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Iron Deficiency Anemia and Difficulty Concentrating in a Nationally Representative Sample of School-Aged Children in the United States.

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ABSTRACT

IRON DEFICIENCY ANEMIA AND DIFFICULTY CONCENTRATING IN A NATIONALLY REPRESENTATIVE SAMPLE OF SCHOOL-AGED CHILDREN IN THE UNITED STATES

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The worldwide prevalence of iron deficiency anemia (IDA) among school-aged children is estimated to be 40%. Anemia has been associated with varying degrees of impaired cognitive functioning due to iron’s role in maintaining the neurochemical environment of the prefrontal cortex (PFC). However, limited research is available related to the prevalence of IDA and its resulting adverse effects on cognitive functioning in school-aged children in the United States. Data from two National Health and Nutrition Examination Surveys (NHANES) from 2013-2016 were analyzed to assess the relationship between iron deficiency anemia and difficulty concentrating in 1,758 children, ages 5-15 years old. Hemoglobin levels were extracted and anemia was characterized by a hemoglobin level less than two standard deviations below standard levels for age and gender. Race/ethnicity and family income may be non-modifiable risk factors for the development of anemia, and as a result, the influence of socio-demographic factors on the prevalence of IDA and difficulty concentrating in schoolchildren was also examined. To understand the nutritional status in this population, average protein intake and iron status were determined and compared to the age-and gender-appropriate RDA.
Using the hemoglobin levels described earlier, an average of 8.3% of school-aged children met the criteria for iron deficiency anemia; however, the presence of anemia was not a statistically significant predictor of reported serious difficulty concentrating. Race/ethnicity was significantly associated with anemia, with a higher prevalence of anemia among non-Hispanic Black children (17.5%) compared to non-Hispanic White children (3.1%). Poverty income ratio was also significantly associated with anemia, with a higher prevalence of anemia among children from low-income households (9.5%) versus middle- (5.4%) and high- (7.3%) income households. Female gender and adolescent age of 14-15 years were common risk factors for low protein and iron intake.

It is expected that this study will prompt further research related to IDA in school-age children, as increased knowledge related to IDA and nutritional status in schoolchildren can better assist health professionals in implementing appropriate dietary recommendations, as well as planning age-appropriate interventions to prevent adverse cognitive outcomes in school-aged children.
IRON DEFICIENCY ANEMIA AND DIFFICULTY CONCENTRATING IN A
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CHILDREN IN THE UNITED STATES

BY

ELIZABETH VOYLES
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Thesis Director:
Dr. Priyanka Ghosh Roy
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CHAPTER 1
INTRODUCTION AND BACKGROUND

Iron is an essential trace mineral that is ubiquitous throughout the body.\(^1\) Iron functions in the body as part of several proteins, including hemoglobin and myoglobin, and serves as a cofactor for enzymes involved in important metabolic body processes. As a result, iron plays a role in oxygen transport, DNA synthesis, procollagen synthesis, mitochondrial electron transport for energy production, amino acid metabolism, and neurotransmitter synthesis.\(^1\)

Iron status is a continuum and can range from iron overload, to normal iron status with various amounts of stored iron, to iron deficiency without anemia (ID) in which there are no mobilizable iron stores, to iron deficiency anemia (IDA), which is characterized by defective hemoglobin synthesis and a decreased capacity of red blood cells to deliver adequate oxygen to body cells and tissues.\(^1\) Unfortunately, according to the World Health Organization, iron deficiency (ID) is the most prevalent nutritional deficiency in developed countries such as the United States,\(^2\) with high-risk groups, such as non-Hispanic Blacks and Hispanics, disproportionately affected.\(^3\)

Because ID may often be asymptomatic, it can develop into IDA. The worldwide prevalence of IDA among school-aged children is estimated to be 40%, and among adolescents it is 30%-55%.\(^4\) This presents a significant public health problem due to the potential of IDA to cause varying degrees of impaired cognitive functioning in children. The adverse effects of IDA on cognitive functioning in children may be partly explained by iron’s role as a cofactor in maintaining the neurochemical environment of the brain’s prefrontal cortex (PFC), which is
involved in executive functions such as sustaining attention over a delay, screening distractions, and shifting and dividing attention according to task demands. The catecholamine neurotransmitters dopamine and norepinephrine regulate PFC-dependent functions and thus are powerful players in working memory and attention. Because the PFC is extremely sensitive to its neurochemical environment, changes in catecholamine levels due to IDA can produce large changes in PFC functions and result in symptomology such as weakened regulation of attention and concentration.

In addition, changes in neurotransmitter metabolism due to IDA may affect brain development and thus contribute to symptoms of iron deficiency in school-aged children such as lack of concentration and decreased cognitive and attentional processes. The effect of micronutrient deficiencies, such as iron, on brain development should be considered since certain areas of the brain are not yet fully developed by the age of 2. As a result, development continues throughout childhood and the school-aged years. The PFC goes through spurts of development from birth-2 years, 7-9 years, and again in the mid-teenage years. Thus, according to the developmental origins of health disease hypothesis, adverse influences during sensitive periods of development can change the structure and function of cells and organs, such as the brain. Since iron is needed to maintain the dopaminergic and noradrenergic systems associated with executive functions of the PFC, IDA in school-aged children has the potential to adversely affect PFC development and executive function due to changes in neurotransmitter metabolism and as a result contribute to adverse symptomology related to working memory, mental flexibility, and concentration in school-aged children.
Purpose of the Study

The purpose of this secondary, cross-sectional study was to determine if IDA is associated with difficulty concentrating in a nationally representative sample of children, aged 5 to 15-years-old, who participated in the NHANES 2013-2016. In addition, since race/ethnicity and family income may be non-modifiable risk factors for the development of anemia, the influence of socio-demographic factors on the prevalence of IDA and difficulty concentrating were also examined. To understand the nutritional status in 5 to 15-year-old children, average protein intake and iron status were determined and compared to the appropriate RDA, according to age and gender-based guidelines. Results from this study may be used to better assist health professionals in implementing appropriate dietary recommendations, as well as planning age-appropriate interventions to prevent adverse cognitive outcomes in school-aged children.

Research Objectives

Research Question 1: What is the association between iron deficiency anemia and difficulty concentrating in American schoolchildren, ages 5-15 years old?

Objective 1: Examine the association between iron deficiency anemia and difficulty concentrating in American schoolchildren.

Research Question 2: What influence do socio-demographic factors, such as race and ethnicity, and socioeconomic status, such as family income, have on the prevalence of American schoolchildren, ages 5-15 years old, affected by iron deficiency anemia and difficulty concentrating?

Objective 2: Examine the influence that socio-demographic factors have on the prevalence of American schoolchildren affected by iron deficiency anemia and difficulty concentrating.
Research Question 3: How does the intake of protein foods in American schoolchildren, ages 5-15 years old, affected by iron deficiency anemia and difficulty concentrating compare to the age- and gender-specific RDA for protein?

Objective 3: Determine the mean protein intake of American schoolchildren affected by iron deficiency anemia and difficulty concentrating and compare it to the appropriate age- and gender-specific RDA for protein.

Research Question 4: How does the average iron intake of American schoolchildren, ages 5-15 years old, affected by iron deficiency anemia and difficulty concentrating compare to the Recommended Daily Allowance (RDA) for iron?

Objective 4: Determine the mean iron intake of American schoolchildren affected by iron deficiency anemia and difficulty concentrating and compare it to the RDA for iron.
CHAPTER 2: REVIEW OF THE LITERATURE

History of Iron Deficiency Anemia

Iron deficiency anemia first became a public health issue during the 1960s when prevalence in infants and toddlers was estimated to be as high as 30%. During that time, it was common practice to switch infants from formula to cow’s milk at 6 months of age. Eventually, studies began to suggest that early introduction of cow’s milk before 1 year of age was directly related to the incidence of IDA in infants because of its poorly bioavailable form of iron, its potential to cause gastrointestinal bleeding in susceptible children, and the fact that it contains calcium and casein, which inhibit iron absorption. Since the iron status of an infant is directly related to his or her levels as a toddler, the American Academy of Pediatrics (AAP) recommended the use of iron-fortified formula in 1969, and this recommendation was later adopted by public health programs such as the Women, Infants, and Children (WIC) program in the 1970s. In addition, the Supplemental Nutrition Assistance Program (SNAP), established in 1964, provided SNAP benefits that could be used to buy iron-fortified breads and cereals as well as iron-rich meat, fish, and poultry, among other things.

Iron fortification of infant formulas and cereals in conjunction with public health program efforts to decrease IDA in infants and toddlers (0-12 months), as well as an increase in the popularity of breastfeeding, was successful in decreasing the prevalence of IDA in infancy from 30% in 1967 to 15% in late 1970. However, despite successful efforts to decrease the prevalence of IDA in infants and toddlers in the 1960s and 1970s, prevalence rates in the last four decades have largely gone unchanged. In fact, in 2005-2008, 15.9% of toddlers aged 1-2
years old in the United States were iron deficient, a percentage higher than the Healthy People 2020 goal of 14.3%.

**Effect of IDA in Infancy and Toddlerhood on Neurodevelopmental Outcomes in School-Aged Children**

Although IDA in infancy and toddlerhood may seem unrelated to cognitive functioning in the school-age years, the role of nutrition in early life is important because it is one factor that can be modified in order to optimize cognitive development in children. As previously mentioned, the PFC undergoes development well into adolescence, and as a result, iron sufficiency or insufficiency in early life has distinct neurobehavioral outcomes in children. Complex executive functions such as working memory, set shifting, multitasking, and attention undergo osseous development, gradually changing from simple to more complex neural circuits. As a result, rapidly growing organs such as the brain are vulnerable to damage if nutrients that support brain growth, such as iron, are not provided in sufficient amounts.

Periods of specification in which the brain undergoes rapid growth and development are called critical or sensitive periods. Critical periods are periods in development with a defined time limit after which repair of an abnormally developed system, resulting from adverse stimuli such as IDA, is no longer possible. Thus, any neurodevelopmental effects may be irreversible. Sensitive periods, on the other hand, refer to broader periods of time when a developing system, such as the brain, is sensitive to stimuli, such as nutrient levels, but effects caused by adverse stimuli, such as IDA, are not permanent. Nutrient effects on brain development, including development of the PFC, can exhibit both characteristics. Poor development in infancy resulting from IDA persists, in most cases, even after iron levels are restored. As a result, it is possible that damage to neurodevelopment caused by IDA in infancy and toddlerhood, as well as
critical periods of PFC development throughout childhood, may result in deficits of attention, cognition, and school performance later.\textsuperscript{20}

A limited number of follow-up studies have examined the long-term outcomes of IDA in school-aged children. Longitudinal follow-up studies indicate that school-aged children who were formerly anemic in infancy are more likely to show soft neurological signs such as clumsiness, inattention and hyperactivity, and have IQs averaging 6 points lower than school-aged children who had not been anemic in infancy.\textsuperscript{21} Furthermore, a study conducted in Israel concluded that 2\textsuperscript{nd}-grade teachers rated school-aged children lower in learning and positive task orientation who had been formerly anemic at 9 months of age.\textsuperscript{22}

In addition, a study of the long-term outcome of IDA in 3,771 school-aged children in Florida found an association between children diagnosed with anemia in infancy and special education placement at 10 years. Researchers based IDA diagnosis on hemoglobin screening in the Women, Infant, and Children (WIC) program and concluded that the risk for special education placement increased by 1.28 for each hemoglobin unit lower at entry into the WIC program.\textsuperscript{23}

Available longitudinal follow-up studies of formerly anemic children throughout the early school-age years and into adolescence, when it is feasible to identify differences in mental functioning, are also limited. One study of importance assessed a group of 191 Costa Rican infants who had severe, chronic IDA and were between the ages of 12-23 months.\textsuperscript{24} Anemic infants were treated with intramuscular or oral iron and then followed and reassessed at 5 years of age\textsuperscript{25} and adolescence (10.9-13.7 years).\textsuperscript{26} Hematology indicators for anemia, including hemoglobin, serum ferritin, transferrin saturation, and mean cell volume, were based on data from the National Health and Nutrition Examination Survey (NHANES). At 5 years of age,
children who had IDA as infants had lower scores on mental functioning than their peers, despite having adequate iron status and growth, indicating that IDA in infancy may be associated with adverse effects in the school-age years.

In addition, when children in the longitudinal study by Lozaff and colleagues were reassessed in adolescence, the formerly iron-deficient infants, who now ranged in age from 10.9-13.7 years, once again tested lower in mental and motor functioning. They had lower scores in reading, writing, and arithmetic; were more likely to have repeated a grade and/or be referred for special services or tutoring; had more externalizing (37% vs 27%) and internalizing (61% vs 42%) problems, more thought problems (13% vs 1%), more attention problems (15% vs 5%), more delinquent behavior (17% vs 2%); and showed a delay or a disruption in a shift in cognitive processing typical of adolescence. Furthermore, children who were anemic as infants performed worse than their iron-sufficient peers on the K-ABC Spatial Memory Task, which measures visual perceptual vigilance, attentiveness, and visual-spatial memory, as well as the Central/Incidental Serial Recall Test, which measures skill in attending selectively to task-relevant information while ignoring task-irrelevant information. In addition, adolescents who had been anemic as infants were more likely to have teachers and parents with increased concerns about anxiety, depression, social problems, and attention problems.

It is not completely obvious how early adverse stimuli, such as IDA, can contribute to long-lasting mental differences in school-aged children. Children who are iron deficient may face a number of other disadvantages in the home and family, such as food insecurity, low socioeconomic status, maternal depression, and low home stimulation that may account for poorer outcomes in the school-age years. However, studies that have controlled for background differences in these factors have not eliminated the effect of severe iron deficiency on adolescent
behavior and development in the school-age years. As a result, the importance of adequate nutrition in infancy and early childhood cannot be underestimated because nutrition is one factor that can be modified in order to optimize cognitive development in children and prevent iron deficiency between 6-24 months of age, which has been associated with lower IQ, slower processing speeds, and deficits in attention and cognition.

More research is needed to determine the extent and outcomes of IDA as well as the association between IDA and difficulty concentrating in school-aged children. Furthermore, the majority of studies which have assessed the association between IDA and altered cognitive functioning in children have been longitudinal follow-up studies and thus have followed formerly anemic infants throughout the school-aged years and into adolescence to identify feasible differences in mental functioning. Few studies have focused on hemoglobin levels in school-aged children and current cognitive functioning. As a result, the purpose of this study is to add to the limited research base and determine the association of anemia and altered cognitive functioning, if any, in school-aged American children.

**Effect of IDA on Neurotransmitter Synthesis**

Neurotransmitters are compounds made in the body that transmit signals from a neuron to a target cell across a synapse. Various amino acids can act directly as neurotransmitters in the body, and others, such as phenylalanine and tyrosine, can be catabolized with the help of enzymes within the brain and nervous system to produce biogenic amines, which act as neurotransmitters. Phenylalanine hydroxylase and tyrosine hydroxylase are iron-dependent, non heme-containing monooxygenase enzymes. They are responsible for the synthesis of the catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine) from amino acids
phenylalanine and tyrosine.\textsuperscript{1} Most importantly, these enzymes are iron dependent. See Figure 1 for mechanism.

![Figure 1: Synthesis of the Catecholamine Neurotransmitters from Phenylalanine and Tyrosine (Iron-Dependent)](image)

Of particular importance are the changes that anemia produces in neural functioning that are related to monoamine metabolism in the brain.\textsuperscript{19} Studies have linked iron deficiency to disturbances in monoamine neurotransmitter systems,\textsuperscript{19} in particular, dopamine, a catecholamine neurotransmitter with production that is dependent on the presence of adequate dietary iron (Figure 1). Dopamine is abundant in the PFC region of the brain and is essential to its function.\textsuperscript{5} The PFC regulates executive functions such as screening distractions, sustaining attention over a delay, shifting and dividing attention appropriately, and concentration.\textsuperscript{5} Most importantly, the PFC is sensitive to changes in its neurochemical environment, and thus changes in catecholamine...
levels, such as dopamine, can produce large changes in PFC regulation of attention and behavior.  

Two animal studies in rodents have attempted to examine the relationship of iron deficiency with altered monoamine metabolism, with a focus on the dopaminergic pathway in the prefrontal cortex of the brain.  

Beard and Connor conducted a study in which male and female rats were fed either an iron-deficient diet (3 mg Fe/kg) or an iron-sufficient control diet (35 mg Fe/kg) from postnatal day (PND) 4 to postnatal day 21. Only data from male rats were included in the final results, and both diets met all other nutrient requirements, with the exception of iron in the iron-deficient diet. Although iron-deficiency had age and brain region-specific effects, at PND 21 iron-deficient rats had diminished prefrontal cortex iron concentrations versus controls, as well as a 50% reduction in the density of dopamine receptors, D₁R and D₂R, and dopamine transporters (DAT) in the prefrontal cortex. The study by Beard and Connor indicates that iron deficiency alters brain iron concentrations, leading to changes in the density of dopamine transporters and dopamine receptors in the prefrontal cortex, and thus indicates a relationship between iron deficiency and altered dopamine metabolism in the brain of rats.

A later study by Li and colleagues also sought to determine the effect of postnatal iron deficiency on dopamine metabolism in the prefrontal cortex of young male rats. As was the case in the study by Beard and Connor, rats were fed either an iron-deficient diet (2-6 mg/kg iron) or a control diet (200 mg/kg iron), and changes in the concentration of iron, dopamine, dopamine transporters, and dopamine receptors in the prefrontal cortex were explored.

Rats fed an iron-deficient diet had significantly lower concentrations of hematocrit and nonheme iron concentrations in the brain versus control rats, however, in contrast to the study...
by Beard and Connor, no significant difference in the concentration of tissue dopamine, dopamine transporters, or dopamine receptors was found in the prefrontal cortex of iron-deficient rats versus control rats. However, extracellular concentrations of dopamine in the prefrontal cortex were significantly lower in iron-deficient rats versus control rats, indicating that available dopamine neurotransmitter available for release into the extracellular space may be limited due to iron deficiency in the prefrontal cortex of iron-deficient rats. An explanation for this phenomenon is the fact that iron is a cofactor for a key enzyme in dopamine synthesis, tyrosine hydroxylase, which is affected by iron deficiency in the prefrontal cortex, thus decreasing the amount of neurotransmitter produced and released into the extracellular space.

In summary, longitudinal observational studies in children have provided useful information about the long-term prognosis of children diagnosed with anemia in infancy and the effects that such a diagnosis may contribute to in the school-age years. Furthermore, although it is difficult to generalize results from rodent studies to infants and school-aged children, randomized controlled trials in rodents have demonstrated a relationship between IDA and altered dopamine metabolism, indicating that IDA may be a contributing biological factor to altered cognition in school-aged children. However, more research is needed to explore the effect of IDA on lack of concentration, decreased cognition, and attention in school-aged children, 5-15 years old, in the United States.

**Socio-Demographic Factors of Schoolchildren Affected by IDA**

Food insecurity can affect dietary nutritional quality and has been associated with income. As a result, food insecurity and anemia are prevalent among low-income families and children. In a longitudinal study of low-income infants in the Massachusetts Women, Infants, and Children (MA/WIC) program, infants living in food-insecure households were 42% more
likely to develop anemia at 18 months versus food-secure infants.\textsuperscript{29} In addition, race/ethnicity was a predictor of anemia risk. Non-Hispanic Black (NHB), Asian, and Hispanic infants were 76%, 39%, and 32% more likely to develop anemia at 18 months of age compared to non-Hispanic White infants.\textsuperscript{29}

In addition, data sets from five NHANES cycles between 2003-2012 noted the weighted prevalence of anemia in the general population to be 14.9% among non-Hispanic Blacks, 5.1% among Hispanics, and 4.0% in non-Hispanic Whites, indicating that race/ethnicity may be a non-modifiable risk factor for the development of anemia.\textsuperscript{3} As a result, this study examined the influence that race/ethnicity has on the prevalence of schoolchildren, ages 5-15 years old, affected by iron deficiency anemia and difficulty concentrating. Also, the influence of socioeconomic status in the form of the family poverty income ratio (PIR), which is calculated by dividing family income by family size, year, and state-specific poverty threshold guidelines, was examined to determine its influence on the prevalence of schoolchildren, ages 5-15 years old, affected by iron deficiency anemia and difficulty concentrating in the United States.

**Food and Nutrient Intake of Schoolchildren Affected by Iron Deficiency Anemia**

*Heme and Nonheme Iron.* Dietary iron is consumed in one of two forms in foods: heme and nonheme. Heme iron is derived from hemoglobin and myoglobin and, as a result, is found in animal products including meat, fish, and poultry.\textsuperscript{1} In fact, the majority, or 2/3 of body iron, is found in hemoglobin, a protein in red blood cells that consists of four heme groups and globin.\textsuperscript{1} Hemoglobin functions as part of red blood cells to transport oxygen in the blood to body tissues. In addition to iron’s role in hemoglobin formation and oxygen transport, an average of 15% of body iron is contained in myoglobin, a heme-containing protein that stores oxygen in muscle cells.\textsuperscript{1}
In contrast to heme iron derived from animal products, food sources of nonheme iron include primarily plant foods, including fruits, vegetables, grains, nuts, and tofu. In addition, most nonheme iron is released from food components in the ferric form ($\text{Fe}^{3+}$) and must be reduced to the ferrous form ($\text{Fe}^{2+}$) by various reductase enzymes at the enterocyte brush border membrane. As a result, nonheme iron found in plant products is less readily absorbed than heme iron consumed in animal products. Dairy products also contain nonheme iron, although dairy products are considered to be a very poor source of dietary iron.

*Fortified Foods.* In addition to iron consumed naturally in foods in the form of heme and nonheme iron, foods such as bread, rolls, pasta, cereals, and flour are often fortified with iron. Food fortification can provide a valuable safety net for the prevention of ID and IDA in children. In addition, food fortification may be especially relevant for school-aged children in low-income families without access to or resources to obtain iron-rich foods such as meat, fish, and poultry. It can also be relevant for school-aged children in which types of meats that are consumed are not always optimal sources of iron such as cold cuts, sausage, and hot dogs.

The 2015-2020 Dietary Guidelines Advisory Committee (DGAC) determined that several nutrients, including iron, were underconsumed in adolescent females compared to the Estimated Average Requirement (EAR) or Adequate Intake Level (AI). Furthermore, inadequate nutrient intake may not be limited to female adolescents but may be prevalent in children of all age groups. When examining the food/food group sources of mean energy intake in children ages 6-11 years old who participated in the NHANES from 2011-2014, O’Neil and colleagues found that the top three food sources of energy included sweet bakery products, milk, and sweetened beverages, while in children 12-18 years old the top three food sources of energy included sweetened beverages, sweet bakery products, and breads, rolls and tortillas. In contrast, iron-
rich protein foods such as poultry, ready-to-eat cereals, meat/poultry/fish mixed dishes, and meats were the 10th, 12th, 18th, and 23rd top-ranking food sources of energy in 6-11 year olds, while in 12-18 year olds they were the 10th, 12th, 14th, and 13th top-ranking food sources of energy. As a result, this study examined the protein intake of schoolchildren, ages 5-15 years old, affected by iron deficiency anemia and difficulty concentrating in the United States and compared it to the age- and gender-specific RDA for protein.

In summary, the purpose of this cross-sectional study is to add to the currently limited research available and determine the association between present hemoglobin levels in school-aged children and altered cognitive functioning. In addition, since studies have determined that ethnic groups such as non-Hispanic Blacks and Hispanics are often disproportionately affected by IDA, in addition to children in families that face food insecurity, this study examined the influence that socio-demographic factors have on the prevalence of school-aged children with IDA who also report difficulty concentrating. Furthermore, nutrition is one factor that can be modified in order to optimize cognitive development in children, and studies have demonstrated that inadequate intake of nutrients such as iron may be prevalent in all age groups. As a result, this study examined the mean intake of protein as well as the average total iron intake of schoolchildren and compared those values to the appropriate age and gender RDA.
CHAPTER 3

METHODS

Study Design and Data

This cross-sectional study used public datasets from children, ages 5-15 years old, who participated in the 2013-2014 or the 2015-2016 cycle of the National Health and Nutrition Examination Survey (NHANES). The use of public datasets that were already prepared and disseminated allowed us to conduct a cost-efficient study, as well as manipulate the data in a format appropriate for this analysis. The National Health and Nutrition Examination Survey (NHANES) is a population-based cross-sectional study designed to collect health, nutrition, and health behavior data of the US non-institutionalized civilian population, aged 2-74 years.

The survey is unique in that it has two parts: a health examination and an interview. Health examinations are conducted in mobile health centers (MEC) that travel to locations throughout the country; health interviews are conducted in participant homes. Health examinations consist of medical and dental examinations, laboratory tests, and physiological measurements. Home interviews consist of questionnaires containing demographic, socioeconomic, and health-related questions such as smoking, physical activity, alcohol and drug use as well as dietary interviews. Findings from the NHANES are often used to develop public health policies, health programs, and health services and increase health-related knowledge in the United States.

Parental consent is obtained via a household consent form if the NHANES participant is 17 years of age or younger, and as a result, no additional consent and/or recruitment procedures
were necessary. Only children with data pertaining to anemia and potential risk factors (age, race/ethnicity/PIR/protein and iron intake) and a response on difficulty concentrating were included. Children with missing results on laboratory tests for defining anemia or no response on age, sex, race/ethnicity, PIR, difficulty concentrating, or protein and iron intake were excluded. The resulting sample consisted of 1,758 children between the ages of 5-15 years old.

Data files from NHANES 2013-2014 and NHANES 2015-2016 containing pertinent demographic, laboratory, disability, and nutrition information were merged and matched according to respondent sequence numbers before analysis for this study, thus establishing one large data file containing all variables of interest from both cycles.

**Dependent Variables**

*Anemia.* Hemoglobin is the most commonly used test for the assessment of iron status in the United States. A diagnosis of anemia was confirmed by a serum hemoglobin (Hb) level less than two standard deviations below normal for age and gender, as defined in Table 1, which lists appropriate as well as anemic age-based hemoglobin levels. Based on these levels, this study categorized population groups as follows: 5- to 11-year-old children (male and female), 12- to 15- year-old males, and 12- to 15-year-old females.
Table 1: Age-Based Hemoglobin Levels in Children

<table>
<thead>
<tr>
<th>Age</th>
<th>Appropriate Mean Hemoglobin Level</th>
<th>-2 Standard Deviations (anemia indicator)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-6 years</td>
<td>12.5 g/dL</td>
<td>11.5 g/dL</td>
</tr>
<tr>
<td>6-12 years</td>
<td>13.5 g/dL</td>
<td>11.5 g/dL</td>
</tr>
<tr>
<td>12-18 years (males)</td>
<td>14.5 g/dL</td>
<td>13 g/dL</td>
</tr>
<tr>
<td>12-18 years (females)</td>
<td>14 g/dL</td>
<td>12 g/dL</td>
</tr>
</tbody>
</table>

Adapted from Wang

**Difficulty Concentrating.** Information on difficulty concentrating was collected from the household interview component of the NHANES. Specifically, the question, “Because of a physical, mental, or emotional condition, [do you/does he/does she] have serious difficulty concentrating, remembering, or making decisions?” was used to identify children with and without difficulty concentrating.

**Independent Variables**

**Demographic Characteristics.** Demographics of interest were race/ethnicity and poverty income ratio (PIR). Race/ethnicity information was collected from the demographic and occupation section of the NHANES family questionnaire (cycles 2013-2014 and 2015-2016) for children ages 5-15 years old. Because the literature suggests that the prevalence of IDA varies by race/ethnicity, race/ethnicity groups of interest included non-Hispanic White (NHW), non-Hispanic Black (NHB), Mexican American (MA), other Hispanic, and other (multiracial).

Poverty income ratio or PIR, which is calculated by dividing family income by family size, year, and state-specific poverty threshold guidelines, was included in the NHANES.
waves from 2013-2014 and 2015-2016. In this study, PIR was classified as PIR 0-1.85 (low), PIR 1.85-3.5 (medium), and PIR > 3.5 (high).35

Dietary Intake Data. The NHANES dietary interview component is called “What We Eat in America” (WWEIA) and is conducted as a partnership between the US Department of Agriculture (USDA) and the US Department of Health and Human Services (DHHS).34 It is designed to obtain detailed dietary intake information from NHANES participants, both children and adults, although proxies are used for children (Table 2).34

Table 2: Table of Proxy and Assisted Dietary Interviews

<table>
<thead>
<tr>
<th>Age of Sample Participant (SP)</th>
<th>Main Respondent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 years old</td>
<td>Proxy</td>
</tr>
<tr>
<td>6-11 years old</td>
<td>Sample participant, with proxy assistant</td>
</tr>
<tr>
<td>12 years +</td>
<td>Sample participant</td>
</tr>
</tbody>
</table>

Source: National Health and Nutrition Examination Survey

Dietary intake data is collected by two 24-hour recalls using the validated USDA dietary collection instrument, the Automated Multiple Pass Method (AMPM).34 The AMPM was validated in a large study by comparing reported energy intake (EI) to total energy expenditure (TEE) and shown to be effective for collecting accurate group energy intake.34 The five-step process of the AMPM is outlined below:

Step 1. Quick List: Participant recalls all foods and beverages consumed the day before the interview (midnight-midnight).

Step 2. Forgotten Foods: Participant is asked about foods consumed that are commonly forgotten during the quick list step.
**Step 3. Time and Occasion:** Collection of time and eating occasion for each food recalled.

**Step 4. Detail Cycle:** Collection of a detailed description, amount eaten, and additions to foods as well as a review of eating occasions and times between eating occasions to elicit forgotten foods.

**Step 5. Final Probe:** Collection of additional foods not remembered earlier.

The first 24-hour recall is collected in person in the MEC (MEC In-Person interview), and the second is collected by telephone 3-10 days later (the Phone Follow-Up [PFU] interview). After the dietary interview, NHANES participants aged 1 year and older are also asked questions about fish and shellfish consumption. Information collected from the two 24-hour recalls is coded and linked to the USDA Food and Nutrient Database for Dietary Studies (FNDDS), which provides nutrient values for every food and beverage reported by participants in the WWEIA dietary intake component of the NHANES. FNDDS nutrient values are updated every two years and used to generate nutrient intake data files for WWEIA.34

From the two 24-hour dietary recall interviews, two types of dietary intake data in four files are included in the NHANES 2013-2014 and 2015-2016 cycles: two individual foods files and two total nutrient intakes files. Each file represents one day of dietary intake data. The number “1” or “2” is included in the third position of the file name to identify the day and the mode of the interview: 1= first day (MEC in-person interview), 2= second day (PFU interview).

Although a second dietary interview is collected by phone 3-10 days after the in-person dietary interview, dietary data used in this study only included data obtained from the MEC in-person interview because of the difference in methodology when collecting this dietary information, versus dietary information in the PFU interview.
Protein and Iron Intake. For each total nutrient intake file (day 1 and day 2) there is one record for each participant. This study only utilized total nutrient intake files from the MEC-In Person interview (day 1) to analyze mean protein and iron intake in anemic and non-anemic children, aged 5-15 years old. Both of these nutrients were then compared to the age- and gender-specific Recommended Dietary Allowance (RDA; Table 3 and Table 4).

Table 3: Age- and Gender-Appropriate RDA for Protein

<table>
<thead>
<tr>
<th>Age</th>
<th>Protein (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-8 years (males and females)</td>
<td>19</td>
</tr>
<tr>
<td>9-13 years (males and females)</td>
<td>34</td>
</tr>
<tr>
<td>14-18 years (males)</td>
<td>52</td>
</tr>
<tr>
<td>14-18 years (females)</td>
<td>46</td>
</tr>
</tbody>
</table>

Source: National Institutes of Health

Table 4: Age- and Gender-Appropriate RDA for Iron

<table>
<thead>
<tr>
<th>Age</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-8 years</td>
<td>10 mg/day</td>
<td>10 mg/day</td>
</tr>
<tr>
<td>9-13 years</td>
<td>8 mg/day</td>
<td>8 mg/day</td>
</tr>
<tr>
<td>14-18 years</td>
<td>11 mg/day</td>
<td>15 mg/day</td>
</tr>
</tbody>
</table>

Source: National Institutes of Health
Inclusion Criteria

As mentioned earlier, children within the ages 5-15 years old who participated in the 2013-2014 or 2015-2016 NHANES cycles and who had complete data for the variables of interest were included in this study. Children with missing or incomplete data for the variables of interest were excluded. The resulting sample consisted of 1,758 children between the ages of 5-15 years old. When analyzing protein and iron intake of children 5-15 years old, the sample size decreased to 251 respondents, due to missing data for these two variables. Figure 2 presents a graphical description of the respondent selection process.

Sample Size

Using a $t$ test, the total number of NHANES participants (children 5-15 years) required for a two-sided (alpha = 0.05) study with a moderate standardized effect size of $r = 0.30$ and $\beta$ of 0.20 ($\beta = 1$-power) was estimated to be 176. The total number of respondents aged 5-15 years old who participated in the NHANES 2013-2016 was 4,562. After eliminating respondents who did not have data for the variables of hemoglobin and/or difficulty concentrating, the final sample size obtained was a total of 1,758 children.

Statistical Methods

All statistical analysis were completed using Statistical Package for the Social Sciences (SPSS) software, produced by SPSS Inc. and acquired by IBM in 2009. The most recent version is SPSS 25.0, released in August of 2017. The significance level, or alpha, was set at 0.05. See Table 5 for research objectives, variables of interest, and statistical tests used in this study.
Figure 2: Respondent Selection Process for Data Analysis, 2013-2016 NHANES
<table>
<thead>
<tr>
<th>Research Objective</th>
<th>Research Objective Subcategories</th>
<th>Independent Variable</th>
<th>Dependent Variable</th>
<th>Statistical Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examine the relationship between IDA and difficulty concentrating in American schoolchildren.</td>
<td>A. Identify the effect of anemia on reported difficulty concentrating.</td>
<td>A. Anemia</td>
<td>A. Difficulty Concentrating</td>
<td>A. Binary Logistic Regression</td>
</tr>
<tr>
<td></td>
<td>B. Determine if there is a significant difference in mean hemoglobin levels of children who report difficulty concentrating versus those who do not.</td>
<td>B. Hemoglobin</td>
<td>B. Difficulty Concentrating</td>
<td>B. Independent-Samples T Test</td>
</tr>
<tr>
<td>Examine the influence of socio-demographic factors on the prevalence of American schoolchildren affected by IDA and difficulty concentrating.</td>
<td>A. Describe the relationship between race/ethnicity and anemia.</td>
<td>A. Race/Ethnicity</td>
<td>B. Anemia</td>
<td>A. Chi-Square Test</td>
</tr>
<tr>
<td></td>
<td>B. Determine if there is a significant difference in mean hemoglobin levels across racial/ethnic groups.</td>
<td>B. Race/Ethnicity</td>
<td>B. Hemoglobin</td>
<td>B. Between-groups one-way ANOVA</td>
</tr>
<tr>
<td></td>
<td>C. Describe the relationship between PIR and anemia.</td>
<td>C. PIR</td>
<td>C. Anemia</td>
<td>C. Chi-Square Test</td>
</tr>
<tr>
<td></td>
<td>D. Determine if there is a significant difference in mean hemoglobin levels across categories of PIR.</td>
<td>D. PIR</td>
<td>D. Hemoglobin</td>
<td>D. Between-groups one-way ANOVA</td>
</tr>
</tbody>
</table>
(Table 5 continued)

<table>
<thead>
<tr>
<th>Research Objective</th>
<th>Research Objective Subcategories</th>
<th>Independent Variable</th>
<th>Dependent Variable</th>
<th>Statistical Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine the protein intake of American schoolchildren affected by iron deficiency anemia and difficulty concentrating and compare it to the appropriate age- and gender-specific RDA for protein.</td>
<td>A. Estimate adequacy of protein intake and anemic and non-anemic children by comparing mean intake to the age- and gender-appropriate RDA for protein.</td>
<td>A. Not applicable</td>
<td>A. Not applicable</td>
<td>A. Descriptive Statistics (Means)</td>
</tr>
<tr>
<td></td>
<td>B. Identify significant differences in mean protein intake of anemic and non-anemic children.</td>
<td>B. Mean Protein Intake</td>
<td>B. Anemia/No Anemia</td>
<td>B. Independent-Samples T Test</td>
</tr>
<tr>
<td></td>
<td>C. Identify significant differences in mean protein intake of anemic children with and without reported serious difficulty concentrating.</td>
<td>C. Mean Protein Intake</td>
<td>C. Difficulty Concentrating/No Difficulty Concentrating</td>
<td>C. Independent-Samples T Test</td>
</tr>
</tbody>
</table>

(Continued on next page)
Determine the average iron intake of American schoolchildren affected by iron deficiency anemia and difficulty concentrating and compare it to the RDA for iron.

- A. Estimate adequacy of iron intake in anemic and non-anemic children by comparing mean intake to the age- and gender-appropriate RDA for iron.
- B. Identify significant differences in the mean iron intake of anemic vs non-anemic children.
- C. Identify possible significant differences in mean iron intake of anemic children with and without reported serious difficulty concentrating.

<table>
<thead>
<tr>
<th>Research Objective Subcategories</th>
<th>Independent Variable</th>
<th>Dependent Variable</th>
<th>Statistical Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Not applicable</td>
<td>A. Not applicable</td>
<td>A. Descriptive Statistics (Means)</td>
<td></td>
</tr>
<tr>
<td>B. Mean Iron Intake</td>
<td>B. Anemia/No Anemia</td>
<td>B. Independent-Samples T Test</td>
<td></td>
</tr>
<tr>
<td>C. Mean Iron Intake</td>
<td>C. Difficulty Concentrating/No Difficulty Concentrating</td>
<td>C. Independent-Samples T Test</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 4

RESULTS

Descriptive Statistics

Of the 16,661 NHANES participants from the 2013-2016 cycles, 4,562 were children between the ages of 5 and 15 years old. After excluding respondents who did not have one or more of the variables of interest, 1,758 (38.5%) were included in this analysis. Nine hundred and eighteen (52.2%) respondents were male and 840 (47.8%) were female. In addition, the included sample was 25.8% non-Hispanic White, 25.9% non-Hispanic Black, 23.0% Mexican American, 10.1% other Hispanic, and 15.1% multiracial/other races. One thousand and eighty-two respondents (61.5%) lived in homes with a low PIR, 349 (19.9%) lived in homes with a medium PIR, and 327 (18.6%) lived in homes with a high PIR. Two hundred and two (11.5%) children reported difficulty concentrating. One hundred and forty-six (8.3%) children were anemic, and of the 146 children who were anemic, 17 (11.6%) also reported difficulty concentrating, while 88.4% did not report difficulty concentrating. Table 6 presents the characteristics of included NHANES participants along all variables of interest.
Table 6: Descriptive Characteristics of NHANES Respondents Aged 5-15 Years Old, NHANES 2013-2016

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>All Respondents 2013-2016 N=1758</th>
<th>Prevalence of Anemia n=146 (8.3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>918 (52.2%)</td>
<td>79 (54.1%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>840 (47.8%)</td>
<td>67 (45.9%)</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td>Non-Hispanic White</td>
<td>454 (25.8%)</td>
<td>14 (9.6%)</td>
</tr>
<tr>
<td></td>
<td>Non-Hispanic Black</td>
<td>456 (25.9%)</td>
<td>80 (54.8%)</td>
</tr>
<tr>
<td></td>
<td>Mexican American</td>
<td>405 (23.0%)</td>
<td>15 (10.3%)</td>
</tr>
<tr>
<td></td>
<td>Other Hispanic</td>
<td>177 (10.1%)</td>
<td>15 (10.3%)</td>
</tr>
<tr>
<td></td>
<td>Other (including multiracial)</td>
<td>266 (15.1%)</td>
<td>22 (15.1%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1758</td>
<td>146</td>
</tr>
<tr>
<td>PIR</td>
<td>Low</td>
<td>1082 (61.5%)</td>
<td>103 (70.5%)</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>349 (19.9%)</td>
<td>19 (13.0%)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>327 (18.6%)</td>
<td>24 (16.4%)</td>
</tr>
<tr>
<td>Difficulty Concentrating</td>
<td>Yes</td>
<td>202 (11.5%)</td>
<td>17 (11.6%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1556 (88.5%)</td>
<td>129 (88.4%)</td>
</tr>
</tbody>
</table>

Estimates apply to children 5-15 y and include all respondents (N=1758) and anemic respondents (n=146). PIR: 0–1.85 (low), >1.85–3.5 (medium), >3.5 (high).

Research Objective 1: Anemia and Difficulty Concentrating in Children, 5-15 Years Old

To identify the effect of the explanatory variable anemia on reported serious difficulty concentrating in children, a binary logistic model was used. The results from this model
indicate that anemia is a non-significant (p = 0.90) predictor of reported serious difficulty concentrating in children 5-15 years old (OR: 1.017, 95% CI 0.599, 1.724).

No statistically significant differences in mean hemoglobin levels of children who reported difficulty concentrating versus those who did not were found. Mean hemoglobin levels in children with and without reported difficulty concentrating were similar: 13.24 g/dL and 13.19 g/dL, respectively (Figure 3).

![Figure 3: Mean Hemoglobin Levels in Children, 5-15 years old, With and Without Reported Difficulty Concentrating, NHANES 2013-2016 (N=1758)]
Research Objective 2: The Prevalence of Iron Deficiency Anemia in Children as it Relates to Socio-Demographic (Race/Ethnicity) and Socioeconomic Factors (Family PIR)

Next, we explored the association between the predictor variables of race/ethnicity and PIR on the prevalence of anemia in children aged 5-15 years old (Table 7).

Table 7: Cross-Tabulation of Iron Deficiency Anemia Status Versus Predictor Variables

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>Children Aged 5-15 Years (N=1758)</th>
<th>Chi Square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anemic</td>
<td>Not Anemic</td>
<td></td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White</td>
<td>14 (3.1)</td>
<td>440 (96.9)</td>
<td>78.63</td>
</tr>
<tr>
<td>(n=454)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>80 (17.5)</td>
<td>376 (82.5)</td>
<td>78.63</td>
</tr>
<tr>
<td>(n=456)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>15 (3.7)</td>
<td>390 (96.3)</td>
<td>78.63</td>
</tr>
<tr>
<td>(n=405)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Hispanics (n=177)</td>
<td>15 (8.5)</td>
<td>162 (91.5)</td>
<td>78.63</td>
</tr>
<tr>
<td>Other (multiracial)</td>
<td>22 (8.3)</td>
<td>244 (91.7)</td>
<td>78.63</td>
</tr>
<tr>
<td>(n=266)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (n=1082)</td>
<td>103 (9.5)</td>
<td>979 (90.5)</td>
<td>6.24</td>
</tr>
<tr>
<td>Medium (n=349)</td>
<td>19 (5.4)</td>
<td>330 (94.6)</td>
<td>6.24</td>
</tr>
<tr>
<td>High (n=327)</td>
<td>24 (7.3)</td>
<td>303 (92.7)</td>
<td>6.24</td>
</tr>
</tbody>
</table>

Estimates of anemia provided apply to children, 5-15 y, NHANES 2013-2016. PIR: 0–1.85 (low), >1.85–3.5 (medium), >3.5 (high)
The bivariate association between anemia status in children aged 5-15 years old and predictors shown in Table 7 indicates that anemia was strongly associated with both race/ethnicity and PIR. The prevalence of anemia significantly (p<0.0001) varied from one racial group to another. The highest proportion of anemia among children 5-15 years old was observed in non-Hispanic Black children (n=80, 17.5%) followed by other Hispanics (n=15, 8.5%), and multiracial ethnicities (n=22, 8.3%). This is opposed to the lowest percentage of anemic children, which was recorded in non-Hispanic Whites (n=14, 3.1%).

The results in Table 7 also indicate that family PIR was found to have a significant (p=0.04) association with anemia status at 5% level of significance. Our results indicate that 9.5%, 5.4%, and 7.3% of children from poor, middle, and rich households were anemic, respectively. Figure 5 represents differences in the prevalence of anemia by family PIR.

**Comparison of Mean Hemoglobin Levels of Children, 5-15y, Across Race/Ethnicity and Family PIR**

One-way analysis of variance (ANOVA) was used to examine mean differences in hemoglobin in relation to the independent variables of race/ethnicity and family PIR. Basic descriptive information which summarizes the association between predictors and mean hemoglobin levels is presented in Table 8.

Results in Table 8 indicate that mean hemoglobin levels differed significantly (p < 0.001) among racial/ethnic groups in all children 5-15 years old at a 5% level of significance. Non-Hispanic Black children had the lowest mean hemoglobin level (12.71 g/dL), followed by multiracial (13.19 g/dL) and other Hispanic children (13.21 g/dL), while non-Hispanic White children had the highest mean hemoglobin level (13.45 g/dL). Figure 4 illustrates the differences in mean hemoglobin levels across racial/ethnic groups.
<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>Mean Hemoglobin Value (g/dL)</th>
<th>Standard Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race/Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic White (n=454)</td>
<td>13.45</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Non-Hispanic Black (n=456)</td>
<td>12.71</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Mexican American (n=405)</td>
<td>13.45</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Other Hispanic (n=177)</td>
<td>13.21</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Other (multiracial) (n=266)</td>
<td>13.19</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td><strong>PIR</strong></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Low (n=1082)</td>
<td>13.15</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Medium (n=349)</td>
<td>13.35</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>High (n=327)</td>
<td>13.20</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

*Estimated hemoglobin levels apply to children 5-15y NHANES 2013-2016*
Figure 4: Mean Hemoglobin Levels in Children, 5-15y, Across Racial/Ethnic Groups (N=1758)

Although hemoglobin levels differed significantly among races in all children, no significant differences in mean hemoglobin levels were found across racial/ethnic groups in anemic children (n= 146, p=0.30) or in anemic children who also reported difficulty concentrating (n= 17, p=0.78).

Results in Table 8 also indicate that mean hemoglobin levels differed significantly (p=0.01) across the wealth index (PIR) in all children 5-15 years old at a 5% level of significance. Children from poor families had the lowest mean hemoglobin level (13.15 g/dL), while children from middle-class families had the highest mean hemoglobin level (13.35 g/dL). Figure 5 illustrates the differences observed in mean hemoglobin levels across the wealth index.
Although significant differences were found in hemoglobin levels of all children by PIR, no significant differences were found in mean hemoglobin levels of anemic children by PIR (n=146, p=0.24) or in mean hemoglobin levels of children with anemia who also reported difficulty concentrating by PIR (n=17, p=0.78).

**Research Objectives 3 and 4: Mean Protein and Iron Intake**

In order to estimate adequacy of protein intake in children 5-15 years old, mean protein intake was determined for anemic and non-anemic children and compared to the appropriate age- and gender-specific RDA. In addition, an independent-samples t test was used to identify significant differences between the mean protein intake of anemic versus non-anemic children. This same sequence of steps was also completed using mean iron intake. Basic descriptive
information which summarizes mean protein and iron intake in these two groups of children (anemic/non-anemic) is summarized in Table 9.

Table 9: Mean Protein Intake (g/day) and Mean Iron Intake (mg/day) of Anemic vs. Non-Anemic Children, 5-15 y, NHANES 2013-2016

<table>
<thead>
<tr>
<th>Categories of Children by Age and Gender</th>
<th>Recommended Amount (g/day)</th>
<th>Mean Protein Intake (g/day)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anemic Children</td>
<td>Non-Anemic Children</td>
</tr>
<tr>
<td>5-8y, male and female (n=125)</td>
<td>19</td>
<td>68.80</td>
<td>59.96</td>
</tr>
<tr>
<td>9-13y, male and female (n=77)</td>
<td>34</td>
<td>76.82</td>
<td>71.43</td>
</tr>
<tr>
<td>14-15y, males (n=27)</td>
<td>52</td>
<td>74.03</td>
<td>81.31</td>
</tr>
<tr>
<td>14-15y, females (n=22)</td>
<td>46</td>
<td>*33.22</td>
<td>62.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Recommended Amount (mg/day)</th>
<th>Mean Iron Intake (mg/day)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anemic Children</td>
<td>Non-Anemic Children</td>
</tr>
<tr>
<td>5-8y, male and female (n=125)</td>
<td>10</td>
<td>13.23</td>
<td>12.93</td>
</tr>
<tr>
<td>9-13y, male and female (n=77)</td>
<td>8</td>
<td>14.92</td>
<td>14.95</td>
</tr>
<tr>
<td>14-15y, males (n=27)</td>
<td>11</td>
<td>15.37</td>
<td>15.66</td>
</tr>
<tr>
<td>14-15y, females (n=22)</td>
<td>15</td>
<td>*10.74</td>
<td>*12.39</td>
</tr>
</tbody>
</table>

Estimated protein and iron intakes provided apply to children 5-15y. Age- and gender-specific RDA for protein and iron adapted from the National Institutes of Health.\(^{36}\) \(p<0.05\). Values below the Recommended Daily Allowance are noted with an asterisk.

Table 9 illustrates that no significant differences were found between the mean protein intake (g/day) of anemic and non-anemic 5- to 8-year-old children (n= 125, \(p=0.42\)), 9- to 13-year-old children (n= 77, \(p=0.99\)), 14- to 15-year-old male children (n=27, \(p=0.58\)), and 14- to 15-year old female children (n=22, \(p=0.18\)). Mean protein intake of non-anemic children was
sufficient according to age and gender recommendations in all age groups. Mean protein intake of anemic 5- to 8-year-old children, 9- to 13-year-old children, and 14- to 15-year-old male children met the RDA. However, 14- to 15-year-old anemic female children were the exception, with a mean protein intake of 33.22 g/day (12.78g/day less than the RDA of 46g/day; Table 9). A graphic representation of mean protein intake of anemic versus non-anemic children is presented in Figure 6.

![Figure 6: Mean Protein Intake of Anemic vs. Non-Anemic Children, 5-15y (n=251)](image)

In addition, Table 9 illustrates that no significant differences were found in mean iron intake of anemic versus non-anemic children in 5- to 8-year-old children (n=125, p=0.90), 9- to 13-year-old children (n=77, p=0.74), 14- to 15-year-old males (n= 27, p=0.94), or 14- to 15-year-old females (n=22, 0.77).
Mean iron intake of anemic and non-anemic children was sufficient according to age and gender recommendations, with the exception of anemic and non-anemic 14- to 15-year-old females. Mean iron intake of anemic 14- to 15-year-old females was 10.74g/day (4.26 g/day less than the RDA), while the mean iron intake of non-anemic 14- to 15-year-old females was 12.39g/day (2.61g/day less than the RDA). A graphic representation of mean iron intake in anemic and non-anemic children is presented in Figure 7.

![Mean Iron Intake in Anemic Versus Non-Anemic Children, 5-15y (n=251)](image)

**Figure 7: Mean Iron Intake in Anemic Versus Non-Anemic Children, 5-15y (n=251)**

**Mean Protein and Iron Intake and Reported Serious Difficulty Concentrating**

In order to examine if anemic children with and without reported serious difficulty concentrating have significant differences in their mean protein and iron intake, an independent-samples $t$ test was performed. Basic descriptive information summarizing mean protein and iron intake in anemic children with and without reported serious difficulty concentrating is presented in Table 10.
Table 10: Mean Protein and Iron Intake of Anemic Children With and Without Reported Serious Difficulty Concentrating (n=21)

<table>
<thead>
<tr>
<th>Category</th>
<th>Recommended Amount (g/day)</th>
<th>Mean Protein Intake (g/day)</th>
<th>p-value</th>
<th>Mean Iron Intake (mg/day)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anemic Children, No Difficulty Concentrating</td>
<td>Anemic Children, Difficulty Concentrating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-8y, male and female (n=6)</td>
<td>19</td>
<td>68.80 (n=6)</td>
<td>No Data (n=0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-13y, male and female (n=7)</td>
<td>34</td>
<td>78.50 (n=6)</td>
<td>66.69 (n=1)</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>14-15y, males (n=6)</td>
<td>52</td>
<td>82.84 (n=3)</td>
<td>65.23 (n=3)</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>14-15y, females (n=2)</td>
<td>46</td>
<td>*29.96 (n=1)</td>
<td>*36.49 (n=1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Recommended Amount (mg/day)</th>
<th>Mean Iron Intake (mg/day)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anemic Children, No Difficulty Concentrating</td>
<td>Anemic Children, Difficulty Concentrating</td>
</tr>
<tr>
<td>5-8y, male and female (n=6)</td>
<td>10</td>
<td>13.23 (n=6)</td>
<td>No Data (n=0)</td>
</tr>
<tr>
<td>9-13y, male and female (n=7)</td>
<td>8</td>
<td>15.52 (n=6)</td>
<td>11.30 (n=1)</td>
</tr>
<tr>
<td>14-15y, males (n=6)</td>
<td>11</td>
<td>21.52 (n=3)</td>
<td>*9.22 (n=3)</td>
</tr>
<tr>
<td>14-15y, females (n=2)</td>
<td>15</td>
<td>*11.02 (n=1)</td>
<td>*10.45 (n=1)</td>
</tr>
</tbody>
</table>

Estimated protein and iron intakes provided apply to children 5-15y. Age- and gender-specific RDA for protein adapted from the National Institutes of Health.\textsuperscript{36} p<0.05. Values below the Recommended Daily Allowance are noted with an asterisk.
Table 10 illustrates that no significant differences in mean protein intake between the two groups of children (anemic and no difficulty concentrating/anemic and difficulty concentrating) was found in 9- to 13-year-old children (n=7, p=0.60) or 14-to 15-year-old male children (n=6, p=0.42). Due to the small sample size when selecting for children with the variables of interest, data comparing the significance of differences in mean protein intake in 5- to 8-year-old anemic children with and without difficulty concentrating, as well as 14- to 15-year-old female anemic children with and without difficulty concentrating, was not available.

Total mean protein intake consumed by children in both groups met the RDA across age groups, the exception being 14- to 15-year-old females. Although data from the present study only included information from two females in this age group due to many respondents having missing or incomplete data, the 14- to 15-year-old anemic female without difficulty concentrating consumed a mean of 29.96 g protein/day (16.04g/day less than the RDA), while the 14- to 15-year-old anemic female with reported difficulty concentrating consumed 36.49g/day (9.51g/day less than the RDA). A graphic representation of mean protein intake in anemic children with and without reported serious difficulty concentrating is presented in Figure 8.

Table 10 also illustrates that no significant differences in mean iron intake between the two groups of children (anemic and no difficulty concentrating/anemic and difficulty concentrating) were found in 9- to 13-year-old children (n=7, p=0.54) or in 14- to 15-year-old male children (n=6, p=0.07). Due to the small sample size when selecting for children with the variables of interest, data comparing the significance of differences in mean iron intake of 5- to 8-year-old children as well as 14-to 15-year-old female children was not available.
Looking at total mean iron intake consumed by children in both groups, 14- to 15-year-old female children, regardless of anemia status or reported difficulty concentrating, were consistently under the RDA for iron. Anemic 14- to 15-year-old female children with no reported difficulty concentrating had a mean iron intake of 11.02 mg/day (3.98 mg/day less than the RDA for iron), while 14- to 15-year-old anemic female children with difficulty concentrating consumed even less, with a mean iron intake of 10.45 mg/day (4.55 mg/day less than the RDA for iron). In addition, 14- to 15-year-old anemic male children with reported difficulty concentrating did not meet the RDA for iron, consuming 9.22 mg/day, or 1.78 mg/day less than the RDA for iron. Figure 9 is a graphic representation of the mean iron intake in anemic children with and without reported difficulty concentrating.
Figure 9: Mean Iron Intake in Anemic Children, With and Without Reported Serious Difficulty Concentrating, NHANES 2013-2016 (n=21)
CHAPTER 5
DISCUSSION

This analysis of anemia, protein, and iron status of a cross-section of a nationally representative sample of American children 5-15 years old participating in the NHANES 2013-2016 showed the following: 1) children with reported serious difficulty concentrating were similar among anemic (11.6%) and non-anemic (11.5%) children; 2) anemia was not a statistically significant predictor of reported serious difficulty concentrating; 3) race/ethnicity was a significant predictor of anemia in children, with a higher prevalence of anemia among NHB children (17.5%) compared to NHW children (3.1%); 4) PIR was a significant predictor of anemia, with a higher prevalence of anemia among children from low-income households (9.5%) versus middle- (5.4%) and high- (7.3%) income households; 5) female adolescent age (14-15 years old) was a common risk factor for low protein and iron intake.

Prevalence of Anemia and Difficulty Concentrating

Anemia is considered to be a public health concern when the prevalence of low hemoglobin concentration exceeds 5.0% in the population.\(^4\) The severity of the public health problem of anemia is classified according to the prevalence of anemia in the population as mild (5.0%-19.9%), moderate (20.0%-39.9%), and severe (≥ 40%).\(^4\) In the present study, the prevalence of anemia in children 5-15 years old was 8.3%, thus representing a public health problem of mild severity.\(^4\) This is of particular concern since studies have demonstrated that IDA can have a multitude of adverse effects on child health and development such as alteration of immune status, adverse effects on morbidity, delayed behavioral and mental development,
below-average school achievement and growth retardation, as well as adverse effects on cognitive functioning that may not be reversible with iron supplementation. This suggests that preventing iron deficiency anemia may be preferable to treating it once it develops. Although anemia in the present study only represented a public health problem of mild severity, health professionals need to remain vigilant in identifying pockets of elevated prevalence in children so that interventions can be developed to focus on the primary prevention of iron deficiency anemia, thus preventing potentially long-lasting developmental effects in children. Although the role that iron plays in neurodevelopment and cognitive functioning is not yet completely understood, it appears that in ID, iron is preferably channeled to hemoglobin synthesis, thus causing the brain to become iron depleted even before the child is anemic.

The correlation between anemia and reported serious difficulty concentrating in this study was weak or non-significant, leading to the conclusion that anemia is not a significant predictor of difficulty concentrating in children 5-15 years old. This is despite the fact that previous studies have found that IDA may be a contributing biological factor to altered cognition in school-aged children, leading to deficits in attention, cognition, and school performance. However, the majority of studies which have examined the role of iron on cognitive functioning in children are longitudinal follow-up studies of formerly anemic infants and toddlers throughout the school-age years and into adolescence. This study is different, and important, in that it involved children who were presently anemic, and although they may be at risk for some of the same adverse cognitive symptoms, this study cannot assess current iron status in anemic children and the long-term prognoses in cognition and behavior. Additional studies with a larger sample size are needed to assess if the non-significant relationship between anemia and difficulty concentrating found in this study is related to a true lack of correlation or,
alternatively, whether an inadequate sample size reduced the ability to find a statistical effect even if the effect existed in the population. In addition, reported difficulty concentrating in children was based on parental/caregiver report, so there may have been underreporting of this phenomenon. As a result, although no significant associations were found in the present study between anemia and difficulty concentrating in children, longitudinal follow-up studies can be conducted to see if IDA in the present study is associated with poor cognitive development and difficulty concentrating in the future.

**Prevalence of Anemia by Race/Ethnicity**

The present study demonstrates that race/ethnicity is a significant predictor of anemia in children 5-15 years old. The highest prevalence of anemia was found in non-Hispanic Black children (17.5%) and was determined to be more than five times that of non-Hispanic White children (3.1%). This finding is slightly higher than previous studies, which have demonstrated that anemia is three times more common in NHB populations versus NHW populations, as well as data from five NHANES cycles between 2003-2012, which noted the prevalence of anemia in non-Hispanic Blacks ranging in age from 0.5-85 years to be 14.9%. As a result, this study indicates that the prevalence of anemia may be higher in non-Hispanic Black children compared to the non-Hispanic Black population as a whole. This is important because, as mentioned previously, anemia in children has been associated with adverse symptomology related to working memory, mental flexibility, and concentration in school-aged children. In addition, other poor health outcomes that have been associated with IDA in children include decreased social interaction, weakness, fatigue, frequent infections, poor appetite, muscle pain, and even increased risk of stroke. For example, a 2007 study found that children with IDA accounted for
more than half of all stroke cases in children without an underlying medical illness, suggesting that IDA is a significant risk factor for stroke in otherwise healthy children.\textsuperscript{41}

The present study also found the prevalence of anemia among other Hispanic (8.5%) and multiracial populations (8.3%) to be more than twice the prevalence of anemia in non-Hispanic Whites (3.1%). The identification of these high-risk groups is consistent with previous studies which have determined that non-Hispanic Blacks and Hispanics are often disproportionately affected by anemia.\textsuperscript{3} As a result, this study augments current knowledge of anemia prevalence among subgroups in the United States and supports findings which state that race/ethnicity is a potential non-modifiable risk factor for anemia.\textsuperscript{3,29} In addition, although the prevalence of anemia in all children 5-15 years old was estimated to be 8.3%, which indicates a public health problem of mild severity, this study shows that anemia may be more prevalent in specific subgroups such as NHB and Hispanic populations. In particular, anemia prevalence in NHB (17.5%) populations may be reaching that of a moderate public health significance (20.0%-39.9%), as opposed to anemia found in all children (8.3%), regardless of race, which indicates a problem of mild public health significance (5.0%-19.9%). This reiterates the idea that health professionals need to remain vigilant in their efforts to identify pockets of elevated prevalence of IDA in order to prevent poor health outcomes in children.

Reasons behind the racial disparity in anemia status are still unclear; however, disparities in income, education, and health care may contribute to the higher prevalence of anemia in NHB children versus that in NHW children. In addition, studies have shown that factors such as childhood BMI and age of menarche, especially in non-Hispanic Black girls, may be predictors of increased anemia prevalence in this population versus that in non-Hispanic Whites.\textsuperscript{42} In particular, the link between childhood obesity and earlier maturation may contribute to the higher
prevalence of anemia in non-Hispanic Blacks, as African American girls have the lowest median age of menarche and the highest rate of childhood obesity. The association between childhood obesity and early maturation has been examined in previous studies, which have found that feminine obesity and early age at menarche may be related to inflammatory reactions associated with obesity that increase cytokines and promote the synthesis of androgen, thereby precipitating early pubertal development. In addition, insulin resistance in obesity is associated with compensatory hyperinsulinemia and decreased levels of liver sex hormone binding protein, which increases estrogen levels and promotes early pubertal development. This is important because studies have found that menstruating earlier is a risk factor for IDA due to rapid depletion of iron stores through blood loss from menstruation.

Existing studies have found that factors such as infant breastfeeding rates, childhood television viewing, fast-food consumption, and family meals could explain a large part of BMI differences among racial and ethnic groups, and as a result, there is a need for interventions that target racial and ethnic disparities in childhood obesity, especially in NHB female children.

Dietary composition is also an important determinant of iron bioavailability and iron status, and as a result, cultural variation in dietary patterns may influence iron availability and body iron stores and contribute to an increased risk for iron deficiency anemia among ethnic populations. Previous studies have found that a traditional Hispanic dietary pattern is more plant-based than the typical American diet, which may predispose Hispanic populations to anemia since plant-based diets are associated with low iron absorption and poor iron status. A 2007 study found that the diet of Hispanic populations included greater amounts of starchy vegetables, rice, milk, and legumes than the diet of non-Hispanic Whites, and mean intakes of red meat, other vegetables, breads and cereals were lower among Hispanics than among non-
Hispanic Whites. This is of significance since iron bioavailability is known to be inhibited by calcium, as well as phytates in legumes, which can reduce iron absorption by up to 80%. This is opposed to highly available heme iron found in animal sources such as red meat, fish, and poultry as well as in fortified grain and cereal products, which have been shown to be more commonly consumed in non-Hispanic White populations. That being said, many legumes can be good sources of iron if consumed with vitamin C, which enhances iron absorption. However, total vitamin C intake has been found to be 1/3 lower in Hispanics versus non-Hispanic Whites. This shows that ethnically related food choices may affect the health status of specific populations and thus increase the risk for anemia in children.

Evidently, disparities of anemia rates among ethnic/racial groups in children is a national health concern that needs to be addressed by public health interventions; however, this is not easy since underlying causes of anemia may vary by race. The present study indicates that non-Hispanic Black children, who had the highest prevalence rate of anemia (17.5%) and the lowest mean hemoglobin level (12.71g/dL), are most severely affected, while non-Hispanic White children, who had the lowest prevalence rate of anemia (3.1%) and the highest mean hemoglobin level (13.45g/dL), are least affected. Ensuring quality medical care regardless of race may help to reduce racial disparity in anemia prevalence, as can nutrition education geared towards families that also respects cultural and ethnic variations in dietary patterns. Specifically, encouraging the importance of dietary vitamin C, as well as supplemental iron, may be important in the Hispanic population, as these have been found to be positive predictors of iron status.
Prevalence of Anemia by PIR

In addition to race/ethnicity, the present study demonstrates that PIR is a significant predictor of anemia status in children 5-15 years old. Children from low-income families had the highest prevalence of anemia (9.5%) as well as the lowest mean hemoglobin level (13.15g/dL) when compared to children from middle- (5.4%) and upper- (7.3%) class families. One reason for this finding may be because families from middle and upper class levels can more easily afford basic requirements needed for optimal health, such as proper medical care and food/nutrition, which families from low-income families may have more difficulty providing. In fact, food insecurity can affect dietary nutritional quality and has been associated with income. As a result, previous studies have indicated that children born into poverty are at a greater risk for nutrition-related deficits. For example, the prevalence of anemia in low-income infants and young children through 2 years of age participating in public health programs is estimated to be between 20-30%, compared to 5% in the nation as a whole. Although the relationship between poverty and poor nutrition status is well established, the exact mechanism by which poverty exerts its influence is less clear. As previously mentioned, children in low-income households may face greater food insecurity and thus poor nutrition and dietary quality. However, other confounding factors which may also be associated with poverty may contribute to the higher prevalence of anemia in low-income versus middle- and high-income families, such as limited access to health care, which affects the ability to detect and treat IDA, poor food preparation facilities, maternal depression, and poor maternal education, all of which are additional factors that can contribute to poor dietary quality in children.

Evidence of how low-income status or poverty is a predisposing nutrition risk factor for children is concerning, since studies have found that poverty in the United States is widespread
and disproportionately affects children. In fact, the poverty rate for children in the United States is thought to exceed that for children in almost all other industrialized countries, with 1 in 6 children under the age of 6 classified as poor. This study supports this finding of a high prevalence rate of poverty in children, as 61.5% of respondents 5-15 years old were classified as living in families with a low PIR. This is concerning because, as mentioned previously, low-income children in the present study had the lowest mean hemoglobin level and the highest prevalence of anemia. More research is needed to examine cognitive effects of this hemoglobin difference in children, as previous studies have found a relationship between measures of poor cognitive development in children and socioeconomic status or income. In particular, children who experience long-term or persistent poverty score lower on IQ tests and other cognitive measures than children who experience transitory poverty, who in turn score lower than children who were never poor. Although the present study did not find significant differences between the mean hemoglobin levels of anemic children who also report difficulty concentrating by PIR, further research is needed with a larger sample size to determine the true relationship between the explanatory variable of family income on the dependent variables of anemia and difficulty concentrating in children.

Programs such as WIC, through the provision of supplemental foods, nutrition education, and health/social service referrals, have addressed risk factors for poor outcomes and thus improved the nutrition status of low-income women and children. However, WIC serves women, infants, and children up to 5 years of age, and as a result, public health interventions need to find ways to address anemia in low-income children who are greater than 5 years of age, as this study has shown that anemia in low-income children from 5-15 years of age is still a persistent public health problem.
Mean Protein and Iron Intake

Mean Protein Intake. Although this study did not find significant differences between mean protein intake of anemic and non-anemic children, or between the mean protein intake of anemic children with and without difficulty concentrating, it is important to note that the present study also examined mean protein intake in 5- to 15-year-old children and evaluated that protein intake for conformity to age- and gender-specific RDAs. Although the majority of respondents consumed adequate protein to meet the RDA, which is the minimum amount of protein necessary to prevent deficiency in 97.5% of individuals, a percent of the population, mainly 14- to 15-year-old females, did not meet the RDA for protein. In particular, 14- to 15-year-old anemic females consumed 12.78g/day less than the RDA, 14- to 15-year-old anemic females without difficulty concentrating consumed 16.06g/day less than the RDA, and the single 14- to 15-year-old female with difficulty concentrating in this study consumed 9.51g/day less than the RDA.

The fact that protein intake was below the RDA in 14- to 15-year-old females may indicate that female adolescence is a risk factor for inadequate protein intake. Reasons for inadequate protein intake may be related to growing independence in making food choices at home and at school, as adolescence affords an opportunity for food selection independent of parental guidance. As a result, adolescents may acquire unhealthy eating behaviors such as skipping meals, undereating, or overeating, all of which may decrease dietary quality. A 2018 study which used NHANES data to characterize protein intake trends from 2001-2014 and assess conformity to protein specific DRIs, such as the RDA, in the US population found similar results to the present study in that adolescent females 14- to 18-years-old had the greatest percentage (23%) of the population, with usual protein intakes that did not meet the current RDA.
Another factor which may play into low protein intake in adolescent females may be the high prevalence of dieting. In a cohort of adolescent females aged 13- to 16-years-old (n=2287), 55%-58% of females reported dieting in the past year. In addition, another study which assessed dietary intake in 2,287 preadolescent at 11 years of age and again in adolescence at 15.5 years of age to examine dieting and disordered eating behaviors in males and females found that the prevalence of dieting and disordered eating in females was high, affecting half of the females in the past year, versus only a fourth of the males. Of particular importance was that the use of extreme weight control behaviors, such as diet pill and laxative use, increased from 8.4% to 20.6% between early adolescence and early young adulthood in females. As a result, dieting in female adolescents may be a contributing factor to low protein intake observed in this study, as previous studies have confirmed the presence of unhealthy weight control behaviors in this population. Individuals who display these behaviors in adolescence are not only at greater risk of iron deficiency anemia and poor growth, but use of these behaviors places adolescents at increased risk for their continued use into adulthood, therefore causing adverse effects on behavioral, physical, and psychological outcomes such as binge eating, altered weight status, and depression. As a result, young people, and females in particular, have a need for support and intervention related to healthful eating and physical activity that can be enjoyed and implemented on a long-term basis, thus steering them away from unhealthy weight control practices.

The physical environment may also contribute to low protein intake in female adolescents as friends, advertising, and promotion of commercial products such as soft drinks, sugary and salty foods, and fast foods may all negatively affect protein intake, as well as dietary quality as a whole. As a result, increased autonomy in making food choices, dieting, and less consumption...
of heme protein sources such as meats paired with overconsumption of energy-dense foods in female adolescents may contribute to the higher percentage of anemia observed in this age group, as well as impair proper growth and development, which rely on adequate dietary protein.\(^1\) As a result, female adolescents may benefit from nutrition education that emphasizes choosing nutrient-dense foods as well as embracing a positive body image.

*Mean Iron Intake.* Differences in the mean iron intake of anemic versus non-anemic children did not reach that of statistical significance; however, it is important to note that in three of the four age groups analyzed (9-13y children, 14-15y male children, 14-15y female children) mean iron intake in anemic children was lower than mean iron intake in non-anemic children. Only in 5- to 8-year-old males and females was the mean iron intake of anemic children (13.23 mg) slightly higher than that of non-anemic children (12.93 mg). This underscores the fact that, when analyzing the mean intake of iron in anemic children with and without difficulty concentrating, anemic children with difficulty concentrating had consistently lower mean iron intake versus anemic children without difficulty concentrating, although the differences once again failed to reach that of statistical significance. The exception to lower mean iron intake observed in anemic children with difficulty concentrating versus anemic children without difficulty concentrating was once again 5- to 8-year-old males and females, who in this case did not have any respondents in the anemic/difficulty concentrating category to analyze.

Although many longitudinal follow-up studies of children and adolescents who were formerly anemic as infants have demonstrated poor cognitive outcomes such as reduced attention\(^25\) and lower scores on reading, writing, and arithmetic,\(^26\) more research like the present study is needed with a larger sample size to examine the current relationship between the explanatory variable of mean iron intake on the dependent variables of anemia and difficulty
concentrating in children, as opposed to longitudinal follow-up studies which track formerly anemic infants throughout the school-age years to determine the influence of anemia in the past on cognitive functioning in the present. There have been some studies that have found that children with a current diagnosis of IDA score lower than controls on a range of psychological tests, but they are limited. Furthermore, early detection of IDA and resulting iron supplementation may benefit anemic children, as studies have demonstrated improved IQ and scholastic achievement after 10 mg/day iron supplementation in the first 2 weeks of detecting IDA and 20 mg/day in the following 14 weeks.

Just as was done with mean protein intake, the present study examined the mean iron intake of children 5-15 years old and evaluated that iron intake for conformity to age- and gender-specific RDAs. Once again, a common theme and potential risk factor for inadequate iron intake was female adolescence. Fourteen- to fifteen-year-old females, regardless of diagnosis (anemic/non-anemic/anemic with difficulty concentrating/anemic without difficulty concentrating), did not meet the RDA for iron. Potential reasons for this could be because, as was previously determined, 14- to 15-year-old females also consume inadequate dietary protein when compared to the RDA. This is significant because iron is found naturally in foods in one of two forms: heme iron, found in animal sources of protein such as meat, fish, and poultry, and nonheme iron, found in plant sources of protein such as beans, nuts, tofu, and legumes. As a result, low protein intake in 14- to 15-year-old females may, in turn, reduce iron intake.

Another potential reason for the greater prevalence of inadequate iron intake in 14- to 15-year-old females is the fact that this population has increased iron needs due to menstruation, as females can deplete their iron stores through blood loss from menstruation. Currently, the American Academy of Pediatrics and the US Preventative Services Task Force recommend
universal screening for anemia at 12 months of age and selective screening at any age in children who are at an increased risk for iron deficiency or IDA, such as severely malnourished children, children born prematurely or with low birth weight, and children with symptoms of IDA. These screening guidelines often miss the large population of anemic female adolescents who would benefit from screening and treatment, if needed.44

Fourteen- to fifteen-year-old anemic males with difficulty concentrating also consumed inadequate dietary iron when compared to the RDA. The sample size here was small (n=3); however, this finding may point to the need for more rigorous screening for IDA in all adolescents, regardless of gender, in order to prevent poor health and cognitive outcomes.

Due to the extremely small sample size of anemic females with (n=1) and without (n=1) difficulty concentrating, more research is also needed to determine the true relationship of the explanatory variable, mean iron intake, on the dependent variable of difficulty concentrating in female adolescents.

**Strengths and Limitations**

This study had a number of strengths. First, it used multiple NHANES waves. Second, it included a diverse population of children from five different race/ethnicities and three income levels, making this a comprehensive study. Third, this secondary analysis of existing data was a cost-efficient way to address potentially important new research questions relating to the effects of iron deficiency anemia in schoolchildren.

This study also had a number of limitations. First, data used to analyze protein and iron intake relied on information from 24-hour recalls. As a result, NHANES respondents (or proxies) relied on memory to self-report dietary intake, which could have potentially led to under- or overreporting of foods and thus affected the accuracy of estimated protein and iron intake.
intake used in this study. Secondly the present study did not assess source specific protein intake, only mean protein intake. Further research on the source-specific protein intake of anemic and non-anemic children is needed, especially since studies have found that source-specific protein intake is below recommended guidelines for dairy (in all ages groups except 1-3 years old) and seafood and meets or falls below recommended guidelines for nuts, seeds, soy products, and legumes.\textsuperscript{51}

An additional limitation was that the current study was limited by the available NHANES question pertaining to serious difficulty concentrating, and as a result, only two cycles of the NHANES (2013-2014 and 2015-2016) were analyzed in this study, as previous cycles did not have this information available. In addition, many respondents were missing data, especially data pertaining to protein and iron intake as well as difficulty concentrating. As a result, the sample size was greatly reduced from the initial 4,562 respondents between the ages of 5-15 years old to 1,758, and the number of respondents who had information for protein and iron intake as well as difficulty concentrating was even smaller, and as a result, small sample sizes did not permit accurate estimates for some age groups. Further research with a larger sample size is needed.

In addition, difficulty concentrating could pertain to a multitude of factors in children such as lack of sleep, stress and anxiety, vision and/or auditory problems, thyroid issues, etc.\textsuperscript{58} However, this study assumed difficulty concentrating in children related to IDA. Further research that controls for potential confounding factors is needed to establish firm associations between IDA and difficulty concentrating in schoolchildren.
Implications for Practice

Findings from this study provide an updated snapshot of anemia, difficulty concentrating, and mean protein and iron intake in American children 5- to 15-years-old. Specifically, this study has confirmed that anemia among children 5- to 15-years-old is a public health problem of mild severity, with 8.3% of children classified as anemic according to age- and gender-specific hemoglobin levels. Also of importance is that high-risk groups such as non-Hispanic Black children and children living in low-income families were disproportionately affected. As a result, improving health disparities in race and the wealth index/household income are influential factors that need to be addressed by public policy makers as well as by pediatricians, dietitians, and other members of the healthcare team.

In addition, female adolescents emerged as a population group that consumed less than the RDA for protein and iron, indicating that this is a population that may specifically benefit from nutrition education to combat disordered eating, discourage skipping meals, and promote overall health, as well as training in media literacy to teach adolescent females to reject messages which equate value with an ideal body image.

Although results in this study pertaining to anemia and difficulty concentrating did not reach that of statistical significance, this study is unique in that it examined children who were currently anemic in order to assess the relationship between anemia and poor cognitive outcomes in children. This is opposed to the majority of studies which have been longitudinal in nature and have examined the relationship between anemia in early life with adverse cognitive outcomes years later. Since this study only assessed children at one point in time, it is unknown if these children will suffer adverse outcomes in the future. As a result, it is expected that this study will serve to prompt further research relating to IDA in schoolchildren, as well as
interventions focused on primary prevention, in order to prevent poor child health outcomes in this population.

Conclusions

Although there has been remarkable progress on our understanding of catecholamine actions on the PFC and executive functions related to working memory and attention, further insight into mechanisms underlying iron deficiency anemia and catecholamine actions in the PFC may be helpful in better understanding and preventing potential adverse cognitive outcomes in school-aged children. Furthermore, awareness of potential risk factors for iron deficiency anemia such as race, family income, and female adolescence can help health professionals design and promote primary prevention interventions to reduce iron deficiency anemia in American schoolchildren.
REFERENCES


