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Factors Associated with Iron Status in a Hutterite Population

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By

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This dissertation is approved as a creditable and independent investigation by a Candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Dedication

This dissertation is dedicated to my husband Tom and my three children, Hannah, Gavin and Garrett. They have given me so much encouragement to reach my goal. I want to thank them for believing in me when I was facing challenges. It makes me happy to share this accomplishment with them.

Abstract

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Several factors are known to influence the absorption of dietary iron. The type of dietary iron consumed, what foods or supplements are consumed with dietary iron can all influence iron absorption. Understanding these factors can lead to better knowledge of what may or may not influence iron absorption. Obtaining accurate dietary data is important in nutrition research. Food frequency questionnaires can be used to estimate intake over a year time period, however, validation of a food frequency questionnaire against the 24 hour recall is essential for assessing the accuracy of the food frequency questionnaire.

The following chapters of this dissertation present papers that have added to work in the field of iron nutrition. It will also add to the studies of the use of food frequency questionnaires in a rural setting. Factors associated with iron status of a Hutterite population, the contribution of tea to iron status in a rural female population and the validation of a food frequency questionnaire for assessment of calcium and bone related nutrient intake in rural populations are presented.

Table of Contents

List of Abbreviations

Ca – calcium

FFQ – food frequency questionnaire

µg/g – microgram per gram

µg/l – microgram per liter

CRP – C-reactive protein

CV – coefficient of variation

FEP – free erythrocyte protoporphyrin

g/dL – grams per deciliter

Hgb – Hemoglobin

kcal – kilocalories

kg – kilograms

mg – milligrams

mg/d – milligrams per day

mg/dL – milligrams per deciliter

NHANES – National Health and Nutrition Examination Survey

r – correlation coefficient

r^2 – coefficient of determination

RDA – Recommended Dietary Allowances

SD – standard of deviation

S-ferritin – serum ferritin

TS – transferrin saturation

List of Tables

List of Figures

Page

Chapter 1

Background

Iron: A Review of Functions

Iron is essential for all individuals because it is vital for several metabolic functions. About two-thirds of iron in the body is found in hemoglobin in the circulating erythrocytes (1). Hemoglobin functions to transfer oxygen from lung to tissues. It has the capability of becoming almost fully oxygenated during the rapid erythrocyte transit time in the pulmonary circulation and then to become mostly deoxygenated as erythrocytes pass across tissue capillaries (2). With moderate anemia, hemoglobin becomes more efficient to take up enough oxygen to provide adequate amounts of oxygen to tissues, despite the reduced oxygen-carrying capacity of blood. However, with severe anemia, the noticeably reduced hemoglobin content decreases oxygen delivery and can lead to persistent lack of oxygen for the tissues leading to fatigue and exhaustion (2). Iron is involved in respiration and energy metabolism reactions including the iron-sulfur complexes of NADH dehydrogenase and succinate dehydrogenase that are required in the electron transport chain. Enzymes involved in the tricarboxylic acid cycle, the gluconeogenic pathway and in DNA synthesis require iron to function as well.

Absorption and inhibitors of iron absorption

The amount of iron stored in the body is well controlled. In general there is very little lost each day unless there is bleeding such as with menstruation or with injuries (3). Three main factors affect iron balance and metabolism: iron intake, iron stores and iron loss. Iron absorption is influenced by several factors including the dietary iron content of the food and meal, the bioavailability of dietary iron, the amount of storage iron and the rate of erythrocyte production (4). Individuals at different life stages absorb differing amounts of iron to maintain iron balance depending on their needs. For, example, adult males need to absorb only about 1 mg/day to maintain iron balance. The average requirement for women of childbearing age is somewhat higher, approximately 1.5 mg/day. Nearing the end of pregnancy, the absorption of 4 to 5 mg/day is necessary to sustain iron balance (1,4).

The bioavailability of iron plays a large role in how well iron is absorbed. Heme iron comes primarily from hemoglobin and myoglobin in meat, poultry and fish. This iron makes up a smaller proportion of iron in the diet, however it is highly bioavailable and is less affected by other dietary factors that may inhibit absorption (4). Nonheme iron consists mainly of iron salts and is absorbed two to three times less efficiently than heme iron. Nonheme iron is found mainly in plants, dairy products and iron-fortified foods. Absorption of nonheme can be influenced by enhancers and inhibitors consumed during the same meal (5). The absorption of nonheme iron depends on the breakdown of the iron salts in the acid

environment of the stomach and ascorbic acid enhances this process (6,7). Factors in meat, fish or poultry products enhance nonheme iron absorption. Hallberg and colleagues found that the addition of 75g of meat to a traditional Latin American meal composed of maize, rice and black beans increased the nonheme iron absorption by 2½ times (8).

Polyphenols (present in tea and some vegetables) have been identified as an inhibitor of nonheme iron absorption (6). Several studies have been completed to show that tea polyphenols inhibit iron absorption (9-13). One study showed that absorption of iron from a breakfast consisting of moderately strong black tea and wheat rolls made from unfortified flour was significantly less compared to the group that consumed the meal with coffee and orange juice (14). Comparable findings were demonstrated when a standard meal composed of a hamburger, string beans and mashed potatoes was consumed with strong tea (13). However, other investigations have found differing results and have concluded that tea consumption was not associated with the iron status (15-18).

Life Stages and Iron Needs

Infants and Children

Numerous factors play into the iron status of an infant. The transfer of iron from mother to fetus occurs mostly during the last trimester of gestation and is stored mainly in the liver and bone marrow. Therefore, the amount of iron present at birth depends on the duration of the gestational period and the weight of

the baby (19). Depending on length of gestation and birth weight, an infant is born with iron stores to help meet its needs until 4 to 6 months of age (20). Iron stores are usually depleted by 6 months of age; this occurs due to the rapid rate of growth when the infant doubles in body weight by 3 to 4 months of age (21). By 6 months of age, most infants have marginal iron stores even when iron intake is regarded as adequate. It is not until 2-3 years of age when the growth rate has slowed down considerably that iron stores start to build up again (21).

Therefore, the timing of the introduction of complementary foods plays a role in the development or prevention of iron deficiency. The bioavailability of iron in breast milk is high, however, its absolute concentration is low, and as a result exclusively breast-fed infants have to rely on their own iron reserves to meet their needs. The introduction of complementary iron rich foods should occur between 4 to 6 months of age to help meet the infant's needs (22). The type of complementary food offered the infant at this stage is important since it has a significant influence on the iron status. Typical grain-based or rice-based complementary foods are poor sources of iron and they may contain phytic acid, which is an inhibitor of iron absorption (23). Iron fortified infant cereals are processed with electrolytically reduced iron to ensure absorption. Research has shown that iron-fortified cereals can prevent iron deficiency anemia (24).

In addition to the rapid growth that occurs with infants and young children, there are several other factors that can make this population more susceptible to iron deficiency. For example, the foods they eat may have a

relatively low iron content, they may have food dislikes especially for iron rich foods, and they may want to drink more juice or cow's milk instead of consuming foods of superior iron content.

Adolescents

Iron needs increase during puberty. Adolescent males gain an average of 10 kg during the peak year of their growth spurt, and their hemoglobin concentration increases at the same time toward values of 14 g/dL (25). More iron is needed to supply the extra blood needed in the larger body size, especially during the growth spurt (21). Adolescent boys generally have a good appetite to meet the extra iron needs.

On the other hand, iron deficiency can be a concern for the adolescent female population. The additional requirement for adolescent females are relatively high due to several factors including the replacement of blood lost during menstruation, a growth spurt that leads to an increase in hemoglobin mass and an increase in tissue (nonstorage) iron (25). Adequate calories in the diet including heme-iron rich food sources should allow females to meet iron requirements.

Women of childbearing age

Iron requirements increase for women of childbearing age to help support blood losses occurring during menstruation. Additionally, during pregnancy, iron

requirements increase due to several factors including more iron that is needed to supply the expanding blood volume of the mother and the rapidly growing fetus and placenta. Extra iron is also necessary to help build iron stores for the mother and fetus, to support an increase in hemoglobin mass and tissue growth, and to make an extra supply in the preparation of blood loss during delivery (21,26). The amount of iron required during the last half of pregnancy is difficult to meet by diet alone, therefore there is risk for iron deficiency (27). Healthy diets which include foods high in heme-iron are important to meet these needs. Iron supplementation is often necessary to meet increased iron needs during pregnancy (27). Married Hutterite women of childbearing age are known to have several pregnancies and they can be spaced closely. This can have a big implication on their iron status and the health of the child.

Iron deficiency

Iron deficiency is the most common nutritional deficiency in the United States, affecting mainly older infants, young children, and women of childbearing age (28). According to the results of the Third National Health and Nutrition Examination Survey (NHANES III, 1989-1994), 9% of children younger than 3 years have evidence of iron deficiency based on iron biochemistry tests, and a third of them are also anemic.

Assessment of iron status

Hemoglobin concentration is the most widespread test used for screening for iron deficiency because of its low cost, the simplicity and speed of the procedure. However, as an indicator of red blood cell population turnover, hemoglobin only detects the late stages of iron deficiency; mild iron deficiency may not affect the hemoglobin to screen for iron deficiency (27). Hemoglobin concentrations are used as a measure of advanced iron deficiency anemia which can have serious negative consequences on health, mental development and work capacity (29-32). Several laboratory tests can be used to assess iron nutrition status, including serum ferritin, free erythrocyte protoporphyrin, zinc protoporphyrin/heme ratio, transferrin saturation, and transferrin receptors. The use of these tests can indicate different aspects of iron metabolism and can differentiate the iron nutritional status ranging from overload to severe deficiency. A decrease in serum ferritin is the first indicator of impaired iron status. It usually indicates reduced liver iron stores. The progression to iron deficiency continues with increased transferrin and protoporphyrin. The most severe deficiency is anemia which is characterized by low hemoglobin (33). There are numerous causes of anemia other than iron deficiency, most notably chronic infection and even mild inflammatory disease (32). For that reason the additional iron status indicators are needed along with hemoglobin to accurately diagnose iron deficiency anemia (34). Low serum ferritin without other abnormal iron tests results only indicates exhausted iron stores. Low iron stores are an essential but

not sufficient condition for iron deficiency. The common cutoff for serum ferritin to identify low iron stores is <10 μg/L for younger children and <12 μg/L for adults (34). In cases of significant or chronic inflammatory conditions, serum ferritin is not useful as an indicator of body iron stores. Serum ferritin is an acute phase reactant and can be significantly elevated by a variety of conditions including mild infections (35,36).

Free erythrocyte protoporphyrin (FEP) is the precursor of hemoglobin; it becomes elevated when the iron supply is insufficient for hemoglobin production (37). Measurement of FEP appears to respond earlier to iron deficiency than does the lowering of hemoglobin concentration. Therefore, FEP can be used as a screening test for iron deficiency in both children and adults, including during pregnancy (38). Mei and colleagues (2003) found that FEP was a better screening tool than hemoglobin in US children aged 1-5 years (37). Free erythrocyte protoporphyrin has limited interpretation because any condition that can result in increased erythrocyte turnover, such as hemolytic anemia or in blockage of heme synthesis other than lack of iron, such as an inflammatory condition, can also result in elevated FEP (39). C-reactive protein (CRP) is an acute phase reactant and CRP level measurements are commonly used to help in the diagnosis of bacterial infections. CRP is analyzed in order to exclude participants with infection, which may cause falsely high serum ferritin values or elevated FEP (36).

Effects of iron deficiency

There are several health consequences of iron deficiency. The most recognized outcome is anemia which is an indication of the severity of iron deficiency (40). The prevention of iron deficiency in children is essential. Iron deficiency anemia has a great impact on psychomotor development, behavior and learning abilities of infants and children of all ages. Researchers have found iron deficiency anemia in young children is related to impaired psychomotor development as well as changes in behavior (29,30). Several studies have demonstrated that even moderate anemia (hemoglobin <10 g/dL) is associated with depressed mental and motor development in children that may not be reversible (29,30,41). Anemia can have a negative effect on learning at all ages. Researchers have demonstrated lower standardized math scores among iron-deficient school-aged children and adolescents, including those with iron deficiency without anemia (42). Even though there is evidence that some of the developmental deficits can be corrected with iron treatment, several studies have provided evidence that differences associated with iron-deficiency anemia in infancy are long-lasting (29). Most follow-up studies have been at early school age (4-8 years), with generally similar results: children who had iron-deficiency anemia in infancy tested lower than peers in overall mental functioning (43). In another follow-up study, 11 to 14 year old children in Costa Rica who had been treated for severe, chronic iron deficiency in infancy still tested lower in arithmetic and writing achievement than their peers who had good iron status in

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Chapter 2

Factors Associated with a Hutterite Population

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Factors Associated with Iron Status in a Hutterite Population

Abstract:

Objective: To describe the iron status and its relationship to food composition, dietary and supplemental iron intake, tea consumption and factors that may influence the iron status of a rural population in South Dakota.

Design: Cross-sectional study. This study examined whether there is a relationship between dietary factors or other factors that influence iron status.

Subjects: Convenience sample of 407 Hutterites living in rural South Dakota.

Main outcome measures: Hemoglobin was expressed as milligrams per deciliter. Serum ferritin was expressed as micrograms per liter. Free erythrocyte protoporphyrin was expressed as micrograms per gram hemoglobin.

Statistical analyses performed: Differences between group means were tested using Students t test, chi-square analysis and Wilcoxon test. Multiple regression models were developed to assess the prediction of iron status indicator concentrations to factors that influence iron status controlling for variables with relevance to the main outcome measure.

Results: Mean hemoglobin, mean serum ferritin, and mean free erythrocyte protoporphyrin values were normal for the population; however there were differences in hemoglobin concentrations between genders within certain age groups. Dietary intake showed inconsistencies among age groups studied. Nonheme iron made up significantly more dietary iron than heme iron ($p < .001$).

Percent nonheme iron had a small negative relationship with hemoglobin ($r^2 = .06$, $p < .001$). Percent heme iron had a significant positive association with hemoglobin ($r^2 = .04$, $p < .001$). Multiple regression analysis showed that vitamin C had a small significant relationship with hemoglobin (partial $r^2 = .02$, $p = .003$). Other factors did not have an influence on iron status indicators.

Conclusions: Based on median hemoglobin, serum ferritin and free erythrocyte protoporphyrin iron status of the population was good. Factors that influenced hemoglobin included percent heme iron, percent nonheme iron and vitamin C.

Factors Associated with Iron Status in a Hutterite Population

Introduction

Iron deficiency is the most common nutritional deficiency worldwide. Although iron deficiency is more prevalent in developing countries, results from the most recent National Health and Nutrition Examination Survey (NHANES 1999-2000) indicated the estimated prevalence of iron deficiency in young children aged 1 – 11 years was 4 – 7% and in adolescent and adult females aged 12 – 49 years was 9 – 16%, similar to NHANES III 1988-1994 (1).

Infants and young children are deemed to be predominantly at risk, due to rapid growth and the diet they consume may have a relatively low iron content, especially when iron is not provided through fortification or supplementation (2). In infants and toddlers, iron deficiency is a particular concern because it can adversely affect child development and behavior and some of this cannot be reversed with iron therapy. Several studies have demonstrated that even moderate anemia (hemoglobin <10 g/dL) is associated with depressed mental and motor development in children that may not be reversible (3-5). Iron deficiency can be a concern for the adolescent female population. Iron needs for adolescent females are relatively high due to several factors including the replacement of blood lost during menstruation, a growth spurt that leads to an increase in hemoglobin mass and an increase in tissue (nonstorage) iron (6-9). Adolescent males also have an increased need for iron to support the increase in muscle mass during the growth spurt (8). Women of childbearing age, especially those who are pregnant are at an

increased risk to develop iron deficiency. During pregnancy, iron requirements further increase to support the needs of both the mother and the growing fetus, to help build iron stores for the mother and fetus, to support an increase in hemoglobin mass, and in preparation of blood loss during delivery (10). Iron is mobilized from maternal stores during pregnancy, resulting in stores remaining low for several months after delivery (11). Therefore it is safe to assume that women who have closely spaced pregnancies are at a higher risk to develop iron deficiency anemia because they may not be able to build up adequate iron stores between pregnancies.

This study focused on the iron status of selected residents of the Hutterite colonies in South Dakota. The Hutterites are a religious group of German descent. They live in isolated colonies and believe in self-sufficiency including producing most of the food that they consume (12). Hutterite eating habits have not been widely studied with respect to dietary iron intake. The main protein sources in the Hutterite diet include poultry, game, and pork which contain iron, but not in as large amounts or as highly absorbable as red meat. Tea, which contains polyphenols that inhibit absorption of nonheme iron, is consumed at most any age. Rice and grain products, which contain phytic acid that also inhibits absorption of nonheme iron, are consumed as well.

The objectives of this study were to describe the iron status and its relationship to food composition, dietary and supplemental iron intake, tea

consumption as well as factors that may influence the iron status in a sample of healthy Hutterites living in South Dakota.

Materials and Methods

Sample and Study design

A convenience sample of four hundred seven (307 females and 100 males) Hutterites from 10 colonies in rural South Dakota participated in the study. The sample was not necessarily representative of the eastern South Dakota Hutterite community at large. The study used a cross-sectional design. Enrollment criteria included being Hutterite and having no overt signs of infection at the time of the visit. Most of the participants were part of a longitudinal study of lifestyle factors on bone mass accretion. The rationale, design and methods of the study as well as selection criteria of the participants have been described in detail elsewhere (13). On the day of the study, participants were provided with a written and oral description of the study and then signed informed consent was obtained. For children with parental consent, child assent was acquired by a research team member using an approved script. Participants were asked to complete a questionnaire capturing demographic and pertinent health information and a food frequency questionnaire (FFQ). All participants were in good health and had no history of disorders known to influence iron status such as inflammatory bowel disease, peptic ulcer disease, inflammatory disorders, cancers and liver disease which may cause increased blood loss and consequently increased iron loss. The

study design and protocol was approved by the South Dakota State University Institution Review Board.

Blood sampling and laboratory analysis

Capillary blood samples were taken in the non-fasting state for measurement of hemoglobin (Hgb), serum ferritin (S-ferritin), free erythrocyte protoporphyrin (FEP) and C-reactive protein (CRP) by trained research team members. Hemoglobin was assayed on site using the B Hemoglobin photometer (Hemocue AB, Ängelholm, Sweden) so participants could get preliminary results. The B Hemoglobin photometer was tested daily against the kit control cuvette to verify accuracy. The capillary blood samples were processed and the serum was spotted in duplicate on filter paper for S-ferritin, FEP and CRP and transported to a USDA Human Nutrition laboratory for analysis. S-ferritin, FEP and CRP were analyzed on an IMMULITE Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). The overall precision of the methods used, reported as CV%, was as follows: S-Ferritin 10%, FEP 25%, Hgb 10% and CRP 10%. CRP was analyzed to serve as an indicator of infection, which may cause falsely high S-ferritin values.

Dietary survey

A questionnaire was developed to capture information regarding factors that could influence iron status. Trained interviewers administered the questionnaires to the participants. A parent or other caregiver assisted children less than 18 years to answer the questionnaire. The consumption of foods that are thought to be good sources of iron such as meat products and fortified grains, the consumption of iron absorption enhancing foods such as citrus fruits and juices, and the consumption of drinks that may inhibit iron absorption, particularly tea were queried. Tea consumption was estimated in cups, and time periods when tea was consumed was reported (with meals or in between meals). Participants were asked if they consumed any vitamin or mineral supplements. If they responded yes, the amount of iron contained in the supplement was recorded. Dietary intake was collected by a brief self-administered food frequency questionnaire (FFQ) designed to be less of a burden on the participants than using a lengthier FFQ that would capture total dietary intake of each participant. The FFQ was developed to target foods high in iron. The FFQ estimated the average amount of dietary iron consumed on a daily basis. The method used to develop the FFQ food list was based on standardized FFQ development (14). Food composition tables (15,16) were used as a basis for the FFQ and the food list was reduced to foods that were commonly consumed by the participants. The food list contained usual portion sizes and the amount of dietary iron of each food item. The participants reported on their frequency of consumption choosing from daily, weekly or monthly

options. The FFQ was completed for children less than 18 years with the help of a parent or other caregiver. Dietary iron intake data did not include nutrients obtained from vitamin and mineral supplements.

Statistics

All statistical procedures were performed using the JMP IN statistical software package (SAS Institute, Version 7.0.1, 2007, Cary, NC). Data were tested for normality using the Shapiro-Wilk W Test. Hemoglobin and free erythrocyte protoporphyrin distributions were skewed, and were log transformed for analysis; however they are presented in tables in the non-transformed form. Significance of differences between laboratory values among the different age groups and sexes were tested using ANOVA, t-tests and Tukey-Kramer HSD. Dietary variables were not normally distributed, therefore, the significance of differences were tested using the Wilcoxon test and chi-square test. Multiple regression models were developed to assess the prediction of iron status indicator levels to factors that influence iron status controlling for variables with relevance to the main outcome measure.

Results

Laboratory measures

Four hundred seven participants provided blood samples for the analysis of Hgb, S-ferritin, and FEP to describe iron status. CRP was measured to identify

possible inflammation. Of those samples, 403 Hgb, 127 S-ferritin, 254 FEP and 337 CRP were deemed valid based on duplicate sample coefficients of variation (CV) less than 25%. The invalid samples were due to obtaining insufficient amounts of sample for accurate analysis leading to large coefficients of variation. None of the participants had CRP >10 mg/L which may indicate an infection, therefore, all samples were utilized.

According to Cook (17), abnormal values for the laboratory measurements are as follows: Hgb less than 11 g/dL for children younger than 6 years, less than 12 g/dL for children from 6 to 11 years and females older than 12 years, and less than 13 g/dL for males older than 11 years; S-ferritin less than 10 µg/l for children less than 6 years and less than 12 µg/l for males and females older than 6 years; FEP greater than 3µg/g Hgb for all ages. The median and range values for Hgb, S-ferritin, FEP and CRP are in Table 1. The 2 infants less than 1 year of age who participated had median Hgb below the cutoff (<11 g/dL). The mean log values for the infants less than 1 year of age were significantly lower than the children between 1 and 3 years of age and 4 and 8 years of age (both $p < .001$). The mean log values for Hgb, S-ferritin and FEP for children between 1 year and 8 years were normal (cut off defined as Hgb < 11 g/dL, S-ferritin < 10 µg/l, FEP >3µg/g Hgb) and did not differ significantly (Hgb $p = .10$, S-ferritin $p = .92$, FEP $p = .29$) between the 1 to 3 year old and the 4 to 8 year old age groups. The males and females in the 9 to 13 year group had normal median values for all three indicators (cut off defined as Hgb < 12 g/dL, S-ferritin < 12 µg/l, FEP >3µg/g

Hgb). The mean log values did not differ significantly (Hgb p= 0.30, S-ferritin p = 0.70, FEP p = 0.64) between males and females in the 9 to 13 year group. Males and females 14 to 18 years of age had normal median Hgb and median FEP (cut off defined as Hgb < 12 g/dL, FEP >3μg/g Hgb); however, the mean log values for Hgb for males was significantly higher than females in the same age group (p < .001). The mean log values of FEP did not differ significantly between genders (p = .28). The median S-ferritin were normal (cut off defined as S-ferritin < 12 μg/l) for females 14 to 18 years old. Males between the ages of 19 to 50 years had significantly higher mean log values for Hgb and S-ferritin than females between ages 19 to 50 years (both, p <.001). Mean log values for FEP for the 19 to 50 year old males and females did not differ significantly (p=.09). In the 51+ age group, median Hgb were normal for both genders. The mean log values for Hgb differed significantly (p <0.001) between genders. The mean log values for S-ferritin and FEP did not differ significantly (p = .10 and p = .59. respectively) between genders.

Abnormal laboratory values

The percentage of participants with abnormal values for the iron indicators according to age and sex are shown in Fig. 1. Values were considered abnormal according to the guidelines defined in the preceding paragraph. Low hemoglobin concentrations were more common than other abnormal iron status indicators in those participants with valid samples. The two children less than 1 year old

(100%) who participated had low Hgb concentrations. In the 1 to 3 year age group three children (13%) had low hemoglobin, and two (25%) had low S-ferritin. Eleven children (16%) had low Hgb and two (11%) had low S-ferritin in the 4 to 8 year old group. Two males (11%) and three females (8%) had low Hgb and 2 females (7%) and one male (8%) had elevated FEP in the 9 to 13 year old group. Only one female (4%) had low Hgb concentrations, and 1 female (6%) had elevated FEP in the 14 to 18 year old group. The percent with abnormal values increased in the 19 to 50 year old group: 29 females (23%) and one male (3%) had low hemoglobin, five females (13%) had low S-ferritin; and nine females (10%) and two males (8%) had elevated FEP. Six females (11%) and one male (11%) had low Hgb in the 50 years and older group. The percentage of abnormal values for the other indicators in this group included four females (17%) with low S-ferritin and three females (10%) with elevated FEP.

Variables

A summary of the dietary iron intake, percentage heme iron and percentage nonheme iron intake, vitamin C intake (mean ± SD) and tea consumption (median and range) for both sexes and different age groups are shown in Table 1. The dietary factors (independent variables) were tested for their association with Hgb, S-ferritin and FEP. Dietary iron intake was not associated with Hgb (Pearson $r^2 = .000, p = .61$), S-ferritin (Pearson $r^2 = .000, p = .73$) or FEP (Pearson $r^2 = .006, p = .22$). In young children, mean dietary iron intake was

greater in the 4 to 8 year age group than infants less than 1 year ($p = .05$). Dietary iron intake was significantly different between children 1 to 3 years and 4 to 8 years ($p = .001$), with the 4 to 8 year group consuming more iron. Males consumed significantly more dietary iron in the 14 to 18 year old group compared to females of similar age ($p = .03$). In the 19 to 50 year age group, there was no difference in dietary iron intake between males and females. In the 51+ age group the women ate significantly more dietary iron ($p = .05$) than the men in the same age group. Both age and sex had significant effects on Hgb (both, $p < .001$), S-ferritin (both, $p < .001$) and FEP ($p = .03$ and $p = .02$, respectively). Since dietary iron intake also varied depending on age and sex, these variables were included in the analyses as covariates. There was no association between dietary iron intake and the iron status indicators while including age and sex in the models (partial $r^2 = .001, p = .49$ Hgb; partial $r^2 = .003, p = .51$ S-ferritin; partial $r^2 = .003, p = .34$ FEP).

The estimated percentages of heme iron and nonheme iron consumed are shown in Figure 2. There was a significant association between Hgb and percent heme iron ($r^2 = .04, p < .001$), indicating the higher the percentage of heme iron consumed, the more the Hgb increased. There continued to be a significant association with Hgb when age and gender were included in the model (partial $r^2 = .02, p = .002$). There was no association between percent heme iron and S-ferritin ($r^2 = .03, p = .07$) or FEP ($r^2 = .01, p = .07$). There was a negative association between percent nonheme iron and Hgb ($r^2 = .06, p < .001$) and

percent nonheme iron and SF ($r^2 = .03, p = .04$) indicating that the higher percentage nonheme iron consumed, the lower Hgb and S-ferritin declined. When age and sex were added to the model, there continued to be significance between Hgb and percent nonheme iron (partial $r^2 = .02, p = .002$) but not with S-ferritin ($p = .71$). Percent nonheme iron did not have an association with FEP ($p = .13$).

The mean (± SD) amount of vitamin C (mg/d) consumed was greater at older ages in both females and adulthood for males (Table 1). Children between ages 4 to 8 years consumed significantly more vitamin C than children between ages 1 to 3 years ($p < .005$). Females in the 14 to 18 year age group consumed significantly more than males in the 14 to 18 year age group ($p = .003$). When the iron status indicators were tested with vitamin C intake, no relationship was found. Since vitamin C intake increased with age and sex, these variables were added to the model and tested with the iron status indicators. Vitamin C intake was significantly associated with Hgb (partial $r^2 = .02, p = .003$).

The median amount of tea consumed each day is shown in Table 1. Older subjects consumed more tea than younger subjects. The 4 to 8 year old children consumed significantly more tea than the 1 to 3 year old children ($p = .017$). The amount of tea consumed each day was tested with the iron status indicators and no relationship was found between tea and Hgb ($p = .24$), S-ferritin ($p = .99$) and FEP ($p = .89$). The relationship did not change when age and sex were included in the model.

Thirty six percent of the participants consumed a multivitamin that contained iron. The median amount of iron in the supplement ranged from 9 mg to 18 mg. There was a significant gender difference in the amount of iron in supplements consumed in the 19 – 50 year old group, with the females consuming more than males (p <.001). There were no associations between consuming an iron supplement and Hgb, S-ferritin or FEP concentrations (p = .59, p = .99, and p = .74 respectively).

A multiple regression model was developed to include all dietary factors that might have an impact on hemoglobin (Table 2). The model included dietary iron (mg/d), vitamin C (mg/d), iron (mg) in a supplement and tea (cups/d) consumed. Age and sex were included as covariates. The only significant association was between Hgb and vitamin C (partial r^2 = .02, p = .003) indicating that as the amount of vitamin C increased, the Hgb concentration increased.

In view of the fact that dietary iron absorption can be influenced by vitamin C or tea consumption, the interaction of dietary iron and vitamin C and the interaction of dietary iron and tea consumed were tested with hemoglobin. No significance was found with either interaction (p =.22 and p =.24 respectively).

Women who have been pregnant had an average of 5 children with a range of 1 child to15 children (data not shown). The number of children they had did not have a significant effect on Hgb, FEP or S-ferritin. The average amount of time between pregnancies was 24 months with a range of 12 to 84 months. There was no relationship between the spacing of pregnancy and the iron status

indicators suggesting that the spacing of the pregnancies did not have an association with the iron status indicators.

Discussion

The population investigated included apparently healthy Hutterites living on South Dakota colonies and is therefore not representative of the entire Hutterite population or people living in rural areas of South Dakota. To our knowledge there are no comparative studies from this population. Overall, based on median Hgb, S-ferritin and FEP the iron status of each population group was considered adequate except for the 0.5 year to 1 year age group. The 2 children tested in that age group had low Hgb and the other iron status indicators were not available to further define their iron status. Hemoglobin alone cannot be used to identify iron deficiency because other factors such as folate deficiency or chronic infection may cause anemia. Therefore, to define iron deficiency at least two of the indicators transferrin saturation (TS), serum ferritin (S-ferritin) and free erythrocyte protoporphyrin (FEP) need to have abnormal values (18). In our study, we tested for S-ferritin, FEP and Hgb. S-ferritin has been demonstrated to be the most sensitive indicator of iron status as it is directly proportional to the body level of iron stores (17). FEP has been studied to be a better screening tool for iron deficiency than is hemoglobin in US children aged 1 to 5 years (19). It can detect iron deficiency at earlier stages (20). Since infection can cause falsely high or S-ferritin or FEP (21,22), it was important to rule out the possibility of

infection in participants by testing C-reactive protein levels. Participants with known infections were excluded from the study and in addition, C-reactive protein levels in all participants were normal.

The median values for the iron status indicators may make it appear that the iron status was good on the colonies; however there were some participants that fell out of the normal range. In the 1 to 3 year old group, 3 (13%) had low Hgb and 2 (25%) had low S-ferritin which indicates low iron stores. There were 9 (13%) children between the ages of 4 and 8 with low Hgb and 2 (11%) with low S-ferritin. Adequate amounts of iron are important at this age to help with proper growth and development (3,4). In the age group of 9 to 13 two males (11%) and three females (8%) had low Hgb while 2 females (7%) and 1 male (8%) had an elevated FEP. The elevated FEP could indicate iron deficiency at a level before a low Hgb (19). During the ages of 14 to 18 many physiological changes are taking place that utilize iron but surprisingly only 1 female (4%) had low Hgb and 1 female (6%) had elevated FEP. During the adulthood years (19 to 50 years) the number of participants with abnormal values increased in the female population with 29 females (23%) with a low Hgb, 2 females (5%) with low S-ferritin and 9 females (10%) with elevated FEP. The sequence for iron deficiency generally progresses from low s-ferritin to low Hgb therefore, these results do not look typical. There were a lot more samples of Hgb than S-ferritin so this may have affected the results. A reanalysis was completed including only those females with both Hgb and S-ferritin. This resulted in 12% with low S-ferritin and 15%

with low Hgb. There could be two explanations to these results. First of all, the S-ferritin samples reliability was questionable due to the high CV making the results unreliable. The second explanation could be that the low Hgb is a result of conditions other than iron deficiency such as chronic infections, or other nutritional deficiencies such as folate or vitamin B_{12} or vitamin A (2,17).

As mentioned previously, in order to identify iron deficiency, at least 2 of the iron status indicators S-ferritin and FEP have to be abnormal (18). Of the results obtained of S-ferritin and FEP, none of the participants had those 2 indicators in the abnormal range. To define iron deficiency anemia, S-ferritin, FEP and Hgb need to be in the abnormal range (18). From the results obtained, none of the participants could be classified as having iron deficiency with anemia as they did not have all three abnormal indicators. The qualities of the iron status indicators S-ferritin, FEP and C-reactive protein in this study were inconsistent. The CV% was high for the samples most likely due to the sampling method utilized. The dried filter paper technique that was used has been found to give reliable results comparable to traditional measurements (23,24). The FEP blood samples were collected onto filter paper and left to dry prior to transport back to the laboratory. For S-ferritin and CRP samples, capillary blood samples were collected in a microtube on site and then transported to the laboratory to be processed. The blood was centrifuged to obtain serum for the S-ferritin and CRP which was spotted using a pipette and dried on filter paper before being transported to the laboratory for processing by experienced laboratory technicians.

There may have been inconsistencies in the amount of serum or blood spotted on the filter paper contributing to the high CV%, therefore, several samples could not be utilized. A limitation to our study was the missing values preventing classification of iron deficiency or iron deficiency anemia.

In children aged 0.5 years to 1 year, the mean dietary iron intake (3.7 mg/d) did not meet the Recommended Dietary Allowances (RDA) for this age group (7). It is difficult to interpret these results due to the small sample size. From the FFQ information the sources of dietary iron for these children included only the nonheme sources from vegetables, grains and oatmeal cereal, but not an iron fortified cereal. One of the participants younger than 1 year was being breastfed and the participant was not taking a multivitamin with iron. The other participant younger than one year was not being breastfed but took a multivitamin with iron. Through personal conversations with some of the mothers on the colony, giving the infants and young children an iron fortified cereal that is recommended to ensure adequate dietary iron once they start to eat solid foods is not a common practice. The introduction of complementary iron rich foods should occur between 4 to 6 months of age to help meet the infant's needs (25,26). There are health consequences of iron deficiency. Several studies have demonstrated that even moderate anemia (hemoglobin <10 g/dL) is associated with depressed mental and motor development in children that may not be reversible (4,5). For the children from 1 year to 8 years the adequate median hemoglobin may be a reflection of the adequate mean dietary iron intake. In

females 14 – 18 years, the mean dietary iron intake did not meet the RDA (15mg/d) (7). These findings are similar to a study done in Finland where they found that the women failed to meet recommendations for dietary iron (27). Although the 14 to 18 year old females in our study did not meet the RDA for dietary iron, their iron status was adequate. During the years of 19 – 50 females are at risk for low hemoglobin due to menstruation and pregnancy. In our study, the dietary iron intake for this group was inadequate with a mean (± SD) of 11(5) mg/d. This less than adequate intake coupled with the demands for extra iron could explain the larger percent (23%) of females with low hemoglobin.

When comparing the hemoglobin between those that consumed adequate amounts of dietary iron and those that consumed inadequate amounts of dietary iron the mean hemoglobin did not differ. When testing for an association between dietary iron and iron status, no association was found (i.e. Hgb, S-ferritin or FEP), results similar to other studies showing that dietary iron intake did not affect iron status indicators (27,28). While it is imperative that dietary intake influences iron status, the differences in bioavailability of iron needs to be considered. The percentage of bioavailable dietary iron that was heme was lower than nonheme iron indicating that more dietary iron came from fortified grains than meat products. In this study, there was a very small negative association between the percent nonheme iron and Hgb and a small positive association between the percent heme iron and hemoglobin. Studies have found that dietary intake is a poor predictor of iron status, in part, due to reporting error in diet (28). The

method used in this study, a food frequency questionnaire, can be prone to reporting bias (29). One limitation of using FFQs in our rural population is the difficulty with estimating what is consumed on average throughout the year since some of the foods consumed vary depending upon season making it difficult to estimate intake on a daily, weekly or monthly basis. For example, fresh garden fruits and vegetables may be consumed in greater amounts when they are in season compared to when they are not readily available; while certain meat products may be consumed more often during the fall and winter months when the supply is more available.

Other dietary factors known to enhance or inhibit iron status were tested for their relationship with the iron status indicators. These include vitamin C, tea consumption and iron supplements. Vitamin C which is known to enhance nonheme iron absorption had an influence on Hgb when age and sex were included in the model suggesting that those who were older and men had more vitamin C in their diet, however; no relationship was found when vitamin C and dietary iron were tested for interaction. Consuming tea on the Hutterite colony is very common. Tea has been known to have a negative effect on the absorption of nonheme iron in the diet because of its polyphenol content (30). Several studies have been conducted to confirm this (31-36). In our study, tea did not have an effect on the iron status indicators which is similar to other researchers that have also found no association of tea to iron status (37-39). An explanation for our finding may be that the amount of tea consumed was not enough to make a

difference. Fewer than 50% of the participants took a multivitamin with iron and there was no relationship between an iron supplement and the iron status indicators.

Because of the high demands for iron during the childbearing years and the usual childbearing practices of Hutterite women having several children, we investigated whether parity had an effect on the iron status indicators. We found that the number of children a women had did not influence the iron status indicators. Some explanations for this could be that this group of women consumed a mean percent heme iron intake of 23 percent (± 9%) while the percent nonheme iron intake was greater at 72 percent (± 24%). They consumed 55 mg (± 44) of vitamin C per day which enhances nonheme iron absorption (40) Mean hemoglobin was 13 mg/dL (± 1) and 17% of the women had Hgb less than 12 mg/dL. Additionally, 6% had elevated FEP and 5% had low S-ferritin indicating that in this group a percentage of women had suboptimal iron stores. Those with lower iron status will absorb more dietary intake iron, either heme or nonheme (41). When outlier data were removed, there was a significant negative association between S-ferritin and the time between pregnancies indicating that serum ferritin declined as the time between pregnancies increased. This was unexpected and could be reflecting the overall S-ferritin status of the female population in that age group.

A limitation with this study is the participants were selected using a convenience sample. Because of the sampling technique used, the findings can

not be generalized to the Hutterite population. Further study is needed before any such findings can be generalizable. The FFQ method utilized in this study could have resulted in reporting bias; mainly due to the difficulty in quantifying the intake over a year. This method was felt to be superior for this cross sectional study over other methods of data collection such as a diet recall, which would not capture the usual intake over a period of time. Another limitation to this study is the inconsistencies of the laboratory results. During the data analysis it was realized that additional blood samples should have been drawn to increase the quantity of reliable laboratory samples.

Conclusion

Based on the results obtained, the overall iron status of the Hutterite population was fairly good. However, there are two groups in particular that could be at risk for low hemoglobin. The very young children could be at risk due to poor dietary iron intake. As it is critical that children consume adequate amounts of dietary iron, it is important that they are offered an iron-fortified food such as iron fortified formula or cereal, especially between 6 months to a year in age. If they can not eat enough dietary iron, an iron supplement should be encouraged after a physician's approval. Women of childbearing age need to consume adequate amounts of dietary iron including food high in bioavailable iron such as red meat products and beans.

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Table 2.1
Selected characteristics of the population by age and gender

Variable	Age and Gender Groups											
	Young children*			Older children and adults**								
	0.5 – 1 Both sexes	1 – 3 Both sexes	4 – 8 Both sexes	9 – 13 M	9 – 13 F	14 – 18 M	14 – 18 F	19 – 50 M	19 – 50 F	51 + M	51 + F
Number of subjects	2	23	69	18	40	5	24	32	130	9	54
Dietary Iron (mg/d)†	4[a] (2)	7[a] (4)	11[b] (5)	12 (6)	10 (5)	19[a] (10)	9[b] (5)	10 (5)	11 (5)	9 (3)	11 (4)
Heme iron† *% dietary iron*	0[a]	13[b] (9)	19[c] (6)	21 (10)	24 (12)	23 (10)	25 (14)	30[a] (5)	23[b] (10)	31 (13)	24 (11)
Nonheme iron† *% dietary iron*	99[a] (.75)	88[b] (22)	77[c] (9)	76 (10)	71 (13)	74 (11)	72 (14)	64[a] (13)	73[b] (23)	58[a] (15)	68[b] (12)
Vitamin C (mg/day)†	0[a]	69[a] (68)	119[b] (66)	147 (104)	125 (82)	254[a] (207)	81[b] (48)	59 (38)	56 (48)	63 (63)	58 (47)
Tea (cups/day)††	0[a]	1[a] (1 – 2)	2[b] (.07 – 3)	2 (1 – 3)	1 (.14 – 4)	2[a] (1 – 2)	1[b] (0 – 3)	2[a] (.14 – 4)	2[b] (0 – 13)	1 (.14 – 4)	2 (.14 – 5)
Iron supplements (mg/d)†† §	---	14[a] (10 – 18)	18[b] (6 – 18)	18 (18 – 18)	18 (18 – 18)	10 (10 – 10)	18 (16 – 18)	15 (12 – 18)	18 (9 – 60)	18 (18 – 18)	18 (4 – 18)
Hemoglobin††	9.9[a] (9.6- 10.3)	12[b] (10.6 – 13.8)	12.4[b] (10.4 – 14.5)	12.9 (11.6 – 17.2)	13.3 (10 – 15.3)	15.7[a] (13.6 – 17.7)	13.3[b] (11.8 – 15.1)	15.4[a] (11.6 – 18)	12.8[b] (10 – 15.7)	15.7[a] (11.5 – 17.8)	13.5[b] (9 – 16)
S-Ferritin††	NA	39 (3 – 61) n = 8	38 (9 – 63) n = 18	40 (18 – 74) n = 4	30 (14 – 45) n = 7	NA	41 (18 – 55) n = 5	115[a] (21 – 829) n = 18	40[b] (8 – 135) n = 39	69 (26 – 413) n = 5	45 (9 – 254) n = 23
FEP††	NA	1.4 (.7 – 2.3) n = 13	1.5 (.7 – 2.8) n = 36	1.7 (.2 – 3.2) n = 13	1.7 (.7 – 3.4) n = 27	1.1 (.9 – 1.2) n = 2	1.9 (.6 – 3) n = 15	1.6 (.2 – 3.4) n = 24	1.9 (.7 – 4.8) n = 91	1.8 (.8 – 2.1) n = 4	1.8 (.8 – 3.6) n = 29
CRP††	.91 n = 1	1 (.8 – 2) n = 18	1 (.7 – 4) n = 61	1 (.8 – 2) n = 13	1 (.2 – 3) n = 33	1 (.9 – 1) n = 3	1 (.5 – 7) n = 21	1 (1 – 4) n = 29	1 (1 – 9) n = 109	1 (2 – 8) n = 7	2 (1 – 8) n = 42

† mean ± SD †† median (range) § including only those taking iron supplements
* for young children, within rows, values with different superscripts are significantly different at $p < 0.05$.
** for older children and adults within age categories and rows, different superscripts are significantly different at $p < 0.05$.

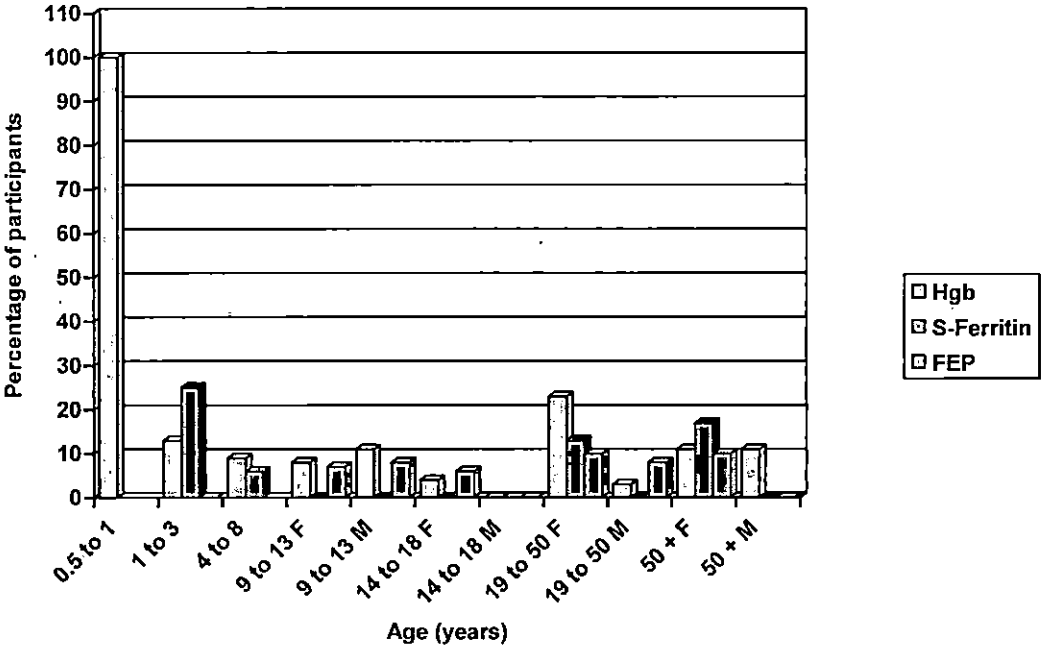

Fig. 2.1 Percentage of participants with low Hgb, low S-ferritin or elevated FEP.
F = female, M = male, Hgb = hemoglobin, S-ferritin = serum ferritin, FEP = free erythrocyte protoporphyrin

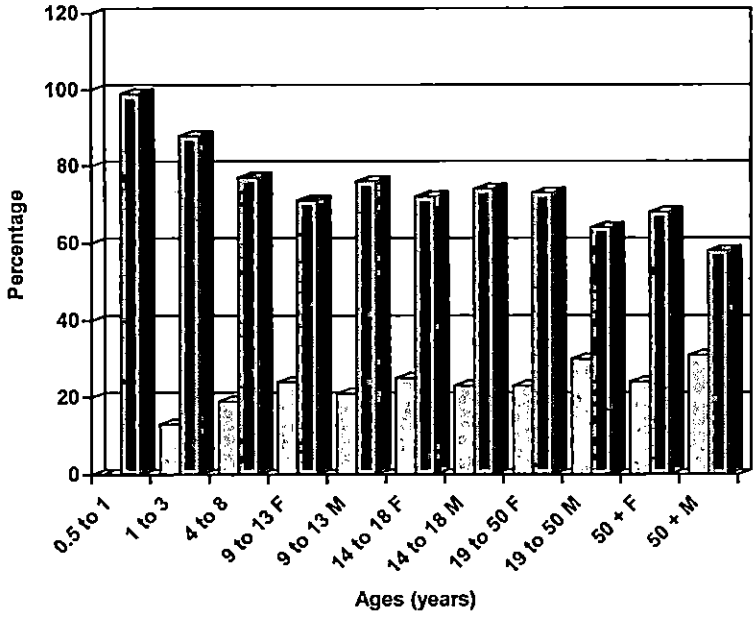

Fig. 2.2 Percentage of heme iron and nonheme iron consumed among participants.
F = female, M = male

Table 2.2 Multiple regression analysis of the relationship between hemoglobin and dietary and supplemental factors adjusting for age and sex.

Hemoglobin $r^2 = .17$, $p < .0001$			
	β estimate	se	*p*
Intercept	12.8227	0.2511	<.0001
Age	0.0257	0.0044	<.0001
Sex	-0.5220	0.0921	<.0001
Dietary iron (mg/d)	-0.0124	0.0149	0.4054
Vitamin C (mg/d)	0.0026	0.0011	0.0152
Iron in multivitamin (mg)	0.0203	0.0117	<.0001
Tea (cups/day)	0.0107	0.0631	0.8652

Chapter 3

Contribution of Tea To Iron Status In a Rural Female Population

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Contribution of Tea To Iron Status In a Rural Female Population

Abstract:

Objective: To investigate the association between tea consumption and iron status in a rural South Dakota female population.

Design: Cross-sectional study. This study will examine whether there is a correlation between tea consumption and iron status.

Subjects: Convenience sample of 136 adolescent girls and women living on Hutterite colonies in rural South Dakota.

Main outcome measures: Hemoglobin was expressed as milligrams per deciliter. Tea consumed was expressed in cups per day. Dietary iron consumed was expressed as milligrams per day.

Statistical analyses performed: Differences between group means were tested using Students t test and chi-square analysis. Multiple regression models were developed to assess the prediction of hemoglobin level to the amount of tea consumed controlling for variables with relevance to the main outcome measure.

Results: The difference in mean hemoglobin concentrations between groups did not differ. The mean intake of tea at mealtime was 2 ± 1 cups. The amount of black tea consumed at mealtime was significantly more than herbal or green tea ($p = 0.05$). The difference in the amount of iron consumed per day between groups was not significant ($p = .11$). No relationship was found between hemoglobin and amount of tea consumed while controlling for variables that influence hemoglobin.

Conclusions: In a seemingly healthy rural female population consuming tea with a whole meal does not have an effect on hemoglobin levels.

Contribution of Tea to Iron Status In a Rural Female Population

Introduction

Iron deficiency is the most common nutritional deficiency worldwide. Although iron deficiency is more prevalent in developing countries, a significant prevalence was observed in the United States during the early 1990s affecting mainly older infants, young children, and women of childbearing age (1). Adolescent girls and women of menstruating age are a population that continues to be at risk of iron deficiency because of the effects of menstruation and pregnancy, which demands increased iron requirements (1-3). Results from the most recent National Health and Nutrition Examination Survey (NHANES 1999-2000) showed the estimated prevalence of iron deficiency in adolescent and adult females aged 12-49 years was 9-16%, similar to NHANES III 1988 – 1994(4).

Several factors contribute to the iron status of an individual including dietary iron intake, if heme iron or non-heme iron is consumed, as well as the consumption of foods and beverages that may inhibit or enhance iron absorption. Consuming tannin-containing beverages such as tea can reduce non-heme iron absorption to a significant extent (5). The inhibitory effects of tea are attributed to their content of phenolic compounds (polyphenols, phenolic monomers and tannins), which reduce non-heme iron absorption by the formation of insoluble complexes with iron in the gastro-intestinal lumen (5-7). Numerous studies have been conducted to confirm this effect (8-14). For example, a controlled study examined a population of mentally handicapped menstruating women who were

long-stay residents of a nursing institute (8). The group of iron-depleted subjects had significantly higher daily and meal-time intake of tea and significantly lower meal-time intakes of vitamin C compared to the group of iron-replete subjects (8). In a more recent study, researchers tested the effect of tea on iron absorption from a meal containing non-heme iron only and found a significant negative effect on iron absorption (9). Tea drinking patterns of a population, such as type of tea and when tea is consumed, may have an adverse outcome on their iron status. However, other investigations have found differing results and have concluded that tea consumption was not associated with the iron status (15-22).

The population group for this study is of Hutterite decent. They use tea as a major beverage choice with their meals and as a refreshment between meals. Hutterites are a religious group of German descent, living in isolated rural colonies who believe in self-sufficiency including producing most of the food that they consume (23). Men, women and children of all ages drink tea throughout their life. Hutterites eat in a communal dining hall with many food items prepared from old-world German recipes. The meals are well-balanced; including a protein source, a starch such as homemade bread or pasta, and a variety of vegetables and fruits from the gardens. This study investigated the association between tea consumption and iron status in a group of apparently healthy menstruating adolescent Hutterite girls and women in South Dakota. We hypothesized that those girls and women who consume tea with meals will have lower hemoglobin than those who do not consume tea with meals.

Methods

Study population

The participants in this study included Hutterite girls and women. During the duration of a longitudinal study of bone health in this population (24), some female Hutterite participants were concerned about their iron statuses, which lead to the development of the current investigation. Hutterites girls and women were recruited from ten Hutterite colonies in eastern South Dakota through brochures alerting them of an opportunity to have their iron status tested. The sample for the present study included a convenience sample of 136 menstruating adolescent girls and premenopausal women between the ages of 12 and 54 years and was not necessarily representative of the eastern South Dakota Hutterite community at large. All participants were in good health and had no history of disorders known to influence iron status such as inflammatory bowel disease, peptic ulcer disease, inflammatory disorders, cancers and liver disease which may cause increased blood loss and consequently increased iron loss. Because pregnancy can influence iron status, participants who were pregnant at the time of the study or were unsure if they were pregnant were excluded. The South Dakota State University Institution Review Board approved the study. Written consent was obtained from all participants.

Assessment of tea consumption and nutrient intake

A questionnaire was developed to capture information regarding factors that could influence iron status. Trained interviewers administered the questionnaires to the participants. The consumption of foods that are thought to be good sources of iron such as meat products and fortified grains, the use of iron supplements and how much iron is contained in the supplement, the consumption of iron absorption enhancing foods such as citrus fruits and juices, and the consumption of drinks that may inhibit iron absorption particularly tea, were queried. Tea consumption was estimated in cups, and when tea was consumed it was reported (with meals or in between meals). Information about the type of tea consumed (black, herbal or green) was obtained as well. Dietary intake was collected by a brief food frequency questionnaire (FFQ) designed to be less of a burden on the participants than using a lengthier FFQ that would capture total dietary intake of each participant. The FFQ was developed to target foods high in iron. The FFQ estimated the average amount of dietary iron consumed on a daily basis. The method used to develop the FFQ food list was based on standardized FFQ development (25). Food composition tables (26,27) were used as a basis for the FFQ and the food list was reduced to foods that were commonly consumed by the participants. The food list contained usual portion sizes and the amount of dietary iron of each food item. The participants reported on their frequency of consumption choosing from daily, weekly or monthly options. Dietary iron

intake data do not include nutrients obtained from vitamin and mineral supplements.

Assessment of iron status and other data

Hemoglobin (Hgb) concentration was used to determine iron status of the participants. This measure reflects the amount of functional iron in the blood. The concentration of the iron-containing protein Hgb in circulating red blood cells is a direct and precise measure to screen for iron deficiency (28). Hemoglobin was assayed using the HEMOCUE B-Hb photometer (29) using non-fasting capillary blood samples. Demographic and medical data, as well as information on menstruation and parity, were obtained through a questionnaire.

Data analysis

The quantity and frequency of tea consumption was estimated from the questionnaire and the mean ± SD for descriptive variables were calculated. Participants were divided into two groups for the analysis: those who drink tea with meals (Group I) and those who do not drink tea with meals (Group II). The dependent variable hemoglobin was normally distributed. The independent variables including the amounts of dietary iron, vitamin C, and iron from a multivitamin were not normally distributed. When these variables were treated as nonparametric data, the analysis did not change. The differences between group means of the variables were tested using Students t test and chi-square analysis.

Multiple regression models were developed to assess the prediction of hemoglobin level to the amount of tea consumed controlling for variables with relevance to the main outcome measure including age, quantities of tea consumed with meals, dietary iron, vitamin C, and quantity of iron in a multivitamin. All statistical procedures were performed using the JMP IN statistical software package (SAS Institute, Version 7.0.1, 2007, Cary, NC).

Results

Description of the sample

The general characteristics of the participants are presented in Table 1. The study participants included menstruating adolescent girls and women. The mean (± SD) age of Group I (drink tea with meals, n = 110) was 30.3 ± 11.5 years and ranged from 12.4 – 54.3 years. Group II (do not drink tea with meals, n = 26) mean age (± SD) was 22.3 ± 10.5 and ranged from 12.3 – 53.6 years. There was a significant difference between the ages of the tea groups with those that drink tea with meals being older ($p = .002$). The group that drank tea had significantly more children than the group that did not drink tea ($p = .006$).

Hematological data

Iron status was measured by hemoglobin concentration. The difference in mean hemoglobin concentrations between Group I and Group II was not significant (p = .13). Mean blood hemoglobin concentrations were normal

(Table 1). Group I mean (± SD) hemoglobin was 13.0 ± 1.3 mg/dL with a range of 9.7 to 15.7 mg/dL. Group II mean (± SD) hemoglobin was 13.4 ± 1.3 mg/dL with a range of 10.4 – 15.6 mg/dL. Hemoglobin levels below 12 mg/dL are considered low (1,30) . Of the participants who drink tea with meals, 18% had hemoglobin below 12 mg/dL and of those who do not drink tea with meals 12% were below 12 mg/dL ($p = .40$).

Dietary Intakes

The mean (± SD) intake of tea at mealtime was 2 (± 1) cups for the tea drinkers. Black tea was consumed significantly more often than herbal or green tea at mealtime ($p = 0.05$).

The mean dietary iron intake for Group I was 10 ± 5 mg/d and ranged from 3 to 33 mg/d. Group II consumed a mean intake of 9 ± 4 mg/d and ranged from 4 to 26 mg/d. There was not a significant difference in the amount of iron consumed per day between the tea groups ($p = .11$). The mean amount of dietary iron consumed each day was roughly 55% of the current recommended dietary allowances for premenopausal women for Group I while Group II consumed on average 48% of their iron needs. When testing for an effect between the amount of dietary iron consumed per day and hemoglobin, no significant correlation was found ($r = .012, p = .21$). Fifty one participants (46%) of Group I took a multivitamin with iron compared with eight participants (8%) of Group II ($p = .07$). Among those who had a hemoglobin less than 12 mg/dL, 39% took a

multivitamin with iron compared to 44% of those with hemoglobin greater than or equal to 12 mg/dL ($p = .65$). The mean amount of iron contained in multivitamins for Group I and Group 2 was 19 mg and 16 mg respectively. The difference in the amount of iron contained in the multivitamins was not significant between the groups ($p = .27$).

Fifty two participants (17%) consumed a vitamin C source with at least one meal a day. The estimated mean amount of vitamin C Group I consumed was 64 ± 52 mg/day with a range of 0 – 304 mg/d. Group II consumed 84 ± 69 mg/d vitamin C with a range of 0 – 308 mg/d. No significant difference was found in the amount of vitamin C consumed between the groups ($p = .23$).

Regression analysis was used to determine if there was an association between mealtime intake of tea and hemoglobin concentration (Fig.). The results indicated that there was not an association with how much tea was consumed at mealtime and hemoglobin concentration ($r = .02, p = .11$). Further multiple regression analysis was used to determine whether hemoglobin concentrations were associated with the amount of tea consumed at mealtime while controlling for age, dietary iron, vitamin C, and quantity of iron in a multivitamin. The coefficient of determination (r^2) was .07, indicating that these variables explained 7% of the variance in hemoglobin. No relationship was found between hemoglobin and amount of tea consumed at a meal ($p = .57$), age of the participants ($p = .08$), amount of dietary iron consumed ($p = .16$), iron in multivitamin ($p = .14$) or the amount of vitamin C consumed ($p = .71$). Since

women of childbearing age have the potential for low hemoglobin, the number of children was added to the model and there was no association between hemoglobin concentration and number of children. The regression model is presented in Table 2.

Discussion

Contrary to studies that suggest drinking tea with meals can lead to decreased absorption of nonheme iron, which may lead to an impaired iron status, our study did not find a relationship between drinking tea with a meal and lower hemoglobin. This study controlled for factors that have been shown to have an influence on hemoglobin status or iron absorption including age, dietary iron intake, amount of iron in a multivitamin and vitamin C intake.

It has been known that tea has a negative effect on the absorption of nonheme iron in the diet because of its polyphenol content (5). Several studies have been conducted to confirm this (8-14). One study involved subjects consuming single meals labeled with an iron radiotracer following an overnight fast. The researchers found that absorption of iron from a breakfast consisting of moderately strong black tea and wheat rolls made from unfortified flour was significantly less compared to the group that consumed the meal with coffee and orange juice (12). Comparable findings were demonstrated when a standard meal composed of a hamburger, string beans and mashed potatoes was consumed

with strong tea (13). Similar tests on green tea resulted in decreased nonheme absorption from a pasta meal accompanied by green tea extract (10).

Polyphenols present in tea are responsible for the inhibiting effect of tea on non-heme iron absorption. It is mainly the galloyl group in the phenolic compounds that specifically binds iron. The polyphenol content of tea differs depending largely on the type of tea, how it was prepared and when it is consumed (31). With this in mind, Mennen et al (17) recently studied the effect of consuming black, green or herbal tea on the iron status in French adults. They found that with a healthy adult population, drinking tea as a part of a total diet does not seem to lead to an increased risk of iron depletion, irrespective of the type of tea consumed (17). These results are similar to the findings in the current study, which did not show a relationship between differing types of tea consumption and hemoglobin status. An explanation for the results in this study could be that the quantities of the differing types of tea consumed are too small to make a significant difference.

Although numerous studies have demonstrated that tea consumption with a meal has an effect (either positive or negative) on nonheme iron absorption, several studies have shown no effect on iron absorption when tea is consumed (17-21). A Canadian study discovered no difference was detected in terms of drinking tea between groups of elderly subjects with an inadequate and those with an adequate iron status, based on serum-ferritin concentrations (19). These results are similar to the data from the British National Diet and Nutrition Survey where

no association between serum ferritin and tea consumption was observed in elderly subjects (21). In 1999, Root et al did not find a relationship between tea consumption and serum ferritin among Chinese women (aged 32-66 years) (20). According to the results from these studies drinking tea with a meal within a whole diet has no effect on iron status in healthy adults. This is in agreement with the findings in the present study.

There could be several explanations for the findings of this study. Humans have the ability to sustain sufficient body iron concentrations regardless of wide variations in physiological requirements and dietary iron availability (32). An adaptive reaction occurs that regulates mucosal iron absorption according to one's iron stores, increasing absorption when iron stores are exhausted and reducing absorption when iron stores are adequate (33). Several researchers have demonstrated that an individual's iron stores, rather than dietary bioavailability of iron, are the main determinant of iron absorption (32,34-36).

Another explanation that tea consumption did not seem to have an effect on iron status is that iron absorption is determined by the bioavailability of dietary iron and the contents of the meal consumed (6,36-40). The meal may contain enhancers as well as inhibitors that determine the bioavailability of non-heme iron. It is a known fact that the bioavailability of non-heme iron is enhanced by ascorbic acid, other acids and some sugars and a substance in meat, fish and poultry (38). Work done by Reddy et al (2000) confirms this statement. Their studies suggested that animal tissue, beef, poultry and seafood, phytic acid and

vitamin C may be the most important factors determining iron bioavailability, not polyphenols (6). Other recent studies (39,40) have confirmed that men and premenopausal women are able to adjust iron absorption according to its bioavailability. When a diet high in heme iron was consumed there was reduced absorption of non-heme iron, while the absorption of non-heme iron increased when a low- bioavailability diet was consumed. Subsequently when the bioavailability of non-heme iron is low due to high tea consumption and low consumption of enhancers such as vitamin C for example, these adjustments inhibit a decline of iron status (41). This research could be applied to our results. The content of the whole meal consumed by the participants may have had more enhancers or heme iron present to increase non-heme iron absorption then polyphenols from tea to inhibit non-heme iron absorption. Generally the meals that are consumed by the Hutterites on the colonies are made of wholesome foods prepared from scratch and very few convenience foods. The meals also consist of enhancers such as a meat source.

A limitation with this study is the participants were selected using a convenience sample. Because of this the findings cannot be generalized to the Hutterite population. Further study is needed before any such findings can be generalizable. It should be noted that tests such as serum ferritin and erythrocyte protoporphyrin are typically used to assess iron status as they can determine initial stages of iron deficiency. This study did test for serum ferritin and erythrocyte protoporphyrin however, the coefficients of variations were high, and the

reliability of the results was questioned. Consequently, the hemoglobin assayed by Hemocue was the test that was utilized.

Conclusion

The results from our study imply that in a seemingly healthy rural Hutterite female population (ages 12 - 54) consuming tea with a whole meal does not have an effect on hemoglobin levels. The type of tea consumed is not of concern. The dietary iron intake and or the bioavailability of dietary iron appear to be sufficient to maintain a favorable iron status in this population.

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Table 3.1 General characteristics of the study population[a]

	Drink tea at mealtime Group I	Do not drink tea at mealtime Group II
Variables	n = 110	n = 26
Age (years)	30.3 ± 11.5[b] (12.4 – 54.3)	22.33 ± 10.5 (12.3 – 53.6)
Hemoglobin (mg/dL)	13.0 ± 1.3 (9.7 – 15.7)	13.4 ± 1.3 (10.4 – 15.6)
Amount of tea at mealtime (cups)	2.1 ± 1.2[b] (.03 – 6)	0
Dietary iron intake (mg/day)	10 ± 5 (3 – 33)	9 ± 4 (4 – 26)
Vitamin C intake (mg/day)	64 ± 52 (0 – 304)	84 ± 69 (.07 – 308)
Amount of iron in multivitamin (mg)	19 ± 9 (0 – 60) n = 42	17 ± 3 (10 – 18) n = 6
Number of children	2 ± 2[b] (0 – 11)	.65 ± 1.7 (0 – 6)

[a]Data are presented as mean ± SD and range.
[b]Group I significantly different than Group II ($p \leq 0.05$)

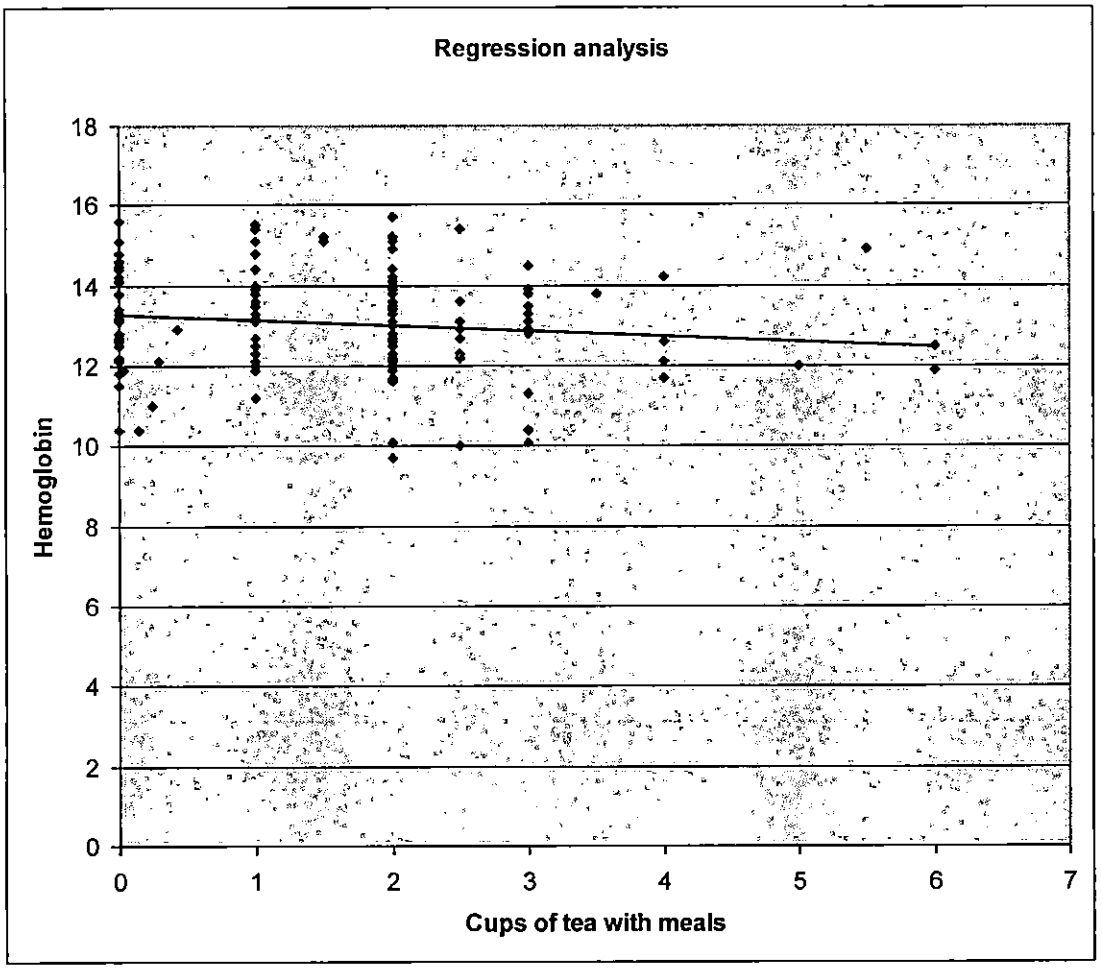

Fig. Association between mealtime tea intake and hemoglobin concentration. Results are based on data obtained from participant questionnaire. Regression line equation: $y = 13.27 - 0.13x$ ($r = .02$, $p = .11$)

Table 3.2 Multiple regression analysis of the relationship between hemoglobin and dietary and supplemental factors adjusting for age.

Hemoglobin $r^2 = .07$, $p = .18$			
	β estimate	se	*p*
Intercept	13.6782	0.4534	<.0001
Tea at meals (cups)	- 0.0548	0.0958	.5683
Age	- 0.0262	0.0150	.0832
Dietary iron (mg/d)	- 0.0391	0.0277	.1608
Vitamin C (mg/d)	0.0008	0.0022	.7056
Iron in multivitamin (mg)	0.0174	0.0118	.1437
Number of children	0.0886	0.0672	.1902

Chapter 4

Validation of a Food Frequency Questionnaire for Assessment of Calcium and Bone Related Nutrient Intake in Rural Populations

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Validation of a Food Frequency Questionnaire for Assessment of Calcium and Bone Related Nutrient Intake in Rural Populations

Abstract:

Objective: To assess the ability of a semiquantitative food frequency questionnaire (FFQ) to measure calcium and bone-related nutrient intakes in a rural South Dakota population.

Design: Intake estimates from FFQ were compared with four 24-hour recalls obtained quarterly over the preceding year.

Subjects: Convenience sample of 100 participants of the SD Rural Bone Health Study were recruited, with 81 completing the FFQ.

Main outcome measures: Calcium and bone-related nutrient intakes were expressed as milligrams per day, milligrams per 1000 kcal, or quartiles.

Statistical analyses performed: Intakes by FFQ and 24-hr recalls were compared using paired *t* test and quartiles were formed to examine cross-classification.

Results: Calcium intakes from FFQ and recalls were 1,287 and 1,141 mg/day ($p=0.01$), but calcium per1000 kcal did not differ. Calcium intake by FFQ correlated with intake by recall when expressed as milligrams per day ($r=0.49$, $p< 0.001$) or milligrams per 1000 kcal ($r=0.59$, $p< 0.001$). Bland-Altman graphs indicated fairly good agreement between methods. Seventy-eight percent of subjects fell into the same or within-one-quartile category when calcium intake

was expressed as milligrams per day and 83% when expressed as milligrams per 1000 kcal. Gross misclassification occurred in 0% to 4% of the nutrients.

Conclusions: Although FFQ may not be a valid indicator of an *individual's* intake, it does adequately classify rural populations into quartiles of calcium and bone related nutrient intakes, making it a useful tool for assessing dietary calcium and bone related intake in rural populations.

Validation of a Food Frequency Questionnaire for Assessment of Calcium and Bone Related Nutrient Intake in Rural Populations

Introduction

The food frequency questionnaire (FFQ) is often the method used for assessing nutrient intake in epidemiological studies. The underlying principle of the FFQ approach is that average long-term diet, such as consumption patterns over weeks, months or years, is theoretically a more relevant determinant of chronic disease rather than intake on a few specific days. Therefore, it may be more useful to give up precise intake measurements obtainable on one or a few days in exchange for more crude information relating to an extended period of time. FFQs must be validated against more detailed and accurate methods of assessment such as diet records (1).

In several studies of their validity, FFQs have been found to be reasonably accurate and are inexpensive to administer and process (2-13). The majority of these studies, however, were completed in more urban populations or with groups of people who may be more keenly aware of their diet including nurses and health professionals (2,5). The educational background of these two groups could influence their diet record keeping and accuracy. In a rural farming population, the Willett FFQ has not been validated.

The assessment of dietary calcium intake is of interest when studying bone health in population groups because dietary calcium (Ca) has long been

considered to play a role in the development of age-related osteoporosis. Several validation studies using FFQs designed to specifically assess calcium intake have been completed (14-19). Angus and colleagues (19) compared a short FFQ with 4-day weighed food records and found that a short, simple questionnaire can be used to rank individuals according to adequacy of calcium intake. In a study completed by Cummings and colleagues (17) two FFQ methods were evaluated for their ability to measure current dietary calcium intake in elderly women. The responses from the FFQs were compared to seven-day food records and the authors concluded that brief food frequency instruments which rate portion sizes on a simple qualitative scale may be suitable for many clinical uses and adequate for some types of epidemiological studies of calcium intake in elderly women. Other studies have similar findings (15-16,19).

To determine the ability of a FFQ to measure intakes of calcium and calcium-related nutrients in rural populations, we compared estimates of intakes of dietary calcium and bone-related nutrients using the Willett semiquantitative FFQ, which has been previously validated in a population of male health professionals (5), with results of four 24-hour dietary recalls obtained over a previous one-year span. We hypothesized that the FFQ may not work as well in our populations due to consumption of foods that may not be captured through the FFQ (i.e., from hunting, fishing, and production of own food products as well as recipes or food items unique to the Hutterites that have been passed down through many generations [20]).

Methods

Subjects

The subjects in this study are a sample drawn from a longitudinal study of lifestyle (rural vs. non-rural) factors on bone mass accretion (21). The South Dakota Rural Bone Health Study consists of rural (n=937), and non-rural (n=337) populations. Within the rural population there were Hutterites (n= 587) and non-Hutterites. To be considered as rural the subject had to have spent 75% or more of their life on a working farm or ranch while working less than 1040 hours per year off the farm. To be classified as Hutterite an individual had to be of Hutterite descent and currently residing on a Hutterite colony (21). The non-Hutterite population was recruited from an eight county area in eastern South Dakota that included at least one participating Hutterite colony. For the duration of the study, all participants completed a 24-hour dietary recall every three months with one recall per season. During the year after the 24-hour recalls were collected, a convenience sample of fifty rural non-Hutterite and fifty Hutterite subjects were recruited to take part in the validation study. The rural non-Hutterite subjects were recruited through phone calls and asked to participate by completing the self-administered semi-quantitative FFQ. The FFQs were mailed to the rural non-Hutterite participants to complete. Written information was included on how to complete the questionnaire and they were instructed to return them in postage paid return envelopes. For the Hutterite population the FFQ information was completed during a visit to the colony. Verbal instructions were provided for

completing the FFQ. All participants were provided with a $5 gift card for their involvement. The South Dakota State University Institutional Review Board approved the study, and the participant's willingness to complete the form was their consent to participate.

24-hour Dietary Recalls

All study participants had completed four 24-hour dietary recalls within the previous year except for one participant who had completed only three recalls. The 24-hour dietary recalls were obtained by trained interviewers approximately every three months. The timing and quantity of recalls provided information throughout all seasons (1). During the first visit for the South Dakota Rural Bone Health Study, trained interviewers displayed measuring cups and spoons, as well as food models to educate participants about how to estimate serving sizes in the 24-hour recall. For the rural non-Hutterite population, the 24-hour recalls were conducted by face-to-face interviews for 1 visit and over the telephone for 3 visits. Because these participants had their 24-hour recall interviews scheduled at different dates and were not in one central location, 3 of the visits were done over the telephone. For the Hutterite population, the 24-hour recall information was gathered through face-to-face interviews for all visits. The large number of participants living in one location made it feasible to do the 24-hour recalls during one trip to a colony every three months. The 24-hour recalls were obtained at random times to help capture a typical intake during the week. The analyses of

the 24-hour recalls were performed using Nutritionist Pro (version 2.3.1, 2004, First Databank, Inc., San Bruno, Ca). For foods that were not in the database, recipes were obtained from the participants and the component foods were entered and the recipes were added to the database. The mean daily intakes of energy, carbohydrate, fat, protein, and bone-related nutrients such as calcium, vitamin D, phosphorus, and magnesium were determined for each participant. Throughout the Rural Bone Health Study, a quarterly quality assessment was performed to assure accuracy and consistency in the analysis of dietary intake records. Each quarter a total of 10 completed diet analyses from each person analyzing diets were randomly selected and reanalyzed by a registered dietitian. The registered dietitian compared the results of the original diet analysis versus the reanalysis, wrote a report of the findings and communicated the results appropriately.

Self-Administered Semi-Quantitative FFQ

Study participants were asked to complete the Willet 97GP 2003 Version self-administered semi- quantitative FFQ (22) (97GP copyrighted at Harvard University). The 20-page semi- quantitative FFQ consisted of 138 foods including low fat and nonfat foods. This tool was designed to group individuals according to levels of average daily intake of selected nutrients over the past year. Participants were asked to report on average their usual eating pattern over the past year of a specific food or dietary supplement. The subjects were required to

indicate the number of times per day, per week or per month, food, or drink items or dietary supplements were consumed. Estimation of serving sizes were indicated in usual household measures (eg, a turkey sandwich, one orange, or a slice of bread) whenever possible, or otherwise typical serving sizes were provided (eg, 8 oz glass of skim milk, or ½ cup blueberries, fresh or frozen). For specific vitamin and mineral supplements, subjects were required to indicate if they currently consume the supplement, have consumed it in the past only or have never taken the supplement. They also were required to indicate how much of the supplement, on average, they consumed per day choosing from a dosage range and how long the supplement had been consumed (a series of ranges from 0-1 years to 10 or more years). The completed FFQs were checked for completeness and then were mailed to the Harvard School of Public Health for analysis.

Statistical Methods

The majority of the distributions for calcium and the bone-related nutrient intakes were skewed to the higher end. Therefore the geometric means and 95% confidence intervals for the means were calculated and a paired *t* test was performed to determine whether they differed. Bland-Altman graphs also were obtained to determine whether the difference between the FFQ and mean intake from the recall varied depending upon the nutrient intake (23,24). Energy adjustment was done by expressing nutrient intake per 1000 kcal. Spearman correlation coefficients were used to compare the two dietary assessment methods

for both unadjusted and energy-adjusted nutrients. Spearman correlation coefficients also were obtained to assess agreement over time between the FFQ and the four 24-hour recall visits. Quartiles were formed for intakes based on both the 24-hour recalls and the FFQ in order to examine their cross-classification using contingency table analysis. For both FFQ and 24-hour diet recalls, separate quartile cut-points were established from their respective distributions of nutrient intake. If a correlation coefficient of approximately 0.60 between the amount of intake estimated by the 24-hour recalls and that intake estimated by the FFQ is expected, at a 5% significance level and 80% power approximately 110 subjects were required to guarantee that the lower limit of the 95% confidence interval of the correlation coefficient was at least 0.40 (1). Our sample size of 81 subjects resulted in a power of 67%. All statistical procedures were performed using the JMP IN statistical software package (version 5.1, 2003, SAS Institute, Cary, NC).

Results

Of the 100 subjects initially invited to participate in the study, 81 (47 females) completed the FFQ. The 81 who completed the study had an average age of 42 years with a range of 17 to 74 years. Forty-five were Hutterite and 36 were rural non-Hutterites. Hutterites reported a greater intake of energy, carbohydrate and fat than non-Hutterites. Nineteen subjects (five Hutterites) chose not to complete the FFQ. There was no difference in age or gender

distribution between those subjects who completed the FFQ and those who did not.

The average nutrient intake calculated from the FFQ was statistically greater than that calculated from the average of the 24-hour recalls for all nutrients total energy and fat (Table 1). When the calcium and bone-related nutrients were adjusted for energy intake, the difference between the means was no longer significant for calcium. The difference between the two means did not vary depending upon mean nutrient intake for the majority of nutrients (Table 1). The Bland-Altman plot for calcium is shown in the Figure. These results indicate that the two methods showed fairly good agreement; however, phosphorus and magnesium showed poor agreement after adjusting for energy, despite fairly good correlation coefficients (Table 2).

The correlation between FFQ and 24-hour recall was 0.55 for energy intake, 0.41 for protein intake, 0.47 for carbohydrate intake, and 0.55 for fat intake (all, $p \leq 0.05$). The correlations for the bone-related nutrients are given in Table 2, with and without adjustment for total caloric intake. To check for agreement over time between the 24-hour recalls and the FFQ, calcium intake (milligrams per day) assessed by the FFQ was correlated with each of the 24-hour recalls. At each of the 4 visits there was correlation of $r=0.41$, $r=0.41$, $r=0.37$, and $r=0.31$, (all $p \leq 0.01$), for the most recent 24-hour recall to the most remote. In order to determine whether the relationship between FFQ and 24-hour recall of bone-related nutrient intake varied by the potential confounders of sex, age or

population group (Hutterite vs. non-Hutterite) the interaction of each of these potential confounders with 24-hour recall intake was tested while modeling for FFQ intake. The interaction term was not significant for any of the minerals, implying that these different variables did not modify the relationship between 24-hour recall and FFQ.

Comparisons of quartiles by each method were used to assess the degree of misclassification. Using calcium as an example, Table 3 summarizes the joint classifications for calcium intake (milligram per day) assessed by FFQ and 24-hour diet records. Seventy-eight percent of subjects when classified by the FFQ for calcium intake (milligrams per day) fell into the same or within-one-quartile category when classified by the 24-hour diet recalls. Thirty-three percent were classified into the same quartile by both methods. Only two subjects (2%) were grossly misclassified. When looking at energy-adjusted calcium intake by 24-hour recalls and FFQ, 83% of subjects fell into the same or into the within-one-quartile category (Table 4) and 44% were classified into the same quartile by the two methods. Only one subject was grossly misclassified by the FFQ. For all nutrients, approximately 2 percent were grossly misclassified into extreme quartiles (Table 5).

Discussion

Other researchers have reported on validation studies using various FFQs for dietary calcium intake (14-19). However, comparisons among studies can be difficult due to differences in sample size; age, sex, racial composition, and educational background of the study group; design of the FFQ (for example the number of food items, the amount of open and closed questions, and length of reference period of the recall); and the method used as the gold standard. All of these factors, in addition to others, may be related to the degree of agreement among methods.

In the validation of nutritional assessment methods, the reference measurement should be as accurate and as precise as possible, and any errors associated with the two methods should be independent (1). For this study, we compared individual nutrient intakes estimated by a semi-quantitative FFQ with intakes calculated from four quarterly 24-hour diet recalls collected by interview over the previous year in a rural population of both Hutterite and non-Hutterite men and women. The 24-hour recalls, which depend on recent memory, may have more reliable and valid mean values for nutrients than those from the FFQ, which depend on long-term memory. The timing and number of 24-hour recalls provided data during all seasons (1). One limitation of using FFQs in our rural population is the difficulty with estimating what is consumed on average throughout the year since some of the foods consumed vary depending upon season. For example, fresh garden fruits and vegetables may be consumed in

greater amounts when they are in season compared to when they are not readily available; while cream-based soups may be consumed more often during the fall and winter months.

Our FFQ validation method is comparable to the validation method used by Subar and colleagues (7). For their study, four 24-hour recalls, scheduled 3 months apart were collected and compared to 3 different food frequency questionnaires. The authors concluded that after energy adjustment, all three methods were comparable for purposes of assessing diet-disease risk.

The estimate of mean calcium intake and other bone-related nutrients recorded in this study using the FFQ were higher than the estimates recorded using the 24-hour recall. Other researchers have reported the usual tendency of FFQs to over-report nutrient intakes more than 24-hour recalls (8,16,25-28). To reduce extraneous between-person variation due to general over-reporting or underreporting of food intake, nutrient intakes per 1,000 kcal were calculated. Our results showed the difference in calcium intake per 1,000 kcal between the FFQ and the 24-hour recalls was not different from zero, consistent with other reports (2,5,7,13,28). However, the difference between estimated intakes obtained from the two methods for the other bone-related nutrients persisted even after adjusting for energy intake. Using the Bland and Altman plots we also found for the majority of nutrients that the difference between the two methods varied depending upon the mean intake of the nutrient.

Despite these results, the nutrients compared well when cross classified by quartiles of intake. Cross-classification according to quartiles of intake showed reasonable agreement between the two methods. For all nutrients, approximately 2% were grossly misclassified into extreme quartiles and is consistent with other reports (5,14). Rimm and colleagues (5) found that on average only 4% were grossly misclassified into extreme quintiles in their study. For epidemiological purposes, the potential of a questionnaire to categorize individual subjects by level of nutrient intake is more important than the capacity to measure group means (1).

Our study sample represents a population of individuals who have lived a rural lifestyle for the majority of their lives. The correlation coefficients of unadjusted calcium intake assessed by the two methods was comparable to those obtained for other validation studies in the general population, which usually reported correlations in the range of 0.45 to 0.79 (4,7,8,13,17,18,28). Longnecker and colleagues (28) studied a rural population and found the energy-adjusted correlation between diet records and the FFQ for calcium was 0.58, similar to our findings.

It is assumed that the most recent 24-hour recall would correlate more closely with the FFQ because it is most recent in memory. Our study found that the correlations between the four dietary recalls visits and the FFQ were similarly correlated. We speculate that for our study the reason the correlations were similar overtime is that there was sufficient time between the last 24-hour recall

and the administration of the FFQ that the participants were not influenced by their memory of their most recent 24-hour recalls.

Strengths and Limitations

The use of 24-hour diet recalls is not a perfect standard for judging the accuracy of assessing dietary intake since it may not necessarily be representative of usual intake. However, the timing and number of 24-hour recalls should help capture variability of usual intake, and is an important strength of this study.

This study has some limitations. One limitation of our study was a relatively small sample size; 81 respondents completed both the 24-hour recalls and the FFQ. This study population is a select group of people from rural areas of South Dakota and is not a representative sample of the general U.S. population. However, we also feel that this study provides evidence that a semi-quantitative FFQ can adequately group rural individuals into appropriate intake categories to study the relationship between diet and disease among populations.

Conclusions

The significant differences in mean intakes between the two methods, and the fact that the differences in the mean intake varied across intake levels indicates that the FFQ may not be a valid indicator of an *individual's* intake. However, these findings do provide evidence that the semi-quantitative FFQ developed by Willett

and colleagues (22) was able to adequately classify the *population* into quartiles of calcium and bone-related nutrient intakes.

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Table 4.1. Calcium and bone-related nutrients based on average intakes from 24-hour recalls and food frequency questionnaire (FFQ). Data are geometric means and 95% confidence intervals for the mean. (n=81)

	Method	Mean	95% CI
Daily Intake			
Calories (Kcal/day) [a]	24 hour recall	1942	1826-2066
	FFQ	2059	1911-2219
Protein (gm/day)	24 hour recall	84 [c]	78-90
	FFQ	92	86-99
Carbohydrate (gm/day) [a]	24 hour recall	226 [c]	211-241
	FFQ	247	227-269
Fat (gm/day)	24 hour recall	78	73-84
	FFQ	77	70-83
Calcium (mg)	24 hour recall	1141 [c]	1041-1251
	FFQ	1281	1162-1412
Vitamin D (IU/day)	24 hour recall	214 [c]	178-258
	FFQ	347	293-411
Phosphorus (mg/day)	24 hour recall	1167 [c]	1085-1255
	FFQ	1480	1380-1596
Magnesium (mg/day)	24 hour recall	250 [c]	229-272
	FFQ	347	322-374
Intake per 1000 kcal			
Calcium (mg/1000 kcal)	24 hour recall	602	546-665
	FFQ	622	565-684
Vitamin D (IU/1000 kcal)	24 hour recall	114 [c]	95-136
	FFQ	168	141-201
Phosphorus (mg/1000 kcal) [b]	24 hour recall	613 [c]	580-648
	FFQ	721	697-745
Magnesium (mg/1000 kcal) [b]	24 hour recall	132 [c]	121-144
	FFQ	169	162-176

Table legend

[a] Positive slope for the relationship between the difference of mean intake by FFQ and 24-hour recall vs. the mean intake by FFQ and 24-hour recall ($p \leq .05$).

[b] Negative slope for the relationship between the difference of mean intake by FFQ and 24-hour recall vs. the mean intake by FFQ and 24-hour recall ($p \leq .05$).

[c] Intake by 24-hour recall significantly different than by intake by FFQ ($p \leq .05$).

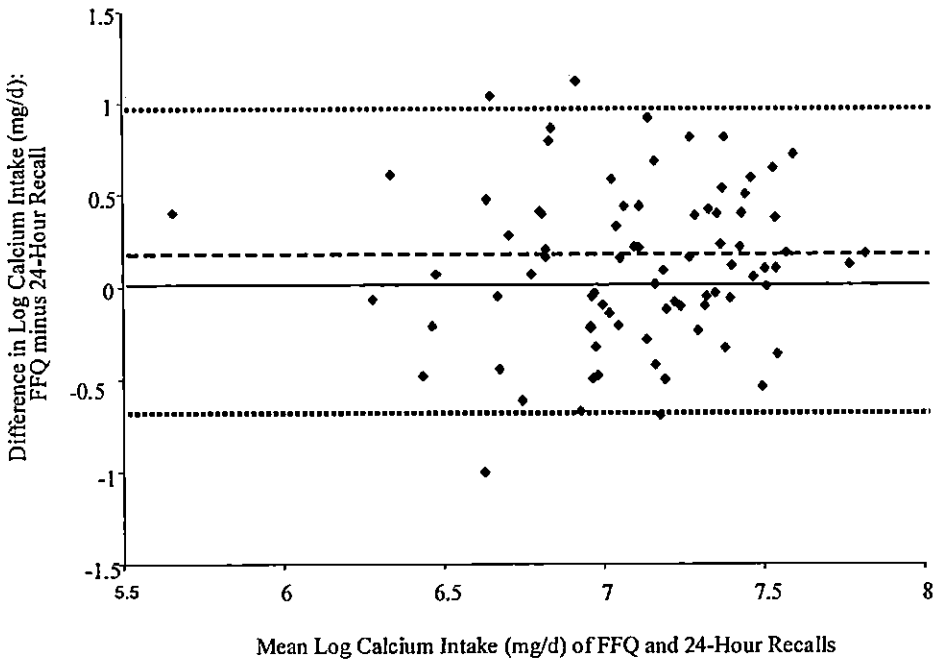

Figure Log

Figure. Difference in the log values of the estimates of calcium intake (food frequency questionnaire [FFQ] – mean of 24 hour recalls) by mean calcium intake based on both methods. The mean (dashed line) and the 95% confidence interval (dotted line) of the individual differences are shown.

Table 4.2. Correlation coefficients between average intakes based on 24-hour recalls and food frequency questionnaires completed by a sample of a rural US population both unadjusted and adjusted for energy intake ($p \leq .05$).

Nutrient	Unadjusted	Partial correlation coefficient[a]	Adjusted[b]
Calcium (mg/d)	0.49	0.42	0.59
Vitamin D (IU/d)	0.55	0.52	0.52
Phosphorus (mg/d)	0.57	0.57	0.41
Magnesium (mg/d)	0.52	0.49.	0.61

[a]Age, sex and population group included in the statistical analysis.
[b]Nutrient intake per 1,000 kcal.

Table 4.3. Cross-classification of calcium intake (mg/day) assessed by FFQ and 24-hour diet records.

	24-Hour Diet Record Quartile [a]				
FFQ Quartile [a]	**1 (low)**	**2**	**3**	**4 (high)**	**Total**
1 (low)	10	5	4	1	20
2	5	7	6	3	21
3	4	4	3	9	20
4 (high)	1	5	7	7	20
Total	20	21	20	20	81

[a] Quartiles 1 to 4 for 24-hour diet records were <880, 880-1247, 1248-1525, and >1525 mg/day.

Quartiles 1 to 4 for FFQ were <979, 979-1346, 1347-1805, and >1805 mg/day.

Table 4.4. Joint classification of adjusted calcium intake (mg/1000 kcal) assessed by FFQ and 24-hour diet records.

	Diet Record Quartile [a]				
FFQ Quartile [a]	**1 (low)**	**2**	**3**	**4 (high)**	**Total**
1 (low)	10	7	3	0	20
2	5	6	4	5	20
3	4	6	8	3	21
4 (high)	1	1	6	12	20
Total	20	20	21	20	81

[a] Quartiles 1 to 4 for 24-hour diet records were <450, 450-596, 597-821, and >821 mg/1000 kcal.

Quartiles 1 to 4 for FFQ were <478, 478-613, 614-863, and >863 mg/1000 kcal.

Table 4.5. Cross-classification of quartiles by FFQ and mean from the 24-hour recalls for bone-related nutrients both unadjusted and adjusted for energy intake.

	% Same quartile	% Same or within one quartile	% Grossly misclassified
Daily Intake			
Calories (kcal/day)	42	85	1
Protein (gm/day)	31	77	2
Fat (gm/day)	41	85	0
Carbohydrate (gm/day)	38	78	1
Calcium (mg/day)	33	78	2
Vitamin D (IU/day)	35	80	2
Phosphorus (mg/day)	40	83	0
Magnesium (mg/day)	32	80	2
Intake per 1000 kcal			
Calcium (mg/1000 kcal)	44	83	1
Vitamin D (IU/1000 kcal)	46	83	4
Phosphorus (mg/1000 kcal)	35	74	4
Magnesium (mg/1000 kcal)	42	89	1

Chapter 5

Discussion and Final Conclusions

The work presented in this dissertation was a result of a concern about the iron status of women on Hutterite colonies in South Dakota. My own interest in the iron status of women and children helped fuel this investigation. The work presented here will add to the knowledgebase of factors that affect iron status. Several factors are thought to influence iron status including dietary iron intake, consuming absorption enhancing foods as well as the number of children a woman has as well as how closely the pregnancies are spaced. Chapter Two was a study to investigate these factors that may influence the iron status. The results indicated that the overall iron status of the population was adequate; however, there were some individuals that had low hemoglobin. Only vitamin C influenced hemoglobin in a slight positive way. Nonheme iron had a small negative effect on hemoglobin. Other factors such as the number of children a woman has did not affect the hemoglobin. There were a small number of individuals with suboptimal iron intake and this could be a concern for the development of iron deficiency.

Tea has been studied for its affect on nonheme iron absorption especially when tea is consumed with meals. In Chapter Three it was found that the amount of tea consumed did not have an effect on hemoglobin. It was felt that the amount of tea consumed was not sufficient enough to have an effect and if a healthy diet is consumed, that may cancel the effects.

Accuracy of dietary intake reporting is important to make proper assessment of dietary intake. While the 24-hour recall is considered the gold standard in dietary intake collection techniques, food frequency questionnaires are utilized to gather intake over a period of time. Validation of the dietary intake technique is important for accuracy. The third study presented (Chapter 4) provided results on a validation of the Willett 97 food frequency questionnaire for calcium and bone related nutrients. It was validated against 24-hour recalls taken four different times in a year in rural populations and the Hutterite population. The results indicated that although the food frequency may not be a valid indicator of an individual's intake, it does adequately classify rural populations into quartiles of bone related nutrient intakes. The results of this study lead to the use of the Willett 97 food frequency in the South Dakota Rural Bone Study.

In conclusion, the overall iron status of the Hutterite population in South Dakota that participated in this study is good; however, a few individuals had low hemoglobin. Factors that are known to influence iron status indicators did not have large significant relationship in our study. Nonheme iron and vitamin C consumption had small significant effects on hemoglobin. In view of the fact that some of the individuals had low hemoglobin and dietary iron intake was suboptimal for some, stressing the importance of adequate dietary iron intake is essential. Tea intake and dietary iron intake did not have an effect on hemoglobin. The work presented in this dissertation is intended to add to the

knowledge base of iron and what factors may affect the iron status particularly in this population.