

# Factors Associated With Iron Status Among WIC Infants and Toddlers in Rural West Virginia

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## Abstract

Iron deficiency severe enough to cause anemia may affect children's ability to grow and learn and, consequently, their lifelong productivity and earnings. This study examined the iron status of infants and toddlers ages 6-24 months with a prevalence of anemia of at least 10 percent participating in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) in West Virginia counties. Blood screening performed especially for this study found that 12 of the 57 infants and toddlers (21 percent) were iron deficient, considerably more than the 4 of 49 (8 percent) with anemia. Because the screening methods routinely performed outside of the study are unable to detect iron deficiency before it progresses to anemia, primary prevention of iron deficiency is the only option that may be universally applied. Expert feeding recommendations—such as introducing iron-rich complementary foods after 6 months of age and limiting consumption of milk among children ages 12-24 months to no more than 24 ounces—are useful for promoting adequate intake of readily-available iron and may help prevent iron deficiency.

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## **Executive Summary**

### **Issue**

Adequate iron status is essential for health and development, and consequently for lifelong productivity and earnings. The WIC Program strives to eliminate iron-deficiency anemia in low income children by providing iron-fortified foods such as infant formula, infant cereals, and adult cereals. Yet several counties in West Virginia have a prevalence of anemia among WIC infants and toddlers (ages 6-24 months) greater than 10% -- higher than the State average, and higher than the 5% that would be expected in a population without iron deficiency. This project examined the iron status in a sample of WIC infants and toddlers in West Virginia.

### **Background**

Iron deficiency develops when iron stores are depleted. Although the terms “iron deficiency” and “anemia” are often used interchangeably, they are not the same. Anemia is a late sign of iron deficiency, but iron deficiency is not the only cause of anemia. Other causes of anemia include infection and inflammation that are unrelated to nutritional status, severe deficiency in intake of certain other nutrients (such as vitamins B6 and B-12, folate, copper, zinc, and protein), and mild hereditary traits such as thalassemia. Iron deficiency severe enough to cause anemia may affect children’s ability to learn and grow, permanently alter behavioral, mental and psychomotor development, decrease resistance to infections, and increase the risk of lead poisoning.

Clinical manifestations of iron deficiency include weakness, muscle fatigue, and decreased cognitive ability -- conditions not specific to iron deficiency. Thus, many children with iron deficiency may not be diagnosed because they are not anemic.

In order to determine the iron status of infants and toddlers and examine the factors associated with iron status, the project recruited parents of infants and toddlers aged 6-24 months in WIC clinics in 10 counties in West Virginia where the prevalence of anemia was greater than 10%. Children were weighed and measured, and parents were interviewed to get a brief medical and feeding history for the child—including whether the child was ever breastfed and for how long, use of vitamin or mineral supplements, dietary intake (collected over 2 days), and information on infections over the past 6 months. Parents were then given a requisition for blood work for the child, to be done in a local hospital or physician's office. After the clinic visit, a check for \$40 was sent to the parent as a thank you gift and compensation for the time they spent participating in the study. Recruitment was very low, however, even after the incentive was increased to \$80.

Infants and toddlers were defined as iron deficient if both serum ferritin and transferrin saturation values were below the criteria established by the Centers for Disease Control and Prevention (CDC); anemia was defined as hemoglobin below 11 grams/liter.

Children with anemia in the presence of iron deficiency were defined as having iron-deficiency anemia; those with low hemoglobin and normal iron status were considered anemic without iron deficiency.

## **Findings**

Iron deficiency was observed among 12 of the 57 (21%) children in the sample for whom laboratory values were available. All cases of iron deficiency were observed among 1-year olds, resulting in a prevalence of 31% among 1-year olds (12 out of 39 toddlers).

This is considerably more than the goal established for Healthy People 2010 for toddlers aged 1-2 years, 5%. It is also nearly 3 times the prevalence of anemia in the sample (4 of 49 children, or 8%)<sup>1</sup>. The much higher prevalence of iron deficiency relative to anemia may be indicative of improvements in iron status in US children: as iron status improves, cases of iron deficiency become milder. In such cases, screening for anemia becomes less effective as a marker for iron deficiency.

High intakes of cow's milk and calcium were associated with poor iron status and low serum ferritin (an indicator of iron stores). While milk and dairy products are excellent sources of nutrients during the second year of life, it is important that they not replace other healthy foods in the diet, especially iron-rich foods. The American Academy of Pediatrics (AAP) recommends that toddlers ages 12-24 months limit milk consumption to no more than 24 ounces per day. Nine of the 39 toddlers (23%) ages 12-24 months drank more than 24 ounces of cow's milk per day, and 5 of the 9 (55%) drank 40-60 ounces per day.

Low meat intake was associated with poor iron status when protein intake was controlled.

Meats are good sources of iron, and can be introduced into the diet after 6 months of age.

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<sup>1</sup> The finding of an 8% prevalence of anemia in counties with a prevalence of 10% or more may be partly due to the small sample and the project's use of venous blood to measure hemoglobin.

However, commercially-prepared jars of baby food “combination dinners” may not be the best way to introduce meats into the diet, since they provide about half the iron and protein per serving than commercially-prepared jars of baby food plain meats.

Vitamin C and iron intakes were not associated with iron status, although iron intake did account for some of the variance in serum ferritin.

There were no discernable relationships between iron status, blood lead levels, and number of infections in the past 6 months. Blood lead levels were related positively to intake of protein and negatively to intakes of phosphorus and vitamin E; iron and calcium intakes were not related to blood lead levels.

## **Conclusion**

Because of very low participation rate in the study, sample size was small and may not be representative of the population. However, the study’s finding of a large prevalence of iron deficiency without anemia is consistent with improvements in iron status in US children.

Iron deficiency was seen exclusively in toddlers aged 12 to 24 months, although infants – particularly those with suboptimal dietary intake—may still be at risk for iron deficiency. The transition to table foods is a vulnerable time for the development of iron deficiency. Current screening methods are unable to detect iron deficiency before it progresses to anemia, so iron deficiency may be a hidden problem among children, especially those

ages 12-24 months. Feeding recommendations from the CDC and the AAP—such as introducing iron-rich complementary foods after 6 months of age and limiting consumption of milk among 12-24 months old to no more than 24 ounces—are useful for promoting adequate intake of readily-available iron and can help prevent iron deficiency.

# **Factors Associated With Iron Status Among WIC Infants and Toddlers in Rural West Virginia**

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## **Introduction**

Adequate iron status is a necessary feature of a healthy, well-nourished population. One of the goals of Healthy People 2010 is to reduce the prevalence of iron deficiency to 5% among children aged one to two years (US Department of Health and Human Services, 2000). Data collected for the 1999-2000 National Health and Nutrition Examination survey (NHANES) indicate that the prevalence of iron deficiency and iron-deficiency anemia in children aged one to two years was 7% and 2% respectively (Centers for Disease Control and Prevention, 2002).

The Centers for Disease Control and Prevention (CDC) monitor the prevalence of anemia in low-income infants (six to 11.9 months of age) and young children using the Pediatric Nutrition Surveillance System (PedNSS). Most PedNSS data come from children participating in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) and reflect hemoglobin values measured in capillary blood using a hand-held instrument (Hemocue Inc., Mission Viejo, CA). The first hemoglobin measurement is done at around six months of age. Anemia is defined as hemoglobin less than the 5<sup>th</sup> percentile value for healthy persons with no evidence of iron deficiency. In infants and toddlers aged six to 24 months, that value is 11.0 g/L (Centers for Disease Control and Prevention, 1998).



Based on data from PedNSS, in 1999 the prevalence of anemia among West Virginia infants younger than 12 months of age was 7.8%, up from 6.8% in 1998. The prevalence of anemia for toddlers aged 12 to 24 months was 8.4 percent. In both age groups, this number is lower than the national average (18.1% for infants younger than 12 months of age and 18.3% for those aged 1 to 3 years) but exceeds the expected prevalence of anemia (5%) for a population of U.S. children without iron deficiency.

A number of counties in West Virginia have a prevalence of anemia that was greater than 10% among WIC children aged six to 24 months. Since iron is generally well absorbed from human breast milk, infant formula, and infant cereal, and WIC provides iron-fortified formula and infant cereal to infants, as well as iron-fortified cereals for children older than 12 months, it is not clear why some infants and young children in the WIC program continue to be anemic. It is possible that some infants and young children are anemic due to factors such as infection or inflammation that are unrelated to nutritional status. Dietary factors that could lead to anemia include inadequate intake of iron or other nutrients required for erythropoiesis, excess intake of dietary inhibitors, a delay in introducing meat into the diet (Roquejo et al., 1999), or excessive intake of unmodified cow's milk.

The objectives of this project were as follows:

1. Determine the prevalence of iron deficiency, iron deficiency anemia (low hemoglobin in the presence of iron deficiency), and normal iron status in a group of

infants and toddlers aged six to 24 months who were served by the WIC program in West Virginia counties where the prevalence of anemia was greater than 10%;

2. Describe dietary patterns and the intake of specific nutrients (iron, calcium, and vitamin C) that influence iron status and serum ferritin in a group of infants and toddlers participating in the WIC program in those counties; and
3. Quantify the relationships among iron status, blood lead levels, number of reported infections during the last six months, and nutrient intake.

## **Background**

### *Iron Status*

Iron is present in all body cells and fulfills several vital functions, including oxygen transport and diffusion and electron transport for the production of energy. Optimal iron status can be defined as sufficient body iron to prevent any limitation in tissue iron supply (Cook, 1999). In the early stages of iron depletion, iron stores are decreased. As long as the production of essential iron-containing compounds is not diminished, there is no apparent physiological dysfunction, but the individual is vulnerable to iron deficiency (Herbert, 1992). Iron deficiency refers to inadequate production of essential iron-containing compounds such as cytochromes and iron-sulfur compounds in the mitochondria of all cells and implies that the vital functions of these compounds are impaired (Dallman and Yip, 1989).

Iron deficiency and anemia are terms that are often used interchangeably, but they are different. Anemia is a late sign of iron deficiency, but iron deficiency is not the only

cause of anemia. Normal synthesis and maturation of red blood cells require a constellation of nutrients that include vitamins B-12, B-6, and folate, copper, zinc, and protein. A severe deficiency of any one of them can cause anemia. More commonly, if not caused by iron deficiency, anemia may be caused by infection, inflammation, or mild hereditary traits such as thalassemia (Yip, 1989).

In determining how to assess iron status, it is important to note that iron is found in two main compartments in the body, storage iron and functional iron. Iron is stored in the liver, bone marrow, and spleen as ferritin. Serum ferritin is in equilibrium with tissue ferritin and represents body iron stores. Infection and inflammation can elevate serum ferritin even in the presence of iron deficiency. Despite this drawback, serum ferritin is often used in epidemiological studies as an indicator for stored iron and a low serum ferritin indicates depletion of iron stores. Individuals with depleted iron stores would be at risk for iron deficiency if their physiological need for iron was greater than their iron intake, but the amount of functional iron may not be impaired. In iron deficiency, stored iron is depleted and transport iron (as measured by transferrin saturation) is reduced (Centers for Disease Control and Prevention, 1998). At this stage, the lack of iron limits the production of red blood cells (as measured by erythrocyte protoporphyrin). Because no single laboratory test is truly reflective of iron status, the CDC used a multiple-indicator model to assess iron status for the 1999-2000 NHANES (Centers for Disease Control and Prevention, 2002). They defined iron deficiency as an abnormal value for at least two of the following three indicators: serum ferritin, transferrin saturation, and free

erythrocyte protoporphyrin. Iron deficiency anemia was defined as low hemoglobin in the presence of iron deficiency.

### *Consequences of Iron Deficiency*

When iron status is compromised, iron stores will be depleted first, then non-storage iron in other tissues will be used before any measurable decrease in hemoglobin. The decrease in iron-containing proteins in the cells is responsible for the clinical manifestations of iron deficiency. These include weakness, muscle fatigue, and decreased cognitive ability (Committee on Nutrition, 1999). These signs are not specific to iron deficiency.

Consequently, many children with iron deficiency will not be diagnosed because they are not anemic. Iron deficiency that is severe enough to cause anemia has adverse consequences that may not be reversible. Iron-deficiency anemia (IDA) has been associated with an increased risk of lead poisoning (Hammad et al., 1996), decreased resistance to infections (Chandra and Saraya, 1975) and permanent alterations in behavioral, mental, and psychomotor development (Moffat et al., 1994; Lozoff et al., 1987).

The interactions between iron status and infection are complex. Infection and inflammation can cause anemia that is unrelated to nutritional status (Yip, 1989). Excess iron stores and iron deficiency are both associated with impaired immune function (Mencacci et al., 1997, Berger et al., 2000). Iron deficiency alters cellular immunity including T lymphocyte proliferation to mitogens and the function of natural killer cells (Walter et al., 1997, Scrimshaw and SanGiovanni, 1997). Cellular immunity is

particularly important for infants because humoral immunity, the other type of immunity, is slower to develop.

The age of six to 24 months is a period of rapid brain growth and cognitive and motor development. A number of studies have documented lower test scores on Bayley Scales of Infant Development in infants with iron-deficiency anemia, and in most of these studies, correction of the anemia did not lead to improvement in test scores (Reviewed by Grantham-McGregor and Ani, 2001).

#### *Factors that Influence Iron Status*

Infants and toddlers aged six to 24 months are particularly vulnerable to developing iron deficiency. They have a rapid rate of growth and blood volume expansion and the need for exogenous iron is high in proportion to body weight. After two years of age, the rate of growth begins to slow and iron stores begin to build up so that the risk of iron deficiency is decreased. Full term infants are born with an iron endowment that is adequate to prevent iron deficiency for about the first four to six months of life. How quickly and to what extent those iron stores are used up depends on the amount of iron stored before birth and the postnatal diet.

Two major factors influence iron absorption - iron stores and the synthesis of red blood cells (erythropoiesis) (Bothwell, 1995). Declining iron stores and stimulation of erythropoiesis both cause iron absorption to increase. Dietary factors play a lesser, but still important role in enhancing or inhibiting iron absorption.

Although the concentration of iron in human milk is low, absorption of that iron is high. The exact mechanism is not known, but is believed to be a low molecular weight component in the whey portion of human milk (Etcheverry et al., 2004). While unfortified infant formula and whole cow's milk have about the same iron content as human milk does, only about 10% of that iron is absorbed compared to about 50% of the iron in human milk. Iron-fortified infant formula is a good source of iron for infants who are not breastfed.

One dietary practice that is known to impair iron status is the introduction of unmodified cow's milk into an infant's diet. Not only is the absorption of the iron in cow's milk very low, but cow's milk may replace foods with higher iron content in the diet, and it may cause losses of small quantities of blood from the gastrointestinal tract (Woodruff et al., 1972). In addition, calcium has been reported to inhibit heme and non-heme iron absorption in animal and human studies (Hallberg et al., 1991; Barton et al., 1983). Epidemiological studies have reported an inverse relationship between milk or calcium intake and serum ferritin (van de Vijver et al., 1999). Pediatric clinical dietitians often see toddlers who consume large volumes of milk to the exclusion of other foods. These toddlers are at risk for iron deficiency.

Other dietary factors that influence iron status include the form of iron (heme or non-heme iron) and the presence of promoters or inhibitors of absorption. Heme iron is supplied in the diet by meats, poultry and fish, and is two to three times more absorbable

than is non-heme iron. Non-heme iron is affected by dietary factors that promote or inhibit absorption. The major promoters are meat proteins and ascorbic acid (vitamin C).

Factors that have been shown in some studies to inhibit non-heme iron absorption include polyphenols in tea and some vegetables and phytates in cereals and legumes. Most of the studies on iron inhibitors were done with single foods, and the inhibitory effects in a mixed meal that also contain factors that enhance iron absorption may be much less important than once believed.

#### *Primary Prevention of Iron Deficiency*

The CDC and the American Academy of Pediatrics have published recommendations for the primary prevention of iron deficiency in infants and toddlers (Centers for Disease Control and Prevention, 1998) (American Academy of Pediatrics, 2004). These recommendations are based on the best scientific knowledge about factors that enhance and impair iron absorption. Although each organization issued its recommendations separately, the recommendations are similar enough that they can be combined. The recommendations are as follows:

- All infants younger than 12 months should receive only breast milk or iron-fortified infant formula for any milk-based part of the diet.
- Encourage exclusive breastfeeding of infants for the first four to six months of life in infants who are breastfed.
- After four to six months, when the infant is developmentally ready, encourage a supplemental source of iron preferably from complementary foods. Iron-fortified

infant cereal and/or meats are a good source of iron for initial introduction of an iron-containing food.

- A breastfed infant who is not able to consume sufficient iron from dietary sources after six months of age should be given iron drops.
- By age six months, encourage one feeding per day of foods rich in vitamin C, preferably with meals, to improve iron absorption.
- Children aged one to five years should consume no more than 24 ounces of cow's milk, goat's milk, or soymilk per day because of the concern that milk will replace iron-rich foods in the diet. Twenty-four ounces of milk per day will meet the dietary intake recommendations for calcium for children from one to eight years of age.

## **Methods**

Infants and toddlers aged 6 to 24 months and their parents were recruited in WIC clinics in 10 counties in West Virginia where the prevalence of anemia was greater than 10%. The WIC clerk or nutritionist informed parents about the study when they came in for nutrition education or their regular visits and gave a stamped, addressed postcard to the parents who were interested. The parents filled out contact information on the postcard and sent it to the investigators. The investigators then contacted the parent and arranged a time to see that child in the WIC clinic. The Institutional Review Board at West Virginia University approved the study and all parents gave informed consent.

At the WIC clinics, the investigators interviewed the parent to get a brief medical and feeding history for the child. Data collected included length of gestation, presence of



chronic or infectious disease, whether the child was ever breastfed and for how long, and use of vitamin or mineral supplements. Dietary intake was assessed using a multiple-pass, 24-hour intake with a standardized script and household measuring utensils. A second 24-hour intake was obtained by phone 3 to 5 days later.

Children were weighed and measured without clothes or a diaper. Weight was recorded in pounds and ounces and length was recorded to the nearest eighth of an inch. Height-for-age, weight-for-age, and weight-for-height z scores were calculated using EpiInfo 2000. For infants who were born prematurely, adjusted age was used to calculate z scores instead of chronological age.

The mother was given a requisition for blood work and instructions as to where the blood would be drawn -- a local hospital or physician's office. While we would have preferred having a trained phlebotomist on site in the WIC clinics, we were not able to identify qualified and willing people to do that in the rural counties in which this study was conducted. All laboratories to which the infants were referred were affiliated with the Laboratory Corporation of America (LabCorp). LabCorp is an independent, national company that provides laboratory analysis for diagnostic, clinical, and research purposes. Approximately 6 ml blood was drawn, divided between an EDTA tube and a serum separator tube and sent to the regional laboratory operated by LabCorp. Blood samples were analyzed for lead by atomic absorption spectrometry, hemoglobin by automatic cell counter, and ferritin by immunochemiluminometric assay. Transferrin saturation was

calculated from measurements of serum iron (colorimetric assay) and transferrin (immunologic assay).

In the interest of minimizing the volume of blood drawn and laboratory expense, we did not measure erythrocyte protoporphyrin. The specificity of erythrocyte protoporphyrin for detecting iron depletion is estimated at 61% compared to 100% for serum ferritin and 93% for transferrin saturation (Centers for Disease Control and Prevention, 1998).

Decreases in transferrin saturation precede changes in erythrocyte protoporphyrin (Cook, 1999).

After the clinic visit, a check for \$40 was sent to each parent as a thank you gift and compensation for the time that they spent participating in the study. During the last 5 months of the study, this incentive was raised to \$80 in an attempt to increase recruitment. At the same time, the method of delivery was changed to provide \$10 in cash at the time of the visit with the remainder to be sent as a check. The cash was provided to enable them to get to the laboratory facility.

### *Iron Status*

Normal iron status was defined as normal values on either serum ferritin (equal to or greater than 15 micrograms/liter ( $\mu\text{g/L}$ )) or transferrin saturation (greater than 15%). The values considered “normal” were based on CDC criteria (Centers for Disease Control and Prevention, 1998). If values for both ferritin and transferrin saturation were below the criteria, the child was considered iron deficient. Anemia was defined as hemoglobin less

than the 5<sup>th</sup> percentile value for healthy persons with no evidence of iron deficiency. In infants and toddlers aged six to 24 months, that value is 11.0 grams/liter (g/L) (Centers for Disease Control and Prevention, 1998). Children with anemia in the presence of iron deficiency were considered to have iron-deficiency anemia. Those with low hemoglobin and normal iron status were considered anemic without iron deficiency.

### *Dietary Intake*

Average intakes from the 24-hour recalls were calculated using Food Processor, Version 7.1 for Windows. There were a small number of children for whom only one day of intake information was available. This day was treated the same as the two-day average for analysis. There was no attempt to account for any variation between weekend and weekday intake.

For infants who were breastfed at the time of the study, it was assumed that the intake of breast milk was 600 ml per day and the mean nutrient content was comparable to that of pooled human milk (National Research Council, 1989). If a breastfed infant was also taking infant formula, the volume of formula consumed was subtracted from 600 ml and the remainder was calculated as breast milk.

Dietary iron, vitamin C, and calcium are nutrients that reportedly affect iron status. The reported 2-day average intake of these nutrients was compared to appropriate Dietary Reference Intakes to determine the proportion of infants who were likely getting adequate intake on the study days. When an Estimated Average Requirement (EAR) was

available, the EAR cut point method was used (Institute of Medicine, 2000). For calcium, and for all nutrients except iron in infants younger than 12 months, there is no EAR established, therefore the Adequate Intake (AI) was used for reference. The AI represents a value that meets the needs of almost all healthy individuals in that group. Although the AI cannot be used to calculate the prevalence of inadequate nutrient intake for groups, if mean intake levels are equal to or exceed the AI it can generally be assumed that the prevalence of inadequacy is low (Institute of Medicine, 2000).

Five dietary factors, based on recommendations from the CDC and the American Academy of Pediatrics (AAP), were examined as independent variables for their influence on iron status:

- consumption of age-appropriate servings of iron-fortified infant or adult cereal
- consumption of age-appropriate servings of meat, fish, or poultry
- consumption of fruit, vegetable, or juice with a meal
- use of a multivitamin/mineral supplement
- age-appropriate use of milk

For infants younger than 12 months of age, “age-appropriate use of milk” was represented by no use of unmodified cow’s milk, since this is a known risk factor for iron-deficiency anemia (Woodruff et al., 1972). For toddlers aged 12 to 24 months, appropriate use of milk was defined as “not more than 24 ounces of milk per day”.

In addition, all intake records were examined for the consumption of tea because the tannins in tea are known to inhibit iron absorption (Charlton and Bothwell, 1983).

There are no specific guidelines for serving sizes for children younger than 24 months of age. A general guideline used by pediatricians and pediatric dietitians is one tablespoon of each food for every year of age (American Academy of Pediatrics, 2004). In addition, USDA's Food and Nutrition Service has issued meal guidelines for the Child and Adult Care Food Program (CACFP) that provides serving sizes for reimbursable meals. We used the more specific serving sizes from the CACFP to establish acceptable serving sizes for use in determining whether the intake recommendations were met or not. The specific serving sizes and types of foods that were considered to have met the guidelines are as follows:

- Iron-fortified cereal - 1 tablespoon of an iron-fortified infant cereal for infants younger than 12 months, 1/4 cup of an iron-fortified hot or cold cereal for those from 12 to 24 months. Cereals listed in the WIC food list were accepted as iron-fortified. Those not on the WIC food list were accepted as iron-fortified if they provided at least 4 milligrams of iron per serving based on information in our nutrient database.
- Meat, fish, or poultry - 1 tablespoon for infants younger than 12 months and 1 ounce for those aged 12 to 24 months. Pepperoni on pizza, meat from canned soups, and commercially-prepared baby food combination dinners were not considered meat servings because they provide minimal amounts of meat. The exact proportion of ingredients in commercially-prepared baby food combination dinners is proprietary

information, but, per serving, they generally provide about 50% of the iron and protein provided by plain meats.

- Fruits and vegetables - 1 tablespoon for infants younger than 12 months and 1/4 cup for those from 12 to 24 months. Baby food desserts and fruit pies, cobblers, and fruited gelatins were excluded.
- Juice - 2 ounces for infants younger than 12 months and 4 ounces for those aged 12 to 24 months. Only 100% juice beverages were included.

Each dietary factor was coded as "yes" (met recommendations) or "no" (did not meet recommendations) and used as a dichotomous independent variable. If the recommendation was met on only one of two days, it was still considered as met.

Independent continuous variables were the growth parameters, number of infections in the past six months, ounces of unmodified cow's milk (averaged over two days), number of servings of meat, fish or poultry; ounces of tea; grams of fiber and protein; and milligrams of iron, calcium, and vitamin C (averaged over two days).

Breastfeeding was coded as a categorical variable, i.e. breastfed for less than six months, breastfed for six months or longer, or not breastfed at all. Iron status was used as an independent variable to test for differences in growth parameters or numbers of infections between children with normal iron status and those who were iron deficient.

The dependent variables were growth, number of infections during the past six months, iron status (normal or deficient), serum ferritin, hemoglobin, and blood lead levels.

Serum ferritin was used as a dependent variable because it represents the storage form of iron and is the first laboratory test to change when iron is depleted. Blood lead levels were of interest in this group because we did not know whether or not these children were being exposed to lead. If they were, those with iron deficiency or with low iron or calcium intake would be at risk for higher blood lead levels.

### *Statistical Analyses*

Student's T-test was used to make comparisons between the group with normal iron status and those that were iron deficient. Backward logistic regression was used to evaluate the dietary variables (dichotomous and continuous) that influenced iron status. All independent variables were initially included in the model. We then dropped the ones that had no significant influence on iron status. This method was used because combinations of variables that are significantly associated with the outcome sometimes show up in a backward regression when they were not apparent in a stepwise logistic regression model. The relationship between serum ferritin and continuous variables (ounces of milk, servings of meat, fish or poultry, number of infections, and dietary intake of specific nutrients) was tested using multiple linear regression. The relationship between nutrient intake and blood lead levels was tested using multiple linear regression. Data were analyzed using SAS, Version 8.0 (SAS Institute, Cary, NC).

## **Results**

Ten counties in West Virginia were identified in which the prevalence of anemia among WIC children was greater than 10% based on data from the 1999 PedNSS. The counties, clustered in four different areas of the state -- west, central, and southeastern parts of the state, and the eastern panhandle—had a total WIC population of 1440 infants and toddlers aged 6 to 24 months. We were able to recruit 87 children from 8 of the 10 counties identified for the study and have at least partial laboratory values for 57. The most common reason that parents gave for not consenting to participate was that they did not want their child to have blood drawn. A venous blood draw is more invasive than a finger stick and is perceived by many people to be more painful. The most common reason that parents gave for participating is that they wanted to know the results of the laboratory tests, particularly the blood lead level.

The complete sample included 32 infants and 55 toddlers, all of them white (Table 1). Mean height-for-age, weight-for-age, and weight-for-height z scores are within normal limits. None of the children were stunted (height-for-age z score less than -2.0) or wasted (weight-for-height z score less than -2.0). Eighteen participants had been born prematurely.



**Table 1. Characteristics of Participants**

|                                  |                                 |
|----------------------------------|---------------------------------|
| <b>Total</b>                     | <b>N = 87</b>                   |
| 6 to 11.9 months/12 to 24 months | 32/55                           |
| Male/female                      | 45/42                           |
| Race                             | White (100%)                    |
|                                  | <b>Mean <math>\pm</math> SD</b> |
| Age in months                    | 14.8 $\pm$ 6.3                  |
| Height in inches                 | 30.2 $\pm$ 3.3                  |
| Weight in pounds                 | 23.1 $\pm$ 4.6                  |
| Mean height-for-age z-score      | -0.03 $\pm$ 1.08                |
| Mean weight-for-age z-score      | 0.25 $\pm$ 1.12                 |
| Mean weight-for-height z-score   | 0.39 $\pm$ 1.25                 |

The 2-day average intakes for various vitamins and minerals were compared to the appropriate Dietary Reference Intakes (DRIs) to determine the adequacy of intake (Table 2)<sup>2</sup>. For nutrients with only an Adequate Intake (AI) reference value (as is the case for most nutrients for infants), mean intake exceeded the AI value (listed in the table). It is likely that the prevalence of inadequacy for these nutrients is low (Institute of Medicine, 2000). For nutrients with an Estimated Average Requirement (EAR), the proportion of children with inadequate intake (intake less than the EAR) is also low for most nutrients. The nutrient most likely to be inadequate is vitamin E in the diets of children aged 12 to 24 months. There was a wide variability in intake of vitamin E in this group. Other studies have also found high prevalence of inadequate intakes of vitamin E among various population groups. Devaney et al (2007) caution that difficulties with nutrient composition databases and dietary assessment methods may underestimate vitamin E intake, and overestimate the prevalence with inadequate intakes.

**Table 2. Dietary intakes appear adequate except for Vitamin E**

| Nutrient          | Age 6 to 11.9 Months<br>N=32 |                      |                 | Age 12 to 24 Months<br>N=55 |                      |                 |
|-------------------|------------------------------|----------------------|-----------------|-----------------------------|----------------------|-----------------|
|                   | Mean Intake (SD)             | Adequate Intake (AI) | # (%) Below EAR | Mean Intake (SD)            | Adequate Intake (AI) | # (%) Below EAR |
| Vitamin A (µg)    | 1178 (485)                   | 500                  |                 | 849 (397)                   |                      | 0 (0)           |
| Thiamin (mg)      | 0.9 (0.4)                    | 0.3                  |                 | 1.1 (0.5)                   |                      | 1 (2)           |
| Riboflavin (mg)   | 1.3 (0.6)                    | 0.4                  |                 | 1.9 (0.7)                   |                      | 0 (0)           |
| Niacin (mg)       | 12 (5.3)                     | 4                    |                 | 19 (7.6)                    |                      | 0 (0)           |
| Vitamin B6 (mg)   | 0.9 (0.5)                    | 0.3                  |                 | 1.2 (0.5)                   |                      | 0 (0)           |
| Vitamin B12 (µg)  | 2.1 (1.0)                    | 0.5                  |                 | 3.7 (2.6)                   |                      | 2 (4)           |
| Vitamin C (mg)    | 129 (63)                     | 50                   |                 | 101 (92)                    |                      | 4 (7)           |
| Vitamin D (µg)    | 6 (5)                        | 5                    |                 | 9 (5)                       | 5                    |                 |
| Vitamin E (mg)    | 15 (7)                       | 5                    |                 | 6 (5)                       |                      | 33 (60)         |
| Folate (µg)       | 126 (54)                     | 80                   |                 | 201 (114)                   |                      | 10 (18)         |
| Calcium (mg)      | 591 (239)                    | 270                  |                 | 1052 (484)                  | 500                  |                 |
| Copper (µg)       | 666 (207)                    | 220                  |                 | 484 (247)                   |                      | 0 (0)           |
| Iron (mg)         | 14 (7)                       |                      | 2 (6)           | 11 (5)                      |                      | 1 (2)           |
| Magnesium (mg)    | 99 (39)                      | 75                   |                 | 157 (56)                    |                      | 1 (2)           |
| Phosphorous (mg)  | 463 (201)                    | 275                  |                 | 942 (353)                   |                      | 0 (0)           |
| Selenium          | 24 (10)                      | 20                   |                 | 44 (21)                     |                      | 1 (2)           |
| Zinc              | 6.5 (3.0)                    |                      | 2 (6)           | 6.5 (3.5)                   |                      | 2 (4)           |
| Energy (kcal/day) | 914 (277)                    |                      | 4(12.5)         | 1312 (393)                  |                      | 3 (5)           |
| Protein (g/kg)    | 3.3 (1.8)                    |                      | 2 (6)           | 4.1 (2.0)                   |                      | 1 (2)           |

*Iron Status*

Partial or complete laboratory values were available for 18 infants and 39 toddlers. Since our outcomes of interest were based on laboratory values, further analyses were done on data from these participants. This group of infants and toddlers is similar to the entire sample in growth parameters and dietary intake. Table 3 shows characteristics and dietary intake for infants and toddlers.

<sup>2</sup> Use of the 2-day average instead of “usual” intake may result in a wider distribution of intakes, and

**Table 3. Characteristics and Dietary Intake of Infants and Children with Lab Values**

|  | <b>Infants (6 to 11.9 months)<br/>N=18<br/>Mean ± SE</b> | <b>Toddlers (12 to 24 months)<br/>N=39<br/>Mean ± SE</b> |
|--|--|--|
| Age (months)                           | 8.4± 0.45  | 19.3±0.55  |
| Weight (pounds)                        | 19.8±0.6   | 25.8±0.6   |
| Length (inches)                        | 27.9±0.3   | 32.6±0.3   |
| Weight for age Z score                 | 0.39±0.3   | 0.27±0.2   |
| Height for age Z score                 | 0.09±0.2   | 0.09±0.2   |
| Weight for height Z score              | 0.41±0.3   | 0.34±0.2   |
| Ferritin (µg/L)                        | 44.2±5.4   | 19.2±2.3***  |
| Hemoglobin (g/dL)                      | 11.6±1.0   | 11.8±1.0   |
| Meat (# of servings)                   | 0.09±0.05  | 1.1±0.1  |
| Unmodified cow's milk (ounces per day) | 0.5±0.5  | 22.5±2.5   |
| Protein (g/kg)                         | 2.4±0.2  | 4.5±0.3  |
| Energy (kcal/kg)                       | 870±205  | 1339±396   |
| Iron (mg/day)                          | 14.5±1.4   | 10.6±0.8   |
| Calcium (mg/day)                       | 554±41   | 956±59   |
| Vitamin C (mg/day)                     | 130±14   | 108±15   |

\*\*\*P<0.0001 compared to younger infants

Table 4 shows the mean values for the laboratory tests and the number (percent) of infants and toddlers that were below the criterion value. In the case of blood lead level, being below the criterion value is preferable.

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overestimate the proportion of children with inadequate or low intakes.

**Table 4. Mean Laboratory Values**

| <b>Lab value</b>         | <b>N</b> | <b>Criterion Value</b> | <b>Mean (SD)</b> | <b>Number (%)<br/>Below Criterion</b> |
|--------------------------|----------|------------------------|------------------|---------------------------------------|
| Serum ferritin           | 57       | 15.0 µg/L              | 27.1 (20.1)      | 26 (46)                               |
| % Transferrin saturation | 57       | 16 %                   | 18.1 (8.4)       | 22 (39)                               |
| Hemoglobin               | 49       | 11.0 g/dL              | 11.8 (0.72)      | 4 (8)                                 |
| Blood lead level         | 55       | <10 µg/dL              | 2.95 (1.6)       | 55 (100)                              |

Hemoglobin was not available for 8 subjects because of insufficient quantities of blood to run all the tests. Of the 49 subjects with complete laboratory values, four (8%) were anemic. This is somewhat lower than the reported prevalence for the selected counties (all greater than 10%). The lower prevalence of anemia could be due to the low number of participants in our study, or to these children self-selecting into the study, but it may also reflect differences in hemoglobin measured in venous blood versus capillary blood. Capillary blood sampling are more prone to false low hemoglobin readings because they sample mostly the blood that has accumulated near the end of the finger or heel, rather than providing a good sample of venous blood (Thomas and Collins, 1982). The Centers for Disease Control and Prevention (CDC) monitor the prevalence of anemia in low-income infants (six to 11.9 months of age) and children using the Pediatric Nutrition Surveillance System (PedNSS). Most PedNSS data come from the WIC program and reflect hemoglobin values measured in capillary blood using a hand-held instrument (Hemocue Inc., Mission Viejo, CA). In contrast, the hemoglobin for this project was based on venous blood samples.

Iron deficiency was defined as having both serum ferritin less than 15 mg/L and transferrin saturation less than 16%. Twelve toddlers, all 12 months of age or older, were iron deficient based on this standard (Table 5). That represents 21% of the entire sample of 57 children ages 6-24 months, but 31% of the 12 to 24 months age group.

Of the four children who were anemic, two were iron deficient (both older than 12 months) and two had normal iron status (one older than 12 months and one younger). Therefore, the prevalence of iron-deficiency anemia was 4.1% (2 of 49) for the entire group and 5.1% (2 of 39) for toddlers 12 to 24 months of age. The one infant who was anemic had low transferrin saturation but normal ferritin<sup>3</sup>. There was no report of a recent infection that may have accounted for an elevated ferritin level, but it is possible that he was truly iron deficient and our tests did not pick it up. There was no difference between male and female children in numbers who were iron deficient.

**Table 5. Iron Status of Participants**

|                      | <b>Normal Iron Status<br/>Number (%)</b> | <b>Iron Deficient<br/>Number (%)</b> | <b>Total<br/>Number (%)</b> |
|----------------------|--|--------------------------------------|-----------------------------|
|                      | 45 (79)                                  | 12 (21)                              | 57 (100)                    |
| <b>Anemia (n=49)</b> | 2 (4)                                    | 2 (4)                                |                             |

Iron status (normal or deficient) was the dependent variable of greatest interest. There were too few children with iron-deficiency anemia in this sample to use that as a dependent variable.

<sup>3</sup> Among toddlers ages 12-24 months, all those with low ferritin and low hemoglobin levels also had low transferrin saturation.

Infant growth is very sensitive to nutritional factors. It is possible that severe iron deficiency can impair growth or that children who are growing poorly due to inadequate intake may be iron deficient for the same reasons. Student's T test was used to compare the group of children with normal iron status to those who were iron deficient. Mean height-for-age, weight-for-age, or weight-for-height z scores were all within normal limits (-2.0 to +2.0). Overall, the iron deficient group was not significantly different from the group with normal iron status in terms of length-for-age, weight-for-age, or weight-for-length z scores. However, when we separated out the toddlers aged 12 to 24 months, there was a significant difference in weight-for-age, but not weight-for-length or length-for-age. Mean weight-for-age z scores were 0.11 in the iron deficient group and 0.42 in the group with normal iron status ( $p=0.04$ ). The mean weight-for-age z scores for both groups were within normal limits.

Immune function is also sensitive to nutritional factors. Severe iron deficiency can impair immune function and lead to increased risk of infections. Likewise, frequent infections can decrease dietary intake and impact iron status. Infection can also affect the identification of iron deficiency by increasing serum ferritin concentrations. There was no difference between the group with normal iron status and those that were iron deficient in number of infections based on parents' report. For both groups the mean was about 1.7 infections per child (range 0 to 4) in the last six months.

Children who were iron deficient took in significantly more calcium ( $1077\pm 87$  mg v.  $759\pm 54$  mg,  $p=0.007$ ), phosphorous ( $1027\pm 76$  mg v.  $717\pm 60$  mg,  $p=0.004$ ), and protein

( $4.9 \pm 0.4$  g/kg v.  $3.5 \pm 0.3$  g/kg,  $p=0.005$ ) compared to those with normal iron status. There were no other significant differences in nutrient intake between groups.

We examined dietary factors (dichotomous and continuous variables) that influenced iron status using stepwise logistic regression analysis (see the listing of variables in the Methods section). The results are shown in Table 6. The models were not adjusted for demographic variables such as age or sex. Whether or not the child met the recommendations from the AAP (dichotomous variables) did not influence iron status, but total amount of milk or meat intake did. For example, in models 1 and 2, ounces of cow's milk and grams of calcium each had a negative impact on iron status. For each one ounce increase in cow's milk intake, the chance of normal iron status decreased by 0.9%. For each one milligram increase in calcium intake, the chance of normal iron status decreased by 0.02%.

In Model 3, meat intake positively influenced iron status while protein intake was a negative influence. If meat intake is held at a fixed value, which implies that the protein is coming from non-meat sources, increasing protein intake by one gram per day decreased the odds of normal iron status by about 0.9%. Likewise, if protein intake is held at a fixed value, each additional serving of meat increased the odds of normal iron status by about 30%. In this model, intake of milk or calcium did not influence iron status.

In Model 4, not breastfeeding at all was associated with better iron status than was breastfeeding for longer than 6 months when controlling for meat and protein intake. It should be noted that each additional serving of meat increased the odds of normal iron status in breastfed infants by about 50%. No other dietary variables significantly influenced iron status.

**Table 6. Dietary Variables Associated with Iron Status**

| <b>Dietary Variable</b>                                    | <b>Odds Ratio</b> | <b>95% Confidence Interval</b> |
|--|-------------------|--------------------------------|
| <b>Model 1</b>   |                   |                                |
| Cow's milk (ounces/day)                                    | 0.94              | 0.899 to 0.989                 |
| <b>Model 2</b>   |                   |                                |
| Calcium (g/day)  | 0.017             | <0.001 to 0.412                |
| <b>Model 3</b>   |                   |                                |
| Meat (servings/day)  | 29.26             | 2.88 to 296.9                  |
| Protein (total g/day)                                      | 0.857             | 0.779 to 0.943                 |
| <b>Model 4</b>   |                   |                                |
| Breastfeeding (not at all v. longer than 6 months)         | 30.3              | 2.1 to 437.9                   |
| Breastfeeding (less than 6 months v. longer than 6 months) | 2.75              | 0.29 to 25.97                  |
| Meat (servings/day)  | 49.6              | 2.98 to 824.4                  |
| Protein (g/day)  | 0.829             | 0.733 to 0.938                 |

We examined the dietary records for intake of tea, another food that interferes with iron absorption. Tea consumption did not influence iron status in our stepwise regression models. Only three children, all older than 12 months of age, consumed any tea on the



recall days and the volumes consumed were small (two to six ounces). However, one of the three children was iron deficient.

Serum ferritin represents the storage form of iron. As such, it is also a dependent variable of interest. It is less important than iron status, because depletion of storage iron does not imply iron deficiency but it does mean that the individual is at risk for developing iron deficiency. The relationship between dietary intake and serum ferritin was tested using multiple linear regression analysis. Intake of protein ( $r^2=0.15$ ,  $p=0.003$ ), calcium ( $r^2=0.15$ ,  $p=0.004$ ), and cow's milk ( $r^2=0.26$ ,  $p<0.001$ ) were negatively associated with serum ferritin and iron intake ( $r^2=0.12$ ,  $p=0.01$ ) was positively associated with serum ferritin. There were no other associations between any dietary or nutrient variables and serum ferritin.

There were no significant associations between nutrient or food intake and hemoglobin. This was an expected finding. Hemoglobin is affected by many factors other than nutrition. It is not particularly sensitive to nutritional status, as it requires a fairly long period of inadequate intake before detectable changes in hemoglobin.

We had a trained psychology graduate student working with us during the first summer to assess the participants' development using the Bayley Scales of Infant Development.

After that time, she was no longer available, nor was anyone else who could administer the test. Twenty children were tested, all within normal limits for all parts of the scale (cognitive, psychomotor, and behavioral). Based on a review of literature, we would only

expect to see developmental delays in children with iron deficiency anemia, not iron deficiency without anemia. Our very limited results were consistent with what others have reported.

Fifty-four children had data on both blood lead level and iron status. The mean blood lead level was  $2.95 \pm 1.6 \mu\text{g/dL}$  with a range from 0 to 7. No child had a blood lead level greater than  $10 \mu\text{g/dl}$ , the cut-off value used by the CDC to recommend treatment. Eight children had blood lead level greater than  $5 \mu\text{g/dL}$ . This level has been associated with cognitive impairment and has been proposed as the new standard for treatment (Lanphear et al., 2000). Mean blood lead level was  $2.8 \pm 1.7 \mu\text{g/dL}$  in the group with normal iron status and  $3.3 \pm 1.7 \mu\text{g/dL}$  in the iron deficient group. The difference was not statistically significant.

Multiple regression analysis of all nutrients revealed that protein was positively associated, and phosphorous and vitamin E were negatively associated, with blood lead levels (Table 7). These 3 factors accounted for about 23% of the variance in blood lead levels. Other nutrients, including dietary iron and calcium, had no impact on blood lead levels.

**Table 7. Nutrients Associated with Blood Lead Levels**

| <b>Nutrient</b> | <b>Parameter Estimate</b> | <b>Standard Error</b> | <b>P value</b> |
|-----------------|---------------------------|-----------------------|----------------|
| Protein         | 0.058                     | 0.02                  | 0.004          |
| Phosphorous     | -0.004                    | 0.001                 | 0.001          |
| Vitamin E       | -0.101                    | 0.03                  | 0.005          |

Children who were breastfed for 6 months or longer (n=8) had higher blood lead levels than did those who were breastfed for less than 6 months (n=17) or not at all (n=32) (4.5  $\mu\text{g}/\text{dL}$  v. 2.9  $\mu\text{g}/\text{dL}$  v. 2.5  $\mu\text{g}/\text{dL}$ , respectively,  $p=0.02$ ). One possible explanation is that the mother's lead burden may be a greater factor in the child's blood lead level than is current lead exposure. Women who were exposed to environmental lead as children tend to deposit that lead in their bones, and that lead is released during periods of bone remodeling such as pregnancy and lactation (Gulson et al., 1998). Alternatively, there may be other differences in environmental risk factors between these two groups.

## **Discussion**

All infants and toddlers in this study were participating in the WIC Program. WIC promotes breastfeeding and provides nutrition education and supplemental foods that are good sources of iron, vitamin C, vitamin A, protein, and calcium – nutrients that tend to be low in the diets of low-income households. The prevalence of anemia has declined country-wide since WIC was implemented in 1974. Improved iron intake during infancy and early childhood, delayed introduction of unmodified cow's milk until one year of age, and increased emphasis on breastfeeding are all factors that are thought to have contributed to the decline in anemia among infants and young children (Fomon, 1987; Yip, 1989).

It is important to note the limitations of this study and how they may have influenced the results. The participation rate was very low at about 4% of the eligible infants and toddlers in the identified counties. This does not allow us to generalize to the population

as a whole. Those that participated may have been different in some very important ways. Their parents may have been more interested in their child's iron status or blood lead levels, or may have been more knowledgeable about nutrition. Dietary intake data that were collected may not reflect usual intake. Using regression analysis in a cross-sectional, observational study is useful for descriptive purposes and for suggesting hypotheses, but does not allow a determination of cause and effect.

In this sample, group, 12 of the 39 toddlers aged 12-24 months were iron deficient, for a prevalence of 31%, almost four times the national average. Two of the 39 toddlers aged 12-14 months were iron deficient and anemic, for a 5% prevalence of iron-deficiency anemia, more than twice the national average for children aged 1 to 2 years in 1999-2000 (Centers for Disease Control and Prevention, 2002). One toddler (of 39) had anemia without iron deficiency, raising the total prevalence of anemia to 8%, although anemia without iron deficiency is likely due to other factors, such as current or recent infection, or statistical anemia (approximately 5%).

Our finding of a high prevalence of iron deficiency without anemia is consistent with the changing picture of childhood iron status. As iron status improves in the population, the cases of iron deficiency become milder, and screening for anemia becomes less effective as a marker for iron deficiency (Schneider et al., 2005).

Iron deficiency was seen exclusively in the children who were aged 12 to 24 months and no longer taking breast milk or iron-fortified infant formula. Iron status is a continuum.

Full term infants have an iron endowment that is generally adequate to prevent iron deficiency for about the first four to six months of life. How quickly and to what extent those stores are used up depends on the amount of iron stored before birth and the postnatal diet. If the intake of available iron is marginally adequate during the first year of life, iron deficiency may not become apparent for several months.

We found no association between current iron intake and iron status, and a weak association with iron stores (serum ferritin). Our findings are similar to those of other investigators using cross-sectional (Soh et al., 2002) and longitudinal (Lind et al., 2003) methods. Soh et al. (2002) reported a positive association between iron intake (estimated by 3-day, weighed food records) and serum ferritin among non-breastfeeding infants and children aged six to 24 months. Lind et al. (2003), in a longitudinal, intervention study, reported a positive association between iron intake (estimated from 5-day food records recorded each month) from 12 to 17 months and serum ferritin at 18 months of age. There was no association between iron intake from six to eleven months and serum ferritin at nine or twelve months.

The transition from primarily a milk-based diet at age six months to a more adult-like dietary pattern after 12 months of age is a time of many changes in sources of nutrients and in concentrations of nutrients in the diet. It is reasonable to note that current iron intake in the children in our study may not accurately reflect prior intake, but our results are similar to those of Lind et al. (2003) that were based on longitudinal data.

Three infants and eight toddlers in our study were taking supplemental iron, either as a part of a multivitamin with iron or alone. The use of these supplements was also not associated with iron status or serum ferritin. This is not surprising, given the absence of an association between current iron intake and iron status. It may be that many of those taking supplements were already taking in adequate amounts of iron from other sources.

Cowin et al. (2001) and Thane et al. (2000) reported that fruit and/or vitamin C consumption was positively associated with serum ferritin, an association that we did not see in our sample. Neither investigator measured iron status. However, Cowin et al. (2001) reported average vitamin C intakes of 41 mg per day (boys) or 37 mg per day (girls) in 18-month-old infants while the average intake in the children in our sample was 108 mg per day and all but two met the EAR for vitamin C. The lack of association between vitamin C and iron status and serum ferritin in our study may be because all participants were taking in more than enough vitamin C to enhance iron absorption. Our subjects were all participating in the WIC program, which provides participants with a supplemental food package that targets particular nutrients including vitamin C and iron. This may account for the better intake in our group compared to that reported by others.

Both volume of unmodified cow's milk consumed and intake of calcium were negatively associated with iron status (normal or deficient) and with iron stores (serum ferritin).

Intake of unmodified cow's milk does not appear to be a major problem among the infants younger than 12 months of age. Although three (16%) had some cow's milk on the days when the diet history was taken, only one of the three had a full serving.

Although mean intake of cow's milk in the toddlers aged 12 to 24 months was not excessive (22.5 ounces per day), 9 of the 39 toddlers (23%) were taking more than 24 ounces per day. In addition, many had other sources of calcium, such as yogurt, cheese, and pudding.

Our finding that calcium intake has a negative impact on iron status and iron stores is consistent with reports by others (Cowin et al., 2001, Thane et al., 2000). This relationship may be due to a variety of factors. Unmodified cow's milk can cause occult blood loss from the gastrointestinal tract of young infants (Fomon et al., 1981), but this effect generally diminishes or disappears in older infants and young children (Jiang et al., 2000). Some investigators have found that calcium interferes with iron absorption in single meals (Monsen and Cook, 1976; Hallberg et al., 1991), but others have found no interference when calcium is part of a mixed diet (Grinder-Pedersen et al., 2004). None of these studies were conducted in children as young as those in our study.

We did find a positive association between meat intake and iron status. This study was cross-sectional in design so we do not have information on history of meat intake or when meats were first introduced. Requejo et al. (1999) reported that preschoolers who were first introduced to meat in their diets after eight months of age were more likely to be iron deficient than were those who were introduced to meat at or younger than eight months. In an intervention study, Engelmann and colleagues (1998) randomly assigned partially breast-fed 8-month-old infants to low meat (10 g/day) or high meat (27 g/day) intake. After two months, mean hemoglobin and ferritin concentrations were significantly

higher in the high meat group, although there was no significant difference in total iron intake. Meat intake seems to play a more important role in maintaining iron status than total iron intake does. This may be related to the proportion of heme iron in meats or to the factor in meat, fish and poultry that enhances absorption of non-heme iron from other foods (or both).

The finding that not breastfeeding was positively associated with iron status, compared to breastfeeding for longer than six months, should not be used to discourage breastfeeding. The rate of breastfeeding was low in our sample. Of the 18 infants younger than 12 months, six (33%) were breastfed initially and two (11%) were still being breastfed at the time of the study (i.e. for six months or longer). Among the 39 toddlers ages 12-24 months, 20 (51%) were never breastfed, 13 (33%) were breastfed initially but for less than six months and six (15%) were breastfed for six months or longer. The American Academy of Pediatrics recommends that breastfed infants be given an exogenous source of iron, either iron-fortified infant cereal or strained meat, at four to six months of age or when they are developmentally ready. They note that recommendations for solid foods are more crucial for breastfed infants than for formula-fed infants to ensure adequate nutrition (American Academy of Pediatrics, 2004). Our finding that breastfeeding for longer than six months was associated with iron deficiency when controlling for current meat intake supports that recommendation.

Traditionally, cereal is the first food that is introduced into an infant's diet, then fruits and vegetables, and only later, at around 8 months, is meat introduced. Some have



recommended that meats be introduced at six months of age before fruits and vegetables are introduced (Krebs, 2000). This is an interesting recommendation that warrants further deliberation. Although none of the infants younger than 12 months of age were iron deficient at the time of the study, suboptimal dietary intake during the second six months of life could contribute to iron deficiency after 12 months of age. Only two infants younger than 12 months had any meat, poultry, or fish that was not a part of a commercially-prepared baby food combination dinner on the days for which we had intake data. Similar findings have been reported in 12-month-old middle class infants in Tennessee (Skinner et al., 1997) and in the Feeding Infants and Toddlers Study (FITS) (Briefel et al., 2004). In FITS, 3% to 4% of infants aged seven to 11 months consumed plain, baby food meats, but 34% to 40% consumed jarred baby food combination dinners, which have less protein and iron per serving than do plain meats, presumably because they contain less meat. Although the infants in our study were not iron-deficient at the time of the study, their dietary patterns may increase their risk for iron deficiency as they get older, particularly if they are breastfed.

While we did not find an association between iron stores and the intake of iron-fortified infant or adult cereal, others have reported a positive relationship (Cowin et al., 2001). Slightly more than half (30/57) of the children in our sample consumed iron-fortified cereals on either day of the dietary recall. This low number was surprising since the WIC program provides such cereals. These cereals are good sources of readily available iron and may help to prevent depletion of iron stores.

### *Blood lead levels*

It is unclear how protein and vitamin E intake may mediate blood lead levels. A search of several databases did not return citations regarding associations between vitamin E and blood lead levels. In rats, low protein diets were associated with higher blood lead levels (Baltrop and Khoo, 1975), but the infants and children in our study had a high protein intake (average 2.3 g/kg for infants younger than 12 months and 4.5 g/kg for those aged 12 to 24 months). These relationships require confirmation in other studies.

The significant inverse relationship between dietary phosphorous and blood lead levels is consistent with other reports (Blake and Mann, 1983). Some investigators have reported associations between blood lead levels and dietary iron or calcium (Mahaffey, 1995). We did not find that association. Our results may be due to differences in lead exposure or dietary intake. Calcium intake in our sample was good with all but three infants meeting the AI for calcium. Our findings are consistent with those of other studies in which the intake of calcium was adequate (Gallichio et al., 2002).

In animal studies, data suggest that low iron diets lead to increased lead absorption (Crowe and Morgan, 1996), probably due to a shared transporter for iron and lead (Bannon et al., 2002). The children in our study likely had adequate iron intake. Only two were taking in less iron than the EAR. Data in humans have demonstrated a significant association between iron deficiency and elevated blood lead levels (Wright et al., 2003). That was not seen in our study. Blood lead levels were generally low in our sample indicating that their exposure to environmental lead was probably low as well (Gallichio

et al., 2002). It is possible that the level of lead exposure in these rural infants may not have been high enough to show significant nutritional effects on lead absorption.

## **Conclusions**

The first objective of this study was to determine the prevalence of iron deficiency, iron-deficiency anemia, and normal iron status in a group of infants and toddlers aged six to 24 months. Although our small sample size (57 infants and toddlers) precludes generalization to the population, the results in this group of children indicate that iron deficiency (21%) is nearly three times more prevalent than anemia is (8%). The prevalence of iron deficiency exceeds the goal for Healthy People 2010 (5% of children aged one to two years).

The greater prevalence of iron deficiency without anemia is consistent with improvements in iron status in US children (Yip, 1989). As iron status improves, the cases of iron deficiency become milder, and screening for anemia becomes less effective as a marker for iron deficiency. Because current screening programs only detect anemia and not less severe cases of iron deficiency, iron deficiency is a hidden problem in this group of toddlers aged 12 to 24 months who participated in the WIC program.

The dietary factors and nutrients that influenced both iron status and serum ferritin were intake of cow's milk and calcium. In our sample, the intake of cow's milk and calcium were negatively associated with iron status and serum ferritin. Although the majority of older infants were not drinking excessive amounts of cow's milk, 9 of the 39 toddlers

(23%) took in more than 24 ounces per day and 5 of them (55%) drank from 40 to 60 ounces per day. While milk and dairy products are excellent sources of nutrients during the second year of life, it is important that they not be allowed to replace other healthy foods in the diet, especially iron-rich foods. That may mean limiting milk and dairy products to three to four servings per day (an amount that meets the AI for calcium in children aged one to eight years) as recommended by the American Academy of Pediatrics.

Meat intake was positively associated with iron status when protein intake was controlled. Children who were breastfed for longer than six months were more likely to be iron deficient when controlling for meat and protein intake. During transition to table foods, infants need a reliable source of dietary iron. Meats are good sources of iron and can be introduced into the diet after six months of age. This is particularly important for infants who are breastfed. However, commercially-prepared jars of baby food combination dinners may not be the best way to introduce meats into the diet since the amount of meat they contain is unknown, but is less than the amount in jars of baby food meats.

The only nutrients that influenced iron status and serum ferritin were calcium and protein, and protein only influenced iron status when meat intake was held constant. Vitamin C and iron intakes were not associated with iron status, although iron intake did account for some of the variance in serum ferritin.

There were no discernable relationships among iron status, blood lead levels, and number of infections. Blood lead levels were related positively to intake of protein and negatively to intakes of phosphorous and vitamin E. Iron and calcium intakes were not related to blood lead levels.

The transition from breast milk or infant formula to unmodified cow's milk and table foods is a vulnerable time for the development of iron deficiency. Most cases of iron deficiency will not be detected with current screening methods. It is important to develop and validate specific, sensitive screening tools for iron deficiency before it progresses to anemia. Until that time, primary prevention of iron deficiency is the only option that can be universally applied. The recommendations from the CDC and the AAP are useful to promote adequate intake of readily available iron and can help to prevent iron deficiency.

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