | 146 | 43.5 | 24 | 46.8 | 1.85 | 14 | 25.91 | 24.67 | 456.57 | 22.83 | 12.96 | Veg |
| 1 | 181 | 154 | 148 | 148 | 142 | 142 | 44 | 25 | 46.8 | 1.83 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | TF |
| 1 | 114 | 108 | 109 | 99 | 102 | 102 | 43 | 30 | 46.8 | 1.87 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | SO4 |
| 2 | 66 | 65 | 68 | 66 | 81 | 81 | 37.5 | 34 | 60 | 2.63 | 15 | 39.38 | 27.67 | 726.25 | 36.31 | 19.69 | SO4 |
| 2 | 94 | 91 | 91 | 93 | 97 | 97 | 40.5 | 27 | 60 | 2.50 | 3 | 7.50 | 5.00 | 124.95 | 6.25 | 3.75 | TF |
| 2 | 96 | 95 | 90 | 84 | 89 | 92 | 39 | 26 | 60 | 2.56 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | SO4 |
| 2 | 43 | 41 | 38 | 39 | 41 | 41 | 37.5 | 24 | 60 | 2.63 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Veg |
| 2 | 83 | 70 | 65 | 66 | 70 | 71 | 38 | 16 | 60 | 2.60 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | TF |
| 2 | 90 | 91 | 91 | 88 | 88 | 90 | 39 | 24 | 60 | 2.56 | 1 | 2.56 | 1.67 | 42.70 | 2.14 | 1.28 | Veg |
| 3 | 95 | 90 | 95 | 103 | 104 | 98 | 41 | 46 | 62.7 | 2.59 | 9 | 23.31 | 18.33 | 474.83 | 23.74 | 11.66 | Veg |
| 3 | 76 | 79 | 82 | 84 | 92 | 92 | 42 | 16 | 62.7 | 2.55 | 16 | 40.73 | 41.00 | 1043.70 | 52.19 | 20.36 | SO4 |
| 3 | 100 | 108 | 102 | 117 | 107 | 114 | 43 | 23 | 62.7 | 2.50 | 17 | 42.53 | 30.67 | 767.20 | 38.36 | 21.26 | TF |
| 3 | 61 | 61 | 61 | 65 | 69 | 71 | 43 | 12 | 62.7 | 2.50 | 10 | 25.02 | 16.67 | 416.96 | 20.85 | 12.51 | SO4 |
| 3 | 67 | 77 | 87 | 97 | 102 | 107 | 40 | 14 | 62.7 | 2.63 | 40 | 105.34 | 110.00 | 2896.74 | 144.84 | 52.67 | Veg |
| 4 | 53 | 46 | 46 | 44 | 43 | 47 | 44 | 21 | 51.8 | 2.03 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | TF |
| 4 | 105 | 93 | 102 | 101 | 103 | 107 | 31.5 | 16 | 51.8 | 2.48 | 2 | 4.97 | 0.67 | 16.56 | 0.83 | 2.48 | Veg |
| 4 | 85 | 87 | 90 | 95 | 98 | 102 | 39.5 | 7.5 | 51.8 | 2.19 | 17 | 37.29 | 37.00 | 811.68 | 40.58 | 18.65 | SO4 |
| 4 | 68 | 69 | 79 | 88 | 93 | 100 | 42.5 | 6 | 51.8 | 2.08 | 32 | 66.72 | 72.33 | 1508.11 | 75.41 | 33.36 | Veg |
| 4 | 108 | 109 | 110 | 116 | 117 | 119 | 39 | 16 | 51.8 | 2.21 | 11 | 24.33 | 24.00 | 530.85 | 26.54 | 12.17 | TF |
| 5 | 68 | 69 | 75 | 81 | 83 | 78 | 37.5 | 9 | 59.1 | 2.59 | 13 | 33.62 | 41.67 | 1077.50 | 53.88 | 16.81 | SO4 |
| 5 | 64 | 68 | 67 | 69 | 68 | 59 | 30.5 | 8.5 | 59.1 | 2.88 | 5 | 14.38 | 14.00 | 402.53 | 20.13 | 7.19 | TF |
| 5 | 83 | 87 | 88 | 88 | 93 | 91 | 40.5 | 12 | 59.1 | 2.46 | 8 | 19.69 | 27.33 | 672.81 | 33.64 | 9.85 | SO4 |
| 5 | 87 | 86 | 91 | 88 | 101 | 101 | 39 | 5.5 | 59.1 | 2.52 | 14 | 35.33 | 29.33 | 740.25 | 37.01 | 17.66 | Veg |
| 5 | 81 | 76 | 79 | 78 | 76 | 76 | 39 | 7.5 | 59.1 | 2.52 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | TF |
| 6 | 119 | 126 | 135 | 135 | 137 | 142 | 40 | 28 | 63.6 | 2.67 | 23 | 61.43 | 66.00 | 1762.86 | 88.14 | 30.72 | TF |
| 6 | 44 | 44 | 46 | 48 | 51 | 53 | 40 | 16 | 63.6 | 2.67 | 9 | 24.04 | 17.67 | 471.88 | 23.59 | 12.02 | TF |
| 6 | 95 | 101 | 104 | 111 | | | 42 | 24 | 63.6 | 2.58 | 16 | 41.31 | 24.00 | 619.72 | 30.99 | 20.66 | Veg |
| 6 | 56 | 59 | 61 | 69 | 75 | 78 | 40 | 15 | 63.6 | 2.67 | 22 | 58.77 | 49.00 | 1308.89 | 65.44 | 29.38 | SO4 |
| 7 | 48 | 47 | 45 | 50 | 55 | 58 | 42.5 | 90 | 65.5 | 2.64 | 10 | 26.36 | 14.00 | 369.04 | 18.45 | 13.18 | Veg |
| 7 | 70 | 71 | 80 | 81 | 83 | 90 | 43 | 65 | 65.5 | 2.61 | 20 | 52.27 | 45.00 | 1176.05 | 58.80 | 26.13 | TF |
| 7 | 68 | 61 | 67 | 66 | 72 | 59 | 42.5 | 50 | 65.5 | 2.64 | 4 | 10.55 | 5.33 | 140.61 | 7.03 | 5.27 | SO4 |
| 7 | 102 | 98 | 104 | 100 | 107 | 112 | 43.5 | 39 | 65.5 | 2.59 | 10 | 25.91 | 12.67 | 328.13 | 16.41 | 12.95 | TF |
| 7 | 76 | 76 | 78 | 85 | 96 | 95 | 42 | 46 | 65.5 | 2.66 | 20 | 53.19 | 41.67 | 1108.04 | 55.40 | 26.59 | Veg |
| 8 | 80 | 84 | 94 | 109 | 116 | 111 | 36.5 | 6.5 | 63.6 | 2.83 | 36 | 101.77 | 97.67 | 2761.04 | 138.05 | 50.89 | SO4 |
| 8 | 54 | 52 | 57 | 67 | 72 | 89 | 36 | <5 | 63.6 | 2.85 | 35 | 99.72 | 48.33 | 1377.15 | 68.86 | 49.86 | Veg |
| 8 | 28 | 30 | 38 | 49 | 63 | 62 | 37 | <5 | 63.6 | 2.80 | 35 | 98.17 | 86.00 | 2412.09 | 120.60 | 49.08 | Veg |
| 8 | 52 | 51 | 67 | 84 | 95 | 98 | 37 | <5 | 63.6 | 2.80 | 46 | 129.02 | 114.00 | 3197.43 | 159.87 | 64.51 | SO4 |
| 8 | 45 | 41 | 42 | 43 | 44 | 46 | 35.5 | 5.5 | 63.6 | 2.87 | 1 | 2.87 | 0.33 | 9.57 | 0.48 | 1.44 | TF |
| 8 | 34 | 34 | 35 | 39 | 42 | 45 | 35 | 5.2 | 63.6 | 2.89 | 11 | 31.83 | 19.00 | 549.82 | 27.49 | 15.92 | TF |
| 9 | 52 | 50 | 57 | 71 | 86 | 87 | 44 | 13 | 57.7 | 2.26 | 35 | 79.17 | 76.33 | 1726.66 | 86.33 | 39.59 | Veg |
| 9 | 52 | 51 | 54 | 58 | 57 | 44 | 41.5 | 6.5 | 57.7 | 2.36 | 6 | 14.18 | 13.33 | 315.04 | 15.75 | 7.09 | TF |
| 9 | 58 | 60 | 76 | 95 | 93 | 93 | 41.5 | 5.6 | 57.7 | 2.36 | 37 | 87.42 | 107.67 | 2543.96 | 127.20 | 43.71 | SO4 |
| 9 | 88 | 84 | 88 | 87 | 93 | 91 | 40.5 | 8.7 | 57.7 | 2.40 | 5 | 12.02 | 7.67 | 184.25 | 9.21 | 6.01 | TF |
| 9 | 54 | 58 | 69 | 67 | 81 | 76 | 41.5 | 7 | 57.7 | 2.36 | 27 | 63.80 | 73.33 | 1732.73 | 86.64 | 31.90 | Veg |
| 10 | 110 | 107 | 105 | 104 | 107 | 98 | 40 | 65 | 59.1 | 2.48 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Veg |
| 10 | 58 | 74 | 60 | 62 | 61 | 61 | 39.5 | 38 | 59.1 | 2.50 | 16 | 40.05 | 15.67 | 392.12 | 19.61 | 20.02 | TF |
| 10 | 68 | 81 | 81 | 74 | 81 | 80 | 38 | 25 | 59.1 | 2.56 | 13 | 33.34 | 47.00 | 1205.52 | 60.28 | 16.67 | Veg |
| 10 | 84 | 81 | 82 | 86 | 85 | 84 | 39 | 15 | 59.1 | 2.52 | 2 | 5.05 | 2.67 | 67.30 | 3.36 | 2.52 | SO4 |
| 10 | 46 | 58 | 58 | 56 | 57 | 61 | 39.5 | 16 | 59.1 | 2.50 | 15 | 37.54 | 46.33 | 1159.67 | 57.98 | 18.77 | TF |
| 11 | 58 | 54 | 60 | 65 | 72 | 80 | 43.5 | 80 | 59.1 | 2.34 | 22 | 51.41 | 33.33 | 779.00 | 38.95 | 25.71 | SO4 |
| 11 | 105 | 108 | 113 | 115 | 118 | 122 | 41 | 29 | 59.1 | 2.44 | 17 | 41.49 | 41.33 | 1008.88 | 50.44 | 20.75 | Veg |
| 11 | 63 | 72 | 64 | 69 | 77 | 76 | 41.5 | 32 | 59.1 | 2.42 | 14 | 33.88 | 31.33 | 758.31 | 37.92 | 16.94 | TF |
| 11 | 74 | 73 | 74 | 78 | 80 | 83 | 43 | 32 | 59.1 | 2.36 | 9 | 21.22 | 13.67 | 322.27 | 16.11 | 10.61 | Veg |
| 11 | 66 | 63 | 56 | 57 | 56 | 61 | 42 | 26 | 59.1 | 2.40 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | SO4 |
| 12 | 40 | 40 | 41 | 43 | 45 | | 35 | 21 | 63.6 | 2.89 | 5 | 14.47 | 10.00 | 289.40 | 14.47 | 7.24 | TF |
| 12 | 35 | 32 | 38 | 44 | 51 | 51 | 31 | 18 | 63.6 | 3.07 | 16 | 49.15 | 36.67 | 1126.36 | 56.32 | 24.58 | SO4 |
| 12 | 48 | 51 | 59 | 65 | 68 | | 35 | 16 | 63.6 | 2.89 | 20 | 57.88 | 53.67 | 1553.01 | 77.65 | 28.94 | SO4 |
| 12 | 41 | 40 | 42 | 42 | 39 | 38 | 37.5 | 8.5 | 63.6 | 2.78 | 1 | 2.78 | 2.00 | 55.65 | 2.78 | 1.39 | TF |
| 12 | 29 | 33 | 36 | 40 | 47 | | 36 | 10 | 63.6 | 2.85 | 18 | 51.29 | 42.00 | 1196.70 | 59.83 | 25.64 | Veg |

**TABLE 6**
Hematocrit values (%) for human subjects

| | | | Week # | | Week of |
|---|---|---|---|---|---|
| Subject # | 1 | 2 | 3 | 4 | 5 menstruation |
| 1 | 45.0 | 44.0 | 43.5 | 36.5 | 43.0 week 4 |
| 2 | 37.5 | 40.5 | 39.0 | 37.5 | 38.0 week 4 |
| 3 | 41.0 | 42.0 | 43.0 | 43.0 | 40.0 week 1&5 |
| 4 | 44.0 | 31.5 | 39.5 | 42.5 | 39.0 week 4 |
| 5 | 37.5 | 30.5 | 40.5 | 39.0 | 39.0 week 4 |
| 6 | 40.0 | | 40.0 | 42.0 | 40.0 week 3 |
| 7 | 42.5 | 43.0 | 42.5 | 43.5 | 42.0 week 3 |
| 8 | 36.5 | 36.0 | 37.0 | 37.0 | 35.5 week 1&5 |
| 10 | 40.0 | 39.5 | 38.0 | 39.0 | 39.5 week 1&5 |
| 11 | 43.5 | 41.0 | 41.5 | 43.0 | 42.0 |
| 12 | 35.0 | 31.0 | 35.0 | 37.5 | 36.0 week 1&5 |
| 13 | 44.0 | 41.5 | 41.5 | 40.5 | 41.5 week 1&5 |

**TABLE 7**
Human subject information

| Subject # | ave ferritin | Fe intake | family hx - chronic diseases |
|---|---|---|---|
| 1 | 24 | high | significant cancer |
| 2 | 25 | moderate | |
| 3 | 22 | high | cancer, NIDDM |
| 4 | 13 | moderate | distant CVD |
| 5 | 8.5 | moderate | |
| 6 | 21 | low | |
| 7 | 58 | moderate | |
| 8 | 5 | high | distant cancer |
| 9 | 8 | moderate | distant CVD |
| 10 | 32 | moderate | significant cancer |
| 11 | 40 | high | distant CVD & cancer |
| 12 | 15 | low | |

(CVD = cardiovascular diseases; NIDDM = type 2 diabetes)

**TABLE 8**

Serum magnesium values (in mg/dl)

| Subj # | mg/dl T=0 | T=30 | T=60 | T=90 | T=120 | T=150 | suppl |
|---|---|---|---|---|---|---|---|
| 1 | 1.8 | 1.9 | 2.0 | 1.9 | 2.0 | | 1.9 veg |
| 1 | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | | 1.9 veg |
| 1 | 1.9 | 1.8 | 1.8 | 1.9 | 1.9 | | 1.9 SO4 |
| 1 | 2.1 | 3.2 | 2.4 | 2.1 | 2.2 | | 2.2 TF |
| 2 | 2.1 | 2.0 | 2.2 | 2.1 | 2.0 | | 2.1 SO4 |
| 2 | 1.9 | 1.9 | 1.9 | 1.8 | 1.9 | | 2.0 TF |
| 2 | 1.8 | 1.7 | 1.8 | 1.9 | 1.9 | | 1.9 SO4 |
| 2 | 2.1 | 2.1 | 2.3 | 2.2 | 2.2 | | 2.1 veg |
| 3 | 2.0 | 1.9 | 2.0 | 1.9 | 2.0 | | 2.0 SO4 |
| 3 | 2.1 | 2.1 | 2.1 | 2.3 | 2.1 | | 2.2 SO4 |
| 3 | 2.1 | 2.1 | 2.1 | 2.1 | 2.0 | | 2.2 veg |
| 3 | 2.3 | 2.4 | 2.2 | 2.4 | 2.3 | | 2.3 TF |
| 4 | 1.8 | 1.9 | 1.9 | 2.0 | 2.0 | | 2.0 SO4 |
| 4 | 1.7 | 1.8 | 1.8 | 1.9 | 1.8 | | 1.8 SO4 |
| 4 | 1.7 | 1.9 | 1.9 | 1.9 | 2.1 | | 2.0 veg |
| 4 | 1.9 | 2.0 | 2.1 | 2.1 | 2.0 | | 2.1 TF |
| 5 | 1.9 | 2.0 | 1.9 | 2.1 | 2.1 | | 1.9 TF |
| 5 | 2.1 | 1.9 | 2.0 | 2.1 | 2.1 | | 2.1 veg |
| 5 | 1.9 | 1.9 | 1.9 | 2.1 | 2.0 | | 2.1 SO4 |
| 5 | 2.0 | 2.0 | 2.0 | 2.0 | 1.9 | | 2.0 veg |
| 6 | 2.0 | 2.0 | 2.1 | 2.0 | 2.0 | | 2.1 TF |
| 6 | 1.8 | 1.9 | 1.9 | 2.0 | | | SO4 |
| 6 | 2.2 | 2.1 | 2.2 | 2.1 | 2.1 | | 2.1 veg |
| 7 | 2.0 | 1.8 | 1.9 | 1.9 | 1.9 | | 2.0 TF |
| 7 | 2.0 | 1.8 | 2.0 | 1.9 | 2.1 | | 2.1 SO4 |
| 7 | 2.0 | 1.8 | 2.0 | 2.1 | 2.2 | | 2.2 veg |
| 7 | 2.0 | 1.9 | 1.9 | 2.0 | 2.0 | | 2.1 TF |
| 8 | 2.0 | 2.1 | 2.0 | 1.9 | 2.2 | | 2.0 veg |
| 8 | 2.0 | 2.0 | 1.9 | 2.1 | 2.0 | | 1.9 TF |
| 8 | 2.0 | 1.9 | 1.9 | 2.0 | 2.1 | | 2.2 veg |
| 8 | 2.8 | 2.1 | 2.0 | 2.3 | 2.9 | | 2.1 SO4 |
| 9 | 2.1 | 2.0 | 2.1 | 2.1 | 2.2 | | 2.2 veg |
| 9 | 2.2 | 2.1 | 2.1 | 2.2 | 2.1 | | 2.3 SO4 |
| 9 | 2.1 | 1.9 | 2.8 | 2.1 | 2.1 | | 2.1 TF |
| 9 | 2.2 | 2.2 | 2.3 | 2.3 | 2.9 | | 2.2 veg |
| 10 | 2.1 | 2.2 | 2.0 | 2.1 | 2.1 | | 2.0 veg |
| 10 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | | 2.1 veg |
| 10 | 2.1 | 2.0 | 2.1 | 2.0 | 2.1 | | 2.0 TF |
| 10 | 2.0 | 1.9 | 2.1 | 2.2 | 2.1 | | 2.2 SO4 |
| 11 | 1.9 | 1.9 | 1.9 | 2.0 | 2.0 | | 2.1 TF |
| 11 | 2.0 | 1.9 | 2.0 | 1.9 | 2.1 | | 2.0 TF |
| 11 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | | 1.8 veg |
| 11 | 2.0 | 1.8 | 1.7 | 2.0 | 2.0 | | 2.0 SO4 |
| 12 | 2.1 | 2.0 | 2.1 | 2.1 | 2.0 | | 2.0 SO4 |
| 12 | 2.0 | 1.9 | 2.0 | 2.0 | 2.1 | | veg |
| 12 | 2.1 | 2.1 | 2.2 | 2.1 | 2.2 | | 2.0 SO4 |
| 12 | 2.2 | 2.1 | 2.2 | 2.0 | 2.2 | | TF |

($SO_4$ = magnesium sulfate, veg = magnesium chelate, TF = taste-free magnesium)

**TABLE 9.**
Urinary magnesium values for human subjects

| Subj # | creatinine (mg) | Mg (mg) | minutes | mg Mg/mg creat | Mg/creat/hr | supplement |
|---|---|---|---|---|---|---|
| 1 | 111.36 | 5.77 | 157 | 0.05 | 0.02 | veg |
| 1 | 97.28 | 8.82 | 156 | 0.09 | 0.03 | veg |
| 1 | 129.60 | 11.11 | 157 | 0.09 | 0.03 | SO4 |
| 1 | 244.00 | 13.00 | 155 | 0.05 | 0.02 | TF |
| 2 | 32.64 | 11.34 | 155 | 0.35 | 0.13 | SO4 |
| 2 | 121.60 | 15.43 | 152 | 0.13 | 0.05 | TF |
| 2 | 121.60 | 12.28 | 155 | 0.10 | 0.04 | SO4 |
| 2 | 54.60 | 9.01 | 155 | 0.17 | 0.06 | veg |
| 3 | 30.96 | 6.12 | 158 | 0.20 | 0.08 | SO4 |
| 3 | 89.76 | 17.54 | 153 | 0.20 | 0.08 | SO4 |
| 3 | 131.12 | 12.90 | 160 | 0.10 | 0.04 | veg |
| 3 | 176.00 | 7.57 | 153 | 0.04 | 0.02 | TF |
| 4 | 60.80 | 10.26 | 129 | 0.17 | 0.08 | SO4 |
| 4 | 69.36 | 11.47 | 156 | 0.17 | 0.06 | SO4 |
| 4 | 126.48 | 18.49 | 158 | 0.15 | 0.06 | veg |
| 4 | 106.56 | 14.67 | 157 | 0.14 | 0.05 | TF |
| 5 | 39.78 | 4.68 | 163 | 0.12 | 0.04 | TF |
| 5 | 86.40 | 5.85 | 72 | 0.07 | 0.06 | veg |
| 5 | 62.40 | 11.97 | 156 | 0.19 | 0.07 | SO4 |
| 5 | 104.00 | 11.90 | 157 | 0.11 | 0.04 | veg |
| 6 | 220.80 | 22.69 | 138 | 0.10 | 0.04 | TF |
| 6 | 140.00 | 14.35 | 100 | 0.10 | 0.06 | SO4 |
| 6 | 19.36 | 4.45 | 155 | 0.23 | 0.09 | veg |
| 7 | 180.00 | 8.85 | 158 | 0.05 | 0.02 | TF |
| 7 | 188.00 | 15.12 | 157 | 0.08 | 0.03 | SO4 |
| 7 | 64.60 | 21.76 | 158 | 0.34 | 0.13 | veg |
| 7 | 34.00 | 13.82 | 156 | 0.41 | 0.16 | TF |
| 8 | 67.76 | 15.22 | 158 | 0.22 | 0.09 | veg |
| 8 | 168.96 | 23.94 | 156 | 0.14 | 0.05 | TF |
| 8 | 125.44 | 19.91 | 157 | 0.16 | 0.06 | veg |
| 8 | 120.00 | 12.30 | 157 | 0.10 | 0.04 | SO4 |
| 9 | 24.64 | 4.04 | 155 | 0.16 | 0.06 | veg |
| 9 | 61.20 | 14.59 | 155 | 0.24 | 0.09 | SO4 |
| 9 | 89.44 | 17.58 | 157 | 0.20 | 0.08 | TF |
| 9 | 79.68 | 19.12 | 156 | 0.24 | 0.09 | veg |
| 10 | 53.56 | 11.89 | 160 | 0.22 | 0.08 | veg |
| 10 | 86.36 | 23.62 | 150 | 0.27 | 0.11 | veg |
| 10 | 112.80 | 15.30 | 152 | 0.14 | 0.05 | TF |
| 10 | 60.76 | 13.63 | 154 | 0.22 | 0.09 | SO4 |
| 12 | 50.75 | 9.86 | 122 | 0.19 | 0.10 | SO4 |
| 12 | 42.70 | 6.91 | 120 | 0.16 | 0.08 | veg |
| 12 | 117.60 | 9.58 | 160 | 0.08 | 0.03 | SO4 |
| 12 | 118.40 | 9.93 | 126 | 0.08 | 0.04 | TF |

# APPENDIX C

## Human Subject Information

Informed Consent of Participants:

As a participant in the mineral absorption study conducted by Dr. Deloy G. Hendricks (797-2124) and graduate assistant Jennifer A. Bowden (797-6259), I understand that I will be asked to participate in the following procedure:

1. Take two mineral supplements in capsule form by mouth, in a dose providing a fraction of the RDA for each mineral. Iron will be provided as a dose of 2 mg (RDA is 15 mg) and magnesium will be given as a dose of 100 mg (RDA is 280 mg).

2. Provide blood samples every 30 minutes for two hours (5 samples) by means of a catheter. The catheter will allow samples to be taken following only one needle stick. It provides an access to the vein that can be used repeatedly. The first blood sample will be 10 mL, all subsequent samples will be 3 mL each. All blood draws will be performed by a phlebotomist from Logan Regional Hospital. Individuals with difficulty in having blood drawn should not participate.

3. Repeat the above procedure one time each week for a total of six weeks.

From this study, I understand that the results will help determine the effectiveness of new mineral supplements. I understand that these supplements have already been studied and patented, and have been shown to be safe in previous studies. I also understand that I will be compensated for participation by receiving $75 if and when I complete the study. I recognize that I can withdraw from this study at any time without negative consequences.

Signature:_____

Name (printed):_____

Address:_____

_____phone:_____

Date:_____

Investigator Signature: _____

***Note: This is a legally binding document. Please consult with your attorney if you have questions about its effect.

Iron/Magnesium Study Participant Questionnaire:

Name: _____

1. Do you have a family history of cancer? If so, please describe (i.e.: type of cancer, relationship).

2. Do you have a family history of other chronic diseases? If so, please describe.

3. To get a better idea of your usual diet, please state how frequently you consume the following types of foods (i.e.: times per day, week, month, etc.). Also include approximate average portion sizes.

| Food Item | Frequency Consumed | Approximate Portion |
|---|---|---|
| A. red meat | | |
| B. any meat / fish / poultry | | |
| C. iron fortified cereal | | |
| D. green, leafy vegetables | | |
| E. any vegetable | | |
| F. any fruit | | |

4. Have you noticed any side effects of the supplements during the dosing period? (Not effects of the blood draws).

Thank you so much for participating in this study. I appreciate your help and cooperation!!!

Instruction Sheet for Iron/Magnesium Study:

1. Eat a meal at about nine or ten PM. Make sure you drink plenty of fluid, and avoid eating foods high in iron and magnesium at this meal. (Also remember to avoid vitamin-mineral supplements.)

2. As soon as you get up in the morning, void, and then drink 8 oz of distilled water. We will provide you with the distilled water on Mondays. Try to drink this an hour before the study begins.

3. At the beginning of the study you will need to void to make sure the bladder is empty.

4. After the catheter is placed for blood sampling, you will receive the mineral dose. This will be given with 8 oz of Tang, to get some sugar into your body. As it will be a mineral free, standardized dose, this will not affect your blood values.

5. The blood samples will again be taken every 30 minutes. Based on the data from the first week, we are estimating that the magnesium will best be reflected in the urine. Therefore, at the end of the blood sampling period, you will need to provide a total void urine sample. This way we can determine how much magnesium was absorbed and cleared.

One other note: Although some people were not able to give all five samples last week, we really need all five for the rest of the study. Otherwise, the data is not helpful. Therefore, I would appreciate your cooperation in helping us to get the necessary information. We also need you to remain relatively sedentary throughout the study.

Thank you again for your help!

# APPENDIX D

Capsule Preparation

Iron capsule preparations for 1000 0 capsules:
(For 2 mg elemental iron in each capsule)

$FeSO_4$ - 10 g $FeSO_4$ (USP, finely ground), 7.5 g Mg Stearate (USP) and 440 g Maltodextrin M-100

Vegetable Fe Chelate - 20 g Vegetable Fe Chelate, 7.5 g Mg Stearate (USP) and 430 g Maltodextrin M-100

Taste Free Fe - 12.5 g Taste Free Fe, 7.5 g Mg Stearate (USP) and 480 g Maltodextrin M-100

Magnesium capsule preparations for 500 00 capsules

$MgSO_4$ - 255 g $MgSO_4$ (USP, finely ground), 4.5 g Mg Stearate (USP) and 440 g Maltodextrin M-100
Each capsule should have 53 mg Mg.

Mg Chelazome® - 250 g Mg Chelazome®, 4.5 g Mg Stearate (USP) and 90.5 g Maltodextrin M-100
Each capsule should have 51 mg Mg.

Taste Free Mg - 312.5 g Taste Free Mg, 4.68 g Mg Stearate (USP) and 78 g Maltodextrin M-100
Each capsule should have 53 mg Mg.

# APPENDIX E

## Statistical Analysis of the Data

**Table 10**
Statistical analysis of $^{59}Fe$ isotope and $FeSO_4$ percent absorption in rats

| Variable | Mean | Std Dev | Std Error | Min | Max |
|---|---|---|---|---|---|
| $^{59}Fe$ | 0.6621 | 0.365 | 0.138 | 0 | 1 |
| $FeSO_4$ | 0.5910 | 0.367 | 0.139 | 0 | 1 |

| (Dif) Mean | Std Dev | Std Error | 2-tail Corr/Prob | t value | Degrees Freedom | 2-tail Prob |
|---|---|---|---|---|---|---|
| 0.0711 | 0.418 | 0.158 | 0.347 / 0.446 | 0.45 | 6 | 0.668 |

**Table 11**
Iron analysis data descriptive statistics for humans

| Variable | Mean | Std Dev | Min | Max | N | Variable Label |
|---|---|---|---|---|---|---|
| SERFE0 | 75.05 | 34.94 | -1 | 207 | 60 | serum iron at |
| SERFE30 | 75.08 | 33.65 | -1 | 210 | 60 | serum iron at 30 min |
| SERFE60 | 77.57 | 32.45 | -1 | 196 | 60 | serum iron at 60 min |
| SERFE90 | 81.12 | 32.73 | -1 | 201 | 60 | serum iron at 90 min |
| SERFE120 | 82.98 | 34.03 | -1 | 207 | 60 | serum iron at 120 |
| SERFE150 | 80.68 | 38.08 | -1 | 197 | 60 | serum iron at 150 |
| HCT | 39.17 | 6.21 | -1 | 45 | 60 | hematocrit of subject |
| WEIGHT | 58.32 | 9.25 | 0 | 65.5 | 60 | subject weight in |
| PEAKABS | 13.58 | 11.74 | 0 | 46 | 60 | peak iron absorption |
| AREAABS | 34.71 | 31.01 | 0 | 129.02 | 60 | area absorption |
| PKPCT | 39.19 | 39.71 | 0 | 159.87 | 60 | peak percent iron |
| AREAPRCT | 17.35 | 15.5 | 0 | 64.51 | 60 | area percent iron |
| FERRITIN | 22.12 | 18.56 | 0 | 90 | 60 | serum ferritin |
| FETYPE | 2.03 | 0.84 | 0 | 3 | 60 | iron supplement type |
| WEEK | 3 | 1.43 | 1 | 5 | 60 | week of study |
| FERRCAT | 1.8 | 0.75 | 1 | 3 | 60 | ferritin level |

**Table 12**
Analysis of variance for iron absorption by supplement type

| Peak percent iron absorption by supplement type for all supplements: | | | | | |
|---|---|---|---|---|---|
| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
| Between Groups | 2 | 723.9929 | 361.9964 | 9.1671 | .0984 |
| Within Groups | 2 | 78.9776 | 39.4888 | | |
| Total | 4 | 802.9705 | | | |

Tests For Homogeneity of Variances
Cochrans C = Max. Variance/Sum(Variances) = .9117, P = .177 (Approx.)
Bartlett-Box F = .914 , P = .344
Maximum Variance / Minimum Variance 10.319

**Table 13**
Analysis of variance demonstrating the decreased absorption in the taste-free iron

| Iron absorption by supplement type using ferrous sulfate and taste-free iron | | | | | |
|---|---|---|---|---|---|
| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
| Between Groups | 2 | 401.5487 | 200.7744 | 2.7985 | .0874 |
| Within Groups | 18 | 1291.3738 | 71.7430 | | |
| Total | 20 | 1692.9225 | | | |

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum(Variances) = .6249, P = .108 (Approx.)
Bartlett-Box F = 1.331 , P = .265
Maximum Variance / Minimum Variance 3.366

**Table 14**
Percent iron absorption by supplement and ferritin category

Peak

| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Main Effects | 4 | 21570.958 | 5392.740 | 4.697 | .003 |
| FETYPE | 2 | 7348.166 | 3674.083 | 3.200 | .049 |
| FERRCAT | 2 | 10831.448 | 5415.724 | 4.717 | .013 |
| 2-Way Interaction | 4 | 12489.416 | 3122.354 | 2.720 | .040 |
| Explained | 8 | 34060.375 | 4257.547 | 3.708 | .002 |
| Residual | 50 | 57402.729 | 1148.055 | | |
| Total | 58 | 91463.104 | 1576.950 | | |

Area

| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Main Effects | 4 | 3297.958 | 824.490 | 5.091 | .002 |
| FETYPE | 2 | 1055.813 | 527.906 | 3.260 | .047 |
| FERRCAT | 2 | 1666.386 | 833.193 | 5.145 | .009 |
| 2-Way Interaction | 4 | 2478.622 | 619.656 | 3.827 | .009 |
| Explained | 8 | 5776.580 | 722.073 | 4.459 | .000 |
| Residual | 50 | 8096.832 | 161.937 | | |
| Total | 58 | 13873.412 | 239.197 | | |

(FETYPE = iron supplement type, FERRCAT = ferritin category)

**Table 15**
Peak percent absorption by ferritin category

vegetable iron chelate

| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | 16635.1719 | 8317.5859 | 9.3399 | .0017 |
| Within Groups | 18 | 16029.7687 | 890.5427 | | |
| Total | 20 | 32664.9406 | | | |

Tests for Homogeneity of Variances:
Cochrans C = Max. Variance/Sum(Variances) = .5678, P = .218 (Approx.)
Bartlett-Box F = .967 , P = .381
Maximum Variance / Minimum Variance 3.257

ferrous sulfate

| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | 5632.6684 | 2816.3342 | 1.2505 | .3165 |
| Within Groups | 14 | 31530.1044 | 2252.1503 | | |
| Total | 16 | 37162.7728 | | | |

Tests for Homogeneity of Variances
Cochrans C = Max. Variance/Sum(Variances) = .7139, P = .045 (Approx.)
Bartlett-Box F = 1.825 , P = .163
Maximum Variance / Minimum Variance 11.490

taste free iron

| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | 1053.0241 | 526.5120 | .9629 | .4006 |
| Within Groups | 18 | 9842.8559 | 546.8253 | | |
| Total | 20 | 10895.8800 | | | |

Tests for Homogeneity of Variances:
Cochrans C = Max. Variance/Sum(Variances) = .6231, P = .111 (Approx.)
Bartlett-Box F = 2.328 , P = .099
Maximum Variance / Minimum Variance 7.337

**Table 16**
Peak percent absorption by supplement - low ferritin category

| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | 17907.7588 | 8953.8794 | 4.9024 | .0185 |
| Within Groups | 20 | 36528.9211 | 1826.4461 | | |
| Total | 22 | 54436.6800 | | | |

Tests for Homogeneity of Variances:
Cochrans C = Max. Variance/Sum(Variances) = .7129, P = .016 (Approx.)
Bartlett-Box F = 5.536 , P = .004
Maximum Variance / Minimum Variance 32.494

**Table 17**
Peak percent absorption by supplement - medium ferritin category

| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | 1687.5710 | 843.7855 | 1.0152 | .3794 |
| Within Groups | 21 | 17454.2885 | 831.1566 | | |
| Total | 23 | 19141.8595 | | | |

Tests for Homogeneity of Variances:
Cochrans C = Max. Variance/Sum(Variances) = .4429, P = .653 (Approx.)
Bartlett-Box F = .247 , P = .781
Maximum Variance / Minimum Variance 1.858

**Table 18**
Peak percent absorption by supplement - high ferritin category

| Source | D.F. | Sum Of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | 242.2520 | 121.1260 | .3188 | .7349 |
| Within Groups | 9 | 3419.5193 | 379.9466 | | |
| Total | 11 | 3661.7713 | | | |

Tests for Homogeneity of Variances:
Cochrans C = Max. Variance/Sum(Variances) = .3719, P = 1.000 (Approx.)
Bartlett-Box F = .020 , P = .980
Maximum Variance / Minimum Variance 1.312

**Table 19**
Correlation Coefficients for iron supplement absorption in humans

| all supplements | | FERRITIN | HCT |
|---|---|---|---|
| | PEAKABS | -0.2418 | 0.5635** |
| | PKPRCT | 0.4492** | -0.0825 |
| | AREAABS | -0.0713 | 0.2090 |
| | AREAPRCT | 0.5844** | -0.2726* |
| ferrous sulfate | | FERRITIN | HCT |
| | PEAKABS | -0.2937 | -0.3216 |
| | PKPCT | -0.4500 | -0.4201 |
| | AREAABS | -0.3202 | -0.4026 |
| | AREAPRCT | -0.3202 | -0.4026 |
| Ferrochel: | | FERRITIN | HCT |
| | PEAKABS | -0.5219* | 0.1228 |
| | PKPCT | -0.4862* | 0.0085 |
| | AREAABS | -0.5076* | 0.0061 |
| | AREAPRCT | -0.5076* | 0.0061 |
| taste free iron | | FERRITIN | HCT |
| | PEAKABS | 0.5224* | 0.0983 |
| | PKPCT | 0.3854 | 0.0004 |
| | AREAABS | 0.5162* | 0.0573 |
| | AREAPRCT | 0.5162* | 0.0573 |

(PEAKABS = peak relative iron absorption, PKPCT = peak percent of doseabsorbed, AREAABS = area relative iron absorption, AREAPRCT = area percent of dose absorbed)
(* - Signif. LE .05 ** - Signif. LE .01 [2-tailed])

**Table 20**
Magnesium analysis descriptive statistics

| Variable | Mean | SD | Min | Max | N | Variable Label |
|---|---|---|---|---|---|---|
| serum Mg data | | | | | | |
| SERMG0 | 2.00 | 0.19 | 1.70 | 2.80 | 46 | serum Mg at baseline |
| SERMG30 | 2.03 | 0.22 | 1.70 | 3.20 | 46 | serum Mg at 30 min |
| SERMG60 | 2.08 | 0.22 | 1.80 | 2.90 | 46 | serum Mg at 60 min |
| SERMG90 | 2.01 | 0.33 | 0.00 | 2.40 | 46 | serum Mg at 90 min |
| SERMG120 | 1.99 | 0.58 | 1.00 | 3.00 | 46 | serum Mg at 120 min |
| SERMG150 | 1.00 | 0.98 | 0.00 | 2.30 | 46 | serum Mg at 150 min |
| MGTYPE | 5.96 | 4.03 | 1.00 | 12.00 | 46 | supplement type |
| PEAK | 1.20 | 0.99 | 0.00 | 2.80 | 46 | peak serum Mg increase |
| urinary Mg data | | | | | | |
| CREAT | 98.21 | 52.69 | 19.00 | 244.00 | 43 | creatinine level |
| MG | 12.81 | 5.14 | 4.00 | 24.00 | 43 | urinary Mg |
| MIN | 149.56 | 17.62 | 72.00 | 163.00 | 43 | collection time (minutes) |
| MGCTHR | 0.06 | 0.03 | 0.00 | 0.00 | 43 | mg Mg/creat/hour |
| MGTYPE | 1.93 | 0.80 | 1.00 | 3.00 | 43 | supplement type |

**Table 21**

Analysis of variance for peak serum Mg increase by supplement type

| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | .0729 | .0364 | .8878 | .4288 |
| Within Groups | 18 | .7386 | .0410 | | |
| Total | 20 | .8114 | | | |

Tests For Homogeneity of Variances

Cochrans C = Max. Variance/Sum(Variances) = .7664, P = .009 (Approx.)

Bartlett-Box F = 4.607 , P = .010

Maximum Variance / Minimum Variance 13.618

**Table 22**

Analysis of variance for urinary magnesium (mg)/mg creatinine/hour by supplement type:

| Source | D.F. | Sum of Squares | Mean Squares | F Ratio | F Prob. |
|---|---|---|---|---|---|
| Between Groups | 2 | .0022 | .0011 | 1.1303 | .3330 |
| Within Groups | 40 | .0396 | .0010 | | |
| Total | 42 | .0418 | | | |

Tests For Homogeneity of Variances

Cochrans C = Max. Variance/Sum(Variances) = .4465, P = .435 (Approx.)

Bartlett-Box F = .477 , P = .621

Maximum Variance / Minimum Variance 1.656