2004

Factors affecting iron status among infants age 6--24 months

Christopher Melonas
West Virginia University

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Christopher Melonas

Thesis Submitted to the
Davis College of Agriculture, Forestry, and Consumer Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Master of Science
In
Human Nutrition & Foods

Cindy Fitch, Ph.D., RD., Chair
Betty Forbes, MA., RD.
Carol Markstrom, Ph.D.
Erron Huey, Ph.D.

Division of Family & Consumer Sciences

Morgantown, West Virginia
2004

Keywords: Iron, Iron deficiency, Anemia, WIC

ABSTRACT

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Christopher Melonas

Iron deficiency has been shown to have a multitude of negative effects on the growing infant. The diet plays a key role in alleviating iron deficiency within a younger population. The objective of this study was to search out specific nutrients in the diet that have some affect on iron status in a population of infants age 6 to 24 months.

This was a cross-sectional study that included 57 rural infants who participated in the WIC program. Dietary intake data was collected by 2 multiple-pass 24-hour intakes done 3 to 4 days apart. Blood was collected by venipuncture and analyzed for hemoglobin, ferritin, and transferrin saturation. Multiple logistic regression, Pearson's Correlation, and independent sample t-tests was used to evaluate iron status and dietary variables.

Twelve children were categorized as iron deficient as defined by serum ferritin $\leq$ 15 ug/L and transferring saturation $\leq$ 15%. Average iron intake for the group met recommendations. On regression analysis, calcium and phosphorous were shown to be the main determining factors affecting iron status, and this was a negative association (calcium, $P < .03$: phosphorous, $P < .01$). The amount of cow's milk consumed, protein intake, riboflavin, niacin, thiamin, folate, vitamin B12, calcium and phosphorous were all shown to negatively correlated with the concentration of serum ferritin (data not shown). These results have shown that there are significant factors in the diet that either enhance or inhibit iron absorption and iron utilization. And each of these nutrients must be looked at carefully in consideration with iron status in infants age 6 to 24 months.

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ACKNOWLEDGEMENTS

A special thanks should be given to the following people who either provided tremendous help, encouragement, moral support and guidance during the production of this research study.

Cindy Fitch, Ph.D., RD
WIC Clinics
Norma Penuela, MS., RD
Betty Forbes, MA., RD
Carol Markstrom, Ph.D.
George Seidel, Ph.D
Erron Huey, Ph.D

Introduction

This research study describes factors related to iron status in rural children between 6 and 24 months of age who are a part of the Special Supplementation Nutrition Program for Women, Infants and Children or WIC. WIC provides nutrition education to mothers and has the ability to encourage better dietary eating habits for the mother and child. WIC provides iron fortified formulas and food vouchers to help obtain iron containing foods. They also provide regular screening for anemia with the use of Hemoglobin (Hb) testing. Hemoglobin testing only looks at anemia and it is not a test for overall iron status. Even with the help from WIC, a number of cases of anemia still arise annually. Iron deficiency may have many detrimental factors from morbidity and mortality to lower mental performance and overall growth of the child, as this thesis will soon describe. This study will look at the many contributing factors associated with iron status and provide insights on the many determinants of iron status.

Iron deficiency is the most prevalent nutritional deficiency worldwide, affecting mainly older infants, young children, and women of childbearing age in industrialized as well as developing countries. It is a condition that is preventable through appropriate dietary measures. Although iron deficiency is more common in developing countries, a significant prevalence was observed in the United States during the early 1990s among certain populations, such as toddlers and females of childbearing age. Iron deficiency commonly remains unrecognized because of subtle symptoms such as pallor, listlessness, and fatigue (Benoist., 2001).

Iron deficiency is of particular concern among infants between the ages of 6 and 24 months. Children between these ages have a rapid rate of growth and development and subsequently have an increased need for iron to provide this growth. At this age, the main nutritional issue is the need to broaden the range of foods and establish healthy eating habits. Persistent iron deficiency among this group is often associated with a multitude of effects such as long lasting diminished mental, behavioral and motor functioning, decreased resistance to infection, increased risk of lead poisoning just to name a few (MMWR., 2002).

The prevalence of iron deficiency anemia has decreased during the past few decades, most likely because of a national increase in breast feeding, standardization of the composition of iron-fortified formulas, and a delay in the introduction of whole milk until the age of 1 year. By providing participants with iron fortified formula, cereal and vouchers for iron containing foods the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) has also played a significant role in this decline (Yip et al, 1987).

The WIC program began in 1974 after a 2 year pilot program. WIC links food assistance and nutrition education to health care for at-risk persons. Infants and children five years and younger are eligible for enrollment in WIC if family income is below 185% of the US poverty income guidelines and if they are at nutritional risk. Nutritional risk may be medically based, such as being anemic or underweight, or having an inadequate diet (Owen et al, 1997).

Iron deficiency should be preventable. Despite the successes demonstrated by WIC, iron deficiency still exists among children. Because of the detrimental effects iron deficiency has on the development of the human body, it needs to be eradicated in every child. So the child's diet seems to be a rational place to begin an analysis of why this devastating problem continues to exist.

Iron Needs During Infancy

Young children are the most susceptible to iron deficiency as a result of an increased iron requirement related to rapid growth during the first 2 years of life and a relatively low iron content in most infant diets when iron is not added by supplementation or fortification. In the normal infant, total body iron changes dramatically during the first 4 months of life. On average, the iron stores in a full-term infant can meet the infant's iron requirements until 4 to 6 months of age. By 4 months of age, neonatal iron stores would have been reduced by half, and exogenous iron is required to maintain hemoglobin concentration during this rapid phase of growth between 4 to 12 months. Absorption of about 0.8 mg of iron per day from the diet is required, of which 0.6 mg is needed for

growth, and 0.2 mg to replace losses. The reference nutrient intake for iron (mg/day) is 4.3 mg (4-6 months) and 7.8 mg (7-12 months) of exogenous dietary iron daily (Booth and Aukett., 1997).

Iron in the Body

Iron is both an essential nutrient and a potential toxicant. It requires a highly sophisticated and complex set of regulatory approaches to meet the demands of cells as well as prevent excess accumulation. A sufficient supply is essential for the functioning of many biochemical processes; including electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cellular growth and differentiation. Iron homeostasis involves the regulation of iron entry into the body, regulation of iron entry into cells, storage of iron in ferritin, incorporation into proteins and regulation of iron release from cells for transport to other cells and organs.

Iron in the body exists in two major forms, functional and stored. The human body contains about 2 to 4 grams of iron: Over 65% of body iron is found in hemoglobin, an iron containing protein, and is involved in oxygen transport from the lungs to the body tissues. Ten percent is found in myoglobin, an iron containing protein, which facilitates diffusion and storage of oxygen in the tissues. About 1 to 5% is found as part of enzymes, and the remaining body iron is found in blood or storage as ferritin or hemosiderin. The total amount of iron found in a person not only is related to body weight but also influenced by a variety of physiological conditions including age, gender, pregnancy, and growth. Iron is a metal and exists in several oxidative states varying from Fe^{+6} to Fe^{-2}, depending on its chemical environment. The only states that are stable in the aqueous environment in the human body and in food are the ferric (Fe^{+3}) and ferrous (Fe^{+2}) forms (Finch et al, 1982: Groff et al, 2000).

Factors Affecting Iron Absorption

The most common factors that contribute to the development of iron-deficiency are rapid growth, insufficient absorption of iron, and blood loss. There are also two major factors affecting iron absorption, body iron stores and erythropoesis (Bothwell., 1995). The regulation of iron absorption is closely tied to the level of iron stores. Absorption increases when iron stores are low and decreases as stores become greater. Declining iron

stores and stimulation of erythropoesis (synthesis of red blood cells) both can cause iron absorption to increase.

Iron bioavailability is determined by overall meal composition and under most circumstances is not a unique property of the food source. There are numerous dietary factors that either enhance or inhibit iron absorption in the gut lumen and the intestinal mucosal cell; type of iron ingested, types of food present, presence of chelators/ligands such as calcium, calcium phosphate salts, ascorbic acid, phytates, polyphenols and competition for absorption of iron with lead and zinc.

Dietary Iron

There are two broad types of dietary iron. About 90% of iron from food is in the form of iron salts and is referred to as non-heme iron. The other 10% of dietary iron is in the form of heme iron, which is derived primarily from the hemoglobin and myoglobin of meat, fish and poultry and is reasonably well absorbed. Since infant diets contain little meat, almost all dietary iron is in the non-heme form that is found predominantly in nuts, fruits, vegetables, grains, dairy products, and also meat.

Iron behaves as though it is derived from one of two common iron pools in a meal. The larger pool, the non-heme iron pool, comprises iron in any soluble inorganic form, non-heme iron found in meat and iron found in vegetables. Many heme iron compounds, primarily hemoglobin and myoglobin in meats, account for approximately 10 to 15% of ingested dietary iron in industrialized countries and constitutes a separate pool with different absorptive capacities and behavior (Lynch, 1997).

Heme iron must be hydrolyzed from the globin portion of hemoglobin or myoglobin prior to its absorption. Heme iron, unlike non-heme iron, remains soluble because of both degradation products of the globin and, if in the small intestine, the alkaline PH found in the small intestine. Absorption of heme iron is influenced by body iron stores. Heme iron absorption is inversely related to iron stores and may range from 15% with normal iron status to 35% in persons who are iron deficient. Iron in the form of heme and constituting the smaller heme iron pool in meat and fish containing meals is much greater absorbed than is non-heme. Heme iron is actually relatively well absorbed under most circumstances. The absorption of heme iron is relatively independent of meal

composition, except when calcium is present, and minutely affected by the inhibitors/enhancers that greatly affect non-heme iron absorption (Lynch, 1997).

Non-heme iron bound to components of foods must be enzymatically liberated in the gastrointestinal tract for absorption to occur. Following its release from food components, non-heme iron is typically present in the stomach in the ferric state. Ferric iron may or may not be reduced depending on the gastrointestinal conditions. If the ferric iron from the non-heme source is not reduced it tends to form compounds that are relatively insoluble and tend to aggregate and precipitate making the iron less available for absorption. Lynch (1997) also reviews some characteristics of non-heme iron absorption. Iron storage status is determined to be the most important factor of the rate at which non-heme iron is absorbed. Specific factors exist in the intestinal lumen that put forth a powerful influence over the body's ability to extract iron from the luminal non-heme iron pool. There are two basic physiological factors that can influence this rate: gastric HCL secretion and the mixing of food in the stomach. Both are necessary for maximal absorption of dietary non-heme iron. Several dietary factors can act as either enhancers or inhibitors of absorption from the non-heme iron pool formed in the stomach and the upper small intestine, and the balance formed between these two factors determines the bioavailabillity of non-heme iron in a meal.

The type of iron given and the composition of the diet also affect iron absorption. The fractional absorption of heme iron, present only in meat, poultry, and fish, is considerably higher than absorption of non-heme iron (approximately 25% compared with 5-10%), and less subject to regulation. The iron status of the individual and dietary composition influence absorption of non-heme iron and to a degree heme iron (Slatkavitz and Clydesdale, 1988).

Chelators/Ligands

The presence of several compounds, known as chelators or ligands, may bind with non-heme iron to either inhibit or enhance its absorption. Chelators are small organic compounds that form a complex with a metal ion. Ligands are compounds that also bind

or complex with minerals. Whether chelated iron or iron that is attached to a ligand is absorbed or excreted depends on the nature of the iron-chelate/ligand complex. If the iron-chelate/ligand complex maintains solubility and the iron is loosely bonded than the iron can be released at the intestinal mucosal cell and absorption is enhanced. Although, if the iron-chelate/ligand is strongly bonded and insoluble, then the absorption of iron will not occur and thus it will be excreted in the feces as part of the chelated compound.

Inhibitors of Iron Absorption

Phytates & Polyphenols

Phytic acid (phytate) a consituent of plant fiber found in dietary grains can form a chelate/ligand complex thus limiting non-heme iron absorption. Gillooly et al. (1983) demonstrated that phytate was indeed a major inhibitor of iron in cereal foods such as wheat, oats, sorghum, unpolished rice and beans. Polyphenols including tannin derivatives of gallic acid (in tea, coffee and cereal grains) have also been shown to be a powerful inhibitor of iron absorption. They appear to be equal in importance to phytates as inhibitors of non-heme iron absorption Polyphenols are thought to act as phytates do forming complexes between the phenolic compounds and iron molecules rendering the iron unavailable to absorption (Gillooly et al, 1983).

Calcium

Calcium is thought to interact with iron and phosphorous and form a chelation and inhibit its absorption at the intestinal mucosa. The addition of calcium, whether it is in the form of milk or inorganic salt, can reduce the percentage rate of non-heme iron absorption. Nevertheless, calcium's seemingless inhibitory effect is complex and the mechanism on which it interferes with iron absorption is poorly understood (Lynch, 1997).

Hallberg and Brune et al. (1991) found a strong dose dependent relation between the amount of calcium in a meal and the reduction in non-heme iron absorption. The relative reduction of the amount of iron absorbed was the same if the calcium was in the form of calcium salt, milk or cheese. There was no observed inhibition seen when the amount of calcium in a meal was <50 mg and the inhibition was maximal when the amount of calcium was approximately 300-600 mg.

Hallberg and Rossander-Hulther et al. (1992) noted a reduction of heme iron by calcium as well, suggesting a common step in the transport of these two kinds of dietary iron. Therefore, the effect was within the mucosal cell and not the intestinal lumen. The observed relationship between the absorption ratio, with and without calcium, and the amount of calcium in a meal had a sigmoidal curve, suggesting some type of one-site competitive binding at a particular receptor.

Hallberg and Gleerup et al. (1991) investigated the inhibitory effect of calcium on iron absorption in 57 healthy human subjects at the Department of Medicine and Clinical Nutrition University at Gateborg, Sahlgren Hospital Sweden. Four studies were made to examine whether the inhibiting effect of calcium was located in the gastrointestinal lumen.

One study tested whether the inhibiting effect was influenced by adding phytate to the test meal, and another study whether the inhibiting effect of calcium was counteracted by ascorbic acid. A third study was also conducted to examine if the effect of calcium was related to a molar ratio competition between iron and calcium or if a certain minimal amount of calcium was needed to induce an effect (iron dose of 0.01 mg and calcium dose of 3 mg, molar ratio of 1:420). A fourth and final study was made to examine the inhibiting effect of calcium given as milk, cheese and a milkshake to three common types of meals.

The presence of phytate in a meal and formation of calcium-iron-phytate complexes is not a prerequisite for the inhibition. The relative increase in iron absorption by ascorbic acid was the same in meals with and without calcium, suggesting that calcium did not influence the balance between enhancing and inhibiting ligands in the gastrointestinal lumen. No inhibiting effect on iron absorption was seen when adding 3 mg calcium to 0.01 mg iron. Previous studies showing a marked inhibition by calcium had a lower molar ratio, but greater amounts of calcium were given. This suggests that a minimal concentration of calcium is needed to achieve an effect. The authors also concluded that the practical nutritional implications of the inhibitory effect of calcium are considerable since addition of milk, milkshake or cheese to common meals such as pizza

or hamburger meals reduced iron absorption by 50-60% (Hallberg and Gleerup et al., 1991).

Lead

Iron deficiency and lead poising have long been postulated to be associated. Poor nutrition and low socioeconomic status are associated with both of these diseases. Thus, factors that predispose children to iron deficiency may also predispose them to lead poisoning and the relationship may not be causative. Lead has implications on iron absorption and also iron utilization. Both lead and iron share a common absorptive receptor, and iron deficiency may increase lead absorption (Barton et al., 1978). Lead has also been shown to inhibit the activity of an enzyme required in heme synthesis, and an enzyme required to incorporate iron into heme (Goyer, 1995).

Enhancers of Iron Absorption

Ascorbic Acid

The influence of ascorbic acid (vitamin C) is most pronounced in inhibitory meals and it is most effective when the meal contains high levels of the two main inhibitors of non-heme iron absorption, phytates and polyphenols (Semba and Bleom., 2002). Ascorbic acid is most effective in promoting iron absorption, especially non-heme iron, only if eaten together with iron. The enhancing effects of ascorbic acid is more profound in the presence of phytates or iron binding polyphenols, but its effect was also seen in their absence as well (Hallber and Hulthen., 2000).

Ascorbic acid acts by maintaining iron in a soluble bioavailable form. As the luminal pH rises once the gastric contents enter the duodenum, iron in the ferric form is mainly soluble only at an acidic pH (Lynch, 1997). These findings suggest that the ability of ascorbic acid to reduce iron and thus prevent the formation of less soluble forms of ferric compounds is probably the important mechanism of action for the proper rate of absorption and is most likely why ascorbic acid is effective in promoting absorption. The predominantly essential interaction between iron and ascorbic acid from the view point of nutritional anemia is the effect vitamin C has on iron bioavailability. There appears to be a direct interaction between these two compounds in the lumen in the upper bowel, and is independent of an individual's overall ascorbic acid status.

Other Organic Acids

Although less well studied than ascorbic acid, many other compounds appear to have the same enhancing effects in single meal studies. All the vegetables associated with good iron bioavailabillity contained appreciable amounts of citric, malic, lactic or tartaric acids (Lynch, 1997). Ballot et al. (1987) also notes that the addition of either citric, malic or tartaric acids to a rice based meal improved iron absorption two to four-fold.

Animal Tissues

Several animal tissues including beef, chicken, fish, lamb, liver and pork improve iron status by supplying highly available heme iron and by promoting better absorption from the non-heme iron pool (Lynch et al, 1989).

When compared with ascorbic acid, animal tissues only present a moderate rise in the percentage of iron absorbed. Although the beneficial factors responsible for animal tissue's ability to better absorb dietary iron might be due to the presence of animal protein but overall remain poorly characterized. The suggestion is presumed to be that the peptides released during proteolytic digestion by pepsin in the antrum of the stomach may increase the ability of inorganic iron to become more soluble (Slatkavitz and Clydesdal, 1988). These particular peptides possibly bind with iron, and help it to remain soluble and improve availability for absorption.

Zinc & Copper

Zinc and copper are essential elements for the maintenance of life and health. Although they are implicated as factors affecting iron absorption they also have roles in iron utilization and mobilization. Dietary factors that contribute to decreased iron absorption also contribute to decreased zinc absorption. Foods that are high in iron are also relatively high in zinc. Also, iron and zinc are digested and absorbed in the same fashion and share similar absorptive pathways. So it is not recommended to ingest larger quantities of these nutrients at the same time. In iron deficiency, zinc protoporphyrin is produced instead of heme, and the zinc protoporphyrin in erythrocytes is increased. Copper also has a role in the absorption and mobilization of iron. The oxidation of ferrous iron (Fe+2) to the ferric state (Fe+3) is carried out by a major plasma copper

protein, ceruloplasmin. Thus, depletion of copper could impair iron absorption and iron mobilization (Ece et al, 1997).

In conclusion all of these factors are prime targets to look at when evaluating iron status in terms of its absorption. However, limitations to many of these studies just mentioned is that they were one meal studies with specific foods to be eaten. Western society, as a whole, has a highly varied diet which could lead to many complex interactions between the inhibitors and enhancers of iron absorption.

Factors Affecting Iron Metabolism and Hematopoesis

There are also numerous dietary factors that affect iron mobilization from iron stores and utilization in hematopoietic processes. The maintenance of normal hematopoietic function requires adequate levels of many nutrients acting in concert. Vitamins such as vitamin A, folic acid, vitamin B12, vitamin B6 and riboflavin are necessary for the production of red blood cells. Vitamins E and C protect mature red blood cells from premature destruction by free radical oxidation. Riboflavin, vitamin A and C also improve intestinal absorption of iron and facilitate iron mobilization from body stores.

Vitamin A

Vitamin A deficiency may induce anemia by impairing the differentiation and proliferation of hematopoietic cells disturbing renal and hepatic erythropoietin synthesis. This will, in turn, redue mobilization of body iron stores and disturbing iron and heme metabolism through sequestration of iron during the acute phase response.

There is a direct correlation between serum retinol and hemoglobin levels, and vitamin A deficiency impairs iron mobilization from iron stores and has some influence on iron's absorption (Lynch, 1997). Vitamin A deficiency also affects the transport of iron, the production of red blood cells, and impaired mobilization of iron stores (Mejia and Chew, 1998).

Semba and Bloem (2002) conducted a comprehensive review of literature to gain further knowledge that a deficiency in vitamin A can cause anemia. They found that although vitamin A deficiency has been recognized to cause anemia, vitamin A

deficiency lacks complete characterization for the exact cause of anemia. Vitamin A appears to be involved in anemia's pathogenesis through many diverse biological mechanisms; such as the enhancement of growth and differentiation of erythrocyte progenitor cells, potentiation of immunity to infection and reduction of the anemia of infection, and mobilization of iron stores from tissues. They concluded in stating that further work be done on the exact biological mechanisms by which vitamin A deficiency causes anemia.

In humans cross-sectional studies show positive correlations between serum retinol concentration and hemoglobin that are more apparent of poorer vitamin A status. Six central American nutrition surveys and biochemical studies in Ethiopia and Bangladesh observed modest, positive correlations between circulating retinol and hemoglobin levels in children, suggesting that serum retinol accounts for 4-10% of the variation in hemoglobin concentration (Fishman et al., 2000).

Vitamin E

Vitamin E (α-tocopherol) is a lipid soluble compound that functions in humans primarily as an antioxidant. Nutritional deficiency of vitamin E is thought to be uncommon as it is widely distributed in foods, particularly vegetable and seed oils such as almond, sunflower, corn, soybean and wheat germ. Vitamin E deficiency induced anemia in infants 6-12 weeks of age has been characterized by red blood cell hemolysis, reticulocytosis, thrombocytosis and edema that resolves promptly following vitamin E treatment (Williams et al, 1975). It has also been recognized that infant formula diets rich in polyunsaturated fatty acids and low in vitamin E especially in the presence of oxidative compounds such as iron, potentiated the severity of deficiency and hemeolytic anemia. To summarize, vitamin E prevents oxidative damage to erythrocytes which can lead to hemolysis (Fishman et al, 2000).

Folate

A deficiency in folate can lead to impaired DNA synthesis, leading to ineffective erythropoiesis, and is the leading cause of megaloblastic anemia in the world. When deficient in folate the synthesis of cell division is prolonged and germ cell mutation is retarded, leading, in the case of marrow, to abnormal red blood cell precursors

(megaloblasts) that have larger than normal cell and nuclear diameters. Megaloblsts undergo grossly disturbed cell proliferation and those that do mature are often ingested and degraded by bone marrow macrophages. Erythropoiesis is thus ineffective, and the rate of delivery of new enterocytes into circulation is depressed and a macrocytic anemia gradually develops (Fishman et al, 2000).

Vitamin B12 (Cobalamin)

A second nutritional cause of megaloblastic anemia is vitamin B12 (cobalamin) deficiency, which can also produce macrocytic anemia as seen in folate deficiency as well as extensive neurological impairment. Deficiency in B12 can lead to impaired metabolism of folate, leading to ineffective erythropoiesis. Cobalamin is an essential cofactor in at least two transmethylation reactions, one of which closely interrelates with folate in DNA synthesis and hematopoiesis. The only natural source of vitamin B12 is its synthesis by certain algae, fungi and bacteria. The best dietary sources are meat products in which B12 has accumulated, either by the animal's ingestion of B12 containing organisms or the synthesis of B12 by the animal's gut flora. Dietary B12 deficiency occurs less frequently than folate deficiency, usually resulting from defective absorption rather than insufficient intake (Fishman et al, 2000).

Riboflavin (vitamin B2)

Deficiency in riboflavin has been associated with the development of normochromic, normocytic anemia that responds favorably to riboflavin supplementation (Alfrey et al., 1970). In vitro and in vivo studies have described a riboflavin dependent mechanism for iron mobilization in which a flavin mononucleotide (FMN)-dependent oxidoreductase catalyses the removal of iron from storage ferritin and makes it available for utilization in heme synthesis. There is also a FMN-dependent oxidase instrumental in the conversion of vitamin B6 to its active form, which ultimately stimulates globin production. Although riboflavin is ubiquitous in food, riboflavin deficiency may be one of the most common vitamin deficiencies among the people of developing nations, particularly in those regions where diets are predominantly rice-based and contain insufficient milk, meat, fish, fresh fruit or vegetables. Thus, riboflavin deficiency may impair iron mobilization, globin synthesis and possibly iron absorption. Supplementation

with riboflavin may enhance the hemoglobin, hematocrit and erythrocyte count response to iron supplementation and improve the hematological status of anemic children and adults (Fishman et al, 2000).

Thiamine (B3), Niacin (B1), Vitamin B6

Each of these three vitamins has been related to the development or treatment of anemia during deficiency and supplementation, respectively, and warrants mention. Although, their public health significance with respect to anemia is largely unknown. Thiamine-responsive megaloblastic anemia is the product of a hereditary disorder of metabolism, part of a syndrome that is also characterized by diabetes mellitus (Nuefeld et al., 1997). Niacin deficiency has produced macrocytic and normocytic anemia in various animal models. Vitamin B6 deficiency, although rare, can disturb heme synthesis and lead to normocytic, microcytic or sideroblastic anemia and with supplementation has been shown effective in correcting various other hematological abnormalities (Fishman et al, 2000).

Iron Deficiency

Anemia is the most familiar consequence of iron deficiency. Anemia, iron deficiency, iron deficiency anemia and iron depletion are often substituted for each other but really are not equivalent. Iron deficiency reflects a range from iron store depletion without health impairment, to depleted iron stores that affect an array of bodily systems. Anemia is a late sign of iron deficiency and iron deficiency may not be the only causal factor of anemia (Benoist, 2001).

Mira et al. (1996) conducted a study at the early childhood centers in Sydney, Australia comparing heme and non-heme dietary intake of children that were iron replete and iron depelete. Iron status was measured by using plasma ferritin concentrations and a three day weighed dietary intake record was completed by the parents of the participating children in the study. The population consisted of 56 iron depleted and 68 iron replete children aged 12 to 36 months. Their results showed that average daily intake of heme iron was significantly lower in the iron depleted group ($t=2.392$, $P=0.018$), The results

also showed a tendency of a lower average daily intake of non-heme iron (t=1.724, P=0.086) and also a lower intake of ascorbic acid (t=1.921, p=0.057) for the iron depleted children. A lower intake of heme iron (<0.71 mg/day) was significantly associated with iron depletion with an odds ratio of 3.0 (P=0.005). This study showed that in young children in developed countries, a lower heme iron intake is a major risk factor for iron depletion.

A study conducted by Kahn et al. (2002) was to determine the prevalence and changes of anemia status in children receiving Special Supplementation Nutrition Program for Women, Infants, and Children (WIC). The main purpose of this study was to determine the prevalence of anemia by age, race or ethnicity and the relationship between anemia, sex, birth weight and weight for height Z score, that could be used to focus appropriate anemia screening in this population. The population consisted of 7053 infants aged 6 to 59 months and the subjects were identified by using a computer database of WIC participants from 3 Chicago, IL sites. The researchers used two definitions of anemia and were considered separately: Anemia 1 used less restrictive Hb cutoff values for the definition of anemia (<11.0 g/dl) for children aged 6 to 23 months and (<11.1 g/dl) for children aged 24 to 59 months. Anemia 2 used more strict Hb cutoff values to determine anemia (<10.5 g/dl) at 6 to 23 months and (<10.6 g/dl) at 24 to 59 months. Analyzed data were grouped by age at initial measurement in the study using two particular methods: The first grouping method, 6 to 23 and 24 to 59 months followed the age separation used by WIC and the CDC to define their cutoff values for anemia. The second grouping method, 6 to 8, 9 to 23, 24 to 35 and 36 to 59 months was used to examine age trends that could affect the progress of anemia.

Infants aged 6 to 8 months were 3.3 times more likely to be anemic than the age group of 36 to 59 months. There was no association among anemia and race, birth weight, sex or height for weight Z score. The anemia rates were proximately halved in the more strictly defined anemia 2 group. Among the children seen for at least 3 visits (n=2926), the prevalence of anemia was 8.5% and 19.1% initially, and these anemic children remained anemic during the study. Additionally, 6.6% developed anemia at a third visit even after having two normal Hb measurements.

In spite of ongoing acceptance of WIC benefits, many children developed anemia or remained anemic even though being in the program. The prevalence of anemia in this population was as high as 17.9%, and by a second visit 10.7% of children had developed anemia and 30.5% remained anemic while participating in WIC. Regardless of frequent screening, food supplementation and nutritional counseling the prevalence of anemia among participants of the WIC program remained high. Although Hb and hematocrit measurements are well standardized and easy to perform they are not ideal screening test for iron deficiency when used alone without the aid of other specific red blood cell tests.

Kapur et al. (2002) conducted a population based study on the prevalence, etiology of anemia and iron status in 545 children that were 9 to 36 months of age. The study was conducted in an urban slum integrated child development services project in north east Delhi, India. Their objective was to assess the severity and possible etiology of anemia and iron deficiency among this age group.

Hemoglobin and serum ferritin concentrations were collected and information on socio-economic, demographic, parasitic infection and dietary intake was collected. The food and nutrient intake were assessed using a ten-item food frequency questionnaire over 3 days. The prevalence of anemia (Hb < 11.0 g/dl) among this population group was 64% and of these 7.8% had severe anemia (Hb < 7.0 g/dl). Using a cutoff value for serum ferritin at 12 ug/L, 88% of the children were estimated to be iron deficient with ferritin concentrations less than 12 ug/L.

Iron intake was approximately one third of the RDA and 98% of the children had intakes below the RDA. Regression analysis showed that milk intake among the group was significant at $p < 0.05$ for the variations seen in hemoglobin concentrations. The milk intake was 410 grams a day, with the amount of milk consumed being negatively associated with iron status. Fourty-five percent of the children showed dimorphic anemia, found by a peripheral blood smear, suggesting folate and vitamin B12 deficiency. Dietary origin and low intake of the vitamins folate and B12 were the main cause of anemia and iron deficiency in this age group (Kapur et al, 2002)

Diet History as a Screening Tool For Iron Deficiency

The use of a diet history questionnaire can be a valuable resource for the clinician to help identify possible risk factors of iron deficiency. Evaluating what the child consumes would help provide a firm foundation for proceeding with further diagnostic tests. There are numerous questions that should be asked about nutrient intake;specific food intake and serving size, amount and kinds of liquid intake, if the child was breast fed or bottle fed, and whether or not the mother was involved with the Special Supplemental Nutrition Program for Women, Infants and Children (WIC).

Bogen et al. (2000) conducted a study that evaluated a parent completed dietary and health history as the first stage of 2-stage screening for iron deficiency anemia (IDA). A cross-sectional study was conducted in inner-city clinics in children 9 to 30 months old, mostly inner city African-American population, having routine screening for anemia as part of their scheduled visit. Parents completed a questionnaire and children had venous blood sampling for a blood count. The questionnaire consisted of 15 items that specifically looked into the diet of the infant; Intake of solid food, intake of beverages and participation in the Special Supplemental Nutrition Program for Women, Infants and Children were considered. Birth history, recent illness, chronic medical conditions, maternal history and any history of anemia were also taken into account. In 282 study subjects the prevalence of anemia (35%), iron deficiency not anemia (7%) and iron deficiency anemia (8%) did not vary significantly by age. Among individual historical and dietary questions, maternal history of anemia and drinking >2 glasses of juice per day identified the highest proportion of children with IDA: 50% sensitivity (95% CI: 16,81) and 77% sensitivity (95% CI: 54,89) respectively. Questions with the highest specificity for IDA were beverage intake (91%; 95% CI: 68,99) and intake of solid food (91%; 95% CI: 68,99). Anemia was defined as Hb < 11.0 g/dl and iron deficiency was defined as ferritin < 10 ug/l or MCV < 70 fl and RDW > 14.5%. In their conclusion, neither individual nor combinations of parental answers to dietary and health questions were able to predict IDA, anemia or ID well enough to serve as a first stage screening tool.

Boutry and Needleman (1996) conducted a cross sectional study based on a review of clinical records. Their study had two aims: The first aim was to investigate the relationship between dietary practices and microcytic anemia among low-income children past the age of weaning. Their second aim was to assess the usefulness of a brief dietary history as a screening tool for microcytic anemia. A total of 305 healthy, African-American inner-city children, with an age range of 15 to 60 months participated in the study. A brief dietary history was taken in the course of primary care visits. Dietary deficiency was defined as: less than five servings each of meat, grains, vegetables and fruit per week, more than 16 oz of milk per day; daily intake of fatty snacks and sweets or more than 16 oz of soft drink. The prevalence of microcytic anemia (Hb <11 g/dl; MCV < 73 fl) was 8% (24 of 305). The prevalence of low Hb (<11 g/dl) with or without microcytosis was 12% (38 of 305). Dietary deficiency was associated with microcytic anemia (chi square=26.8). As a screening test for microcytic anemia, dietary deficiency had a sensitivity of 71% (17 of 24), specificity of 79% (222 of 281), and negative predictive value of 97% (222 of 229). Microcytic anemia was associated with a deficient diet among low-income African-American children. A brief dietary history correctly identified children at low risk for microcytic anemia 97% of the time.

In conclusion, there is some evidence that a record of a dietary history as a first stage screening tool may be used to evaluate some types of iron deficiency and its adverse consequences. To better evaluate and identify this problem, a combined two stage screening process including a dietary history and complete blood work-up would better suit the criteria for establishing and diagnosing iron deficiency, iron deficiency anemia, iron deficiency not anemic or iron sufficient not anemic.

Wharf et al (1997) wanted to determine the effects of dietary, physiological, and environmental factors on body iron levels in infants aged 4-18 months. A total of 181 healthy infants in age groups 4, 8, 12, and 18 months participated in the study at the department of medicine Kansas University Medical Center in Kansas City, Kansas. The design of this study included daily iron intake of the infants measured by a diet history obtained by interview using a standardized question sheet. Capillary blood samples were

analyzed for hemoglobin, mean corpuscular volume, hematocrit, zinc protoporphyrin, and plasma ferritin concentration.

Infants recruited for this study had adequate body iron levels and there was a low prevalence of iron deficiency anemia. Because of this low prevalence of IDA plasma ferritin concentration was selected as the incidence of iron status to be used for the regression analysis to identify predictive factors for body iron content. The authors concluded that hemoglobin and mean corpuscular volume are too insensitive and transferrin saturation requires a larger blood sample. Plasma ferritin reflects iron stores and a reduction in its level is the earliest sign of iron depletion in the body. Iron stores and thus ferritin levels are the last to rise even when iron depletion is reversed, although plasma ferritin levels can be falsely elevated in the presence of infection.

Main determinants of iron stores in the 4 month old infants were birth weight (+ve($p<0.001$) and body weight (-ve($p<0.005$). In the 8 month old infants, main determinants of iron stores were intakes of cow's milk (-ve($p<.05$), belonging to a smoking household (-ve($p<.05$), and quantity of commercial baby food consumed (+ve($p<.05$). Also in this age group there was a gender effect on iron stores (girls>boys, $p<0.01$) and the gender effect remained at 12 months (girls>boys, $p<0.05$) but at 18 months only non-heme iron intake was a significant factor (-ve($p<0l.05$). In concluding, at 4 months of age, birth weight and body weight exerted the greatest influence on iron stores, whereas by 8 months components of the weaning diet influenced iron stores the most (positively associated with baby food, negatively associated with cows milk). There was also a gender effect (girls>boys) possibly reflecting their growth rates. At 12 and 18 months of age the only significant factors were again gender (girls>boys) and non-heme iron intake which was negatively associated with iron stores (Wharf et al., 1997).

Cowin et al (2001) wanted to investigate the association between composition of the diet at 18 months of age and ferritin and hemoglobin levels. It was a cross sectional study with a total of 796 healthy children taking part in the Avon Longitudinal Study of Pregnancy and Childhood at the University of Bristol, Bristol UK. The diet was assessed by a 3 day unweighed food record. A heel prick capillary blood sample was taken for measurement of ferritin and hemoglobin. Ferritin levels were negatively associated with

the amount of cows milk consumed (r =-.02463, p<0.001) and calcium intake (equivalent to a 4-5% drop in ferritin levels for a 100 mg increase in energy adjusted calcium intake). Hemoglobin levels were positively associated with energy adjusted vitamin C intake and were higher in children who consumed any type of fruit (p=0.024) or any type of vegetable (p=0.030). The prevalence of low hemoglobin levels was higher in those children who consumed no meat or poultry (p=0.044). In conclusion, higher levels of milk and dairy product consumption are associated with lower ferritin levels in children of this age and over consumption of these foods should be avoided (Cowin et al, 2001).

So far we have how associations between the diet and nutrient intake and their effects on iron status. We have also seen many different types of methods used in obtaining and diagnosing iron status. In this present study we will look at intakes of specific nutrients with regards to iron status, in similar populations that have been discussed.

The research objectives for the present study were to establish the relationship between iron deficiency without anemia, iron deficiency with anemia, and overall iron status in a population of infants between the ages of six and twenty-four months who are served by the WIC program in rural West Virginia. The research hypothesis for this study are as follows: 1. The prevalence of iron-deficiency without anemia will be greater than that of iron deficiency anemia and some occurrences of anemia will be caused by factors other than anemia: 2. There will be an underlying relationship, whether positive or negative, between iron status and intakes of specific nutrients including iron, zinc, blood lead, number of infections, copper, phosphorous, calcium, folate, ascorbic acid, riboflavin, cobalamin, vitamin B6, vitamin E, vitamin A, protein and total caloric intakes: 3. Health status will also be a predictor of iron status, whether positive or negative.

Study Design and Subject Recruitment

This was a cross-sectional study to determine and analyze the iron status and quantitative nutrient intake of a group of infants in areas of rural WV where the prevalence of anemia is greater than the state average. Dietary patterns, quantitative nutrient intake, and health status was compared among the participants. Subjects for this study were recruited from WIC clinics in selected counties where the prevalence of anemia was greater than 10%. Selection criteria for the participants to be analyzed for the study include being free of disease and within the age limits of 6 to 24 months. The WIC employees notified parents of potential participants that were eligible for the study and gave them direction on how to be enrolled. They gave the participants wanting to participate in the study an addressed stamped postcard to fill out. The parents then sent a postcard to the study coordinator signifying they would like their child/children to participate in the study. Parents allowing their children to participate in the study were also instructed to sign a parental or guardian consent form (Appendix A).

Assessment of Dietary Intake

A graduate research assistant contacted each client via a telephone call, and then set up a time that they were able to meet with them during their next WIC appointment. During the scheduled appointment the research assistant followed a scripted procedure (Appendix B) collecting pertinent information on the child's medical and nutritional history as well as a diet history using a multiple pass 24-hour diet recall (Appendices C-D). A follow up 24-hour dietary recall was obtained by telephone call three to five days later. The multiple pass format allowed the researcher to gather a quick list of foods ingested on a specified day, specific times and occasions the food was eaten, and also the approximate amount of food eaten.

The 24-hour dietary recall was analyzed for nutrient composition using the computerized dietary analysis program Food Processor for Windows version 7 (ESHA Research, 1998) . Nutrient analysis consisted of an average of the two-day intake, and the quantitative intake for all nutrients was compared to the current intake recommendations for this age group as issued by the National Academy of Science and Institute of

Medicine. Dietary sources of iron and quantitative intakes of nutrients that enhance or inhibit iron absorption were also examined.

Assessment of Iron Status

A blood sample was collected on the day specified by an experienced professional. Local blood labs in the area performed the blood draw according to their lab procedures. Six milliliters of blood by a venous puncture was obtained from each child by an experienced phlebotomist and Laboratory Corporation of America performed the lab analysis. Each blood sample was analyzed for a complete blood count (hemoglobin, hematocrit, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], red cell distribution width [RDW]), serum iron, total iron-binding capacity, percent transferrin saturation, and serum ferritin. Iron status was determined by abnormal test results for serum ferritin ($\leq$ 15 ug/L), transferrin saturation ($\leq$ 15%), and anemia defined as hemoglobin (< 11 ug/dl) (MMWR, 1998).

Diagnosis of Iron Deficiency

The diagnosis of iron deficiency is based primarily on laboratory measurements. However, the tests used commonly have limitations due to their poor sensitivity or specificity, or because they are modified by conditions other than iron deficiency. The practice of using a battery of assays improves the precision of defining iron nutrition in a population. However, there are two pitfalls confounding this issue. One is the difficulty detecting mild iron deficiency and the other is identification of inflammation as a cause of changes in laboratory tests results that are not due to iron deficiency.

Ferritin is a storage compound of iron, and serum ferritin levels normally correlate with total iron status. As iron stores are depleted, serum ferritin concentration declines and is the earliest marker of iron deficiency, especially when used in conjunction with other tests to assess iron status. Serum ferritin has high specificity for iron deficiency, especially when combined with other markers such as hemoglobin. However, serum ferritin is an acute-phase reactant that can become elevated in the setting of inflammation, chronic infection, or other diseases.

Transferrin saturation indicates the proportion of occupied iron-binding sites and reflects iron transport rather than storage. Transferrin saturation is calculated from two measured values: serum iron concentration divided by total iron binding capacity (TIBC) expressed as a percent. Transferrin stauration implies low serum iron levels relative to the number of available iron binding sites, suggesting low iron stores. It decreases before anemia develops, but not early enough to identify iron depletion. Transferrin saturation is influenced by the same factors that affect TIBC and serum iron concentration and is less sensitive to changes in iron stores than is serum ferritin. For the present study iron deficiency was defined as both serum ferritin $\leq$ 15 ng/ml and transferrin saturation $\leq$ 15%. Iron deficiency anemia was defined as both serum ferritin $\leq$ 15 ng/ml and transferrin saturation $\leq$ 15% and hemoglobin < 11.0g/dl.

Experimental Design

Subjects were analyzed as a whole group, and also broken down into two age groups: 5.55 to 11.9 months and 12 to 25.65 months. Iron status, as measured by two abnormal lab values, was examined in relationship to dietary intakes of total iron, zinc, copper, phosphorous, calcium, folate, ascorbic acid, riboflavin, cobalamin, vitamin B6, vitamin E, vitamin A, total protein and total caloric intake. Blood lead concentrations and number of infections were also analyzed. The dependent variables were iron status as measured by abnormal values for ferritin and transferrin saturation. The independent variables were the quantitative intakes of each of the specific nutrients.

Statistical Analysis & Interpretation

Assessment of lab values for iron and quantitative intakes of nutrients that enhance or inhibit iron absorption was thoroughly examined. The relationship between these nutrients and results of the iron tests was analyzed by using multiple and univariate regression and Pearson's correlation. Univariate analysis provided insight on how well each independent variable by itself predicted iron status. Multivariate analysis analyzed the relationship as a collection or in combination with all the variables. Pearson's correlation determined the relationship between each independent variable against each dependent variable. All analysis was conducted at alpha level of .05 and if p was less than alpha level than the data was statistically significant.

RESULTS

Sample Characteristics

The number of children enrolled was 87, and 30 were excluded due to lack of a blood analysis. In the end, 57 subjects participated in the study. Iron status was measured within these 57 subjects as a group and also broken down within two age groups (age group 1: 5.55 to 11.9 months (n=18), and age group 2: 12 to 25.65 months (n=39)). Overall 45 children were classified as iron replete, and 12 children were classified as being iron deficient and within these 12 children 4 were anemic. Moreover, the children who were classified as being iron deficient were all older than 11.9 months and thus fell into age group 2 (n=12). Iron deficiency was classified as 2 iron indices being low; serum ferritin $\leq$ 15 ng/ml, iron saturation $\leq$ 15%. As for the anemic children, two were anemic without iron deficiency and two were anemic with iron deficiency. The presence of infection was less than 10% in this population and statistical analysis showed no relationship between health status and iron status.

Hematological mean indices for corpuscular volume, red blood cell distribution width, transferrin, total iron binding capacity and blood lead levels for the group as a whole are shown in Table 1. These mean values are all within normal limits. Anthropometric statistics such as weight, height, age, weight for height z score, height for age z score and weight for age z score are shown for the group in Table 2.

Table 1: Hemotalogical indices of all subjects

	Mean	**s.d.**	**min**	**max**	**n**
MCV (fl)	78.16	3.82	64.00	85.00	50
RDW (%)	13.66	1.31	11.70	18.20	50
Transferrin (mg/dl)	279.16	38.71	200.00	373.00	50
TIBC (mcg/dl)	367.70	75.27	240.00	632.00	56
Blood Lead (mcg/dl)	2.91	1.66	0	7.00	55

*9 subjects didn't have sufficient blood analysis for MCV,RDW and TfR
*3 subjects didn't have sufficient blood analysis for TIBC
*4 subjects didn't have sufficient blood analysis for blood lead levels

Table 2: Sample mean characteristics of the whole group

	Mean	s.d.	min	max	n
Age (months)	15.83	6.02	5.55	25.65	57
Weight (lb)	23.81	4.46	4.50	38.50	57
Length (inches)	31.06	2.72	25.00	36.50	57
WAZ	.2948	1.18	-1.70	4.13	57
HAZ	.09	1.00	-1.75	2.03	57
WHZ	.3802	1.30	-1.35	4.20	57

*WAZ: Weight for age Z score
*HAZ: Height for age z score
*WHZ: Weight for age z score

Nutrient Intakes

Descriptive statistics for all groups for dietary intakes of calcium, phosphorous, iron, zinc, copper, vitamin A, vitamin E, vitamin B12, vitamin B6, riboflavin, niacin, thiamine, protein and total energy are shown in appendices E-G. Milk intake levels for each of the groups are also shown in appendices E-G.

Assessment of Iron Status

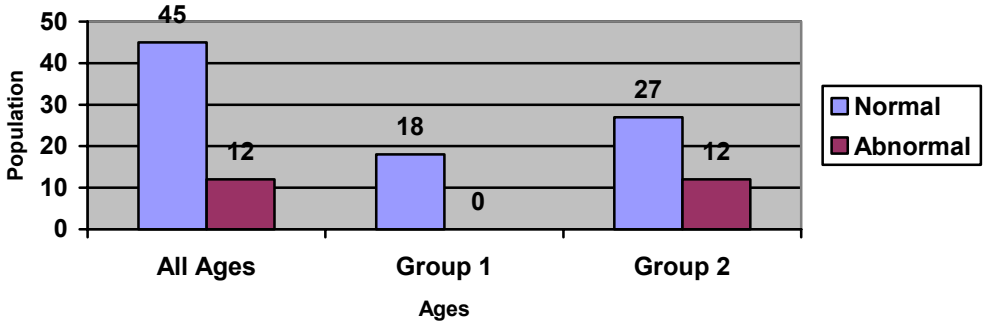
Twelve out of the fifty-seven children were classified as having abnormal iron status (Fig 1, Table 3). When we broke this down further into groups: group 1 (ages 5.5 to 11.9 months) had no children that were classified as being iron deficient, group 2 (ages 12.0 to 25.65 months) had 12 out of 39 subjects classified as being iron deficient (Fig 1, Table 3). Four out of these fifty-nine children also were classified further as being anemic, two were iron deficient with anemia, and two were anemic without iron deficiency.

Table 3: *Mean iron status indices of all subjects*

	Mean	s.d.	min	max	n
Whole Group					
Hb (g/dl)	11.7	0.72	10.10	13.20	49
Ferritin (ng/ml)	27.09	20.89	2.00	86.00	57
Iron Sat (%)	18.00	8.43	4.00	39.00	56
Age Group 1					
Hb (g/dl)	11.58	0.59	10.5	12.7	17
Ferritin (ng/ml)	44.17	22.93	12.00	86.00	18
Iron Sat (%)	19.44	8.47	5.00	36.00	18
Age Group 2					
Hb (g/dl)	11.89	0.77	10.10	13.20	32
Ferritin (ng/dl)	19.20	14.36	2.00	62.00	39
Iron Sat (%)	17.32	8.43	4.00	39.00	38

* Age group 1: 5.55 to 11.9 months
* Age group 2: 12.0 to 25.65 months
* *Whole group: 5.55 to 25.65 months*

Fig 1: Assessment of Iron Status



Abnormal Iron status assessed as Ferritin < 15 ng/ml, Trans Saturation < 15%

The researchers also found specific associations between certain nutrients and serum ferritin. Calcium, phosphrous, folate, vitamin B12, niacin, thiamine, riboflavin, protein measured in grams per day, protein measured in grams per kilogram, total energy and the amount of milk (oz) consumed daily all were shown to negatively affect serum ferritin (table 4). No other nutrients were found to be significantly related to serum ferritin.

Table 4*: Associations between serum ferritin and specific daily nutrient intakes of the Group as a whole (all ages)*

	R value	**p value**
Milk (oz/day)	-0.465	0.0001
Length	-0.423	0.001
Calcium	-0.46336	0.00012
Phosporous	-0.46190	0.00011
Protein (g/day)	-0.39260	0.0015
Protein (g/kg)	-0.316	0.017
Energy	-0.28033	0.03
Folate	-0.28934	0.02
Vit B12	-0.32700	0.01
Riboflavin	-0.38244	0.02
Niacin	-0.34185	0.006
Thiamin	-.026836	0.03

*Pearson Correlations comparing associations between nutrients consumed in the diet and serum ferritin.

* P value significant at less than 0.05 (two-tailed)

** No other significant associations found for any other nutrients*

Table 5: *Associations between serum ferritin and specific daily nutrient intakes of age group 2 (ages 12.0 to 25.65)*

	R value	p value
Calcium	-0.39350	0.01
Vit B12	-0.29939	0.05
Riboflavin	-0.33207	0.03

*Pearson correlations comparing associations between nutrients consumed in the diet and serum ferritin of the children after being grouped by age

*P value significant at less than 0.05

**No other significant associations found for any other nutrients*

There were no significant correlations in age group 1 (ages 5.55 to 11.9 months) after statistical analysis was run on the two age groups sperately. However, within age group 2 (ages 12.0 to 25.65 months), serum ferritin was shown to be negatively affected by calcum, riboflavin and vitamin B12 (Table 5).

Univariate logistic regression analysis of the group as a whole, showed that both phosphate ($p < .03$), and calcium ($p < .01$) were significantly negatively associated with iron status. No other variables were significant. When the group was broken down into the two specific age groups, there were no other significant associations found within the univariate and multivariate models.

The researchers decided to compare the means between the iron deficient group and the iron replete group to give further insight on the role of nutrition in iron status. Independent sample student t-tests revealed that when the means of the two groups were compared there were significant differences between calcium, phosphorous, milk intake, and protein intake (table 6).

***Table 6**: Comparison of the means between the iron deficient group and the iron replete group*

	Mean (groups)		p value	95 CI: of the difference
	Deficient	**Replete**		
Milk (oz/day)	28.67	11.67	0.013	29.78 to 4.22
Protein (g/day)	53.96	39.42	0.020	26.62 to 2.26
Protein (g/kg)	5.0	3.5	0.007	2.46 to 0.44
Phosphorous (mg/d)	1026.75	717.01	0.004	508.96 to 110.54
Calcium (mg/d)	1076.60	758.93	0.005	530.08 to 105.27

*Independent sample student t-test comparing means of iron deficient group with iron replete group

*Significant at alpha level 0.05

**Equal variances not assumed*

Discussion

In industrialized societies, where dietary iron intake and bioavailability are relatively high, there should be, in theory, no iron deficiency. However, children are still vulnerable to iron deficiency due to their high requirements for growth particularly between the ages of 6 to 24 months. Persistent iron deficiency among this age group is often associated with a multitude of negative effects. Children between these ages have a rapid rate of growth and development and because of this, a good supply of iron is needed to provide for this growth.

The aim of this study was to determine if there was an underlying relationship between iron status and intakes of specific nutrients. With regards to the first hypothesis, iron deficiency without anemia was greater than that of iron deficiency with anemia so this hypothesis was accepted. There were certain nutrients found in this study that did affect iron status, so this hypothesis was also accepted. The third hypothesis was rejected due to fact that there was no significant relationship found with health status affecting iron status.

The children in this study were rural infants in West Virginia and all were participating in the WIC program. There were 10 counties that were included in the study all of which would fall into the rural designation of being in the country: Braxton,

Greenbrier, Pocahontas, Hampshire, Morgan, Calhoun, Gilmer, Jackson, Mason and Pleasants. Infants in the WIC program are provided infant cereal and juices at ~four to six months of age. Iron-fortified infant formula is also provided for non breastfed infants until the age of one year. Factors that have been thought to contribute to their success in lowering anemia in the past include: improved iron intake owing to the receipt of vouchers for iron containing foods, extensive nutrition counseling, and frequent screening for anemia.

Children were classified as iron deficient when two iron indices were abnormal (ferritin < 15 ng/ml, iron saturation < 15%). Out of the 57 children in the study, 21% percent of the children in this population were classified as being iron deficient without anemia. Four children were classified as anemic (Hb < 11.0), and out of these two were anemic due to iron deficiency. The children were studied as a group, and also broken down into two age groups. The 12 children that were classified as iron deficient were all older than 12 months and thus fell into age group 2. Therefore, most of the discussion related to groups will be between the iron deficient and iron replete group.

When we talk about iron and an infant's diet one has to consider the subtle differences in the type of milk consumed between the ages of 6 to 24 months. The children in this population were breast fed or received iron-fortified formula until the age of 1 year. Most formulas are fortified with a reduced form of iron and this reduced form of iron is highly bioavailable and to an extent not subject to the inhibitors of iron absorption. This is probably why there were no children less than 12 months of age found to be iron deficient. But this is not to say that these children do not ingest other forms of iron in the diet, since children begin to incorporate solid food into their diets between four to six months of age. Cow's milk for the most part is incorporated into the diet after 1 year of age, and would replace the formula in the majority of WIC children.

Low iron stores represent a state of vulnerability for progression to iron deficiency and iron deficiency anemia. The body's regulation of iron metabolism is very effective in working against the development of iron deficiency if the diet supplies

sufficient absorbable form. Ferritin levels reflect total iron stores and have to decline to low levels before the production of hemoglobin is impaired. Iron stores and thus ferritin levels are the last to rise, even when the iron depletion is reversed. But the main disadvantage of using plasma ferritin is that it can be falsely elevated in the presence of infection. In this study the presence, although only orally stated, of infection was less than 10% and this percent is based on children having infections within a 3 month period prior to being in this study, and statistical analysis showed no difference between the iron deficient and iron replete groups. Previous studies have shown that using ferritin as a iron status parameter for the indication of iron status has been justified (Malope et al, 2001: Gibson, 1999).

Regression analysis showed that the only factors significant for determining iron status were calcium and phosphorous. Both of these nutrients were elevated above the recommended intakes for their respected age groups (appendices E-G). After reviewing the dietary intakes, the analysis showed that the majority of calcium and phosphorous was being supplied to the diet by cow's milk for the children over the age of 12 months.

Milk forms a major part of the diet of young children and might be expected to have an important influence on their iron status. Cow's milk does contain significant amounts of phosphorous and calcium, 227 mg and 290mg per 8 ounces of milk respectively. This comparison was also shown to be significant when the means of these nutrients was compared between the two iron status groups (Table 6).

Indeed, among those children consuming cow's milk the analysis showed a strong negative association between the volume of cow's milk consumed and ferritin levels (Table 4). This might be due either to calcium inhibition of iron absorption (Hallberg et al., 1992) or intestinal occult blood loss, and the possible chelation of iron with calcium-phosphorous complexes. In many of the cases seen when cow's milk has a negative impact on iron status, the age when it is consumed is between 6 and 12 months and the negative affect in this study was seen in children greater than 12 months. In this study, no child consumed cow's milk until the age of one. If one was to consider that these children were drinking milk when consuming meals containing iron and that the iron in these

meals was probably in the non-heme form, it is logical to consider that iron-inhibiting components of the milk would negatively affect iron absorption.

Ferritin was also shown to be negatively affected by the amount of protein consumed which seems reasonable since cow's milk does contain protein. Protein intake for the iron deficient group was measured at 53 grams per day; this is a staggering amount since it is comparable to what a person weighing 145 pounds should have. This association was seen for protein intake measured by grams a day and measured by grams per kilogram body weight. There was also a significant relationship found when the means of the two groups were compared (table 7). By measuring protein intake by grams per kilogram body weight, we can see a better relationship to the amount of protein consumed because protein needs are better addressed by an individuals weight than by age. Protein in the form of animal tissues have been shown to improve iron status by supplying highly available heme iron and by promoting better absorption from the non-heme iron pool (Lynch et al, 1989). But as in this case, cow's milk is not animal tissue, and iron in cow's milk is in the non-heme form which its absorption can be inhibited.

There were many B vitamins that were negatively correlated with serum ferritin (Table 4). Folate and vitamin B12 are usually implicated in nutritional anemias with relation to megaloblastic anemia, and in these cases the two vitamins are usually deficient in the diet. These vitamins act by aiding in DNA synthesis thereby taking part in erythropoesis. Being deficient in either of the two would result in inefficient red blood cell formation which could result in impairing hemoglobin production. Dietary deficiency was not the case in this study, folate and vitamin B12 concentrations in the diet were actually above the DRI's. This increased intake was seen both in the iron deficient group and iron replete group. Riboflavin, niacin and thiamin were all also negatively correlated with ferritin. Riboflavin dependent enzymes are needed to catalyze the removal of storage iron from ferritin, and niacin and thiamin have been shown to result in anemia in various animal models as well. But, as with folate and B12, none of these three vitamins were deficient, all three were also elevated above the DRI's for their respective age groups also. As for all of these B vitamins, there was a positive correlation between calcium and all of the B vitamins that were significant. One would assume that calcium

and those B vitamins were coming from the same source because of their positive correlation with eachother.

Dietary intake data were collected by using a multiple pass 24-hour diet recall. A follow up 24-hour recall was also later obtained. These methods have been shown to be an effective way of obtaining dietary information but again there are many discrepancies that can result from obtaining this information orally, such as, misinformation on specific foods, serving size, types of foods and amount of foods just to name a few.

There were not many noteworthy differences in intake of dietary nutrients between the iron deficient group and iron replete group (Appendices E-G), except for the amounts of phosphorous, calcium, protein and cow's milk consumption. These were all highly significant in determining iron status and ferritin levels. Cow's milk is a poor source of iron, and it also has inhibitors of iron absorption (calcium and phosphorous). Calcium and phosphorous tend to complex and aggregate with non reduced iron in the intestinal lumen and is thus rendered unabsorbable. Protein in most cases enhances iron status but in this case significantly negatively reflected iron status to the high consumption of cow's milk. Intakes of all other nutrients related to iron status were found not to be significant in determining iron status, and serum ferritin. Ascorbic acid intake was above the DRI's for every group, so there were sufficient enhancers of iron absorption being ingested.

Perhaps WIC should start implementing strategies controlling the amount of milk that is available to their clients or counsel them on a designated amount to consume since milk is available for the clients until the children are 5 years of age. They should also inform their clients not to allow consumption of cow's milk when ingesting high amounts of non-heme iron. Instead of drinking milk with their meals they should consume some type of citrus fruit with ascorbic acid, since vitamin C is known to enhance non-heme iron absorption.

Iron deficiency can be eradicated if the cause is that of a dietary nature. People should be aware of the devastating consequences of iron deficiency and anemia, and should be knowledgeable on the methods of stopping it. In an economically challenged population, WIC is the perfect resource to aid in the education of this problem. The

relationship between dietary intake and iron status is a complex and unpredictable one as those foods which are rich sources of iron or the promoters of iron absorption may also be a rich source of inhibitors of iron absorption. The effect of any particular nutrient on iron status will depend on the balance of nutrients in the diet, and on the physiology and lifestyle of the person consuming it.

In conclusion, this study has shown that there were significant associations between nutrient intake and their relation to iron status. This study has shown that calcium and phosphorous appear to be the main determinants of iron status in this population. It was also shown how the intake of cow's milk, the amount of B vitamins and protein ingested were all correlated with indicators of iron status. No other significant differences were found with relation to other nutrients. The researchers conclude that limiting the amount of cow's milk to 24 ounces a day would further alleviate the discrepiences found with iron status. All infants and young children should be encouraged to eat a wide variety of nutrient dense foods in the knowledge that they have a high requirement for iron. By tackling the problem from a dietary perspective, the development of good eating habits, which will continue to provide for the nutrient needs of the growing child is encouraged.

Contributions of Study

This study will add to the collected pool of data on the relationships between nutrient factors and their significance on iron status. The children in this study were from a rural background and that is of significance because most of the literature to date among this same population place these children more from an urban background. Moreover, there needs to be further research among this population and iron status because the majority of findings are still showing conclusive evidence that iron deficiency is still a significant problem in industrialized nations.

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APPENDIX A
(Parental or guardian consent form)

WestVirginiaUniversity

Davis College of Agriculture, Forestry and Consumer Sciences

Parental or Guardian Consent and Information Form

Factors Associated with Iron Deficiency among WIC Infants in Rural West Virginia

Introduction.
I, _____, have been asked to allow my child, _____, to participate in a research study that examines the factors that are associated with iron deficiency among infants who are participating in the Special Supplemental Food Program for Women, Infants, and Children (WIC). Cindy Fitch, Ph.D., RD, who is conducting this research at West Virginia University, has explained the study to me. I understand that the money to pay for this study is being provided by the United States Department of Agriculture.

Purposes of the Study.
I understand that the purpose of this study is to learn more about the risk factors associated with iron deficiency and the relationships among iron, diet, lead poisoning, and infant growth and development.

Description of Procedures.
I understand that this study will be performed at the WIC clinic that my child attends and that approximately 600 children from around the state will be included in the study. If my child participates his/her length and weight will by measured. His or her mental development and motor skills will be measured by a series of tests that are designed for infants. I will be asked to give a history of my child's usual diet. A person who is experienced in drawing blood from infants will draw six milliliters (about 1 teaspoon) of blood from a vein. The amount of iron and lead in my child's blood will be analyzed in a laboratory. These results will be made available to me through the WIC nurse or dietitian and my child's personal care physician. I understand that if my child participates in this study, these interviews and measurements will require about 2 hours of my time.

Benefits.
I understand that this study is not expected to be of direct benefit to my child, but the knowledge gained may be of benefit to others.

Risks and Discomforts.
I understand that my child may experience some pain or discomfort when his/her blood is drawn. There is a slight risk of infection, bruising, and swelling at the site where the blood is drawn.

Division of Family and Consumer Sciences
702 Allen Hall
PO Box 6124
Morgantown, WV 26506-6124
Phone: 304-293-3402
Fax: 304-293-2750

Parental or Guardian Consent and Information Form
Factors Associated with Iron Deficiency among WIC Infants in Rural West Virginia
West Virginia University, Division of Family and Consumer Sciences

Contact Persons.
For more information about this research I can contact Dr. Fitch at 304-293-3402, ext. 1768. For information regarding my child's rights as a research subject, I may contact the Executive Secretary of the Institutional Review Board at 304-293-7073.

Financial Considerations
I understand that there will be no cost to me for participation in this study or for any of the supplies that are used in the study. I understand that I will be compensated $80.00 for the time I spend in the interviews with Dr. Fitch or her student and having my child's blood drawn. If I complete part, but not all, of the interview session, I understand that I will receive $10.00 for my time.

Voluntary Compensation
If my child is injured as a result of this research, treatment will be available. The investigator, sponsor, West Virginia University, or other associated affiliates will not voluntarily provide compensation for his/her injuries.

Alternatives
I understand that I have the option not to allow my child to participate in this research study.

Voluntary Participation
I understand that participation in this study is voluntary. If my child participates, I may withdraw my child from this study at any time. Refusal to participate or withdrawal will involve no penalty or loss of benefits for my child or me, and will not affect my child's participation in WIC services in any way. I have been given the opportunity to ask questions about the research, and I have received answers concerning areas I did not understand. Upon signing this form, I will receive a copy.

Confidentiality
I understand that any information obtained as a result of my child's participation in this research will be kept as confidential as legally possible. I understand that these research records, just like hospital records, may be subpoenaed by court order or may be inspected by federal regulatory authorities. My name or that of my child or any information from which we might be identified may not be published without my consent.

I willingly consent to my child's participation in this research.

Signature of Parent or Guardian	Date	Time
Signature of Investigator or Investigator's Representative	Date	Time

APPENDIX B
(Scripted Procedure & individual intake forms)

24 Hour Recall (Script for In Person Interview)

1. I'd like you to tell me everything (NAME) had to eat and drink all day yesterday, (DAY), from midnight to midnight. Include everything (he/she) ate and drank at home and away, including snacks, drinks, bottles, or breast milk.

[WHEN RESPONDENT STOPS, ASK: Anything else?]

Now I'm going to ask you for more detail about the foods and beverages you just listed. I will be using this notebook to find the specific questions I need to ask. When you remember anything else (NAME) ate or drank as we go along, please tell me.

When I ask about amounts, you can use these measuring guides: the cups and spoons for volume of foods; the ruler for length, width, and height of foods; and the sample baby food jars that we have with us.

2. About what time did (NAME) begin to (eat/drink) (FOOD ON LIST)? [OR CONFIRM IF RECORDED ON QUICK LIST]

3. Please tell me what you would call this occasion. [BREAKFAST, BRUNCH, LUNCH, DINNER, SUPPER, SNACK, FEEDING, OTHER (SPECIFY) - OR CONFIRM IF RECORDED ON QUICK LIST]

4. Did (NAME) have (NEXT QUICK LIST ITEM) at (TIME) with his/her (OCCASION) or was that at another time? (CONFIRM IF OBVIOUS OR RECORDED ON QUICK LIST. IF SAME TIME AND OCCASION, GO TO STEPS; IF AT ANOTHER TIME, ASK QUESTION 2)

STEPS

Step 1	Transfer quick list food to the food/drink column. Check off food in quick list as it is transferred.
Step 2	Go to column 4 for food probes. Be sure to request food labels if respondent cannot answer probes.
Step 3	Go to column 5 for amount question
Step 4	Return to question 2 for next food recorded on quick list.

REVIEW

5. Now let's see if I have everything. I'd like you to try to remember anything else (NAME) ate or drank yesterday that you haven't already told me about, including anything (NAME) ate or drank while waiting to eat.

6. Did (NAME) have anything to eat or drink between midnight yesterday and (NAME'S) (TIME) (FIRST OCCASION)?

7. Now at (TIME) for (THIS OCCASION) (NAME) had (FOODS), did (NAME) have anything else?

8. Did (NAME) have anything to eat or drink between (NAME'S) (TIME) (THIS OCCASION) and (TIME) when (NAME) had (NEXT OCCASION)?

9. Repeat 7 and 8 for each occasion except last occasion. For last occasion go to 10.

10. Now at (TIME) for (LAST OCCASION) (NAME) had (FOODS), did (he/she) have anything else?

11. Did (NAME) have anything to eat or drink after (NAME'S) (TIME) (LAST OCCASION) but before midnight last night?

12. Was the amount of food that (NAME) ate yesterday about usual, less than usual, or more than usual?

13. What is the main reason the amount (NAME) ate yesterday was (less/more) than usual?

14. Now I'd like you to think about all of the plain drinking water that (NAME) had yesterday, regardless of where (he/she) drank it. By plain drinking water, I mean tap water or any bottled water that is not carbonated, with anything added to it.

15. How many fluid ounces of plain drinking water did (NAME) drink yesterday?

16. How much of this plain drinking water came from your home? Would you day all, most, some, or none?

17. What was the main source of plain drinking water that did not come from your home? Was it tap water, water from a drinking fountain, bottled water, or something else?

18. Is (NAME) on any type of formula or special diet for a health-related reason?

19. How often, if at all does (NAME) take any vitamin or mineral supplement? Would you say every day or almost every day, every so often, or not at all?

20. What types of supplements does (NAME) usually take - a multivitamin; a multivitamin with iron or other minerals; combination of specific vitamins? (list all that apply).

Interviewer Observations

Do not read these questions to the respondent.

1. Who was the main respondent for this interview? (Mother, father, sister, brother, grandparent, aunt, uncle, someone else)

2. Who else helped in responding for this interview?

3. Did you or the respondent have difficulty with this intake interview?

4. What was the reason for this difficulty?

Is data retrieval necessary for daycare/baby-sitter or other caretaker? If yes, record information for follow-up phone call.

Individual Intake Forms

ID Number_____

Column1 Quick List of Food Items	√	**Column 2** Time	**Column 3** Occasion
A.		am pm	
B.		am pm	
C.		am pm	
D.		am pm	
E.		am pm	
F.		am pm	
G.		am pm	
H.		am pm	
I.		am pm	
J.		am pm	
K.		am pm	
L.		am pm	
M.		am pm	
N.		am pm	
O.		am pm	
P.		am pm	
Q.		am pm	
R.		am pm	
S.		am pm	
T.		am pm	

ID Number_____ Page 2

Food/Drink with Additions	**Column 4** Description of Food/Drink and Ingredient Amounts	**Column 5** How much of this (FOOD) did (NAME) actually (eat/drink)?
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
18.		
19.		

12. Amount of food Usual Less More

13. Reason _____

15. Ounces of plain drinking water _____

16. Amount from home _____

17. Source of water outside of home _____

18. Special formula or diet?
_____

19. Vitamin or minerals Daily Occasionally Not at all

20. Types of supplements (brand)
_____

Interviewer Observations

1. Main respondent _____
2. Additional respondent _____
3. Any difficulty _____
4. Reason _____
5. Information for follow-up phone call _____

_____

APPENDIX C
(Diet history information sheet)

DIET HISTORY

Infant's Name:_____ Date: _____

Infant's Age: _____ Infant's weight: _____

1. How is your baby's appetite? Good Fair Poor

2. In the past two weeks, has your baby had: Vomiting? Yes No
   Diarrhea? Yes No
   Constipation? Yes No

3. Is your baby taking vitamins or minerals (including iron or fluoride)? Yes No

4. What type of water do you use? City Well Spring Cistern Other

5. Do you feed your baby with: A bottle? Yes No
   A feeder? Yes No
   A cup? Yes No
   A spoon Yes No

6. Do you put cereal or other foods in your baby's bottle? Yes No

7. Is food given to your baby directly from a jar? Yes No

8. How were you feeding you baby when you left the hospital? Breast Bottle Both

9. Are you breastfeeding your baby now? Yes No

10. If you have stopped breastfeeding, how many weeks did you breastfeed?____ Weeks
    Why did you stop? _____
    _____

11. Does the child currently drink cow's milk? Yes No

12. How many glasses of cow's milk does the child drink each day? (Estimated number of 8 ounce glasses of milk per day) _____

13. Was the mother participating in WIC before or while she was pregnant? Yes No

14. Was the mother taking prenatal vitamins? Yes No

APPENDIX D
(Mean nutrient intakes of all subjects)

Table A: *Daily mean nutrient intakes of all subjects (5.55 to 25.65 months)*

	Mean	s.d	min	max	n
Protein (g/day)	42.42	23.02	8.64	149.85	57
Protein (g/kg)	3.70	1.87	1.00	12.0	57
Energy (kcal/day)	1188.00	405.85	521.41	3042.00	57
Calcium (mg/d)	830.48	361.68	204.86	2159.00	57
Phosphorous (mg/d)	784.96	394.07	127.13	2573.00	57
Zinc (mg/d)	6.32	2.57	1.38	19.11	57
Copper (ug/d)	0.55	0.24	0.16	1.77	57
Iron (mg/d)	11.82	5.126	1.62	24.66	57
Folate (ug/d)	173.91	94.31	56.65	515.66	57
Vit B12 (ug/d)	3.16	2.27	0.28	14.80	57
Vit B6 (mg/d)	237.87	144.96	0.61	624.00	57
Vit A (ug/d)	893.68	401.55	16.49	2222.00	57
Vit E (mg/d)	7.8	6.04	1.09	23.56	57
Vit C (mg/d)	113.47	82.46	9.84	540.12	57
Riboflavin (mg/d)	1.72	0.73	0.33	4.61	57
Niacin (mg/d)	16.94	7.56	4.62	49.68	57
Thiamin (mg/d)	1.04	0.43	0.18	2.52	57

APPENDIX E
(Mean nutrient intakes of age group 1)

Table B: *Daily mean nutrient intakes of group 1 (5.55 to 11.9 months)*

	Mean	**s.d**	**min**	**max**	**n**
Protein (g/day)	20.66	7.23	8.64	32.90	18
Energy (kcal/day)	868.05	199.09	521.41	1324.00	18
Calcium (mg/d)	566.73	177.23	238.05	995.13	18
Phosphorous (mg/d)	436.77	167.71	127.13	741.01	18
Zinc (mg/d)	6.05	1.71	1.38	8.64	18
Copper (mg/d)	0.64	0.15	0.43	0.93	18
Iron (mg/d)	14.48	6.00	1.62	24.66	18
Folate (ug/d)	112.23	20.98	58.32	141.58	18
Vit B12 (ug/d)	2.12	0.95	0.28	4.32	18
Vit B6 (mg/d)	228.41	181.60	0.79	593.00	18
Vit A (ug/d)	1009.00	380.22	567.46	1897.00	18
Vit E (mg/d)	13.70	5.70	3.65	23.56	18
Vit C (mg/d)	127.50	59.76	37.45	271.33	18
Riboflavin (mg/d)	1.37	0.53	0.33	2.46	18
Niacin (mg/d)	11.83	5.39	4.62	26.14	18
Thiamin (mg/d)	0.88	0.38	0.18	1.77	18

APPENDIX F
(Mean nutrient intakes of age group 2)

Table C**: Daily mean nutrient intakes of group 2 (ages 11.9 to 25.65 months)**

	Mean	s.d.	min	max	n
Protein (g/d)	52.76	20.57	27.99	149.85	39
Energy (kcal/d)	1340.00	391.08	656.86	3042.00	39
Calcium (mg/d)	958.97	360.02	204.86	2159.00	39
Phosphorous (mg/d)	950.34	361.72	557.32	2573.00	39
Zinc (mg/d)	6.45	2.90	3.53	19.11	39
Iron (mg/d)	10.66	5.22	2.92	22.38	39
Copper (ug/d)	0.51	0.26	0.16	1.77	39
Folate (ug/d)	203.21	101.43	56.65	515.66	39
Riboflavin (mg/d)	1.89	0.75	0.93	4.61	39
Thiamine (mg/d)	1.11	0.44	0.58	2.52	39
Niacin (mg/d)	19.37	7.27	8.61	49.68	39
Vit B12 (ug/d)	3.65	2.54	0.50	14.80	39
Vit B6 (mg/d)	213.86	119.17	0.61	624.00	39
Vit A (mg/d)	838.72	404.30	16.49	2222.00	39
Vit E (mg/d)	5.05	3.83	1.09	18.38	39
Vit C (mg/d)	106.81	91.23	9.84	540.12	39