Relationships Between Iron Status and Measures of Attention & Memory

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M.Sc. Thesis

Lakehead University

Thunder Bay Ontario

Submitted in partial fulfillment of the degree of

Master of Science in Kinesiology

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Date: May 2007

Your file Votre référence
ISBN: 978-0-494-31841-6
Our file Notre référence
ISBN: 978-0-494-31841-6

ACKNOWLEDGEMENTS

Life is a strange thing. We eat. We sleep. We work. Occasionally, among other things, we take journeys. These journeys are never constant. Changes occur along the way. There are rivers to cross, mountains to climb, and walls to go through. There are good and bad times. There is happiness and there is sorrow. Sometimes the journeys are short, but other times they are long. They are so long that at times we stray from the path, but who is to say that the path we were on was the right path in the first place. The bottom line is that we have to get to the light no matter how far or how dark it is, we must get to the promise land.

At this time I would like to thank Dr. Ian Newhouse for being my thesis supervisor. I would like to thank all committee members for their input and support. Finally, I would like to thank all members of the data collection team.

Everyone mentioned had a role to play to allow this journey to come to a conclusion. Thank you.

ABSTRACT

Iron plays a role in cognition. Iron deficiency can lead to abnormal dopaminergic neurotransmission and deficits in attention and memory. The objective of the present study was to assess the relationship between iron status, attention and memory in 71 healthy 10th grade female adolescents (mean $\pm$ SD, 15.4 $\pm$ .56 years). Participants were selected from public high schools in Thunder Bay Ontario, Canada. Main outcome measures were: serum ferritin, hemoglobin (measuring iron status) and Trail Making (parts A & B), Digit Span (forward, backward, total), and Motor Free Visual Perception Test-3 (measuring cognition). Vitamin B_{12}, and albumin were assayed primarily for control purposes. Using serum ferritin $\leq$ 20 µg/L as a cutoff value, 58% of participants (n = 42) were iron deficient. One case of anemia (Hb < 120 g/L) and two cases of B_{12} deficiency ($\leq$ 150 pmol/L) were observed. Low albumin (< 35 g/L) was not observed. Statistical analyses included: bivariate Pearson and partial correlation, multivariate regression, between subjects t-test, and relative risk. There were no statistically significant relationships noted between serum ferritin, hemoglobin, vitamin B_{12}, or albumin and any test of attention or memory. Results suggest that serum ferritin $\leq$ 20 µg/L is not a threat to attention and memory.

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CHAPTER 1

INTRODUCTION

Over the last 40 years there has been a vast accumulation of literature regarding the effects of iron deficiency, the most frequently displayed single-nutrient disorder affecting humans, on behaviour (Lozoff & Brittenham, 1986; Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998; Webb & Oski, 1973; Werkman, Shifman, & Shelly, 1964). The estimated prevalence of iron deficiency in the world is interesting considering that iron is the most abundant metal in the earth's crust (Walter, 1993). According to the World Health Organization, between 66 and 80% of people world-wide are affected by iron deficiency (World Health Organization, 2003). A majority of these individuals may be iron deficient unknowingly. Iron is an inexpensive metal, but is a critical component of blood, a connective tissue vital to human life. Iron is an essential nutrient. An adequate supply is critical to the function of several biochemical processes. Electron transfer reactions, gene regulation, binding and transport of oxygen, and cell growth and differentiation require iron (Beard, 2001). However, excess iron accumulation can be toxic to cells and result in disease. Iron needs tend to be inverse of intake. The physiologic demand for iron is lower in males than in growing children and in women during their reproductive years. However, iron intake is considerably higher in men than in children or women (Clarkson & Haymes, 1995; Clement & Asmundson, 1982). Also, the amount of iron consumed can be many times greater than the amount absorbed. Only approximately 18% of dietary iron is absorbed (Wardlaw, Hampl, & DiSilvestro, 2004).

Throughout evolution humans obtained iron from food and consumed vitamin C rich fruits, which improved iron absorption (Rossander, Hallberg, & Bjorn-Rasmussen, 1979). These eating habits ensured that a sufficient supply of iron was bioavailable (absorbed and used by the body). Roughly 10,000 years ago humans began growing and eating grains containing less bioavailable iron (Walter, 1993). This practice started the iron deficiency epidemic. Persons with inadequate iron intake, limited rates of iron absorption or high rates of iron loss can develop a reduced concentration of hemoglobin in red blood cells. This extreme condition of iron insufficiency is called iron deficiency anemia. It results in general sluggishness, loss of appetite, and a reduction in the capacity to sustain even mild exercise (Schoene, Escourrov, & Robertson., 1983). Individuals exhibit these symptoms because a significant decrease in the iron content of red blood cells reduces the blood's oxygen-carrying capacity (Beard & Tobin, 2000).

Anemia in infancy, adolescence and pregnancy is frequently a result of iron deficiency (Peirano et al., 2001). The peak prevalence of iron deficiency occurs during infancy. It is estimated that 25% of babies in the world have iron deficiency anemia and an even higher proportion have iron deficiency without anemia (Roncagliolo et al., 1998). Iron deficiency prevails in underprivileged countries, affecting mostly infants. It is also common in industrialized nations, predominately in women (Peirano et al. 2001). Women have a considerably higher risk for developing iron deficiency compared to men. Rapid adolescent growth and the onset of menstruation place women in jeopardy. An average 28-day menstrual cycle increases iron loss by 0.44 mg/day (Beaton, Thein, Milne, & Veen, 1970). Women who lose between 40 and 60 mL of blood per period lose between 0.6 and 1.0 mg/day of iron (Hallberg Hogdahl, Nilsson, & Rybo, 1966).

Inadequate iron intake frequently occurs in women adding to the risk (Clarkson & Haymes, 1995).

Although iron deficiency anemia is often thought of as a condition restricted to the anatomical properties of blood, its wide spanning impact can have systemic effects on the body (Peirano et al., 2001). There is evidence suggesting an association between iron deficiency anemia and lower mental and motor developmental test scores during infancy and early childhood (Soewondo, 1995). Studies performed by Walter, Kovalshys, and Stekel (1983), Lozoff et al. (1987), Lozoff, Wolf, and Jimenez (1996), and Idjradinata and Pollitt (1993) have all shown that iron deficient anemic infants score lower than their iron sufficient counterparts on the Mental Development Index (MDI), and the Psychomotor Development Index (PDI) of the Bayley Scales of Infant Development (BSID). Infants treated for iron deficiency anemia continue to have lower cognitive scores years later (Lozoff, Jimenez, & Wolf, 1991). DeAndraca et al. (1991), Lozoff., Jimenez, Hagen, Mollen, and Wolf (2000), Walter (1993), and Walter, DeAndraca, Castillo, Rivera, and Cobo (1990) have all noted that 4 to 12 year old children who had been anemic as infants or toddlers displayed lower cognitive performance than peers up to ten years after iron status was corrected with iron therapy. It has been hypothesized in the literature that a person's level of cognitive functioning, including attention and memory, could be affected by iron status (Pollitt, Hathirat, Kotchabhakdi, Missell, & Valyasevi, 1989). This reasoning would help explain the link between iron deficiency and lower developmental test scores.

A physiological connection between iron deficiency, attention, and memory is a possibility. Several neurological metabolic processes require iron. These processes include: neurotransmitter synthesis, myelin formation, and brain growth (Youdim, 2000; Erikson, Pinero, Connor, & Beard, 1997). The dopaminergic system is the only neurotransmitter system in the central nervous system that has been consistently sensitive to experimental changes in iron status (Beard, 2001). A prominent feature of iron in the human brain is its sharp deposits in regions known to contain high concentrations of dopamine (Youdim, Ben-Shachar, & Yehuda, 1989). These regions include the globus pallidus, substantia nigra, ventral pallidum, thalamus, interpenduclar nucleus, caudate nucleus and putamen. Iron deficient individuals show a decrease in dopamine neurotransmission (Youdim et al., 1989). Iron is a cofactor for tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis (Youdim et al., 1989). Iron deficiency has been shown to alter dopamine D_1 and D_2 receptor density and activity in animals (Erickson, Jones, Hess, Zheng, & Beard, 2001). In the frontal lobes, dopamine controls the flow of information from other areas of the brain. Dopamine transmission is involved in the prefrontal cortex (Durstewitz & Seamans, 2002). The prefrontal cortex is essential for short-term/working memory and attention control (Glickstein & Schmauss, 2001; Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000) both which require the activation of dopamine receptors (Fan, Fossella, Sommer, Wu, & Posner, 2003). Prefrontal cortex neurons encode working memory information via sustained firing patterns (Durstewitz & Seamans, 2002). Dopamine via D1 receptors modulates activity of prefrontal cortex neurons and therefore performance in working memory tasks (Durstewitz & Seamans, 2002). Dopamine dysfunction in this region of the brain can result in symptoms (inappropriate impulsivity, over-activity, and inattention) (Shaywitz, Cohen, & Bowers, 1977) leading to decline in neurocognitive functions such as memory, attention, and problem-solving (Schmauss 2002). Reduced dopamine concentrations in the prefrontal cortex are thought to contribute to attention deficit disorder (ADD) and some symptoms of schizophrenia (Schmauss 2002). Considering iron's role in dopamine synthesis, and the evidence relating iron to various functions of cognition, iron deficiency may impact the utility of attention and memory resulting in altered behavioral expression in a variety of cognitive tasks.

Purpose

The purpose of this study is to determine the relationship between iron status (serum ferritin and hemoglobin), visual attention and memory in 14 to16 year old healthy adolescent females by measuring performance on the Trail Making Test (parts A & B), Motor Free Visual Perception Test (MVPT-3) and a Digit Span Test (forward, backward, total).

The hypothesis of the researchers is that a statistically significant positive relationship will be found between iron status and cognitive test scores of participants. As iron status increases so should cognitive performance. This relationship should result in a group difference between those who are iron deficient and those who are not, accompanied by a substantial risk for altered cognitive performance. Implications of positive relationships should be considered. Discovery of positive relationships between iron status and attention and memory would provide rationale for continuing related research. A positive relationship would underscore an increased need for screening and prevention of iron deficiency in adolescent populations.

Significance of the Study

It has been stated in the literature that more studies are needed to help clarify the relationship between iron deficiency and cognition in the adolescent age group (DiSilvestro, 2005). There are currently an inadequate number of published studies available to understand the link between iron status and cognitive performance in this group (Taras, 2005). The majority of findings linking iron deficiency to cognitive ability have been generated through investigations of participants from unindustrialized countries. Of thirty known studies dealing with iron, cognition, school performance, or supplementation Groner, Holtzman, Charney, and Mellitis (1986), Bruner, Joffe, Duggan, Casella, and Brandt (1996) and Halterman, Kaczorowski, Aligne, Auinger, and Szilagyi (2001), are the only three articles that studied North American participants between the ages of seven and twenty-four. No investigators have attempted to examine Canadian participants. Groner et al. (1986) looked at pregnant females. Only two studies (Bruner et al.; Halterman et al.) investigated non-pregnant female adolescents. More recently there has been some suggestion that iron deficiency in teenagers and young women can be corrected and academic function restored by supplementation (Murray-Kolb, Whitfield, & Beard, 2004). If this finding is reliable, iron supplementation may enhance cognitive performance. But, until more research accumulates and appears in peer reviewed journals, more evidence should be gathered to determine if fluctuating iron levels are correlated with cognitive test performance in adolescent female populations. Millions of people suffer from iron deficiency in North America and throughout the world. This fact illuminates the importance of gathering more knowledge to better understand the relationship between iron status and cognitive function.

Definitions

Albumin: Albumin is a small plasma protein produced by the liver which helps regulate blood volume and can also function as a transport protein. Low levels of albumin can signal periods of malnutrition or high blood loss. Albumin is sensitive to hydration levels and decreases in response to inflammation. This protein is found in egg white, milk, green plants, seeds, and animal blood (Merriam Webster, 2003).

Attention: A limited capacity, or pools of capacity, to process information (Schmidt, 1988).

B_{12} Deficient: B_{12} level $\leq$ 150 pmol/L.

Cognition: A general term for the higher mental processes by which people acquire knowledge, solve problems, and plan for the future (Carlson & Buskist, 1997). Cognitive functions include attention, perception, thinking, judging, decision making, problem solving, memory, and linguistic ability.

Ferritin: "A protein that binds iron and stores it within the cells, especially the liver. A small amount of it circulates in the blood. This circulating ferritin reflects the amount of stored iron in the body therefore, measurement of serum ferritin can be used to estimate iron stores" (Manore & Thompson, 2000, p. 344). Ferritin is composed of 24 polypeptide subunits (Beard, 2001).

Hemoglobin: The iron-protein compound that increases oxygen carrying capacity of blood by approximately 65 times. Whole blood hemoglobin concentration is a quantitative measure of the amount hemoglobin in the blood. If this value is low then the body has insufficient hemoglobin available to make normal red blood cells (Merriam Webster, 2003).

Iron Sufficient: Serum ferritin > 20 μg/L, normal hemoglobin (120 – 160 g/L).

Iron Deficient not Anemic: Serum ferritin ≤ 20 μg/L, normal hemoglobin (120 – 160 g/L).

Iron Deficient Anemic: Serum ferritin < 12 μg/L, hemoglobin < 120 g/L.

Low Albumin: Albumin level < 35 g/L.

Memory: The persistence of the acquired capability of responding (Schmidt, 1988).

RNI: The RNI (Recommended Nutrient Intake) is a Canadian based nutritional recommendation for intake of specific nutrients. The RNI accounts for the needs of 98% of the healthy population. Those with higher than average nutrient requirements are included under the RNI (Manore & Thompson, 2000).

Vitamin B_{12}: Member of the water soluble B vitamin group, B_{12} is needed for the synthesis of hemoglobin and for the proper function of the nerves. Vitamin B_{12} is found only in foods of animal origin (i.e., eggs, meat, poultry, milk, and milk products) (Merriam Webster, 2003).

Limitations

1. Serum ferritin can be artificially raised by factors independent of iron status (i.e., inflammation or infection). Health status of participants was monitored and evaluated for this study's exclusion criteria. Responses did not suggest a confounding effect. However, we did not statistically eliminate inflammation or infection from this study.

2. Literature involving the effects of iron deficiency on development has depended heavily on standardized tests. The Trail making, Digit span, and MVPT tests provide legitimate measures of attention and memory. However these tests do not provide direct evidence of central nervous system function at a neurophysiologic level (i.e., evoked action potentials).

3. All blood samples were taken at the same time of day. Logistically, cognitive testing had to be spread over the school day. Time of day can potentially influence cognitive test results due to normal biorhythms and effects of previous classes. Based on timetabling within each high school, it was possible that some students were exposed to academically demanding circumstances (i.e., a school test) prior to our study's cognitive testing. It can be speculated that engaging in challenging or stressful academic activities could have potentially lead to premature mental fatigue, and loss of concentration or interest during the tests of attention and memory. On the other hand, challenging academic circumstances

could have primed participants for optimal performance on the attention and memory tests.

4. Socioeconomic factors likely influence cognitive functioning but, were not considered as confounding factors. The use of more sensitive screening procedures during recruitment could have reduced those factors. Although, in order to generate interest and participation in this investigation, excluding probes into participant's economic stability may have boosted volunteer enrolment.

5. Fluctuating iron parameters during the menstrual cycle represent a possible source of error when evaluating iron status. Dates of menses were not controlled however, menstrual cycle stage was recorded and included as a predictor in multivariate analysis.

6. Precision and consistency in participant diet recording along with researcher interpretations and data entry, in the software program (Foodworks college edition), can influence accuracy and reliability of dietary analyses.

Delimitations

1. In this study we sampled a Canadian population in Thunder Bay, Ontario. Participants were healthy 10th grade females (mean $\pm$ SD, 15.4 $\pm$ .56 years) free of disease, contraceptive use, pregnancy, and the use of medication known to cause

acute or persistent blood loss from the gastrointestinal tract. Participants were in the Public Education System.

2. Participating high schools included: Hammarskjold, Port Arthur Collegiate, Hillcrest, Sir Winston Churchill, Fort William Collegiate, and Westgate.

3. Iron status indicators were: serum ferritin, and hemoglobin (Hb).

4. Groups, for between-subject analysis, were formed based on serum ferritin levels as follows: iron deficient ($\leq$ 20 µg/L), iron sufficient (> 20 µg/L).

5. Control variables were: vitamin B_{12} and albumin.

6. Attention and memory were evaluated by performance on the following cognitive tests: Trail Making (parts A & B), Digit Span (Forward, Backward, Total) and the Motor Free Visual Perception Test - 3.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The literature which relates iron to cognition (including attention and memory), and cognitive performance, will be examined under the following headings: (a) Physiological role of iron, (b) Iron consumption and need, (c) Iron deficiency: Prevalence and outcomes, (d) Attention, (e) Iron and attention deficit, (f) Attention assessment instruments: Trail Making, Motor Free Visual Perception Test – 3 (MVPT – 3), (g) Memory, (h) Memory assessment instrument: Digit Span, (i) Cognition, behaviour and iron status, (j) Iron status and cognitive ability in adolescent females, (k) Summary

Physiological Role of Iron

Iron is needed by hundreds of proteins in the body. Iron is an essential trace mineral (a dietary element needed by the body in very small amounts). As a component of hemoglobin, iron's major role is in the transportation of oxygen. Other iron compounds include myoglobin (functions similar to hemoglobin within muscle cells) and cytochromes. Cytochromes are components in the electron transport chain of aerobic energy release (Bothwell, Charlton, Cook, & Finch, 1979). Cytochromes are a division of a family of enzymes called cytochrome P-450 dependent enzymes. The P-450 enzymes are involved in processes such as drug metabolism and steroid hormone synthesis (Dallman, 1986). Non-heme iron compounds include iron sulphur proteins (i.e., nicotinamide adenine dinucleotide and succinic dehydrogenase). Iron is an essential cofactor for many enzymes. These include heme enzymes in the mitochondria, and the flavoproteins (Wardlaw et al., 2004). Another iron-dependent enzyme, required for deoxyribonucleic acid (DNA) synthesis, is ribonucleotide reductase. This suggests that iron plays an important role in growth, reproduction, healing and immune functions (Yip, 2001). Iron acts as a cofactor for enzymes involved with catecholamine metabolism and is a key component in the dopaminergic and serotoninergic neurotransmission systems (Dallman, 1986; Peirano et al., 2001). Iron has been identified as a factor in the preservation and construction of myelin (Youdim, 2000; Walter, 2003). Neurotransmitter levels and brain growth are likely affected by deficiency in iron (Youdim, 2000).

Management of iron is influenced by a number of factors. The most influential of these is body iron stores. When stores are low, the intestine becomes more efficient at iron absorption. If stores are high, iron absorption is inhibited (Heinrich, 1970). In addition, iron absorption is affected by the particular iron compound consumed along with other dietary components and by physiological factors such as production of stomach acid (Yip, 2001). Intestinal cells manufacture an iron-binding protein called ferritin in inverse proportion to body iron stores (Beard & Dawson, 1997). If iron stores are low, little ferritin is made. This process elevates iron absorption because ferritin acts a barrier to iron entering the bloodstream (Finch, 1994). If iron stores are high, large amounts of ferritin are made. Ferritin then binds iron and prevents it from ever entering the bloodstream (Finch, 1994). Once iron is absorbed from the intestine, it can be stored in the liver.

Iron is transported out of the liver to other locations in the body by a transport protein called transferrin (Bothwell et al., 1979). The delivered iron is released to various molecules such as iron containing enzymes, hemoglobin, myoglobin, and ferritin (to store iron and prevent toxicity). In an iron overloaded state, ferritin is converted to hemosiderin, a water-insoluble iron-binding protein (Finch, 1994). This process helps protect against iron toxicity. However, the primary defense against iron toxicity is ferritin in the intestine (DiSilvestro, 2005). Iron is lost everyday via the GI tract, urine, and skin. Menstruating women lose iron during their menstrual cycles.

Iron is categorized into two main classes: heme (in meat from animals) and non-heme (from vegetables, grains, and supplements). Heme iron gets absorbed more effectively than non-heme iron. Meat has advantages as an iron source. The amount of iron in meat exceeds the amount in the majority of other foods (Yip, 2001). Meat contains a protein factor increasing non-heme iron absorption from other foods eaten at the same time (Yip, 2001). Vitamin C (ascorbic acid) improves iron absorption by chelating it into a more absorbable form (Rossander, Hallberg, & Bjorn-Rasmussen, 1979). The likelihood of iron absorption can be decreased by intake of phytic acid and some polyphenols such as the tannins in tea (Zijp, Korver, & Tijburg, 2000). However, the iron absorption promoted by vitamin C and meat more than compensates for any inhibitory effects of tea on iron absorption (Zijp et al., 2000). One evaluation of a cross section of studies by Temme & Van Hoydonck (2002) concluded that tea consumption can only affect iron status if this status is low or poor. Varying levels of iron absorption have been reported when combined with ingestion of multi-vitamin-mineral supplements. These discrepancies are likely due to the presence of other nutrients and additives in the supplements, especially calcium and magnesium (Babior, Peters, Briden, & Cetrulo, 1985; Neufeld, Ramakrishnan, Rivera, & Villalpando, 2001; Reid, Lopez, Galaviz, &

Isorrd, 2001). Supplements may not provide as much absorbable iron as consumers believe.

Iron Consumption and Need

Recommended iron intake is based on losses versus gains or balance of iron in the body. It is assumed that 18% of iron in the diet will be absorbed (Wardlaw et al., 2004). A considerable source of iron loss is attributable to menstruation, therefore iron intake recommendations tend to differ with age and gender (DiSilvestro, 2005). It is recommended that adult women take in 18 mg of iron per day and adolescent females 15 mg. Men need about 8 mg per day. The recommended values do not account for women with exceptionally high menstrual blood losses (DiSilvestro, 2005). These individuals may require supplements to meet extra needs. Young adult and teenaged females are at risk for iron deficiency due to iron losses plus a lower average meat consumption in contrast to their male counterparts (Allen, 2002).

Pregnant, elderly and vegetarian are three populations with specific iron needs. Due to the physiology of pregnancy, expecting women avoid menstrual blood loses. Under these conditions iron remains a concern. In pregnancy a substantial quantity of iron is invested in making a new bloodstream and for other body changes (Marx 1997; Wardlaw et al., 2004). In an early childbirth, O'Keeffe et al. (2002), and Rao & Georgieff (2002) have demonstrated that premature infants are disposed to iron difficulties. Iron stores help sustain many initial needs after birth. Most iron stores are formed during the last trimester of pregnancy. If pregnancy concludes in a premature birth the iron storage period does not reach optimal length (O'Keeffe et al., 2002). In addition, low birth weight infants can experience a growth acceleration or "catch up" period that increases iron demands (Trachtenbarg & Golemon, 1998). Iron deficiency is a concern in these children, although over-treating with iron can put these children at risk for iron overload (Rao & Georgieff, 2002). If maternal iron deficiency develops mother-infant interactions may suffer. Perez et al. (2005) showed that iron deficient anemic mothers tended to be less responsive and emotionally available than controls. Corwin, Murray-Kolb, and Beard (2003) observed that postpartum anemia may be a risk factor for postpartum depression. There may be value to iron supplementation during pregnancy, however due to ethical limitations of the research this area is difficult to evaluate (DiSilvestro, 2005).

Serum ferritin concentrations tend to rise after the age of 65 years, either because of increased iron storage or development of progressive inflammatory disease (Cook et al, 1976). However, iron needs are a concern in elderly populations. It has been noted by Balducci, (2003); Beard, Richards, Smicklas-Wright, Bernardo, and Kordish (1996); Bohmer et al., (2003) and Coban, Timuragaoglu, and Meric (2003) that iron intake, development of chronic disease, and GI blood losses all contribute to iron status issues in elderly samples. These researchers demonstrate that if anemia is sustained it can play a role in mortality, poor health, fatigue, functional dependence and increased risk of cardiovascular or neurological problems. Although, not every elderly population is susceptible to high rates of iron deficiency. In a study of 1016 non-institutionalized U.S elderly participants Fleming et al. (2001) observed a small (3%) incidence of low iron stores (serum ferritin < 12 μg/L).

Vegetarians must be careful to address iron consumption and need. Vegetarians choose not to consume red meats, these individuals do not get adequate amounts of heme iron in their diets. Fordy and Benton (1994) noted ferritin levels of vegetarians were significantly less than non-vegetarians ($p < .0001$). Adding to this issue, the high fiber or oxalic acid (forms insoluble compounds with calcium and iron) content of the usual vegetarian diet can reduce iron absorption (DiSilvestro, 2005). Vegetarian diets do supply a substantial amount of vitamin C enhancing iron absorption (Wardlaw et al., 2004). Ascorbic acid aids in absorption by reducing the metal (iron) from the Fe^{+3} state to Fe^{+2} (Yip, 2001). To help iron consumption meet needs vegetarians should take in foods containing considerable amounts of vitamin C at the same time as they consume non-heme iron foods.

Individuals wishing to increase their iron consumption by multi-vitamin-mineral supplements should be aware that these sources may not provide as much bioavailable iron as claimed on the label. The difference is likely due to interactions with other nutrients present such as calcium and magnesium (Babior et al., 1985; Reid et al., 2001). The most widely used and cost efficient iron supplement is ferrous sulfate (Nestel & Alnwick, 1996). This source of iron may lead to GI distress and stomach aches in some people (DiSilvestro, 2005).

Iron Deficiency: Prevalence and Outcomes

Iron deficient populations exist throughout the world. It is not just the famished developing nations that suffer from rampant iron deficiency, but industrialized nations as well. Iron deficiency remains the most common nutritional deficiency in the world (Yip, 2001). Iron deficiency has been linked to behavioral and learning problems among children (Booth & Aukett, 1997; Lozoff et al., 1991; Lozoff et al., 2000; Oski, 1993;

Pollitt, 2001) and adolescents (Halterman et al., 2001). Iron deficiency is the major cause of anemia in infancy, adolescence and pregnancy. The peak prevalence is found during infancy. It is estimated that 25% of babies in the world have iron deficiency anemia, the most severe stage of the disorder (Roncagliolo et al., 1998). Many individuals display iron deficiency without anemia. Looker, Dallman, Carroll, Gunter, and Johnson (1997) found that 9% to 11% of American adolescent girls and women of childbearing age were iron deficient (abnormal value for 2 of 3 tests of iron status: erythrocyte protoporphyrin; > 1.24 μmol/L, transferrin saturation; < 14%, serum ferritin; < 12 μg/L). This percentage corresponds to 7.8 million U.S. females. There are probably many more individuals who go undetected for iron deficiency.

Although iron deficiency anemia is a hematological diagnosis, its effects are not only expressed in the blood. Researchers such as Peirano et al. (2001) have noticed that this condition could have more universal effects on the body. Evidence provided by Soewondo (1995), suggested a relationship between iron deficiency anemia and lower mental and motor developmental test scores in early childhood. Other works by Walter et al. (1983), Lozoff, Brittenham, et al. (1987), Lozoff, Wolf, et al (1996), and Idjradinata and Pollitt (1993) all demonstrated that iron deficient anemic infants scored lower than iron replete matched pairs on the Mental Development Index (MDI), and the Psychomotor Development Index (PDI) of the Bayley Scales of Infant Development (BSID). In addition, Lozoff et al. (1991) has noted that infants treated for iron deficiency anemia continue to have lower cognitive scores years later. In a longitudinal follow-up Walter (1993) observed that 4 to 8 year old children, previously anemic as infants or toddlers displayed lower cognitive performance than peers several years after iron treatment. Other projects have followed children at older ages. In a population based study of women, infants, and children participants in Florida, Hurtado, Claussen, and Scott (1999) reported an inverse relationship between hemoglobin level in infancy and the risk of mild to moderate mental retardation at 10 years of age. In another study, Seshadri and Gopaldas (1989) conducted four experiments with older children and adolescents. Their findings suggested that supplementing iron deficient anemic children and adolescents improves scores on attention, memory and concentration tests.

Speculation has surfaced that an individual's level of cognitive functioning, including attention and memory, could be affected by iron status (Pollitt, Hathirat, Kotchabhakdi, Missell, & Valyasevi, 1989; Pollitt, 1993). These findings help explain the link between iron deficiency and lower developmental test scores. Research is available showing that iron deficiency in the presence of absence anemia can compromise the developmental growth of a child or adolescent (Grantham-McGregor, & Ani, 2001; Taras, 2005). The majority of research conducted has been on infants and children. There is a void in study linking iron status to cognitive function in the adolescent group. A review on cognitive and behavioral development by DiSilvestro (2005) indicated that more information needs to be gathered in this population.

Attention

Cognition is a general term for the higher mental processes by which people acquire knowledge, solve problems, and plan for the future (Carlson & Buskist, 1997). Cognition depends on the ability to imagine or represent objects and events that are not physically there at a given instant. The umbrella of cognition encompasses several functions including: perception, thinking, judging, decision making, problem solving, memory, linguistic ability, and attention. Attention is relatively easy to define subjectively. The classical definition of attention was proposed by William James who said, "attention is the taking possession of the mind in clear and vivid form of one out of what seem several simultaneous objects or trains of thought." (James, 1890).

During a period of attention, individuals focus on the stimulus of the task and take no notice of other environmental stimuli. Generally a person can only attend to a few things at a time. Trying to attend to more than one thing at a time, such as driving and talking on a cellular phone, may result in reduced efficiency. There are distinct and measurable neurological and physiological aspects of attention. The ability to achieve a state of attention may be limited by mental or physical dysfunctions (Barkley, 1990). Short-term illnesses such as the flu can adversely affect the individual's ability to concentrate. Attention is an essential component of learning. There is evidence that very young human infants have a natural ability and tendency to attend to particular instances of auditory or visual stimulation (Hans, 1993). During stages of development, a child's attention span increases.

Limitations to the subjective definition of attention by James (1890) have lead to a recent view of attention with its own functional anatomy, circuitry and cellular structure. It is now possible to view attention much more concretely as an organ system (Fan, McCandliss, Sommer, Raz, & Posner, 2002). A major advantage of viewing attention as an organ system is to trace the ability of children and adults to regulate their thoughts and feelings. Posner and Petersen (1990) proposed that the sources of attention form a specific system of anatomical areas which can be broken down into three networks.

These networks carry out the functions of alerting, orienting, and executive control. Alerting is defined as achieving and maintaining an alert state; orienting is the selection of information from sensory input; and executive control is defined as resolving conflict among responses. The Attention Network Test (ANT) was designed to evaluate alerting, orienting, and executive attention (Fan et al., 2002).

Iron and Attention Deficit

It is possible that iron is associated with attention-deficit/hyperactivity disorder (ADHD). This disorder is characterized by inappropriate impulsivity, over-activity, inattention, and altered executive functions (American Psychiatric Association, 1994). The symptoms of ADHD may be related to a dysfunction or imbalance in dopaminergic function (Ernst et al., 1999; Jucaite, Fernell, Halldin, Forssberg, & Farde, 2005). Iron is a cofactor for tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis (Youdim, Ben-Shachar, & Yehuda, 1990). Iron deficiency has been shown to alter dopamine D_1 and D_2 receptor density and activity in animals (Erickson, Jones, Hess, Zheng, & Beard, 2001). Consequently, brain iron stores might influence dopamine-dependent functions and, therefore, expression of ADHD (Konofal, Cortese, Lecendreux, Arnulf, & Christine, 2005). Literature indicating the role of iron in ADHD is expanding. Following is a review of what has been reported.

Sever, Ashkenazi, Tyano, and Weizman (1997) conducted a preliminary report of iron treatment in children with ADHD. Participants were 14 boys aged 7 to11 years with ADHD from Israel. ADHD was established according to Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) criteria. Iron status was measured by hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, serum iron, iron-binding capacity, and serum ferritin. All boys had normal iron status. Severity of ADHD symptoms was measured by parent and teacher scores on the Connors' Rating Scale. Effects of short term (30 days) iron administration on behaviour were assessed using Student's paired t-test. Pearson correlation was used for correlation analysis. Results showed a statistically significant increase in serum ferritin levels (25.9 $\pm$ 9.2 μg/L to 44.6 $\pm$ 18 μg/L, $t = 4.14$; $p < .005$) and a statistically significant decrease (indicating less severe ADHD symptoms) on the parent Connors' Rating Scale (17.6 $\pm$ 4.5 to 12.7 $\pm$ 5.4, $t = 3.34$; $p < .005$). There was no correlation between these two factors ($r = 0.17$; $p > .55$). The teacher score remained unchanged. A placebo effect was not ruled out. The authors concluded that effects of iron supplementation on behavioural and cognitive symptoms in ADHD children merits further investigation using a placebo-controlled study.

Using a controlled group comparison design Konofal, Lecendreux, Arnulf and Mouren (2004) evaluated iron deficiency in boys ($n = 45$) and girls ($n = 8$) aged 4 to 14 years with ADHD versus iron deficiency in an age and sex matched control group ($n = 27$). ADHD participants were diagnosed based on DSM-IV criteria. The setting was a pediatric hospital in Paris, France. Main outcome measures were serum ferritin and Connors Parent Rating Scale scores. Hemoglobin, hematocrit, and serum iron were additional iron status measures. No participants were anemic. Statistical analyses were performed using t-test and Π^2 test for between group comparisons. Correlations between symptom severity and serum ferritin levels were computed using Pearson correlation. Mean serum ferritin levels were lower in the ADHD children than controls (23 $\pm$ 13 μg/L vs. 44 $\pm$ 22 μg/L; $p < .01$). This was the first clinical study demonstrating abnormally low serum ferritin levels in children with ADHD. Using a 30 μg/L serum ferritin cutoff value, 84% of ADHD children and 18% of controls had low serum ferritin. Low serum ferritin levels were correlated with more severe ADHD symptoms on Connors' Parent Rating Scale ($r = -0.34$; $p < .02$) and greater cognitive deficits ($r = -0.38$; $p < .01$). The children with the most severe iron deficiency were the most inattentive, impulsive, and hyperactive. The researchers concluded that low iron stores contribute to ADHD. However, this conclusion was challenged. In a review letter D'Amato (2005) noted that Konofal et al. (2004) did not distinguish between those who had been taking medicines for ADHD and those who had not. It is possible that the observed low iron stores may have been due to factors associated with these medications (i.e., decreased appetite and therefore decreased intake of iron rich foods). Also, a double-blind placebo-controlled study linking iron to ADHD had yet to be conducted.

Konofal, Cortese, Lecendreux, Arnulf and Mouren (2005) assessed the behaviour of one French male child (3 years old) with ADHD. The child met DSM-IV criteria for ADHD. Behaviour was assessed by clinical interviews and questionnaires. Serum ferritin and hemoglobin were used to measure iron status. Parent and teacher observations of the child were recorded using the Connors' Parent and Teacher Rating Scale. Observations occurred before and after iron treatment. At baseline the Parent and Teacher scores were 30 and 32 respectively. The child had low serum ferritin (13 μg/L). After 8 months of iron treatment (80 mg/day, ferrous sulfate) his serum ferritin increased to 102 μg/L. Parents and teachers reported behavioural improvements. Connors' Rating Scale scores improved to 19 and 13 respectively. Although authors studied only one participant and relied mostly on qualitative methods, no other study had addressed the effectiveness of iron supplementation in iron-deficient children with ADHD. Sever et al. (1997) studied participants with normal iron status whereas Konofal et al. (2004) did not use iron treatment. The results of Konofal et al. (2005) suggested the potential effectiveness of iron treatment for children with ADHD who have low ferritin levels. Konofal et al. (2005) provided rationale for double-blind placebo-controlled studies using iron in this population.

To further determine the role of iron deficiency in ADHD Millichap, Yee, and Davidson (2006) measured serum ferritin in a battery of laboratory tests (including complete blood count) in children attending an ADHD clinic. Participants were Americans (Chicago) aged 5 to 16 years. Sixty-eight patients (54 male, 14 female) were identified with ADHD. These individuals were compared to control participants ($n = 27$) and U.S. national control data. The mean serum ferritin level in participants with ADHD was not statistically different from controls (39.9 ± 40.6 μg/L vs. 44 ± 22 μg/L, $t = 0.86$; $p > .20$). Forty-four percent of participants had serum ferritin below 30 μg/L; 18% were below 20 μg/L; and 7% were below 12 μg/L. No participants showed evidence of anemia. A comparison of 12 patients with the lowest serum ferritin (< 20 μg/L) and 12 with the highest levels (> 60 μg/L) showed no statistically significant difference in severity or frequency of ADHD. These results were opposite of Konofal et al. 2004 regarding serum ferritin levels in an ADHD population. Findings of Millichap et al. (2006) suggested that a proportion of children with ADHD may have relatively low serum ferritin levels, but a causative role for low serum ferritin in ADHD cannot be established.

Antalis et al. (2006) investigated Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. ADHD participants (n = 35) and control individuals (n = 112) were drawn from the Purdue University student population. Participants were 20 to 27 years of age. A case-control design balancing gender, age, body mass index, smoking, and ethnicity was used. Participants provided a blood sample, a urine sample, a questionnaire about their general health, and a dietary intake record. Biochemical measures analyzed included markers for several nutrients, antioxidants, oxidative stress, inflammation, and fatty acid profiles. Hemoglobin, hematocrit, mean corpuscular volume and serum ferritin were among these measures. Using a Student's t-test, serum ferritin was significantly greater in ADHD versus controls (113.54 $\pm$ 49.35 µg/L vs. 71.13 $\pm$ 44.89 µg/L; $p < .04$). There were no statistical differences in any other iron status indicators. Antalis et al. studied older participants (20 to 27 years) compared to Konofal et al. (2004) (4 to 14 years) and Millichap et al. (5 to 16 years). Ratio of males to females was similar across studies. The results of these three studies conflict regarding serum ferritin concentrations in ADHD versus control participants. The possible contribution of iron status to ADHD has only been recently reported. More research should be conducted to confirm any conclusions linking iron status to ADHD. A double-blind placebo-controlled trial is needed.

ATTENTION ASSESSMENT INSTRUMENTS

Trail Making Test

The Trail Making Test (TMT) was originally part of the U.S. Army Test Battery for the selection of military forces. The Trail Making Test measures visuomotor tracking (maintaining a cognitive task, associated with movement, over time), visual-spatial ability (processing of visual orientation or location in space), and divided attention (ability to divide attention between two or more concurrent tasks) (Snyder & Nussbaum, 1998). The test has two parts. Part A requires a participant to connect, by pencil, 25 numbers, randomly arranged on a sheet of paper, in increasing order. In part B, 25 numbers and letters are connected in alternating order (i.e., 1-A-2-B-3-C-4-D). Scoring is expressed in terms of the time required to complete the test (Leclercq & Zimmerman, 2002). This test has become widely used because it is simple to administer, has potentially useful clinical meaning, and provides a format open to attentional interpretation of results (Leclercq & Zimmermann, 2002). Correlational studies support the TMT as a general measure of attention (McCaffrey, Ortega, Orsillo, & Nelles 1992). As a general measure of attention, it can be speculated that if a relationship exists between iron status and attention, decreasing performance on the TMT will occur in individuals who are increasingly iron deficient.

Motor Free Visual Perception Test – 3 (MVPT – 3)

The Motor Free Visual Perception Test (MVPT) is used across a wide range of ages in different settings. Schools, rehabilitation centers and other clinical practices have used this tool (Colarusso & Hammill, 2003). The MVPT – 3 assess visual perceptual ability (the process through which sensory information derived from light is interpreted for object recognition or spatial orientation) (Snyder & Nussbaum, 1998), while avoiding motor involvement needed to enable a response. The test was designed to assist in screening, diagnostic and research endeavors. Previous editions included the MVPT (1972), MVPT – R (1996) and MVPT – V (1997). The MVPT-3 uses all aspects of the previous tests while introducing new items and procedures following feedback from over 500 users of the previous editions (Colarusso & Hammill, 2003). Normative data is available for teenaged populations making it appropriate for use in this investigation. The horizontal test plate format allows quick administration and easy scoring. Teachers, psychologists, occupational therapists, and educational specialists have been identified as potential users of this test (Colarusso & Hammill, 2003). The MVPT provides a quick, reliable and valid measure of visual processing in children, adolescents, and adults (Colarusso & Hammill, 2003; Strong et al., 1999; Wang, Doherty, Rourke, & Bellugi, 1995). A study by Burtner, Qualls, Ortega, Morris, and Scott (2002) suggested moderate test-retest reliability for the MVPT-R with stability in visual perceptual scores for individuals with learning disabilities. The MVPT-R was administered to 38 individuals with identified learning disabilities and 37 controls on two separate occasions within a 2.5 week window of time. Inter-rater reliability agreement between examiners was 99%. Intra-class correlations for perceptual quotient scores ranged from .63-.79 and perceptual age scores ranged from .69-.86. Pearson product moment correlations for perceptual quotient scores ranged from .70-.80 and perceptual age scores ranged from .77-.87. The MVPT – 3 may also identify individuals who have an attentional deficit which interferes with their ability to attend to a portion of the visual field. This deficit would cause them to “miss” answer choices presented in one part of the visual field even though vision per se is normal. Some learning-disabled persons show a similar visual attentional deficit (Mercier, Hebert, Colarusso, & Hammil, 1997).

Memory

Memory can be described as the cognitive processes of encoding, storing, and retrieving information (Carlson & Buskist, 1997). The capacity of individuals to retain and utilize information in various ways for various periods of time characterizes memory. Cognitive psychologists have developed conceptual information processing models to describe the structure of memory. According to this view memory exists in three forms: sensory, short term, and long-term (Atkinson & Shiffrin, 1968). The characteristics of each differ, suggesting that they differ physiologically as well. Sensory memory is very limited. It provides temporary storage until the newly perceived information can be stored in short-term memory. Short-term memory, analogous to working memory, contains representations of information that have just been perceived (i.e., an item’s name or information retrieved from long-term memory). Short-term memory is where thinking occurs because it allows us to remember what we have just perceived in relation to what we already know (Haberlandt, 1994). Although the capacity of short-term memory is limited, rehearsal of information increases the likelihood of remembering it indefinitely (the information will enter long-term memory) (Carlson & Buskist, 1997). Researchers have failed to identify specific sites in the brain as locations of individual memories, but certain brain areas are critical for memory to function. Immediate recall is the ability to repeat short series of words or numbers immediately after hearing them and is thought to be located in the auditory associative cortex (Rapp, 2001). Short-term memory is located in the deep temporal lobe (Rapp, 2001). Long-term memory involves exchanges between the medial temporal lobe, various cortical regions, and the midbrain (Rapp, 2001).

MEMORY ASSESSMENT INSTRUMENT

Digit Span

Digit span is the measure of how many sequential digits can be taken in, stored, processed, and recalled in the correct order (Snyder & Nussbaum, 1998). Digit span assessment is conducted visually and orally. An individual's digit span typically increases one digit per year from birth to age seven (Strub & Black, 1985). A typical five year old will have a digit span of five. To utilize phonics beyond memorizing a few individual sounds, a child must have an auditory digit span close to six (Strub & Black, 1985). A 7 year-old's digit span is normally seven and usually will not increase more than 1 or 2 digits throughout adulthood. The average digit span for individuals over the age of seven is considered to be in the range of 5 to 9 digits, represented as 7 +/- 2 (Miller, 1956). Digit span is a common measure of short-term memory used in many intelligence tests (Snyder & Nussbaum, 1998). There is support in the literature for using digit span as a measure of visual and auditory short term memory (Vance & Singer, 1979). Digit span is a reliable tool for assessing short term memory with younger children (Gathercole & Adams, 1993), and a reliable predictor for reading and math achievement (Arcia, Ornstein & Otto, 1991).

Digit spans relating to specific problems and achievement have been noted. A link seems to be found between poor readers and low digit spans (Spafford, 1989; Koppitz, 1975). In addition to reading and reading related disabilities, a low digit span has been associated with a specific spelling disability. Newman, Fields & Wright (1993) assessed 368 English children (mean age 13.6 years) by analysis of variance in a longitudinal study comparing cognitive abilities in reading (n = 28), spelling disabled (n = 10), and control (n = 330) groups. Digit span was lower in the reading ($p < .01$) and spelling ($p < .05$) groups compared to controls (6.8 and 8.3 vs. 9.1). Using one way analysis of variance, post hoc t-tests, and chi-square tests to analyze neuropsychological performance, Rumsey & Hamburger (1990) found a two-digit difference (7 ± 1 vs. $5 \pm .92$; $p < .05$) between a control group of students (n = 15) and a "dyslexic" group of students (n = 15) matched for age, sex, and educational level with the dyslexic group having the lower average. A similar two-digit difference (7 ± 1 vs. $5 \pm .92$; $p < .05$) was also found between the dyslexic and autistic (n = 10) groups. The autistic group performed better. Jaquith (1996) looked at the affects of short term memory on standardized achievement scores. This researcher compared the auditory and visual digit spans of 546 students, from a private school located in the Southeastern United States, to their scores on the Stanford Achievement Test (SAT). The data showed that as digit span increased, so did performance on the SAT.

Cognition, Behaviour and Iron Status

In the study of cognition, behaviour and iron status, some investigators make a distinction between iron deficient participants who are not anemic (normal hemoglobin values) and those who have iron deficiency anemia. Iron deficiency anemia has been studied in more detail than non-anemic iron deficiency in children. Upon review by

Taras (2005) there is evidence that non-anemic iron deficiency may be associated with poorer cognitive outcomes. Articles selected for the review met specific criteria. Subjects were school-aged (5 to 18 years old), articles were published after 1980 in a peer reviewed journal, and findings included at least one outcome measure from the following list: school attendance, academic achievement, a measure of cognitive ability (i.e., memory), and attention. It appears results are more dramatic in the presence of anemia. It cannot be concluded that individuals with non-anemic iron deficiency are at greater risk of decreased cognitive performance (Taras, 2005).

Iron levels can range on a continuum of good to poor. Research has been conducted along the entire spectrum of values. Within normal limits investigations linking iron to tests of cognitive performance have produced non-statistically significant findings (Idjradinata & Pollitt, 1993; Oski, Honig, & Helu, 1983; Sungthong, Mo-suwan, & Chongsuvivatwong, 2002; Tucker & Sandstead, 1981). Furthermore, low iron status may have no effect on contributing performance factors such as mood or depression (Hunt & Penland, 1999). However, for years it has been suspected that poor iron status and iron deficiency correlates with poorer cognitive and behavioral performance (Pollitt, Saco-Pollitt, Leibel, & Viteri, 1986). Despite the strides made in describing the effects of iron deficiency, it has been a challenge to isolate the cognitive and behavioral outcomes of iron deficiency alone. Finding significant relationships between anemia and poor development shows promise in identifying at-risk populations, although these associations cannot establish cause-and-effect relationships.

Literature review has consistently shown that anemia seems to be related to decline in cognitive function (DiSilvestro, 2005). As a whole most of the following studies presented show consistent evidence for poorer cognitive and motor development, poorer scholastic achievement, and increased behavioral and disciplinary problems in iron deficient individuals. These studies tend to fall into the following categories: correlational/case control, longitudinal, and therapeutic intervention trials. Results of several of these studies may have been confounded by poor socioeconomic status. Due to higher standards of living, North American studies tend to have fewer problems with the issue of socioeconomic confounders, parasitic infections, and elevated blood lead levels. However, poverty, lack of stimulation in the home, lack of maternal warmth, poor maternal education (De Andraca et al., 1990; Idjradinata & Pollitt 1993), intelligence quotient (IQ) (Lozoff et al., 1991), maternal depression and more absent fathers (de Andraca et al., 1990) may all have confounding affects unlikely to be controlled for in one study.

Relationships between iron status and cognitive development can be traced back to the early 20th century. Waite and Neilson (1919), provided evidence of an association between iron status and mental function while investigating cognitive task performance deficits in Australian children (ages 6 to 14 years) suffering from hookworm disease, which lead to iron deficiency. Hemoglobin was measured in 196 children. Standardized tests included: Binet-Simon, Porteus mazes, and a Dot-Counting test. Hemoglobin was reduced by 14.2% in those with hookworm compared to those not infected. Iron deficient participants displayed poorer performance on the tests (greater retardation in score by months). Iron deficient participants were slower to complete tasks by an average of two seconds (Binet Test; 12.96 vs. 10.92). Significance levels and statistical techniques were not reported.

The following investigations linking cognition or behaviour to iron status were conducted in the 1970's. In study of American junior high male and female students (n = 193) with low socioeconomic status, Webb and Oski (1973), investigated the relationship between hemoglobin and cognitive performance. The measure of cognition was the Iowa Test of Basic Skills. This achievement battery was routinely used in the Philadelphia Public School system. Multifactor analysis of variance was used to analyze relationships between anemia, age, sex, and composite achievement scores. Anemic subjects (n = 92) tested significantly lower than non-anemic controls (5.08 ± 1.25 vs. 6.73 ± 2.07; $p < .025$). Data revealed a fairly strong correlation between iron status and the test of cognitive performance ($r = +.67$). In discussion and commentary researchers noted that confounders (i.e., race, poverty) may have potentially altered results. To address this socioeconomic issue Oski and Honig (1978) recruited participants similar in respect to racial distribution or mean educational level of parents. These investigators randomly assigned 24 iron deficient anemic U.S participants (9 to 26 months) to control and experimental groups (n = 12). Using a pre-test – post-test design and iron treatment (mean 6.8 days) to restore hemoglobin above 120 g/L, the experimental group showed a significant (96.25 ± 9.82 vs. 109.83 ± 9.18; $p = .01$) increase in cognitive function (Mental Development Index). The control group did not attain a statistically significant increase in their mean score. Although group numbers were small and the difference in test scores between the groups did not reach statistical significance, these results suggested that iron deficiency produces developmental alterations in infants that can be reversed with therapy.

In the early 1980's Tucker, Sandstead, Swenson, Sawler, and Penland (1982) observed no reliable relationship between serum ferritin and cognitive task performance (work fluency, imagery) in a longitudinal study of seven male university students who displayed consistent decrements in serum ferritin due to repeated phlebotomy over time. Due to the small number of participants involved the possibility of correlations being influenced by range restriction cannot be ruled out. Electroencephalographic (EEG) performance was also analyzed in relation to ferritin. An association between ferritin concentration and EEG asymmetry was found by multiple regression analysis in four of seven participants. For example, one participant displayed the following statistics: Work Fluency (max Rsquare = .93, $p < .01$), Imagery (max Rsquare = .74, $p < .05$). Contrary to the non-statistically significant relationship between iron status and cognitive task performance observed by Tucker et al. (1982), iron status was significantly linked to tonal memory backward ($r = -0.30$, $p < .05$) and word fluency ($r = -0.30$, $p < .05$) by Tucker, Sandstead, Penland, Dawson, & Milne (1984). These investigators examined the relationship between iron status and brain function in a sample of 69 university students (17 male, 52 female). Statistically significant relationships were consistent with EEG asymmetries. Associations between iron parameters and cognitive tests were assessed with zero-order Pearson correlations. Gender differences were controlled by partial correlation. Serum ferritin and serum iron determined iron status. In conclusion the authors suggested that body iron stores are relevant to specific neurophysiological processes supporting attention and that iron status influences psychological functioning.

Therapeutic intervention trials linking iron to cognition from the 1980's can be identified. In an effort to determine if iron deficiency in the absence of anemia would effect behavioural development Oski et al. (1983) studied non-anemic U.S infants ranging from 9 to 12 months old (n = 38). The test of cognitive performance was the Mental Development Index. By paired t-test it was found that following iron supplementation (7 days) iron deficient participants improved significantly ($p < .05$) in test score (+21.6 points). No significant changes in scores were observed in iron sufficient patients. Differences between iron deficient and iron sufficient groups failed to achieve statistical significance. Alternatively, a between group difference was noted in a double blind placebo-control prospective cohort study of 196 infants (birth to 15 months of age) in Chile, by Walter, De Andraca , Chadud, and Perales (1989). These investigators assessed the relationship between iron status (hemoglobin, mean cell volume, hematocrit) and psychomotor development (BSID, MDI, PDI, IBR) in 39 anemic, 30 control, and 127 non-anemic iron deficient children. Short-term (10 day) and long-term (3 months) oral iron therapy was utilized. Participants possessed no neonatal complications, chronic or congenital disorders, inadequate growth, or development. By analysis of variance, anemic infants had significantly lower Mental (96.4 ± 1.3 vs. 102.1 ± 1.8; $p < .001$) and Psychomotor Development Index scores (90.0 ± 2.0 vs. 101.2 ± 2.1; $p < .001$) than controls. No difference was noted between the effect of oral iron or placebo after 10 days or three months. Results of these studies suggested that when iron deficiency develops into anemia, but not before, decreases in developmental test performance appear and persist for at least three months despite correction of anemia with iron therapy.

Evidence suggesting iron supplementation may be effective in producing treatment effects increased in the 1990's and into the new millennium. Idjradinata and Pollitt

(1993) conducted a randomized double-blind trial to monitor the effects of iron supplementation (4 months) on performance in the Bayley scales of mental and motor development in 12 to 18 month old Indonesian infants. Participants were classified by iron status (hemoglobin, transferrin saturation, serum ferritin) as iron deficient anemic (n = 50), non-anemic iron deficient (n = 29) and iron sufficient (n = 47). Analysis of variance with repeated measures was calculated with maternal education used as a covariate. Before intervention, the mean developmental scores (88.8 $\pm$ 2.1 vs. 102.4 $\pm$ 2.7 vs. 105.4 $\pm$ 2.2) of the iron deficient anemic infants were significantly ($p < .01$) lower than the other groups. Following treatment the change in test score was significant ($p < .001$) in the IDA group (88.8 $\pm$ 2.1 to 108.1 $\pm$ 2.2). There was also no post-treatment difference between test score of the IDA treated group and iron sufficient infants suggesting that performance in the Bayley scales of development can be improved to the level of performance of iron sufficient infants by treatment (ferrous sulphate). Van Stuijvenberg et al. (1999) matched 115 South African children (age 6-11) with a control group (n =115) and after 12 months, of a diet supplemented with iron fortified biscuits, there was a significant between-group treatment effect in serum ferritin (control: 33.6 µg/L vs. intervention: 22.7 µg/L; $p < .0001$), hemoglobin (control: 129 g/L vs. intervention: 127 g/L; $p < .05$), and in cognitive function measured by change in the digit span forward task (control: 0.25 $\pm$ 0.83 vs. intervention: 0.61 $\pm$ 1.01; $p < .05$) which measures short-term memory. Prevalence of anemia (Hb < 120 g/L) was high but, similar across control and intervention groups (24% vs. 29%). Gender split was not noted. Iron supplementation was also associated with improved cognitive performance by Murray-Kolb et al. (2004). These researchers investigated the relationship between iron status and cognition in iron sufficient (n = 30), iron deficient (n = 53), and iron deficient anemic (n = 30) young women (average age 21 years). This intervention study used a blinded, placebo controlled format. Eight computerized cognitive performance tasks measuring attention, memory, and learning from the Detterman's Cognitive Abilities Test were administered at baseline and after 16 weeks. Performance was significantly better and time to achieve the tasks was significantly shorter in the women with the highest iron status (ferritin 41.6 – 170.0 μg/L) compared to the women with the lowest (ferritin 1.1 – 6.5 μg/L) iron status ($p < .05$). Performance was affected even in the absence of anemia. Analysis of variance indicated an improvement in performance as well as average time to complete the attention (100 msec vs. 250 msec), memory (1,250 msec vs. 1,050 msec), and learning tasks (17,000 msec vs. 11,000 msec) in those women with improved iron status. These results demonstrated a relationship between iron status and information processing in adult women. Together these supplemental trials support the use of iron treatment to improve cognitive function in iron deficient individuals.

Lozoff and colleagues have conducted longitudinal research involving iron status and cognition spanning 15 years. An objective of this research was to assess if iron deficiency in infancy identifies children with developmental risk years after successful iron treatment. In a follow-up study of 191 Costa Rican infants successfully treated for iron deficiency in infancy Lozoff et al. (1991) assessed developmental outcome at five years of age. Analysis of variance was the primary statistical technique. This study had good covariate control. Covariates included: sex, birth weight, mothers IQ, height & education, breastfeeding, and absence of father. Cognitive measures included: Woodcock Johnson psychoeducational battery, Goodenough-Harris Draw-a-man test, Beery Developmental test of Visual-Motor Integration, (VMI) Bruininks-Oseretsky Test of Motor Proficiency. There were no current differences in any measure of iron status (hemoglobin, serum ferritin, transferring saturation). However, children who had moderately severe iron-deficiency anemia as infants, (Hb $\leq$ 100 g/L) had lower scores on tests of mental and motor functioning at school entry than the rest of the children even after controlling for covariates. For example, the mean ($\pm$ SD) adjusted Woodcock-Johnson preschool cluster score for the children who had moderate anemia in infancy (n = 30) was 448.6 $\pm$ 9.7, as compared with 452.9 $\pm$ 9.2 for the rest (n = 133) of the children (p <.01) and the adjusted visual-motor integration score was 5.9 $\pm$ 2.1, as compared with 6.7 $\pm$ 2.3 (p <.05). These results suggest that children who have iron-deficiency anemia in infancy are at risk for a long-lasting developmental disadvantage as compared to their peers with better iron status. These results represented a point of clinical interest supporting early treatment of anemia in infancy. In further longitudinal follow-up Lozoff et al. (2000) continued to investigate the long term effects of iron deficiency in infancy by again revaluating participants from the previous investigations whose average age was currently 12.3 years. All participants were now free from iron deficiency. Eighty-seven percent (n = 166) of the original 191 Costa Rican participants were enrolled. Those with chronic severe iron deficiency in infancy (n = 48) were compared to those with good iron status before and/or after iron therapy in infancy (n = 114) by analysis of covariance. Covariates included sex, mothers IQ, and HOME (Home Observation of for Measurement of the Environment). Results showed that children who had severe chronic iron deficiency in infancy scored lower on measures of cognitive function. After controlling for background factors, differences remained statistically significant in many of the very large and comprehensive battery of cognitive tests which included: Wide Range Achievement, Bender Gestalt visual motor, Bruininks-Oseretsky motor proficiency, Central/incidental learning, Attention capacity underlining, K-ABC Spatial memory, Tactual performance, computerized cognitive abilities: learning, reaction time, stimulus discrimination, Sternberg search, tachistoscopic threshold, self-paced probe recall parent and teacher behavior check list. For example, formerly iron-deficient children did more poorly on the K-ABC Spatial Memory Task. The raw score for the ID group averaged 12.8 ± 0.4 vs. 13.9 ± 0.4 in the good iron status group ($p < .03$). These results suggest that severe chronic iron deficiency in infancy identifies children who demonstrate developmental risk greater than 10 years after iron treatment. Most recently, Lozoff, Jimenez, and Smith (2006) continued a longitudinal follow-up of the same Costa Rican participants. These individuals were now up to 19 years of age. Seventy-eight percent ($n = 185$) of the original participants were evaluated. The objective of Lozoff et al. (2006) was to assess change in cognitive functioning after iron deficiency in infancy, depending on socioeconomic status (SES; middle vs. low). Individuals who had chronic iron deficiency in infancy (Hb $\leq$ 100 g/L) were compared with those who had good iron status as infants (Hb $\geq$ 120 g/L) before and/or after therapy. Cognitive change over time (composite of standardized scores at each age) was the main outcome measure. Hierarchical linear modeling was the primary statistical approach. For middle-SES participants, scores averaged 101.2 points in the group with chronic iron deficiency versus 109.3 points in the group with good iron status in infancy and remained 8 to 9 points lower through 19 years (95% confidence interval [CI], -10.1 to -6.2). In low-SES participants, the gap increased from 10 points (93.1 vs. 102.8; 95% CI for difference, -

12.8 to -6.6) to 25 points (70.4 vs. 95.3; 95% CI for difference, 20.6 to 29.4). The authors concluded that the chronic iron deficiency in infancy group did not catch up to the group with good iron status in cognitive scores over time. There was a larger gap for those in low-SES families. The combined results of this longitudinal research underline the importance of preventing iron deficiency in infancy. Long term effects of iron deficiency during infancy may be permanent.

Iron's link to cognition and behaviour has been studied in diverse populations. With a few exceptions, impaired cognitive and motor development, decreased scholastic achievement, and increased behavioral and disciplinary problems appear to accompany iron deficiency. Using a cross-sectional design, Hutchinson et al. (1997) studied 9 to 13 year old fifth grade Jamaicans (n = 417: boys, n = 383: girls). The sample was randomly selected from 16 schools. Iron status was determined by hemoglobin. Cognitive achievement was measured by the Wide Range Achievement Test (WRAT). Anemia (Hb < 110 g/L) was correlated with attendance ($r = 0.20$; $p <.001$) and achievement scores in reading ($r = 0.13$; $p <.001$) and spelling ($r = 0.12$; $p <.01$). Participants who were anemic had lower scores. In multivariate analyses of factors associated with attendance controlling for socioeconomic status, anemia predicted poor school attendance ($B = - 4.40$; $p <.01$). Otero, Aguirre, Porcayo, & Fernandez (1999) examined 33 Mexican children (6-12 years of age) who were iron deficient (serum iron < 60 :g/dl) but not anemic and 33 controls with normal iron status. Groups were matched by age sex, and sociocultural level. Tests given included the WISC-R for IQ and computerized tests of learning as well as an electroencephalogram (EEG). Analysis by ANOVA showed that iron deficient children had lower values in several WISC items compared to children with normal iron levels (information: 5.9 ± 2.3 vs. 8.6 ± 2.9; p = .0007, comprehension: 5.1 ± 2.5 vs. 10.6 ± 4.3; p = .0004, verbal IQ: 85 ± 23 vs. 106 ± 18; p = .001, performance IQ: 83 ± 15 vs. 102 ± 16; p = .0001, and full scale IQ: 83 ± 14 vs. 104 ± 15; p = .0001). Iron deficient children showed slower activity in EEG's (absolute power delta and theta bands) as well suggesting developmental lag and/or central nervous system dysfunction. Hurtado et al. (1999) used a logistic regression analysis to associate anemia in a large sample of American 10 year olds (n = 5411) with increased likelihood of mild to moderate mental retardation (odds ratio: 1.28; 95% CI: 1.05-1.60). This effect was independent of birth weight, maternal education, sex, race-ethnicity, mother's age, and child's age. Hemoglobin was the only measure of iron status.

Some authors have noted no association between iron and measures of cognition or behaviour. To examine the possibility that low iron status had cognitive functional consequences Fordy and Benton (1994) sampled 297 male (n = 130) and female (n = 167) students between the ages of 17 and 27 years (mean age 20.5 years) from the United Kingdom. Hemoglobin and serum ferritin were measures of iron status. Cognitive tests included: digit span, reaction time, sustained attention and a questionnaire measure of mental health. One and two-way analyses of variance were used in statistical analysis. Participants were classified into three groups by ferritin level (≤ 5 μg/L, 5.1 – 20 μg/L, and > 20 μg/L). Results showed no association between low ferritin and poor performance on the tests. Harahap, Jahari, Husaini, Saco-Pollitt, & Pollitt (2000) studied the effect of an energy (1171 kJ) and mircronutrient supplement (12 mg iron) on iron deficiency anemia, physical activity, and mental and motor development in Indonesian children (n = 36). Iron status (hemoglobin, ferritin, transferrin saturation) was measured before and after six months of treatment. Mental and motor development was evaluated with the Baley Scale. Physical activity was measured by four hour continuous observation at home and day care centres. Results by t-test showed that anemic children ($n = 18$) displayed greater improvement in motor development ($t = 2.16$; $p < .05$) than controls ($n = 18$). Regarding physical activity the main effect of IDA status with repeated measures ANOVA was statistically significant ($F = 4.66$, $p < .05$), but the interaction term of time and IDA was not. This analysis showed that changes were significantly ($p < .05$) larger in the IDA group than among non-IDA participants. However, none of the mental development tests showed inter-group differences suggesting iron status had no effect on cognitive performance. Evidence is available that iron status has no effect on cognition or behaviour, however the majority of research results suggest the opposite.

In conclusion it seems profound iron deficiency early in the lifespan has deleterious effects on cognitive and behavioural development. Iron intervention may be useful in improving cognitive function. The relationship between cognition, behaviour, and iron status in older populations is not clear. The narrow range of studies in populations of later childhood and adolescence, which will be presented now, make it difficult to derive any final conclusions.

Iron Status and Cognitive Ability in Adolescent Females

The relationship between iron status and cognitive performance in the adolescent age group has not been finalized. Limited published authors have investigated heterogeneous populations using heterogeneous methods. There is room for knowledge acquisition on this topic. Following is a summary of the relevant research already conducted. A table highlighting some recent studies conducted to assess the relationship between iron status and cognitive abilities in adolescent and non-adolescent populations is also presented (Table 1).

Groner et al. (1986) examined the relationship between iron status and cognition in a group of 38 pregnant American females between the ages of 14 and 24 years. Participants had hematocrits greater than or equal to 31%. A double blind, randomized protocol was used. Participants were considered at risk due to poverty. Serum ferritin and hemoglobin, hematocrit, and mean corpuscular volume were used to measure iron status. Cognitive ability was evaluated by a set of six standardized tests for short term memory and attention (four subsets of the WISC, Consonant Trigrams, and Rey Auditory Verbal Learning Test). At baseline and after one month post intervention there was no correlation between iron status and test performance. Treated group performance improved significantly (pre: 10.0 ± 2.6 vs. post: 1.3 ± 1.5; $p < .005$) on the Digit Symbol test (short-term memory), calculated by paired t-test, and a test of attention (Consonant trigrams) also determined by paired t-test (pre: 9.8 ± 2.2 vs. post: 1.1 ± 2.5; $p < .05$). While the experimental group improved significantly on the Digit Symbol test, the difference between the changes of both groups (experimental: 1.3 ± 1.5 vs. control: 0.3 ± 1.7) did not reach statistical significance ($p < .08$). The change in Consonant trigrams was greater for the experimental group (experimental: 1.1 ± 2.5 vs. control: -0.7 ± 2.4; $p < .05$). Due to the physiological changes encountered during pregnancy, the author concluded that findings cannot be generalized to other groups. There were only 9 participants in the control group following dropout ($n = 10$). The experimental group was also small ($n = 16$) raising concern regarding statistical power and effect size. Groner et al. conceded that similar investigations should be performed among non-pregnant teenagers and young adults.

To address the lack of generalization of findings due to pregnancy, Ballin et al. (1992) documented subjective response to iron therapy in non-pregnant female adolescents (ages 16 to 17) from a high school in Israel. A double-blind placebo control design was used to compare an iron supplemented group (n = 29) to a placebo group (n = 30). After two months the percentage of self-reported improvement by the iron group, using medical questionnaire, was significantly higher in lassitude (25% vs. 2.5%; $p < .05$), concentration (30% vs. 2.5%; $p < .025$), and mood (20% vs. 0%; $p < .05$) versus the placebo group. The fact that this was a double-blind control study gives the results some merit despite speculation of the limited importance and generalization of subjective parameters.

Concern over pregnancy or self-reports were not an issue in a double-blind placebo controlled trial by Bruner et al. (1996). In a sample of 98 adolescent American girls from Baltimore (ages 13-18 years), Bruner et al. used serum ferritin and hemoglobin to study non-anemic iron deficiency. Cognitive ability was determined by several standardized tests including: Brief Test of Attention (BTA), Symbol Digits Modalities (SDMT), Visual Search and Attention (VSAT), and the Hopkins Verbal Learning Test (HVLT). Initially, ferritin was not correlated with scores on the cognitive tests. Data were analyzed by stepwise regression analysis following iron treatment. Results showed that those treated with iron (n = 37) performed significantly better ($p < .02$) than the placebo group (n = 36) over baseline in the HVLT measuring verbal learning and memory (R^2 = 0.25, R^2 change = 0.07, Baseline score: 1.79). The authors concluded that further research is needed to assess whether such cognitive effects are limited to neuropsychological measures or are also evident in academic performance.

To satisfy this recommendation, Abalkhail and Shawky, (2002) determined cognitive ability by academic school performance using actual school tests. Iron status (hemoglobin) was measured in a group of 800 Saudi girls and boys between the ages of 9- 21 years (mean 14 yrs). Nutritional habits (i.e., breakfast intake) were then assessed in 84 (42 boys and 42 girls) participants. Results showed a significantly ($p < .05$) higher prevalence of anemia among those failing (zero to < 70% of marks) school exams versus those with excellent (≥ 90%) results (33.8%; 95% CI: 23.0 – 44.6 vs. 17.2%; 95% CI: 12.6 – 21.8). Proportions and 95% confidence intervals were computed. Differences between proportions were reported as statistically significant when the 95% confidence interval did not overlap. Hemoglobin was the only measure of iron status. These findings cannot be generalized to individuals with non-anemic iron deficiency.

Similarly, in a study of 452 eighth grade Jamaican girls between 13 and 14 years of age, Walker, Grantham-McGregor, Himes, Williams, & Duff (1998), used hemoglobin as the only parameter of iron status in comparison with school performance (Wide Range Achievement Test [WRAT]). Multiple regression analysis showed a significant difference ($p < .001$). Anemic girls displayed more poor reading ($R^2 = 0.21$), and spelling ($R^2 = 0.26$) achievement compared to those not anemic. Because this study utilized hemoglobin as the only indicator of iron status, findings cannot be generalized to individuals with non-anemic iron deficiency either.

Lynn and Harland (1998) matched 208 and 205, 12 to 16 year old participants from the United Kingdom. Cognitive ability was determined by IQ utilizing the Ravens

Progressive Matrixes. Hemoglobin and serum ferritin were used to assess iron status. The initial correlation between ferritin and IQ was not statistically significant. The correlation ($r = 0.17$) between hemoglobin and IQ although small was statistically significant ($p = 0.01$). Iron treatment improved IQ performance (IQ rise = 3.07 points) significantly ($t = 2.34$; $p = .02$) in those with low iron stores, which were defined as a serum ferritin < 12 μg/L versus a placebo group (IQ difference: 5.8 points). The employment of serum ferritin as an indication of iron status enabled consideration of larger iron status ranges and helped contribute to the identification of more subtle effects of iron deficiency. Data collection included non-adolescent participants and both male and female sexes. Therefore, generalizing findings specifically to adolescent females is problematic and more controversial.

In a large group from a national representative sample ($n = 5398$) of school aged children and adolescents between 6 and 16 years of age Halterman et al. (2001) investigated the relationship between iron deficiency and cognitive test scores in American girls ($n = 2731$). Cross-sectional data were obtained from the National Health and Nutrition Examination Survey III. This database contained measures of iron status including serum ferritin, hemoglobin, transferrin saturation, and free erythrocyte protoporphyrin. Several age groups were assessed. One of these groups was comprised of females between the ages of 12 and16 years. Cognitive ability was appraised by standardized test scores on the Wechsler Intelligence Scale for Children (WISC-2) and the Wide Range Achievement Test (WRAT- reading and math). Test score averages were calculated using t-tests for means and Π^2 for proportions. Multivariate analysis was performed by logistic regression. Outcomes revealed that math score averages were lower in iron deficient participants, whether or not anemia was present verses those with normal iron status (86.4 and 87.4 vs. 93.7; $p < .05$). Relative risk analysis showed that iron deficient participants had more than twice the chance of scoring lower in Math (odds ratio: 2.3; 95% confidence interval, 1.1 – 4.4), compared to those who had normal iron status. As data from this study came from a nationally representative sample, findings can be generalized to the entire American adolescent population.

Table 1.
Recent Studies of Iron and Cognition Including Females.

Study	Participants	Design	Iron Parameters	Cognitive Measures
Lozoff et al., (2006)	185 Costa Ricas; 15-19 years; no acute or chronic health problems	Longitudinal	Hb, (≤ 100 g/L in infancy = anemic) TSAT, SF	Cognitive change over time (composite scores at each age).
Findings: Chronic ID group in infancy did not catch up to good Fe group in cognitive scores over time (Hierarchical linear modeling). Widening gap for those in low SES families.				
Murray-Kolb et al., (2004)	183 American Women; 18-35 years (Penn State)	Intervention trial (blinded, placebo, controlled); 16 wk.	Plasma iron, TIBC, SF, serum transferrin receptor	Detterman's CAT (attention & memory)
Findings: Performance better & time to finish shorter in highest quintile Fe status vs. lowest quintile.				
Halterman et al., (2001)	5398 American child. & ado.; 6-16 yr.; 1114 of total were females; 12-16 years.	Cross sectional comparison group (from National Health survey)	Hb, SF, TSAT, FEP	WISC-2, WRAT (reading, math)
Findings: Math score ave. lower in ID (2 abnormal values for age) whether or not anemic (Hb < 120 g/L for 12-16 yr olds) vs. those with normal Fe status (t-tests, Π^2, logistic regression).				
Hurtado et al., (1999)	5411 American; child.; up to 10 years	Longitudinal retrospective	Hb only	SE placement; criteria used by Florida Dept. Ed. for mild/moderate mental retardation
Findings: Anemia (Hb ≥ 2 SD below age & sex-specific references) associated with increased likelihood of mild to moderate mental retardation (logistic regression). Effect independent of birth weight, maternal education, sex, race-ethnicity, mother's age, and child's age.				
Bruner et al., (1996)	98 American; teenage girls; 13-18 years	Intervention trial (DB, placebo, controlled); 8 wk.	Hb , SF	BTA, SDMT, VSAT, HVLT
Findings: Fe treated (n = 37) performed better (stepwise regression) than placebo group (n = 36) in HVLT (verbal learning, memory).				
Fordy & Benton, (1994)	297 (130 male, 167 female); United Kingdom; 17-27 years	Post-test comparison group	Hb , SF	DS, RT, sustained attention, questionnaire of mental health
Findings: Fe treated (n = 37) performed better (stepwise regression) than placebo group (n = 36) in HVLT (verbal learning, memory).				

Abbreviations: Ave. = average, BTA = Brief Test of Attention, child. = children, CAT = Cognitive abilities Test, DB = Double Blind, DS = Digit Span, FEP = free erythrocyte protoporphyrin, Fe = iron, Hb = hemoglobin, HVLT = Hopkins Verbal Learning Test, RT = Reaction Time, SE = Special Education, SES = socioeconomic status, SF = serum ferritin, SDMT = Symbol Digits Modalities Test, TSAT = Transferrin saturation, TIBC = Total Iron Binding Capacity, vs. = versus, VSAT = Visual Search Attention Test, WISC = Wechsler Intelligence Scale for Children, wk. = weeks, WRAT = Wide Range Achievement Test.

Summary

Iron deficiency is a prevalent public health concern. Critical assessment of the literature reveals that iron deficiency has been linked to behavioral and learning problems among children. Iron deficiency in infancy appears to identify children with developmental risk years later. Most short-term follow-up studies show that low scores persist in spite of complete hematologic correction. After extended periods of observation it is not clear to what extent these impairments are reversible. Iron status may be associated with attention-deficit/hyperactivity disorder (ADHD). There is apparent room to attain knowledge in the study of iron deficiency related to cognition in adolescents. Works in the field are numbered and widespread conclusions are difficult to make considering the populations studied have been diverse. These populations have extended from third world underdeveloped countries to affluent first-world countries. The majority of findings linking iron deficiency to various cognitive abilities have been generated through investigations conducted beyond North America. A number of these studies collected data from samples allowing inclusion of both males and females. Some investigators have mixed together non-adolescents with teenagers in their statistical procedures. Studies from underdeveloped nations present confounding challenges in methodology related to poor socioeconomic conditions (i.e., parasitic infections). Generalizing findings has been a challenge considering a uniform battery of tests measuring cognitive performances accompanied by homogeneous statistical analyses have not been employed. The groundwork linking iron deficiency to attention or memory in adolescents has been provided. However, definite conclusions have not been established. The need for ongoing research is apparent.

CHAPTER 3

METHODOLOGY

Participants

Seventy-one healthy female volunteers, between the ages of 14 and 16 years, were self-selected for this study. All participants were Thunder Bay high school students. The volunteer nature of participation classified the sample as a non-probability sample.

Participant recruitment proceeded as follows:

1. Ethics approval from both Lakehead University (Ethics Procedures and Guidelines for Research Involving Humans) and the Lakehead Public School board (Code of Ethics) was obtained to allow research to be conducted involving student participants.

2. Electronic notification (email) was provided to secondary school principals, by the Lakehead Public Schools Educational Officer, acknowledging that the study was approved and participant recruitment was underway.

3. Meetings with Principals were arranged by the researchers to explain the investigation in detail and negotiate how the study protocol would occur at each institution.

4. Recruitment/Orientation presentations were made by the researchers to all grade ten classes. The significance of the study and potential risks were addressed. Individuals

expressing an interest in participation were given consent forms (Appendix A) to be signed by themselves and their legal guardians. Students were reminded to return the signed forms.

5. Participant screening was accomplished based on responses to health survey questionnaire (Appendix B). Those possessing health attributes compromising the studies' protocols were excluded from the investigation. These attributes are included in the following exclusion criteria.

6. Exclusion criteria included: male gender, grade level other than 10th, contraceptive use, pregnancy, presence of disease, and the use of medication known to cause blood loss from the gastrointestinal tract. There were no other restrictions to participation.

Participant standardization

Delimiting recruitment of female students to the 10th grade made certain that all participants were exposed to similar instruction, assessment, evaluation, and reporting leading up to inclusion in this study. All participants in this study functioned at a similar achievement level. In Ontario, a well-designed system of assessment, evaluation, and reporting based on clearly stated curriculum expectations and achievement criteria allows teachers to focus on highly standardized achievement for all students (Ontario Ministry of Education and Training, 1999). This system promotes consistency in professional practice across Ontario (Ontario Ministry of Education and Training, 1999). Teachers must use standardized assessment and evaluation strategies (Ontario Ministry of

Education and Training, 1999). Restricting enrollment to a single grade reduced individual differences and maturation effects. Valid analyses between participants could be conducted following formation of sub groups based on our definition of iron deficiency.

Procedure

Agreement was met between the researchers and principals to authorize data collection. Testing was performed on separate days at each high school. Testing began at 9:30 am. Participants donated blood samples after being instructed to fast overnight, and refrain from vigorous physical exertion 24 hours prior to the tests. Participants were called by the school's intercom system to meet at the room allocated for testing. Participants filled out a supplemental information sheet (Appendix C) divulging the date of their last period and ethnicity. Answers to these questions were sought to improve interpretation of results. Participants were seated and asked individually to enter a confined area (created by dividers from the school), within the room, to have their blood samples drawn. A certified phlebotomist performed the procedure. Approximately 6 mL of blood was collected in Vacutainer tubes by antecubital venipuncture, using Eclipse blood collection needles. Participants were seated when samples were taken. Single-use needles were collected and discarded in a Vacutainer sharps collector. Blood samples for hemoglobin and serum ferritin assays were each collected in 3 mL tubes (Vacutainer plus Plasma Separator Tube [PST] with lithium heparin, plasma separator gel and hemogard; Vacutainer K2 containing the anticoagulant ethylene diamine tetra-acetic acid [EDTA], Becton Dickinson Canada Inc. Oakville ON). Samples were refrigerated for storage at

2^{o}- 8^{o} C. For safety and observation of side effects (e.g., dizziness or fainting), participants stayed seated following the blood test. Blood samples were analyzed for levels of serum ferritin, hemoglobin, vitamin B_{12}, and albumin. The assays performed were those currently practiced by the Thunder Bay Regional Health Science Centre. Hemoglobin was analyzed by a Coulter Counter instrument (Coulter Electronics, Hialeah FL). Serum ferritin concentrations were assessed by the Quantimune ferritin immunoradiometric assay (Bio-Rad Laboratories, Mississauga, Ontario). These iron indicators allowed us to classify participants into three groups (iron sufficient, iron deficient not anemic, or iron deficient anemic). Vitamin B_{12} was evaluated by radioisotopic assay (Becton Dickinson, Oakville ON). Albumin status was determined by colorimetric assay (BioAssay Systems, Hayward CA).

Following recuperation from the blood sampling participants proceeded through a cycle of standardized tests measuring attention and memory. Students who could not be accommodated immediately were given a time to return after their regularly scheduled class to allow minimal disruption of school activities. Students were scheduled every 10 minutes. The schedule was devised to allow a smooth shuttle of students to and from testing. We avoided keeping students out of class for an extended period of time. Testing stations were positioned in the rooms so that they were either completely separated by walls or were spaced far apart. Students could not interfere with each other. Separate stations were utilized to improve consistency of delivery. There was one examiner and one test at each of the three stations. The same researcher administered the same test to each participant. Participants were seated during testing.

At the first testing station participants performed the Trail Making Tests (TMT - parts A & B). The Trail Making Test is a paper and pencil test. It is a visual attention task measuring visuomotor tracking and divided attention. Part A of the test required the participant to connect 25 numbers randomly arranged on a sheet of paper, in increasing order (Appendix D). In Part B, 25 numbers and letters were connected in alternating order (1-A-2-B-3-C-4-D) (Appendix D). The test was scored based on the amount of time needed for completion. After completing the TMT, a participant moved on to the next station to perform another task. The time allocated for switching stations allowed participants a short break from each activity and hopefully helped maintain attentiveness. This next attention task was the Motor Free Visual Perception Test – 3 (MVPT-3). This test measured various aspects of visual-perceptual abilities such as visual analysis, visual discrimination and discrimination of figure ground. The format looked like a visual multiple choice. The participant was presented with a line drawing and was then instructed to choose the matching drawing from a set of four similar drawings. The drawings were presented horizontally (Appendix E). Scoring was based on the number of correct answers. Once the MVPT-3 had been completed, the participant moved on to the last station. The final task was the Digit Span Test (forward, backward, total). This test measured the number of digits the participant could absorb and recall in correct serial order after seeing them presented on a computer monitor. In this short-term memory task, the participant had to remember a small amount of information, for a relatively short time, prior to recall. Numbers were presented slowly in one second intervals by a computer program. Two trials were performed at each level. Each succeeding level was made more difficult by the addition of one more digit to remember. For example, the numbers 4-7 (which represented a trial) would flash one at a time. The subject would then repeat the numbers back orally to the test administer. Another two digit sequence would follow (e.g., 6-3). In order to advance to the next level the participant had to be correct on at least one of the two trials. Trials on the next level would be one digit longer (e.g., 5-8-2). If both trials at a level were incorrectly recalled the test was stopped. One point was scored for each correct trial. Once this task was completed the same task was presented again. Although, this time the participant had to repeat the digits in backward order. The correct response to a display of 2-4 was to say "four-two". Backward scoring was the same as forward scoring. To get a total score, both forward and backward points were added together.

A 3-day dietary analysis, including one weekend day, was used to assess participant eating habits. Participants recorded all intakes (solid and liquid) in a log (Appendix F1). Participants received instructions on the method of recording food intake quantities and were asked to continue their usual dietary practices. Written instructions and examples to enhance clarity and accuracy of reports were also made available (Appendix F2). Following return of the logs, data were entered and assessed using Foodworks college edition software (version 1.0). Body Mass Indexes (BMI's) were also calculated.

Variables

The variables in this study were measured numerically. These variables are classified as quantitative. The independent or predictor variables are characterized as continuous. These variables included: serum ferritin, hemoglobin, vitamin B_{12}, and albumin. Vitamin B12 and albumin served as control variables in partial correlation. Vitamin B_{12} is found only in foods of animal origin. Vitamin B_{12} functions in the synthesis of red blood cells (Merriam Webster, 2003). B_{12} deficiency can lead to impaired DNA synthesis and development of abnormal macrocytic (abnormally large) red blood cells that cannot effectively transport oxygen and remove carbon dioxide (Hebert, 1996). Deficiency in vitamin B_{12} could manifest in a type of anemia, called pernicious anemia, not as a direct result of poor iron status (Hebert, 1996). Albumin is a small plasma protein produced by the liver. Albumin helps regulate blood volume and can also function as a transport protein (Tortora, 1999). Low levels of albumin can signal periods of malnutrition or high blood loss (Bulow & Madsen, 1986). The dependent or criterion variables in this investigation were the scores on the tests of attention and memory. Some of these variables were continuous (e.g., Trail Making {parts A & B}). Others were discrete (e.g., MVPT-3, and Digit Span {forward, backward, total}). Additional descriptive variables included in this study were: height, weight, and body mass index. These variables were continuous.

Statistical Analysis

A correlational research design provided the statistical foundation for the study. This design involves collecting data in order to determine whether, or to what extent, a relationship exists between two or more quantifiable variables (e.g., serum ferritin and Trail Making A). A derived correlation coefficient represents the strength and direction of the relationship.

Possible relationships between iron status (serum ferritin, hemoglobin), and the measures of visual attention and memory (Trail Making A & B, MVPT-3, and Digit Span forward, backward, total) were investigated using the Pearson Product Moment Correlation. Vitamin B_{12} and albumin were statistically controlled in partial correlation. The 95% confidence interval (.05 level of probability) was selected as the threshold of statistical significance. As a secondary objective, vitamin B_{12} and albumin were assessed by correlation with the measures of visual attention and memory.

Besides correlation, other similar statistical analyses were performed. This approach was expected to produce the same conclusions. The scope of this study crosses the boundaries of clinical physiology, cognitive neuropsychology, and education. We believed the use of different statistical techniques expressing the same relationship would benefit the readership of diverse audiences (e.g., researchers, clinicians, educators). Multivariate linear regression was used to determine which if any variables were statistically significant predictors of cognitive performance. To evaluate between group differences independent t-tests were used to compare iron deficient and iron sufficient participants based on cognitive performance on attention and memory tasks. Relative Risk was used to numerically express the excess risk of scoring below the group average in cognitive test performance. Risk was determined by the conditional probability of being either iron deficient (ferritin $\leq$ 20 μg/L) or iron sufficient (ferritin > 20 μg/L) and scoring below or above the middle (median) of the distribution for each test of attention and memory. Statistical equations used are listed in Appendix G.

CHAPTER 4

RESULTS

Two hundred females from six Thunder Bay public high schools showed interest in the study. Following recruitment and screening, signed consent forms were received from 71 female students {Westgate (n = 6), Fort William Collegiate Institute (n = 7), Hillcrest (n = 9), Port Arthur Collegiate Institute (n = 11), Churchill (n = 14), and Hammarskjold (n = 24)}. There were no drop outs during the study. The 71 participants underwent blood assays for serum ferritin, hemoglobin, vitamin B_{12}, and albumin. Following blood samples participants completed the Motor Free Visual Perception Test, Digit Span Test (forward, backward, and total), and Trail Making Test (part A and part B).

Blood analyses revealed iron deficiency (serum ferritin $\leq$ 20 µg/L) in 58% of participants (n = 42). No participants displayed elevated serum ferritin levels. One participant was anemic (hemoglobin <120 g/L). The average for serum ferritin was 19.8 $\pm$ 9.8 µg/L. The average for hemoglobin was 136.2 $\pm$ 7.2 g/L. Although two participants were vitamin B_{12} deficient ($B_{12} \leq$ 150 pmol/L), their serum ferritin and hemoglobin levels were normal. No participants had low albumin (< 35 g/L). Mean digit spans were within normal ranges based on average age (15.4 $\pm$ .56 years). Average Trail Making (part A) scores were at the 90^{th} percentile. Part B scores fell between the 25^{th} and 50^{th} percentile of normal. Motor Free Visual Perception scores registered at an 11 year old age equivalent. Normative data are presented in Appendix H. The descriptive statistics for the group are listed in table 2.

Table 2.
Statistics Describing Age, Serum Ferritin (SF), Hemoglobin (Hb), Motor Free Visual Perception Test (MVPT), Digit Span Test (forward, backward, and total), Trail Making Test (part A and B), Vitamin B_{12}, Albumin, and Body Mass Index (BMI).

	N	Mean	Std. Dev.	Min.	Max.	Range
Age (yrs.)	71	15.4	0.56	14	16	2
SF (µg/L)	71	19.80	9.8	4.30	49.40	45.10
Hb (g/L)	71	136.21	7.2	120	153	33.00
MVPT (pts.)	71	42.41	4.3	32	50	18
Digit Span Forward (pts.)	71	9.51	2.0	4	14	10
Digit Span Backward (pts.)	71	7.44	2.1	3	13	10
Digit Span Total (pts.)	71	16.96	3.6	9	25	16
Trail Making A (sec.)	71	14.18	4.2	6.84	26.41	19.57
Trail Making B (sec.)	71	55.72	15.8	30.55	128.76	98.21
B12 (pmol/L)	71	355.87	129.8	148	658	510
Albumin (g/L)	71	43.71	2.5	37.40	49.50	12.10
BMI	62	21.90	3.0	17.83	33.40	15.52
Valid N (listwise)	71					

Normal blood values: (SF) 20-150 µg/L, (Hb) 121-160 g/L, (B_{12}) 151-900 pmol/L, (albumin) 35-50 g/L.

Bivariate correlations (table 3) and scatterplot graphs (Appendix I1) were used to evaluate the relationship between serum ferritin and, hemoglobin, versus the measures of cognition (Motor Free Visual Perception Test, Digit Span Test {forward, backward, total} and Trail Making Test {part A & B}). The 95% confidence interval was used to highlight statistically significant correlations.

Table 3.
Correlations: Serum Ferritin and Hemoglobin, Versus Measures of Attention and Memory.

	MVPT	Digit Span Forward	Digit Span Backward	Digit Span Total	Trail Making A	Trail Making B
Serum Ferritin	r = -.197 p = .102	r = -.128 p = .291	r = +.040 p = .745	r = -.049 p = .686	r = -.010 p = .937	r = +.113 p = .353
Hemoglobin	r = -.122 p = .314	r = +.090 p = .475	r = -.047 p = .699	r = +.023 p = .847	r = -.003 p = .979	r = -.094 p = .438

Note:* indicates significance at the .05 level.

Serum ferritin produced correlational coefficients in the negative direction with the Motor Free Visual Perception, Digit Span Forward, Digit Span Total, and Trail Making A tests. Serum ferritin had a positive correlational coefficient with the Digit Span Backward and Trail Making B tests. Statistical significance ($p < .05$) was not reached in any of the cases (table 3). Partial correlations were calculated to statistically control B_{12} and albumin (table 4). Direction of relationships remained the same as the initial correlations. Magnitudes were similar. Statistical significance was not reached. Correlations were recalculated with outliers (cases with residuals > 3 standard deviations, SPSS) removed from the iron status and cognitive performance variables. Outliers have the potential to increase or decrease correlations. In the instances where outliers were identified, serum ferritin displayed a negative correlational coefficient with the Digit Span Total test and a positive correlational coefficient with the Trail Making B Test. These correlations did not reach statistical significance. These results were not beyond the play of chance.

Table 4.
Partial Correlations: Removing Effects of B_{12} and Albumin.

	MVPT	Digit Span Forward	Digit Span Backward	Digit Span Total	Trail Making A	Trail Making B
Serum Ferritin	r = -.189 p = .122	r = -.123 p = .299	r = +.049 p = .691	r = -.044 p = .724	r = -.011 p = .928	r = +.113 p = .281
Hemoglobin	r = -.112 p = .364	r = +.098 p = .427	r = -.025 p = .838	r = +.041 p = .742	r = -.018 p = .881	r = -.072 p = .561

Note:* indicates significance at the .05 level.

Hemoglobin produced correlational coefficients in the negative direction with the Motor Free Visual Perception, Digit Span Backward, Trail Making A and Trail Making B tests (table 3). Hemoglobin produced a positive correlational coefficient with the Digit Span Forward and Digit Span Total tests (table 3). In partial correlation, the direction of relationships between hemoglobin and the measures of cognition remained the same as the initial correlations. Again no statistical significance was reached. Magnitudes were very similar. After outlier identification and removal, hemoglobin had a positive correlational coefficient with the Trail Making B test. Statistical significance was not reached.

Vitamin B_{12} and albumin were used in bivariate correlational analysis with all measures of attention and memory to add more depth to the investigation. No significant correlations ($p < .05$) involving B_{12} and the tests of visual attention or memory were noted (table 5). Removal of outliers from the calculations did not change the outcome. Correlations with albumin also showed no significance (table 5). Again, removal of outliers from both variables did not change the outcome.

Table 5.
Correlations: Vitamin B_{12} and Albumin Versus Attention and Memory Tasks.

	Trail A	Trail B	Digit Forward	Digit Span Backward	Digit Total	MVPT
B12	r = +.084 p = .489	r = -.012 p = .924	r = -.026 p = .832	r = -.066 p = .887	r = -.053 p = .661	r = -.001 p = .993
Albumin	r = +.022 p = .856	r = -.181 p = .924	r = -.012 p = .920	r = -.100 p = .409	r = -.066 p = .589	r = -.100 p = .410

Note: *indicates significance at the .05 level

We used a multivariate analysis to determine which if any blood parameters would represent statistically significant predictors of cognitive test performance. Regression coefficients (B) were calculated for serum ferritin, hemoglobin, vitamin B_{12}, albumin, and menstrual cycle phase. Iron status can be influenced by menstrual phase (Kim, Yetley, & 1993). To monitor menstrual phase we documented recent participant periods. Of the iron deficient participants (n = 42), ten were in the menstruation phase of their cycle at the time of venipuncture, while 5 of 29 iron sufficient participants were assayed during the menstruation phase. Cycle phase was coded (0 = not menstruation phase, 1 = yes menstruation phase). Each test of cognition was entered as the dependent variable for each model. Not one model was able to explain a statistically significant amount of the variance in any test of cognition. Not one blood parameter was a statistically significant predictor of performance on any test of attention or memory. Predictors, regression coefficients, t-values, and significance levels are presented in table 6.

Table 6.
Predictors of Cognitive Performance Tests.

	MVPT			Digit Span Forward			Digit Span Backward		
	B	t	Sig.	B	t	Sig.	B	t	Sig.
SF	-.007	-1.41	.162	-.002	-1.15	.254	.001	.498	.620
Hb	-.005	-0.661	.511	.003	0.965	.338	-.001	-.306	.760
B_{12}	.001	0.248	.805	-.008	-0.414	.680	-.009	-.457	.649
Alb.	-.130	-0.607	.546	-.008	-0.085	.932	-.007	-.733	.466
CP	-.006	-0.052	.959	.304	0.487	.628	.539	.827	.411

	Digit Span Total			Trail Making A			Trail Making B		
	B	t	Sig.	B	t	Sig.	B	t	Sig.
SF	-.001	-.354	.725	-.005	-.103	.919	.230	1.14	.256
Hb	.002	.362	.719	-.008	-.115	.909	-.209	-0.752	.455
B_{12}	-.001	-.499	.619	.003	.734	.466	.006	0.421	.682
Alb.	-.008	-.477	.635	.001	.075	.940	-1.16	-1.48	.142
CP	.843	.757	.452	-.651	-.491	.625	-2.75	-0.570	.571

Note: SF = Serum Ferritin, Hb = Hemoglobin, B_{12} = Vitamin B_{12}, Alb = Albumin, CP = Cycle Phase (0 – not menstruation phase; 1 – menstruation phase), B = regression coefficient

We evaluated group differences in attention and memory test scores between iron deficient (n = 42) and iron sufficient participants (n = 29). Independent t-tests were used to compare scores. Differences were not statistically significant in any tests (table 7).

Table 7.
Between Subjects T-Test.

	Iron deficient Group (n = 42) (Means ± SD)	**Iron Sufficient Group (n = 29) (Means ± SD)**	**t-test**	**Significance**
MVPT	42.8 ± 4.0	41.9 ± 4.8	$t_{(68)}$ = -.786	p = .383
Digit Span Forward	9.7 ± 2.01	9.3 ± 2.04	$t_{(68)}$ = -.710	p = .906
Digit Span Backward	7.3 ± 2.0	7.6 ± 2.2	$t_{(68)}$ = .480	p = .475
Digit Span Total	17.0 ± 3.3	16.9 ± 3.9	$t_{(68)}$ = -.119	p = .358
Trail Making A	14.1 ± 4.1	14.2 ± 4.4	$t_{(68)}$ = .090	p = .530
Trail Making B	54.9 ± 13.4	56.9 ± 18.8	$t_{(68)}$ = .524	p = .788

Table 8 shows relative risk (RR) counts. These counts were used in determining the conditional probability of participants to score below the middle of the distribution (median) for each cognitive test coupled with being iron deficient (ferritin ≤ 20 μg/L) or iron sufficient (ferritin > 20 μg/L). In all instances iron status did not contribute to a statistically significant risk. The confidence intervals for each test of cognition embraced the relative risk value associated with no difference (RR = 1.0). Iron status did not affect position in the distribution of scores. The relative risks and 95% confidence interval for each test of cognition were: MVPT (RR: 1.06; 95% CI: 0.7-1.7), Digit Span Forward (RR: 1.02; 95% CI: 0.7-1.6), Digit Span Backward (RR: 1.04; 95% CI: 0.7-1.6), Digit Span Total (RR: 1.23; 95% CI: 0.7- 2.2), Trail Making A and Trail Making B, (RR: 1.06; 95% CI: 0.7-1.7).

Table 8.
Relative Risk.

Trail Making A	-	+
	Below 13.43 sec.	Above 13.43 sec.
Iron Deficient -	n = 20	n = 21
Iron Sufficient +	n = 15	n = 14
Trail Making B	-	+
	Below 54.34 sec.	Above 54.34 sec.
Iron Deficient -	n = 20	n = 21
Iron Sufficient +	n = 15	n = 14
Digit Span Forward	-	+
	Below 9	Above 9
Iron Deficient -	n = 14	n = 21
Iron Sufficient +	n = 9	n = 13
Digit Span Backward	-	+
	Below 7	Above 7
Iron Deficient -	n = 13	n = 20
Iron Sufficient +	n = 10	n = 14
Digit Span Total	-	+
	Below 17	Above 17
Iron Deficient -	n = 17	n = 18
Iron Sufficient +	n = 14	n = 10
MVPT	-	+
	Below 42.5	Above 42.5
Iron Deficient -	n = 20	n = 21
Iron Sufficient +	n = 15	n = 14

A 3-day dietary analysis was used to analyze nutrient intakes and eating patterns. Despite frequent reminders via school visits, announcements and phone calls, several subjects did not complete and return their food diaries. Forty-one participants, representing greater than half the sample, did complete the request. Over the three days observed, iron intakes ranged from 4.5 to 30.8 mg/day. Mean iron intake was 15.7 $\pm$ 5.9

mg/day. The RNI for iron in this population is 15 mg/day. Carbohydrate intakes ranged from 42 to 70% of caloric intake. In a healthy diet carbohydrates can make-up between 50 and 70% of energy intake. Protein intakes ranged from 8 to 24%. It is generally recommended that 0.8 g/kg of protein should be supplied in the diet to support growth and repair (Pellet, 1990). Fat intakes ranged from 21 to 38%. Common health guidelines recommend fat intake should not exceed 30% of the total daily energy intake. Using the dietary analysis we were able to test the relationship between iron intake and iron status. We calculated a bivariate correlation between iron intake and actual iron status (table 9). The correlational coefficient was not statistically significant (r = .062, p = .70).

Table 9.
Iron Intake Versus Iron Status.

	Iron intake (mg)	**Significance**
Iron status (serum ferritin)	r = .062	p = .70

Note: Daily iron intake (mean $\pm$ SD, 15.7 $\pm$ 5.9 mg/day).

CHAPTER 5

DISCUSSION

Within this chapter separate sections will be devoted to discussion of the following topics: prevalence of iron deficiency in sample, cognitive test results, serum ferritin, hemoglobin, vitamin B_{12}, albumin, t-tests and relative risk, menstrual cycles, dietary analysis, and limitations. All evidence provided by our methods suggests that serum ferritin below 20 µg/L does not dispose an individual to inferior cognitive performance.

Correlations between iron status (serum ferritin, and hemoglobin) and attention and memory (Motor Free Visual Perception Test, Digit Span Test {forward, backward, total} and Trail Making Test {part A & B}) were not statistically significant. Correlation coefficients between albumin and the tests of cognition did not achieve statistical significance. Furthermore, the correlation coefficients between vitamin B_{12} and the tests of cognitive ability were not statistically significant. No blood parameters were statistically significant predictors of cognitive performance. In all cognitive tests, there was no significant difference in performance between females who were iron deficient (serum ferritin $\leq$20 µg/L) and females who were not. Iron deficient participants did not show any increased risk of performing below the level of their iron sufficient counterparts.

Prevalence of iron deficiency in sample

In discussion of iron deficiency prevalence rates an important factor to consider is the cutoff value used to identify low iron status. The related literature reveals that both serum ferritin cutoff values (< 20 μg/L and <12 μg/L) have been used in the past with no conclusion reached on a decisive standard for identification of non-anemic iron deficiency (Manore & Thompson, 2000). Fifty-eight percent of the participants were iron deficient (≤ 20 μg/L) in the present investigation. Other researchers who investigated iron deficiency in female adolescent samples (Bruner et al., 1996; Halterman et al.., 2001) have used a cutoff of 12 μg/L to define iron deficient participants. Using this value in the present study revealed a 25% rate of iron deficiency. This prevalence rate is noticeably higher than the 16% observed by Bruner et al. and the 8.7% noted by Halterman et al. in comparable samples of normal populations with similar health attributes (free of disease). A clear reason for these differences is difficult to explain. An answer could involve Canadian versus American dietary composition. Unfortunately, dietary intakes were not reported in the other studies and cannot be compared. Prevalence of anemia across studies is more similar. Anemic participants accounted for 1.9% of the Bruner et al. sample and 1.5% of the Halterman et al. sample. In the present study the rate of anemia was 1.4%. All three of these figures register lower than the prevalence of adolescent iron deficiency anemia reported by Cook et al. (1976), Ballin et al. (1992), Nelson (1996), and Morad (2005). These authors noted anemia rates of 4.8%, 8.1%, 0-22%, and 5-8% respectively. Differences among reports may be attributed to eating habits and socioeconomic status in different countries. Ballin et al. studied iron state in female adolescents from Israel. Estimates by Nelson came from English females. Morad's report of anemia in the Western world did not distinguish between males and females, although it is reasonable to expect that the female rate anemia would not lower the estimate. The 4.8% prevalence of anemia in American adolescent females noted by

Cook et al. is still higher than Bruner et al., Halterman et al., and this study. A possible explanation for the lower rates of anemia in these studies could lie in the age of the samples. The oldest participants in the Bruner et al. sample were 18 years old. Halterman et al. did not include anyone over 16 years of age. In the present study most participants were 15 years old. As menstruation proceeds throughout adolescence a small negative iron imbalance may result in higher rates of anemia as females reach the late teens.

Cognitive test results

Regarding test score performance it is interesting to note that participants functioned within normative values for all cognitive tasks except for the Motor Free Visual Perception Test. According to normative data, the 15 year-old participants were performing at the age equivalent of 11 year-olds or less. The reason for this pattern is not clear. A possible explanation could lie in the nature of the task. Unlike the other cognitive tasks in the battery, the MVPT utilizes a multiple choice format. Guessing, random answering, and perseverance are examinee behaviours cited which can influence results on the test (Colarusso & Hammill, 2003). Other undetected developmental or psychological factors that can affect performance on this test include: poor visual acuity, abnormal saccades, and attentional neglect (Colarusso & Hammill, 2003). The MVPT took the longest to complete in the battery. Examiners noted participant mannerisms on MVPT score sheets (raw data not shown) suggesting distractibility or poor attention (i.e., wandering eye movements) on several occasions. When this behaviour is apparent it is advised that testing be discontinued and resumed at a later date (Colarusso & Hammill, 2003). Due to logistical restraints we did not have this option. As multiple factors affect performance on the MVPT, results of this test should be interpreted with caution.

Serum ferritin

In this study there were no statistically significant relationships noted in any of our analyses between serum ferritin and cognitive tests of attention or memory. Moreover, evidence of non-statistically significant relationships was shown by dispersed scatterplot graphs (Appendix I1). Conversley, Bruner et al. (1996) noted a significant ($R^2 = 0.25$, R^2 change = 0.07; $p < .02$) relationship with a standardized test of verbal learning and memory (increased iron status group performed better) whereas Halterman et al. (2001) found a relationship ($p < .05$) with Math. Iron deficient participants had an increased risk of scoring below average (odds ratio: 2.3; 95% CI, 1.1-4.4) compared to a normal iron group. The results of our study do mirror those regarding task performance observed by Tucker et al. (1982). Tucker et al. found no reliable relationship between serum ferritin and cognitive task performance (work fluency, imagery) in a longitudinal study of seven university students. In another study, Tucker et al. (1984) also noted that iron status was not significantly related to 4 of 6 tasks of cognitive performance. However, statistical significance was observed with two cognitive tasks (tonal memory backward: $r = -0.30$; $p < .05$, and word fluency: $r = -0.30$; $p < .05$). It should be noted that of 69 participants Tucker et al. (1984) included 17 males and all were university students. All our participants were high school females. The low correlations exhibited by serum ferritin in the present study needs to be addressed. It is possible that serum ferritin is not a good predictor of cognitive performance. The multivariate results of this study provide support. Serum ferritin did not significantly predict any cognitive test scores. However, measuring serum ferritin has merit. It is a commonly used, reliable and sensitive indicator of iron stores in healthy individuals (Cook, Lipschitz, Miles, & Finch, 1974; Jacobs, 1977). Although, a drawback to ferritin is that levels will rise fairly rapidly after the onset of an inflammatory event and stay elevated as long as it persists (Lipschitz, Cook, & Finch, 1974). Inflammation raising serum ferritin is unlikely in this study for several reasons. No participants demonstrated elevated or extreme serum ferritin ($\geq$ 300 μg/L). The maximum serum ferritin level observed in this study was 49.4 μg/L. An inflammatory event would likely have been accompanied by low levels of albumin. Albumin is a negative acute-phase reactant (Moshage, Janssen, Franssen, Hafkenscheid, & Yap, 1987). Not one case of hypoalbuminemia (albumin < 35 g/L) was observed. Alcohol consumption has also been suggested to independently raise serum ferritin (Leggett et al., 1990). Our sample was below the legal drinking age. Alcohol intake was likely not a factor as there were no reports of alcohol intake in the dietary analyses.

Evidence in other areas can be found which challenge the worthiness of serum ferritin as an independent research variable. Millichap et al. (2006) found that low serum ferritin (< 20 μg/L) was not related to severity, symptoms or frequency of ADHD. Other authors have noted that low serum ferritin (< 20 μg/L) did not effect exercise performance (Beard & Tobin, 2000; Newhouse, Clement, Taunton, & McKenzie, 1989; Nielsen & Nachtigall, 1998) or antioxidant function (Gropper, Kerr, & Barksdale, 2003). However, very recently Foroughi et al. (2007) found that serum ferritin is an important and independent predictor of the development of diabetes. Risk of diabetes was markedly elevated in participants with clinically raised (but not indicative of iron overload) ferritin (odds ratio:

3.2; 95% CI, 1.3-7.6). This finding may have important implications for understanding the aetiology of diabetes. The determination of valid reference values for serum ferritin differs in various populations (e.g., male, female, pregnant, elderly, and diseased) and the range of so-called normal is wide. A serum ferritin threshold effect may be a key to establishing the clinical significance of non-anemic iron deficiency. Serum ferritin levels < 12 μg/L reflect exhausted iron stores (Heinrich, 1981). At levels < 10 μg/L anemia develops (Hallberg & Hulthen, 2002; Kotagal & Silber, 2004). However, a clinically subnormal or reduced serum ferritin cutoff value concurrent with a demonstrated functional consequence in the absence of overt anemia has not been identified (Beard & Tobin, 2000; Millichap et al., 2006).

Hemoglobin

There was no statistically significant relationship between hemoglobin level and any test of cognition in this adolescent female sample. These findings concur with the non-significant findings noted by Bruner et al. (1996). This author found no significant effect with regards to iron status and performance on three tests of attention and all but one component of a multicomponent test of verbal learning and memory. Our results also confirm some of the non-significant findings of Halterman et al. (2001). With a similar age, and percentage of participants displaying low hemoglobin (< 120 g/L) no effect was found in a performance examination (block design), a reading test, and digit span. Halterman et al. had a much larger sample of teenage females (n = 1114) to work with corresponding to more cases of anemia (n = 17) to study. The literature has consistently shown that anemia seems to be related to decline in cognitive function (DiSilvestro, 2005; Grantham-McGregor & Ani, 2001). In this study we observed only one case of anemia (Hb < 120 g/L). Essentially this is a study of iron deficiency in the absence of anemia. Based on the low (1.4%) prevalence of anemia it is not unusual that we observed no relationship between normal values of hemoglobin and cognition. A larger sample accompanied by a larger range of hemoglobin values and higher prevalence of anemia may have produced more insightful findings related to hemoglobin status.

Vitamin B_{12}

Vitamin B_{12} served as a control variable in partial correlation. Its measurement excluded the likelihood of Pernicious anemia as a product of a B_{12} deficiency, not iron deficiency. Two cases of B_{12} deficiency were observed. Both participants had normal iron status. Anemia (Hb < 120 g/L) was noted in only one participant. This individual exhibited normal B_{12} levels. Pernicious anemia was not a factor. In this study there were no statistically significant relationships noted in any of our analyses between vitamin B_{12} and any test of attention or memory.

Albumin

Albumin levels were measured to provide statistical control in partial correlation and broaden exploration of participant health. Albumin may be useful when interpreting iron status. Decreasing levels of this plasma protein have been linked to periods of malnutrition or high blood loss (Bulow & Madsen, 1986). In both of these circumstances iron status would inevitably be affected. However, not one participant displayed low albumin in this study. In this study there were no significant relationships noted in any of our statistical analyses between albumin and attention or memory. By these methods a sensible conclusion to draw is that albumin did not influence cognitive performance. This finding agrees with Dik et al. 2005 regarding albumin and cognition. Dik et al. assessed whether levels of several inflammatory proteins, including albumin, were associated with cognitive decline. The sample consisted of 1,284 participants in an Amsterdam longitudinal aging study (aged 62 to 85 years). General cognition was assessed using the Minni-Mental State Examination (MMSE). Memory was assessed by the Auditory Verbal Learning Test (AVLT). Statistical analyses included analysis of covariance, linear regression, and logistic regression adjusted for age, sex and education. Albumin was not associated with cognitive decline. However, comparing results in older persons to adolescents represents a point of concern.

T-tests and relative risk

When we formed groups based on iron status (iron deficient vs. iron sufficient), between subject t-tests showed no statistically significant differences among cognitive test scores. This finding complements the non-significant correlational statistics. A correlation coefficient is a measure of how two variables relate and t-tests examine how two groups are different. Correlations and t-tests use different statistical approaches to answer the same question (Myers & Well, 2003). A combination of low, non-significant correlations and no statistically significant group differences seem to suggest that within the scope of this study, iron status had no significant influence on cognition as measured by multiple tests of attention and memory. In comparison, Bruner et al. (1996) noted a significant difference (stepwise regression, $R^2 = 0.25$, R^2 change $= 0.07$; $p <.02$) between groups on a test of verbal learning and memory. Halterman et al. (2001) found no significant difference in digit spans, as did the current study. Halterman et al. noted a difference (t-test, $p < .05$) between group math averages. Iron deficient females performed more poorly in the math section of the Wide Range Achievement Test – Revised (87.4 $\pm$ 15.6 vs. 93.7 $\pm$ 17.1) and had greater than twice the risk of scoring below average in math (odds ratio: 2.3; 95% CI, 1.1-4.4) compared to those with normal iron status. We did not measure math performance and are not in a position to add support or argue against those findings, however we evaluated risk.

Relative risk analysis showed that iron status did not result in an excess risk, to placement in the distribution of scores, for any test of cognition. Iron deficiency or sufficiency did not statistically elevate the risk to score below the median in each set of scores. The confidence intervals for every test of cognition embraced the relative risk (RR) value associated with no difference (RR = 1.0). The researchers chose the median as the reference point for evaluating risk due to interest in assessing average performance in comparison to the group. Due to its stability in the face of outliers the median was the logical choice as the measure of central tendency. Halterman et al. (2001) also assessed risk in comparison to average group performance. In our sample it is probably safe to conclude that iron status (based on serum ferritin dichotomy) does not increase the risk of below average cognitive performance.

Menstrual cycles

A possible source of error when studying menstruating females is the cyclical variation of iron status parameters (Kim et al., 1993). Women display varied iron parameters because of individual differences, and cyclic variations. In one study, during the monthly cycle hemoglobin values taken from the menstruation phase to luteal or late luteal phase ranged from 133 g/L – 130 g/L and serum ferritin from 17.2 µg/L – 24.0 µg/L (Kim et al., 1993). Kim et al. identified the menstruation phase (days 1 to 5) as iron's low point in women's blood. In the present study, menstrual cycles of the 42 iron deficient participants were assessed to distinguish which ones were in the menstrual phase at the time blood was extracted. Ten of the 42 iron deficient participants gave blood samples during the menstruation phase. Conversely, 5 of 29 iron sufficient participants were assayed at this time. A wide spectrum of iron status parameters were observed no matter which cycle phase blood was taken in. In this study we attempted to incorporate menstrual cycle data into the multivariate analysis as a predictor of cognitive performance. Data were nominally coded (0 = no menstrual phase, 1 = yes menstrual phase) according to the date of participant's last period. In all models menstrual cycle was unable significantly predict cognitive scores. Given, our menstrual data was based on self-report of last period (participants may have remembered incorrectly) and actual blood and iron losses were not measured we cannot cast doubt on the utility of menstrual cycle data in future iron study analyses. Menstrual cycle data were not presented, analyzed or discussed by Bruner et al. (1996) or Halterman et al. (2001).

Dietary Analysis

We conducted dietary analyses to test the relationship between iron intake and iron status. The three-day analyses were completed by 41 participants. A bivariate correlation between iron intake (dietary iron in mg) and iron status (serum ferritin) resulted in a non-statistically significant ($p = .70$) correlation coefficient ($r = .062$). The relationship between iron intake and iron status in this sample was not strong or direct (see scatterplot graph Appendix I2). The very weak correlational magnitude suggests that iron intakes are not related to iron status or iron stores (serum ferritin reflects iron storage). This result may be linked to the prevalence of iron deficiency in the sample. As many of our participants were iron deficient (58%) it can be speculated that the majority of dietary iron consumed by these individuals was used to support metabolic processes requiring iron and not for iron storage. Assuming a negative iron balance was present in the majority of participants, the quantity of dietary iron consumed would not be reflected in iron stores. An alternative explanation for the low correlations could be that participants were careless (did not follow instructions) in reporting their food intakes. Also, a 3-day approach, although practical, is not as likely to provide accurate estimates of nutrient intakes compared to dietary analyses of longer durations.

CHAPTER 6

CONCLUSIONS

The purpose of this study was to determine the relationship between iron status, visual attention and memory in 14 to16 year old healthy 10^{th} grade female students. Visual attention and memory were measured by performance on standardized cognitive tests. Reasoning for a relationship centers around the role of iron in cognition. The mechanism by which iron affects dopamine neurotransmission therefore altering attention and memory was presented. The hypothesis of the researchers was that a statistically significant relationship would be found between iron status and cognitive test scores. It was thought this relationship would result in a group difference, between those who were iron deficient and those who were not, accompanied by a substantial risk for altered cognitive performance. After multiple statistical analyses, a statistically significant relationship was not observed between iron status and attention or memory. There is no support for the original hypothesis. Data support the null hypothesis. As related to iron status, there was no difference in cognitive test scores.

The following conclusions are drawn:

1. There was no correlation between iron status (serum ferritin, hemoglobin) and any tests of attention and memory (Trail making A & B, Digit Span {forward, backward, total}, and Motor Free Visual Perception Test).

2. Iron deficient cognitive performance was no different than iron sufficient performance. Iron deficient participants did not have an increased risk of below average test scores.

3. Iron deficiency is highly prevalent in menstruating females between 14 and 16 years of age.

Recommendations for future research include the following:

1. As the relationship between iron status and performance on tests of attention and memory is likely complex, future researchers should explore creative methods of data collection and statistical analysis. Limiting scope by conforming to the methods of others to achieve comparability across studies may limit further understanding of iron deficiency.

2. A study comparing iron status to performance on The Attention Network Test (ANT) designed to evaluate alerting, orienting, and executive attention (Fan et al., 2002) may provide further insight relating iron to cognitive function.

3. More studies assessing the link between iron status and cognition in adolescent populations should be conducted. Further research is required to assess whether cognitive effects are limited to standardized tests or are also apparent in functional academic settings.

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Appendix A: Participant Consent

Dear Potential Participant and Parent/Guardian:

Thank you for showing interest in this exciting investigation. I, _____________ (please print name) hereby volunteer to participate in this research study entitled "Relationships Between Iron Status and Measures of Attention and Memory".

Dr. Ian Newhouse, Dr. Jim McAuliffe, and Donna Newhouse of Lakehead University, along with Dr. Chris Lai are working together on a study investigating relationships between iron status, attention, and memory. The purpose of this study is to determine whether or not an association exists between iron status, attention, and memory in 14 to16 year old healthy 10^{th} grade female students. Your participation in this research will allow us to identify groups at risk to iron deficiency in the Thunder Bay area. Iron deficiency is the most common nutrient deficiency in the world. Billions may be affected. The data you provide may help direct future health recommendations in this region and abroad. You will be making a valid contribution to science by participating in this study. As a benefit, your health will be assessed free of charge. Such assessment can contribute to goal planning and achievement of your greatest potential as a student.

As a participant you will undergo the following:

1. One blood test; samples will be measured for serum ferritin, hemoglobin, vitamin B_{12} and albumin.
2. Three cognitive tasks involving reaction time key pressing on a computer and pencil and paper tests; 30-45min. in total.
3. Record of what you ate over a 3-day period.
4. You may also be asked to participate in other research involving iron supplementation. Such participation is entirely your choice.

Blood testing will be conducted by certified individuals. Side effects and risks due to participation are minimal. All data provided will remain confidential and securely stored at Lakehead University for seven years. No individual will be identified in reporting results. Participants will be referenced by number. You are entitled to a briefing of individual results and a summary of the study. As a volunteer it is your right to withdraw from the study at any time.

If you have any questions concerning the study, we can be reached at the Lakehead University School of Kinesiology (343-8544).

Thank You,

My signature on this form indicates that I have read and understood the covering letter and my daughter will participate in the study entitled "RELATIONSHIPS BETWEEN IRON STATUS, AND MEASURES OF ATTENTION, AND MEMORY", by Dr. Ian Newhouse, assessing links between iron level, and cognition.

I understand the following:

My daughter is a volunteer. She can withdraw from the study at any time even after signing this form. There is no risk of physical or psychological harm in this research. The data provided by my daughter will remain confidential. I am entitled to a summary of the study, following its completion. My daughter will donate one blood sample and then complete three attention and memory tasks. She will also record what she has eaten over a 3-day period.

________________________ ____________________

Signature of Parent/Guardian Date

________________________ ____________________

Signature of Witness Date

I have explained study procedures to the participant. I believe she has comprehended what is involved.

________________________ ____________________

Signature of Researcher Date

Appendix B:
HEALTH AND PHYSICAL ACTIVITY SURVEY

Name:	Grade:	Age:

Please answer questions by checking "yes" or "no" and fill in blanks when applicable.

	Yes	No
➢ Are you pregnant?	____	____
➢ Are you taking any contraceptives at this time? (birth control medications)?	____	____

➢ Can you please list any special medical conditions you have.______________________

➢ At what age did your periods begin? ____

➢ Can you please list any medications you are taking:

____

➢ If you are physically active please describe the type of exercises you participate in (i.e., running, strength training etc.)

____

Check off the answer that best applies to you.

Frequency:
Over a typical seven-day period (one week), how many times do you engage in physical activity that is sufficiently prolonged and intense to cause sweating and a rapid heart beat?
- o At least three times
- o Normally once or twice
- o Rarely or never

Intensity:
When you engage in physical activity, do you have the impression that you:
- o Make an intense effort
- o Make a moderate effort
- o Make a light effort

Perceived Fitness:
In a general fashion, would you say that your current physical fitness is:
- o Very Good
- o Good
- o Average
- o Poor
- o Very Poor

Appendix C: Supplemental Information

Name: ______________________

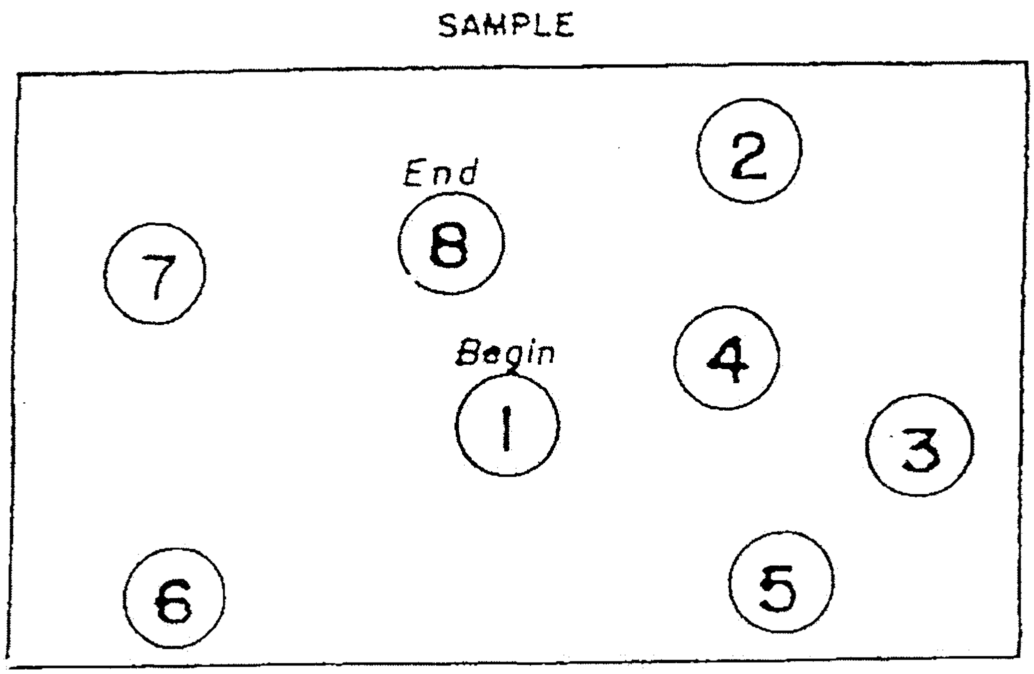
1.) When was the date of your last period?

____

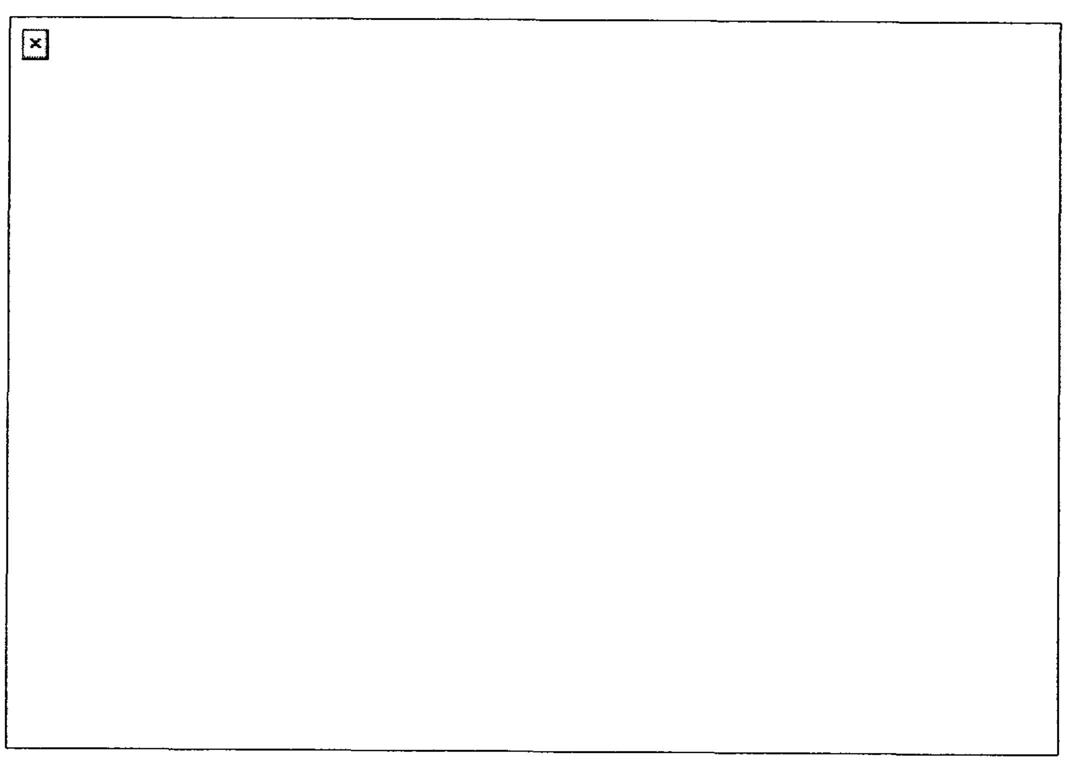
2.) What is you ethnic background? (i.e., Caucasian [White], African Canadian, Aboriginal, etc.)

____

Appendix D: Trail Making Test (part A)



Trail Making Test (part B)



Appendix E: MVPT-3

Appendix F1: Dietary Intake Log

Name:	Weight:	Height:	Age:	Day of the week:

MEAL	Description of Food or Drink	Brand Name	Amount
Breakfast			
Lunch			
Dinner			
Snacks			

Appendix F2: Instructions for Dietary Analysis

How to fill out the Dietary Intake Log.

It is important to honestly record all that you eat or drink during the day for three consecutive days. Be sure to record **2** weekdays and **1** weekend day as your three consecutive days.

To ensure success when filling out the log, it is very important that the specific **kinds** and **amounts** of food recorded are accurate. You should be **very detailed** in the **description of food or drink** column. Include details like brand names, and whether the food consumed is light or smooth etc. when you can. Following are some examples to help you represent clearly what you consume.

Name: Ianna **Weight:** 108 lbs. **Height:** 5'5" **Age:** 15 **Day of the week:** Monday

MEAL	Description of Food or Drink	Brand Name	Amount
Breakfast	Captain Crunch	Kellogg's	2 cups
	2% milk		1 cup
	banana		1 large
	whole wheat bread	Wonderbread	1 slice
Lunch	Peanut butter sandwich	Dempsters	2 slices white bread
		Skippy (light smooth)	2 tsp peanut butter
	Chocolate chip granola bar	Nature Valley	1 bar
	Orange juice	Frutopia	1 glass
Dinner	Spaghetti	Primo	3 cups
	meatballs		1 cup
	carrots		½ cup
	lettuce	romaine	2 cups
	Salad dressing	Kraft	1 tsp
	pop	Pepsi	1 can
Snack	Ice cream, vanilla	Ben & Jerry	1 cup
	Frosty	Wendy's	small

Appendix G: Statistical Analysis Equations

Pearson Correlation: $r = \frac{E(Zx\ Zy)}{N}$

Partial Correlation: $X = a_x + b_x A$

Multivariate Linear Regression: $Y' = a + b_1X_1 + b_2X_2 + \ldots\ldots b_kX_k$

Between Subjects/Independent t-test: $t_{X_1 - X_2} = \frac{(X_1 - X_2) - :_{X_1 - X_2}}{\Phi_{X_1 - X_2}}$

Risk Ratio : $RR = \frac{a/(a+b)}{c/(c+d)}$

Null = 1.0

	Below median	**Above median**
Iron Deficient	**a**	**b**
Iron Sufficient	**c**	**d**

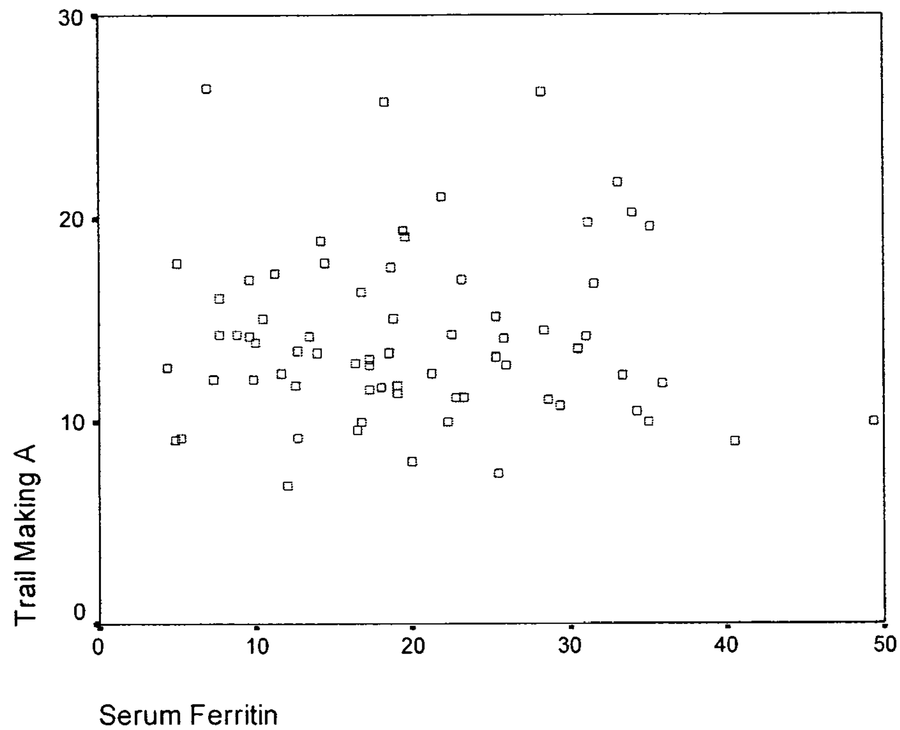
Data Analysis. Following testing protocols all data all was entered and analyzed using SPSS (Statistical Package for the Social Sciences) 10.0 for Windows.

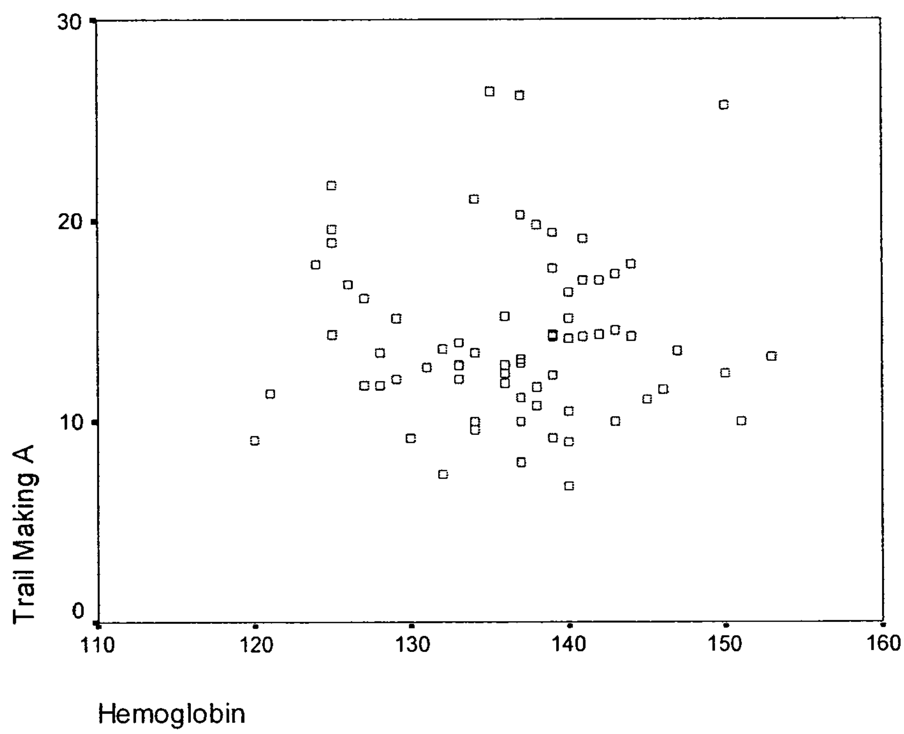
Appendix H: Age Equivalent Normative Data for 15 Year Olds

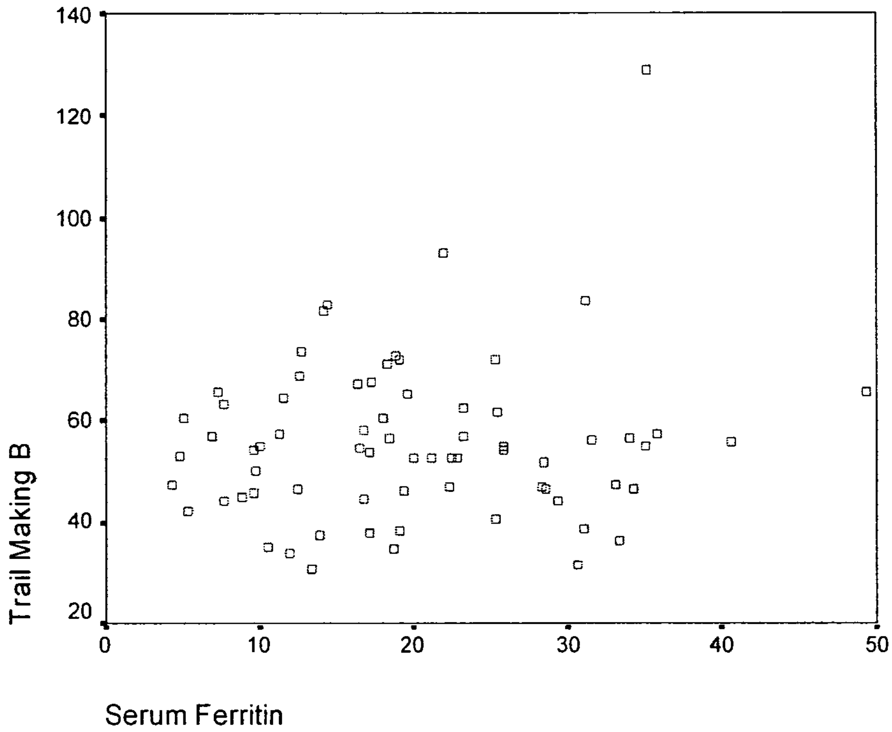
Test	Normal Scoring/Range
Digit Span Forward (a)	**(7 ± 2 digits) mean 6.99**
Digit Span Backward (a)	**(7 ± 2 digits) mean 5.44**
Trail Making A (b)	**90^{th} percentile: 15 sec.**
Trail Making B (b)	**50^{th} percentile: 47 sec.** **25^{th} percentile: 59 sec.**
Motor Free Visual Perception (c)	**54 pts.** **≤ 49 pts. equivalent to 11 year old or younger**

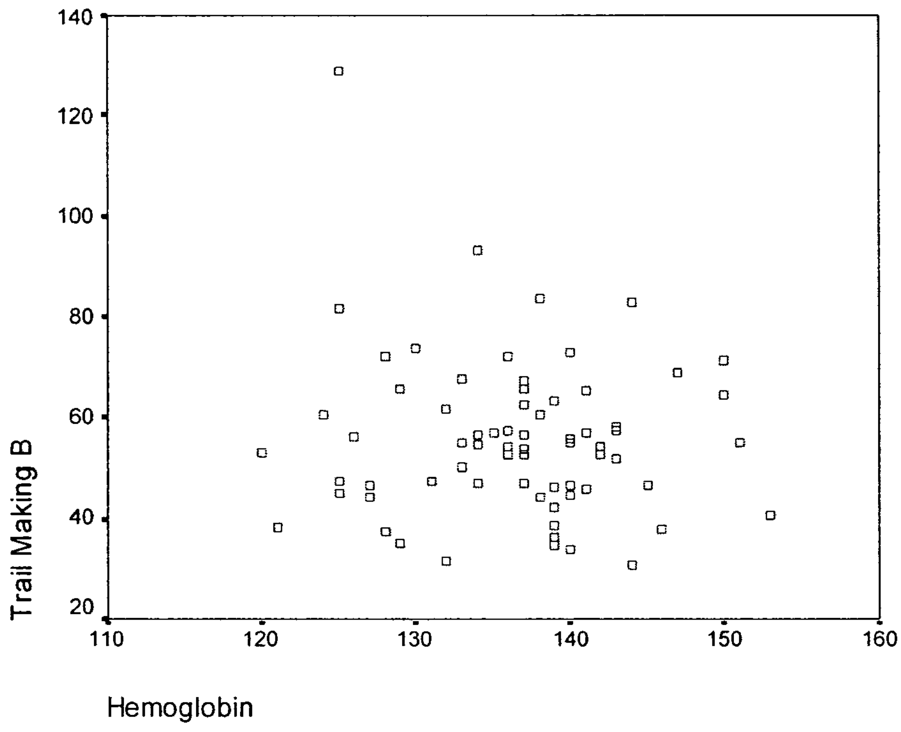
(a) These data are taken from "Wechsler Adult Intelligence Scale – Third Edition: Canadian Technical Manual," by David Wechsler, 2001. **(b)** These data are taken from "A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary," by Spreen & Strauss, 1991. **(c)** These data are taken from "Motor Free Visual Perception Test Manual – Third Edition," by Hammill & Colarusso, 2003.

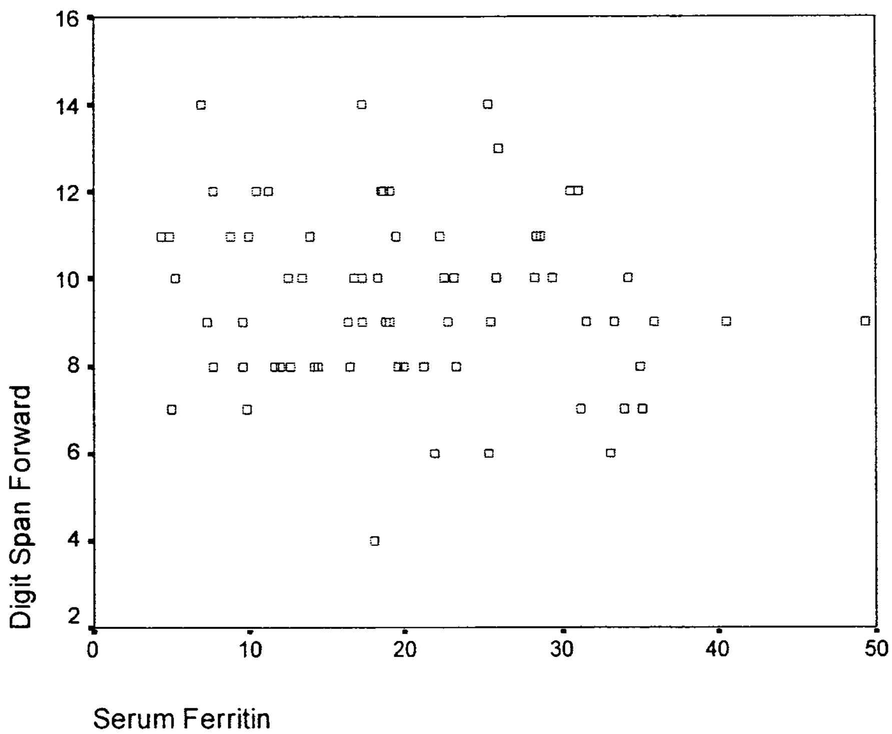
Appendix I1: Scatterplots of Serum Ferritin and Hemoglobin vs. Tests of Attention and Memory

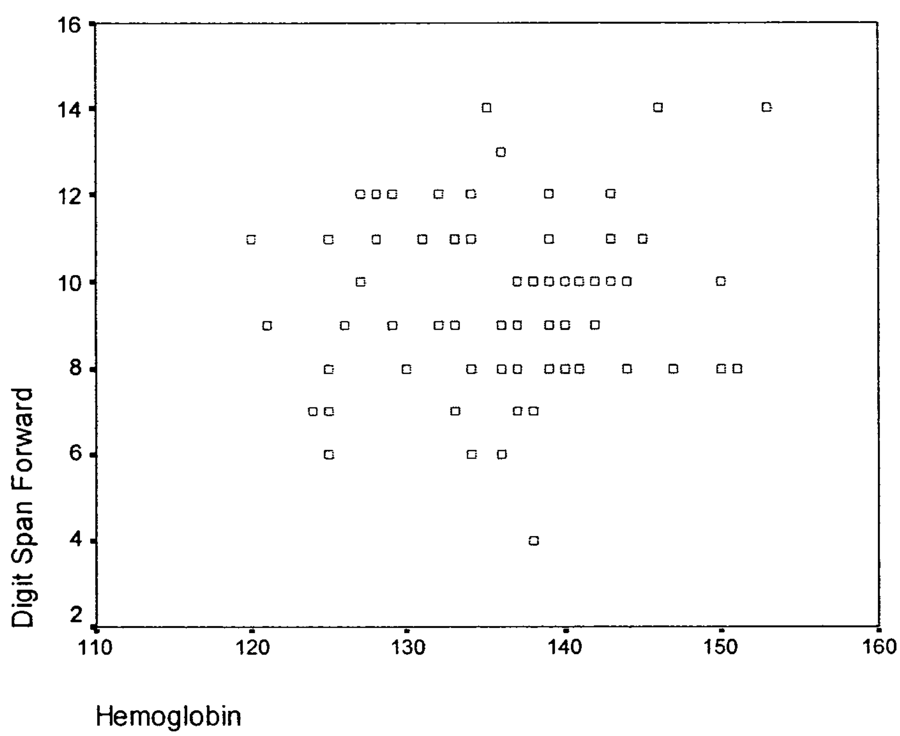


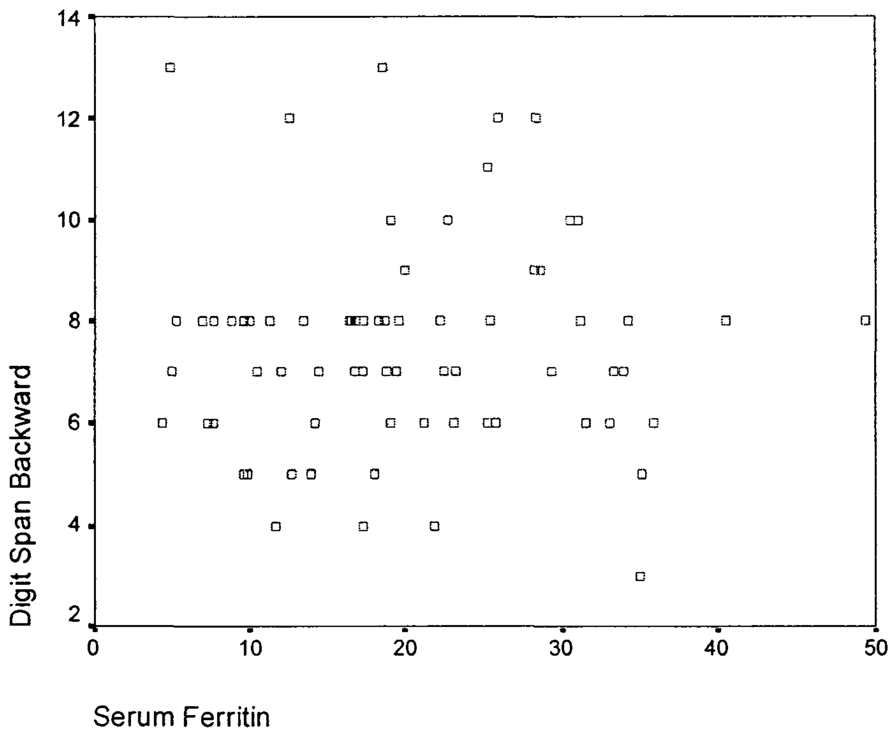



Trail Making B
140
120
100
80
60
40
20
0
10
20
30
40
50
Serum Ferritin

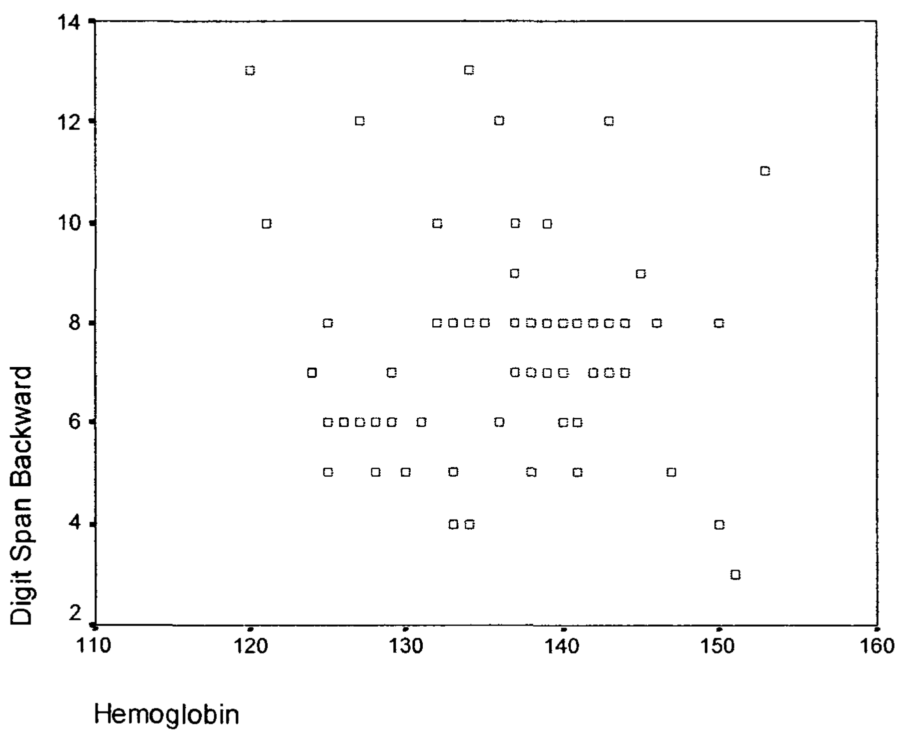

Trail Making B
140
120
100
80
60
40
20
110
120
130
140
150
160
Hemoglobin


16
14
12
10
8
6
4
2
0
10
20
30
40
50
Digit Span Forward
Serum Ferritin

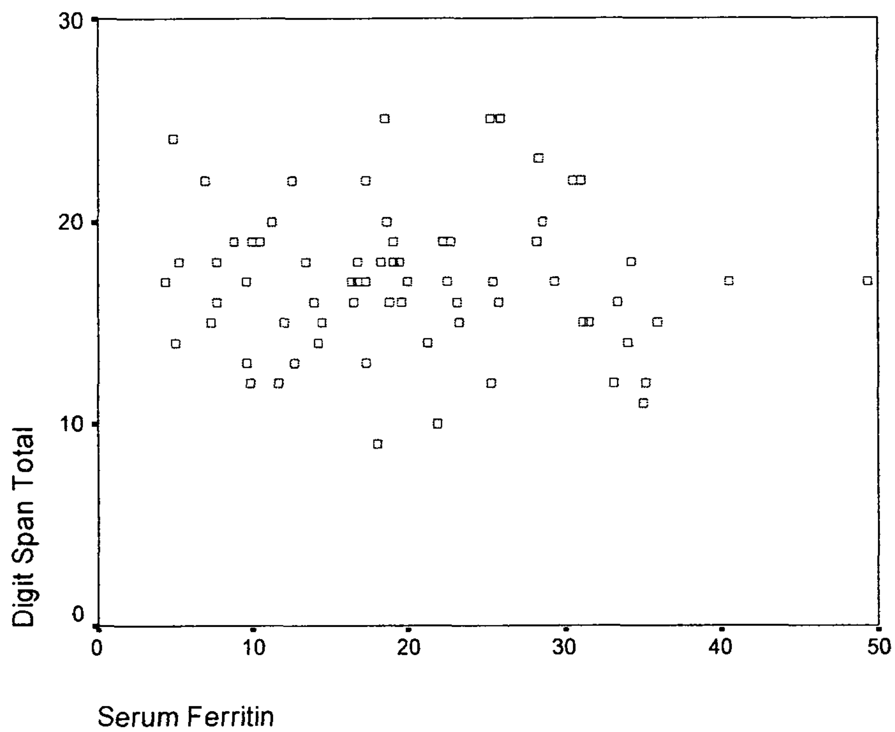

16
14
12
10
8
6
4
2
110
120
130
140
150
160
Digit Span Forward
Hemoglobin

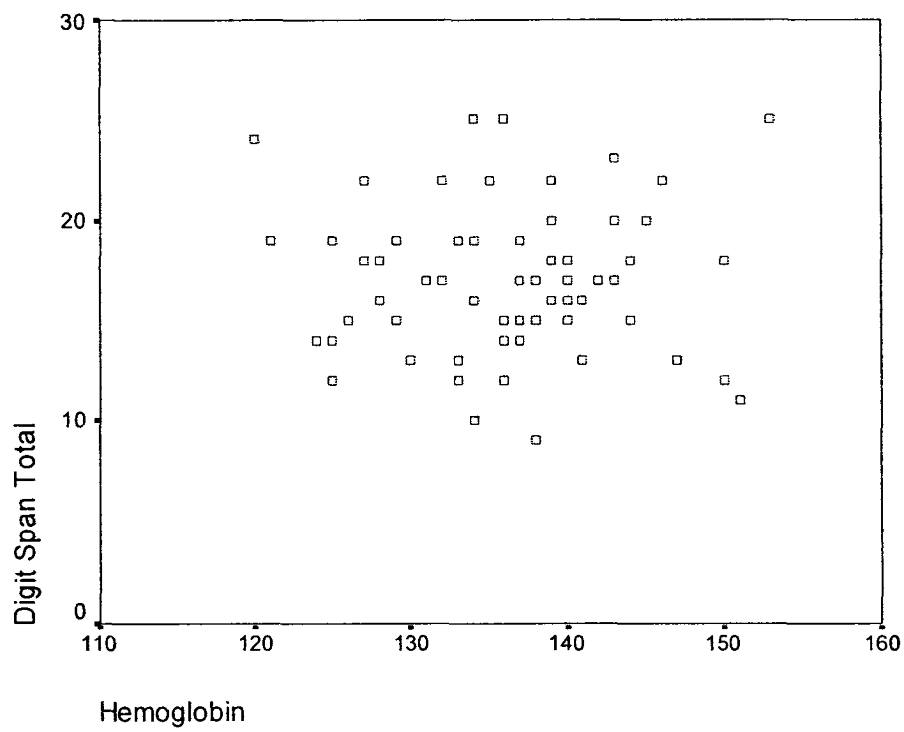


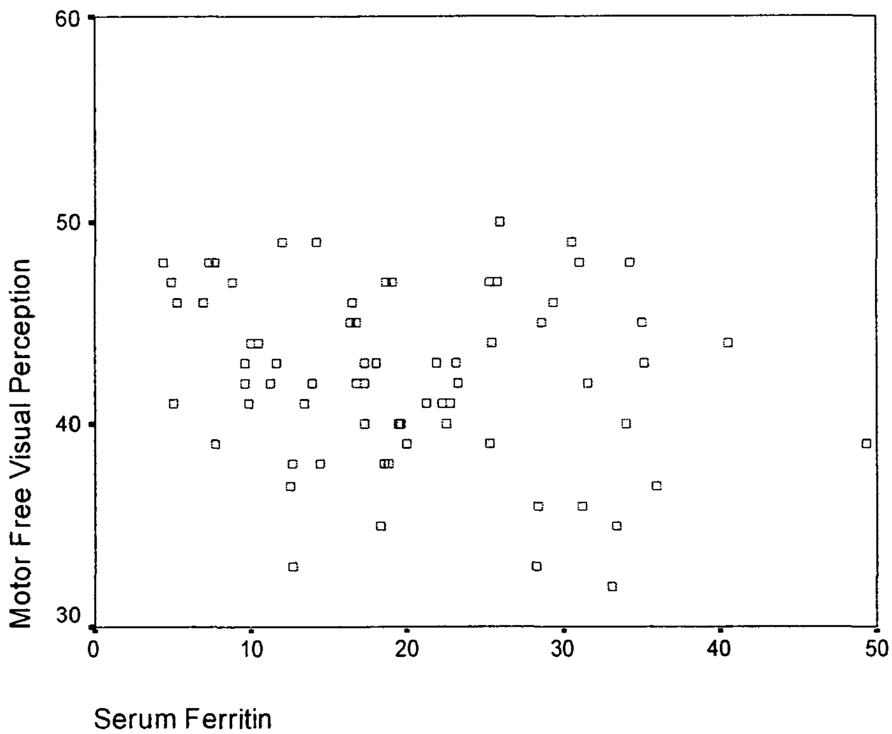
Digit Span Backward
2
4
6
8
10
12
14
0
10
20
30
40
50
Serum Ferritin

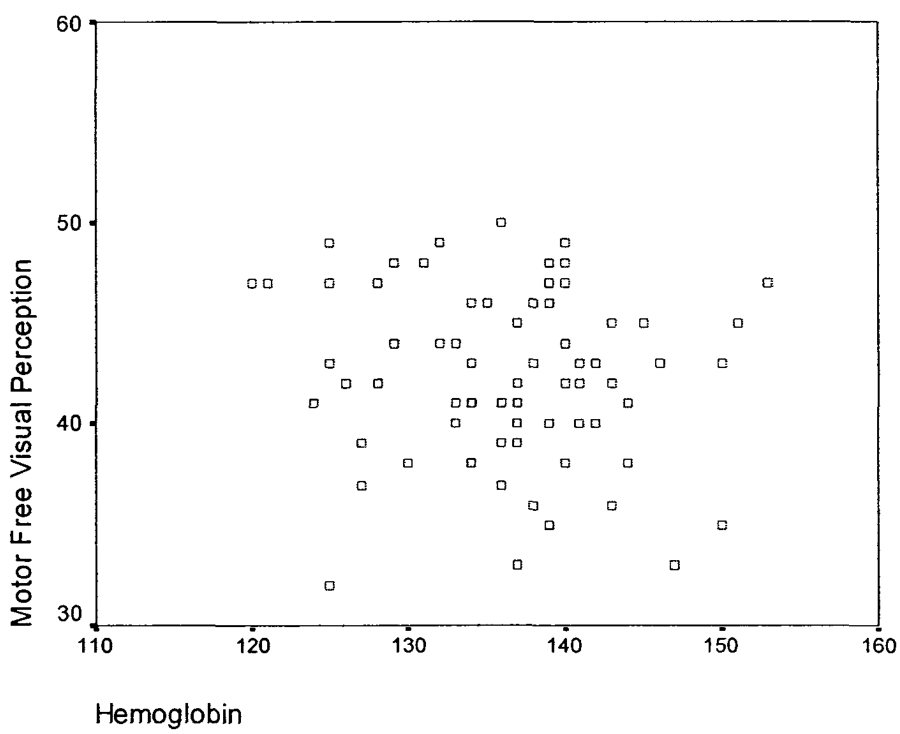


Digit Span Backward
2
4
6
8
10
12
14
110
120
130
140
150
160
Hemoglobin


Digit Span Total
0
10
20
30
0
10
20
30
40
50
Serum Ferritin


Digit Span Total
0
10
20
30
110
120
130
140
150
160
Hemoglobin


60
50
40
30
Motor Free Visual Perception
0
10
20
30
40
50
Serum Ferritin


60
50
40
30
Motor Free Visual Perception
110
120
130
140
150
160
Hemoglobin

Appendix I2: Scatterplot: Iron Status Versus % RNI