Lakehead University

Iron Status and Cognitive Performance in Adolescent Females

by

Tracey Larocque

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ABSTRACT

Cognitive impairments have been associated with iron deficient anemic infants and young children. The relationship in individuals with only a deficit in their iron stores is less clear. Adolescent females are particularly at risk for being iron deficient, however, the research on the relationship between iron status and cognition in adolescent females is limited. Iron supplements have been frequently used to improve iron status and findings suggest they may also improve cognitive impairments. The purpose of this study is to examine the relationship between iron status and cognition in a group of Canadian, adolescent females. Twenty-one, 14-16 year old, iron deficient (ferritin $\leq$ 20 μg/l and hemoglobin $\geq$ 120 g/l) but non-anemic adolescent females participated in an 8-week, double blind, randomized controlled trial. One hundred milligrams of ferrous gluconate (2 x 50 mg) was administered daily to participants randomly assigned to the active group (N=12).). The control group (N=9) was administered a placebo. Participants completed the Trail Making Test A and B, Motor Free Visual Perception Test-III, Digit Span, and the Covert Orienting of Visual Attention Task before and after the supplementation period. A 3-day dietary analysis was also conducted pre and post treatment. Cognitive deficits were not apparent pre supplementation and performance was not significantly different between the groups post supplementation. Also, ferritin levels improved in both groups. This study does not support previous findings of a relationship between low iron status and cognitive impairment. The lack of cognitive impairment at the pre-test and the difference between brain iron stores and systemic iron stores may be plausible explanations for the failure to find a relationship between iron status and cognition in this study.

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CHAPTER ONE
INTRODUCTION

There has been considerable interest in the relationship of iron status to cognition and brain functions (Beard, Connor, & Jones, 1993). Studies of attention and memory in particular have noted poorer performances in individuals who were considered iron deficient as compared to their iron sufficient counterparts (Groner, Holtzman, Charney, & Mellitt, 1986; Bruner, Joffe, Duggan, Casella, & Brandt, 1996; Lynn & Harland, 1998). Even though deficient levels of iron may impair cognitive performance researchers have suggested that there may be an improvement after iron supplementation (e.g., Groner et al., 1986).

Iron deficiency is the most prevalent nutritional disorder in the world today (Bruner et al., 1996). The World Health Organization estimates that iron deficiency affects more than two billion people worldwide although the majority of this population lies within developing countries (Viteri, 1997). In the United States, it is estimated that 22% of women have chronic iron deficiency while up to 12% of children under the age of two and approximately 14% of adolescent females are affected by iron deficiency (Carley, 2003, Johnson, 2006). In Canada, Newhouse, Clement & Lai (1993) reported that 39% of their adult female participants were deficient in iron. Seoane, Roberge, Page, Allard and Bouchard (1985) reported that 14% of the 15-19 year olds they studied were iron deficient and more recently the National Institute of Nutrition (2002) indicated that the prevalence might be as high as 39% in Canadian adolescent females.

Studies have examined the cognitive effects of iron deficiency in infants and young children (Beard et al., 1993; Pollitt, 1993) but little research has targeted

adolescent females. Bryan, Osendarp, Hughes, Calvaresi, Baghurst, & van Klinken (2004) found only nine randomized controlled trials of iron treatment and cognition. Only three of these involved adolescent females. Females in this age group are of significant importance as i) puberty predisposes teenage girls to an increased risk of iron deficiency due to the high requirements related to their rapid growth spurt; ii) the onset of menses and continual levels of menstrual blood loss place adolescent girls at greater risk for being iron deficient; and iii) social pressures tend to increase the prevalence of eating disorders in this age group thereby resulting in a poor dietary intake of iron (Halterman, Kaczorowski, Aligne, Auinger, & Szilyagi, 2001).

Attention abnormalities or deficits have been associated with a low iron status (Voeller, 2004; Konofal, Cortese, Lecendreaux, Arnulf, & Mouren, 2005). Groner et al. (1986) reported a frequent mention of a lack of concentration within their iron deficient participants. Concentration or vigilance is the ability to sustain attention over an extended period of time (Strub & Black, 1977). William James (1890) defined attention as "taking possession of the mind in a clear and vivid form of one out of what seem several simultaneous objects or trains of thought. Focalization, concentration, and consciousness are of its essence. It implies withdrawal from some things in order to deal effectively with others" (p. 403). Attention was previously viewed as a limited capacity resource (Bourne, Dominowski, & Loftus, 1979). Research is now becoming more specific in targeting the different areas of the brain associated with attentional processes (Fan & Posner, 2004). Attention can now be viewed as a separate system that has differentiated structures with its own anatomy, circuitry and neurochemical modulators.

Assessing attention can be conducted by visual attention tasks. Visual attention plays an important role in gaining knowledge of our external environment. The ability to allocate our attention to different areas in our visual field efficiently is essential in identifying which external stimuli are relevant to a specific task from those which are not (Zangemeister, Stiehl, & Freska, 1996). The visual system has nerve pathways to both cortical and subcortical areas (Jiang, Stein & McHaffie, 2003; Roberts, Robbins & Weiskrantz, 1998) and may or may not involve movement of the eyes (Posner and Cohen, 1984). The effect of what an iron deficient state has on this system is still elusive; however, myelination of neurons and specific neurotransmitters within the attentional system that are related to iron are targeted as possible factors (Beard & Connor, 2003).

Memory disturbances have also been associated with a low iron status (Sen & Kanani, 2006; Youdim & Yehuda, 2000). Schacter (2004) defines memory as "... the ability of living organisms to retain and utilize acquired information. The term is closely related to learning... in that learning implies retention (memory) of information" (p. 643). There are different features to memory that are distributed across several locations in the brain (Schacter, 2004). These areas are similar to those related to visual attention and are also areas where the same neurotransmitters function (Beard & Connor, 2003).

Supplementation has been a key strategy in normalizing iron status (Viteri, 1997). Iron supplements have not only improved iron status but this improvement has resulted in better performances in many different cognitive tasks in those who were iron deficient (Grantham-McGregor & Ani, 2001). There are many different forms of iron supplements but the ferrous compounds are the preferred choice as they have a greater bioavailability than other ferric forms of iron (Tsang, 2004).

Iron deficiency may have a profound effect on one's ability to attend to and retain information and adolescent females may be at greater risk for becoming iron deficient; however, there is limited research that examines iron deficiency, supplementation and cognitive performance in an adolescent population. Further research is therefore necessary to improve the knowledge of the relationship between iron status and cognition in adolescent females.

A. Purpose

The goal of this study is to examine the effect of iron supplementation on cognitive performance in a group of grade 10, Canadian adolescent females. Initially, iron status will be assessed. Next, those identified as iron deficient but non-anemic will be assessed on cognitive performance using selected tasks of attention and memory. Finally, iron status and cognitive performance will again be assessed following a supplementation period in which one group of participants receives an iron supplement and the other group receives a placebo.

B. Hypothesis

With regards to the current state of the literature, it is expected that administering an iron supplement to adolescent females with a deficit in their iron stores will improve their iron status. Also, since poor attentional and memory processes have been mediated by this nutrient deficiency, it is possible that the iron deficient state will result in cognitive deficits. It is then possible that an improvement in iron status may, in turn, improve their performance in the cognitive tasks selected.

CHAPTER TWO
REVIEW OF LITERATURE

In order to understand the association between cognition and iron deficiency, it is important to have a general insight to the workings of the brain. The following is a brief introduction to the anatomy and functions of selected portions of the brain dealing with the areas of cognition.

A. The Cognitive Brain

The frontal lobe and its relationship and connections to other areas of the brain, such as the temporal and parietal cortices, have been considered paramount in the area of cognition that relate to attention and memory (Han, Jiang, Gu, Rao, Mao, Cui, and Zhai, 2004; Gazzaniga, 2004; Aalto, Bruck, Laine, Nagren, and Rinne, 2005). There are four functional divisions of the frontal lobe: The Motor Cortex, Premotor Cortex, Prefrontal Cortex, and the Cingulate Cortex.

The Motor Cortex (Brodmann area 4) is specialized for the control, manipulation, and the execution of voluntary movements of the limbs and face and also for orienting movements of the neck and eyes.

The Premotor Cortex (Brodmann areas 6 & 8) is the area in which the motor cortex receives its instructions from and thus influences the motor cortex. There are direct projections from the primary and secondary somatosensory areas and also projections from the somatic association areas of the parietal lobe.

The Prefrontal Cortex (Brodmann areas 9, 46, 11, 12, 13, 14) begins the area of cognitive processes and can be divided into 2 subdivisions: The Dorsal Prefrontal Cortex (9 & 46) and the Ventral Prefrontal Cortex (11,12,13, 14). Outputs from these areas are

connected to the premotor cortex and the dorsomedial eye field. There are also heavy projections that have a direct connection from the dorsal prefrontal cortex to the deep layers of the superior colliculi and a connection to the superior colliculi through the basal ganglia. The superior colliculi is a bilateral area in the midbrain that has been implicated in visual orientation and search mechanisms (Jiang, Stein, McHaffie, 2003). The main inputs to the dorsal prefrontal cortex are from the parietal cortex. One of the functions of the dorsal prefrontal cortex is acting on information from the parietal cortex (Passingham, 1995). Area 7a of the parietal cortex processes information about the direction of gaze and the spatial position of objects in the environment (Anderson, 1987). The ventral prefrontal cortex has its connections with the amygdala and the hypothalamus. These structures are largely involved in memory processes (Gazzaniga, 2004) and with changes in autonomic responses such as blood pressure, gastrointestinal motility, pupillary dilatation, and respiration (Kaada, 1960). The main inputs are from the temporal lobes. The functional significance of the ventral prefrontal cortex is that it receives external information about the environment from the senses (Roberts, Robbins, Weiskrantz, 1998). Visual information is conveyed from the infero-temporal cortex and auditory information is conveyed from the superior temporal cortex. Goldman-Rakic (1987) suggests that the role of the prefrontal cortex as a whole is to initiate responses when there are no external cues or when there were external cues present but not at the time the response is executed.

The functional significance of the Anterior Cingulate (Brodmann's areas 24, 25, & 32) is with executive control and selective attention. This area is consistently activated with tasks that involve significant exertion during target detection or conflict resolution

(Raz, 2004). The dorsal prefrontal cortex is interconnected to the anterior cingulate and areas 24 & 32 are interconnected with the amygdala (Barbas and Pandya, 1989). The amygdala then influences the ventral portion of the prefrontal cortex directly to areas 12, 13, 14 and indirectly via the thalamus (Barbas & De Olmos, 1990). The amygdala has been connected to areas of memory that are necessary in some attentional processes.

B. Iron

Iron is used for the synthesis of hemoglobin, myoglobin, and numerous enzymatic functions (Lauffer, 1992). The major function of iron is with the transport of oxygen and carbon dioxide to and from the working tissues in the body accomplished by the iron-containing compound hemoglobin. Hemoglobin is the red hemoprotein that is used in the production of red blood cells. Myoglobin is the red iron containing protein in muscle fibres. It is similar in function to hemoglobin with a higher affinity for oxygen. It also functions in the production or formation of cytochromes. Cytochromes are proteins with an iron-containing heme group that function in the electron transport chain as carriers of electrons. They are capable of alternating between an oxidized and a reduced form, which is important in aerobic cellular respiration (Tortora & Grabowski, 1996).

I. Iron Absorption

The majority of the process involved in iron absorption is conducted in the small intestine. The stomach contributes hydrochloric acid that is necessary to remove protein-bound iron and reduce it from its ferric state to the more absorbable ferrous state. Beard, Dawson and Piñero (1996) divide iron absorption into 3 phases: iron uptake,

intraenterocyte transport, and storage and extraenterocyte transfer. The first phase involves heme-and nonheme-iron binding to specific mucosal membrane sites. It is then transferred within the mucosal cell by a transferrin-like protein and is either retained by the mucosal cell as mucosal ferritin or is transported to the basolateral membrane and bound to transferrin. There are many factors that control this process. Intraluminal factors act as enhancers or inhibitors that affect the amount of iron available for absorption; mucosal factors, such as the amount of mucosal surface or intestinal motility, will affect the amount of iron binding; and somatic factors, which include erythropoiesis, hypoxia, and inflammatory processes, may also influence the rate of iron absorption.

The difference between heme and non-heme iron is the source it is derived from. Heme iron comes from red meat products but can also include poultry, pork, fish and seafood; non-heme iron comes from other non-meat sources in our diet such as vegetables, grains and supplements (Hunt, 2003). The differentiation is in the bioavailability or absorbability of these types. Bioavailability is defined as "the degree to which a substance is absorbed or becomes available at the site of physiological activity" (Conway, Geissler, Hider, Thompson, & Powell, 2006, p. 1). Depending on the iron status of the individual and the amount of enhancers and inhibitors in the diet, approximately 15-40% of the heme-iron intake may be absorbed whereas only 1-15% of the nonheme-iron may be absorbed (Hunt, 2003). There are also differences between the enhancers and inhibitors between these two groups. With heme-iron, animal proteins enhance its absorption and calcium inhibits it (Hallberg, Rossander-Hulthen, Brune, & Gleerup, 1993). Non-heme iron has many more factors that affect its absorption. Those that inhibit absorption include calcium, bran, phytic acid in wheat and soy products,

(Simpson, Morris, & Cook, 1981), hemicellulose, cellulose, pectin (Baig, Burgin, & Cerda, 1983), tannins in tea (Disler, Lynch, Charlton, Torrance et al, 1975), and other metal ions and minerals (Hallberg et al., 1993). Those that enhance absorption include vitamin C and the heme-iron sources (DiSilvestro, 2004).

The second and third phases involve storage and transport. After the intraenterocyte fate of iron is decided any excess of the enterocyte's iron requirement is then transported. Ceruloplasmin, a serum copper protein, is responsible for the oxidation of iron necessary for its binding to transferrin at the basolateral membrane (Harris, 1995). Transferrin is the most significant iron transport molecule in vertebrates and is responsible for the extraenterocyte transport of iron from the basolateral surface of the mucosal cell to the peripheral tissues and the redistribution of iron to various body compartments and stored as ferritin (Beard et al., 1996). The major storage sites in the body are primarily the liver, but also include the spleen and bone marrow, which function in erythropoiesis or red blood cell formation.

II. Iron Status

A number of biochemical indicators are used to determine iron status: transferrin saturation rate; total iron binding capacity; free erythrocyte protoporphyrin; ferritin; and hemoglobin. The latter two are used consistently and are currently the most efficient indicators of iron status in iron interventions (Mei, Cogswell, Parvanta, Lynch, Beard, Stoltzfus, & Grummer-Strawn, 2005). Ferritin is the first and most sensitive indicator of the amount of iron that is stored in the body (Food and Nutrition Board, 2002). Its concentration reflects the size of the storage iron compartment that usually falls in the

range of 20 – 300 mg/L with each mg/L representing 10 mg of storage iron (Beard et al., 1996). A level below 12μg/L has indicated a total depletion in iron stores that will ultimately inhibit supply to the functional compartment resulting in anemia. A continuous decline in the concentration of ferritin levels is associated with significant decreases in the other parameters (Hallberg, et al., 1993). The level of hemoglobin in the body is used as a primary indicator of anemia. One of the characteristics of anemia is impaired hemoglobin synthesis. The normal range for hemoglobin levels is between 120-160 g/L (Nissl, 2005) and the World Health Organization (WHO) proclaims that hemoglobin levels that drop below 120 g/L usually indicate anemia (Cesari, Penninx, Lauretani, Russo, Carter, Bandinelli, Atkinson, Onder, Pahor & Ferruchi, 2004).

Iron deficiency is a level of iron below what is necessary to maintain iron balance. Iron balance is the difference between iron intake and retention and the body's iron requirements (Food and Nutrition Board, 2002). Iron deficiency is generally classified in 3 levels: iron depletion, indicated by a decline in the stores of iron but supply of iron to the functional compartment is not limited; iron deficient, characterized by a limited supply of iron to the functional compartment but not enough to cause anemia; and iron deficient anemic, when there is a significant decrease in the supply of iron to the functional compartment that results in a measurable decline in the production of red blood cells.

Iron deficiency most commonly results from excessive blood loss, poor dietary intake of iron, and/or poor absorption of iron (Carley, 2003). Signs and symptoms of being iron deficient systemically have been cited as fatigue, irritability, dizziness, problems with the immune system and fighting off infections, muscle weakness and poor

work capacity (Quilici-Timmcke, 2004; Bruner et al., 1996). It is possible that impaired oxygen delivery due to decreased hemoglobin concentration in the blood affects the production of red blood cells (Food and Nutrition Board, 2002). A decrease in the metabolism of oxygen would be the result of depleted oxidative enzymes.

The body is capable of maintaining a sufficient level of iron quite well (Beard et al., 1996). The storage iron levels in the body are the trigger for the rate of absorption in the small intestine. When storage iron starts to deplete, the rate of absorption increases and when storage levels are high, the rate of absorption decreases (DiSilvestro, 2004). The body also has the ability to reuse iron that is already present in the body. When the red blood cells have lived their lifecycle, the iron contained within them is recovered and used again or stored as ferritin. Unless an individual is genetically predisposed to a condition that affects the rate of absorption or has acquired a condition that results in abnormal blood loss, then iron deficiency in an apparently healthy individual is likely going to be the result of the amount of dietary iron intake, the bioavailability of that iron and the extent of iron losses (Beard et al, 1996; Viteri, 1997; Pierano, Algarin, Garrido, Pizarro, Roncagliolo, & Lozoff, 2001). The resources to consume an adequate amount of iron in a more developed nation is reasonably accessible but those who practice a vegetarian diet or unhealthy diet habits may not get the required amount of iron to meet the body's requirements (DiSilvestro, 2004).

III. Iron in the Nervous System

Iron is diffusely distributed in the brain. High concentrations have been found in areas such as the globus pallidus, substantia nigra, thalamus, ventral thalamus, dentate

gyrus, red nucleus and cingulate nucleus, and in some cases even higher than in the liver (Youdim, 2000; Beard & Connor, 2003). The highest concentration of transferrin, which binds and transports iron, is found in the hippocampal and cortical regions (Youdim, 2000). Other areas that have known dopamine neuron projections are the striatum, nucleus accumbens, and the anterior cingulate (Diamond, Briand, Fossella, & Gehlbach, 2004).

Iron's function in the brain is still not fully elucidated but animal studies have clearly shown that iron deficiency can have profound effects on the structure and function of neurons, neurotransmitters and overall function of the central nervous system (Youdim, 2001). Animals have been used to determine these effects with the thought that the animal model is an appropriate comparison to a human iron deficient state (Yehuda & Youdim, 1989). The consequences of iron deficiency in the nervous system result in impaired cognitive functions. These include an inability to concentrate, a lack of focus, learning disabilities, disturbances in attention and perception, and memory problems (Saloojee & Pettifor, 2001; Quilici-Timmcke, 2004, Youdim, 2000, Soemantri, Pollitt, & Kim, 1985). Iron balance is not only crucial for the proper functioning of the body but also an imperative factor in the functioning of the brain.

Like the systemic system, iron balance is well regulated and almost all of the iron in the brain is maintained throughout life (Youdim, 2000). A deficiency results when the intake of dietary iron is continuously lower than the body's requirements. The difference between the two systems is that turnover in the brain is much slower than that in the liver. It takes longer for the brain to become iron deficient than the body, but conversely, it takes longer for the brain to return to sufficient levels from a deficient state. In the rat

model, deficient levels of brain iron can persist even after supplementation, but is largely age dependent (Yehuda & Youdim, 1989). Although brain iron in the newborn rat could not recover, brain iron in the young and adult rat could be recovered after supplementation but at a much slower rate than recovery in the liver.

Pierano et al. (2001) have documented that iron is largely involved in the production and maintenance of myelin. Myelin is the fatty layer that surrounds neurons known as the myelin sheath and functions to speed the propagation of impulses along the fibres. Youdim's rats that were iron deficient were also hypomyelinated (Yehuda & Youdim, 1989). With the suggestion that the iron deficient state he imposes on rats might have the same consequences in the human brain, hypomyelination may be a common underlying factor in abnormal neural functioning.

Neurotransmitters have a large role in the function of the brain (Posner& Dehaene, 1994; Fan, & Posner, 2004). Neurotransmitters are "chemical agents that are released by a presynaptic cell when excited which then crosses the synapse to either stimulate or inhibit the postsynaptic cell" (Stedman's Concise Medical Dictionary, 1997). Iron aids in the production of these neurotransmitters (Youdim & Yehuda, 2000). Dopamine, norepinephrine, and serotonin are neurotransmitters that are synthesized from iron and appear to be involved in cognitive functions. These functions include attention, memory, learning and problem solving, and are largely associated with the frontal lobes of the brain.

Dopamine plays a large role in controlling the flow of information within the frontal lobes from other areas of the brain and is also identified as the primary neurotransmitter involved in 3 major pathways arising from the midbrain (Dreyer, 2006).

Dopamine receptors are also present in large amounts in the frontal cortex, the anterior cingulate, the hippocampus, and the hypothalamus. Research has shown that dopamine disorders in the frontal lobes cause a decline in neurocognitive functioning (Youdim & Yehuda, 2000). Right frontal lobe damage has been associated with poor performance on visual problem tasks and left frontal lobe damage has been associated with poor performance on cognitive flexibility and conceptual structuring tasks (Gilandas, Touyz, Beumont, & Greenberg, 1984). Dopamine is also associated with the pleasure system of the brain. It provides enjoyment and encouragement to motivate us to continue doing activities that require concentration and sustained attention, a concept referred to as brain reward (Routtenbourg, 1990).

Iron has also been identified as a significant factor in the production of serotonin and norepinephrine. Serotonin has many functions in the brain such as temperature regulation, mood and behaviour control, and endocrine regulation. It is also involved with memory and learning processes of cognition (Zablocki, 2000). The noradrenergic system has been shown to be most active in an alert state and important in modulating the alertness capabilities that follow warning signals (Raz, 2004). This system has also been shown to have a connection with arousal levels that have been linked to attention deficits. Grantham-McGregor & Ani (2001) noted that anemic children had higher scores in anxiety and depression and had greater social and attentional problems. Satcher (2000) stated that anxiety disorders were considered to be an abnormal version of arousal and also that females are more susceptible to these disorders, although the reasons are not fully understood.

Brain iron deficiency that is present in the infant or young child stage may result in behavioural disturbances or cognitive impairments that are irreversible (Beard & Connor, 2003; Pierano et al., 2001). Iron balance in 'critical' periods of brain development thus becomes very important. It is well recognized that the first decade, or even more so, the first 5 years of a child's life is the most crucial period of brain development (Youdim, 2000). The individual is therefore most vulnerable to any adverse conditions that may affect the developmental processes during this period. This period is also where myelin deposition on the neurons is at its peak.

The next stage of life is adolescence, a period covering roughly the whole second decade of life. This period should not be overlooked as another important stage in the overall development of the individual. Much of the research in iron status and cognition has involved infants or toddlers under the age of two (Sheard, 1994); however, certain aspects of the brain are not fully developed at this age, in fact, it has been documented that the myelin sheath actually continues to develop through adolescence and into adulthood (Bryan et al., 2004). Adolescence may therefore be another critical period to recognize and one that becomes more important especially if the individual was iron sufficient as an infant. In the rat model, cognitive impairments could be improved at this age (Yehuda & Youdim, 1989). Detection of iron status abnormalities in adolescence, and particular in the female adolescent, is necessary not only to improve any state of iron deficiency but may help improve any attention or memory deficits that may be present.

It is clear that iron deficiency is not only a concern from a nutritional standpoint relating to hematological effects but also has enormous implications with respect to its effects on cognitive aspects such as attention, memory and learning processes.

C. Related Nutritional Factors

I. Vitamin B12

Vitamin B12 functions in red blood cell formation and maintenance of the nervous system (Nissl, 2005). It is found in meat and other animal products, thus a deficiency may arise in those who are vegetarians. Low levels have been implicated in anemia unrelated to iron and may also indicate a folic acid deficiency.

II. Folate

Folate has been shown to be involved as a cofactor in methylation reactions in catecholamine synthesis and metabolism and also functions as maintenance factor of another key cofactor used in the synthesis of serotonin and the catecholamine neurotransmitters dopamine and norepinephrine (Fernstrom, 2000). It also aids in the function of red blood cells and when deficient, may cause anemia unrelated to iron. Low folate levels can indicate abnormal liver function or eating disorders such as anorexia nervosa (Nissl, 2005). Both Vitamin B12 and folate have also been implicated in cognitive deficits (Bryan et al., 2004).

III. Albumin

Albumin has been used to evaluate overall nutritional status, evaluate liver function, and may signal periods of high blood loss and gastrointestinal malabsorption syndromes (Payne, 2004).

The following table presents the normal values for hematologic parameters that have been previously mentioned.

Table 1: Normal Values for Selected Hematologic Indices

	Ferritin	Hemoglobin	Vitamin B12	Folate	Albumin
Normal Values	20–160 µg/L	120-160 g/L	100–700 pmol/L	2–20 ng/ml	35-55 g/L

(Information extracted from Nissl, 2005; Payne, 2004)

D. Adolescent Females

Several studies have examined the cognitive effects of iron deficiency in infants and young children, but little research has targeted adolescent females. This is a stage in life where numerous physiological changes are occurring and a time where outside influences can have an enormous impact on a potentially vulnerable population.

The risk for iron deficiency increases in an adolescent population and particularly with female adolescents. The National Institute of Nutrition (2002) indicates that the prevalence may be as high as 39% but also cautions that this value may be underestimated. Testing for deficient iron stores is not routinely conducted unless the individuals are high-risk infants or considered disadvantaged, thus, the true prevalence may not be known.

Adolescence is generally identified as a period between the onset of puberty and adulthood. In females, this period is typically by the age of 12 until the age of majority (Merriam -Webster, 2002). At this time, there is a large growth spurt with an overall expansion in the total blood volume, thereby increasing the requirement for iron from a preadolescent level of approximately 0.7 mg Fe/d to levels as high as 2.2 mg Fe/d during adolescence (Beard, 2000).

The onset of menses and continual levels of blood loss monthly, results in an additional requirement of iron. Beard (2000) indicated that an average of 84 ml per

period would result in an approximate 0.56 mg Fe/d increased need. The amount of blood loss differs between individuals and may be greater in those who suffer from dysmenorrhea (painful periods) or menorrhagia (heavy periods) (Quilici-Timmcke, 2004). Blood loss is indicated as one of the primary causes of iron deficiency but is not limited to menses. Gastrointestinal ulcers and hemorrhoids, non-steroidal anti-inflammatory drugs (NSAID's), and intrauterine devices to prevent pregnancy have all shown to cause bleeding (DiSilvestro, 2004).

There are other non-physiological factors that also tend to afflict adolescent females. Dieting, eating disorders, and vegetarianism, can all contribute to a low dietary intake of iron. Dieting has become one of the growing trends among teenage girls (McVey, Tweed & Blackmore, 2004). Jones, Bennett, Olmsted, Lawson, & Rodin (2001) reported that approximately 23% of adolescent females were dieting to lose weight and 27% of girls aged 12-18 exhibited disordered eating attitudes and behaviours that tended to increase throughout adolescence. Dieting may be the healthful alternative for those who require strict dietary intakes but for the majority of this population it seems to be a way of life, one that McVey, Tweed & Blackmore (2004) report as being associated with eating disorders and all the chronic health problems that are related to them.

Eating disorders among adolescents are typically referred to as anorexia and bulimia. Harris, Eberly, & Cumella (2004) report that one to three percent of adolescent females have bulimia and one percent have anorexia. These two devastating conditions are characterized by an inadequate dietary intake that typically results from a distorted self-image, shame, and low self-esteem. Various nutritional deficiencies are obviously going to be apparent with these conditions and iron is likely going to be one of them.

Vegetarianism cannot be ruled out as a factor partly responsible for the prevalence of iron deficiency. The *U.S. News & World Report* (1993) published a survey estimating that more than 12.4 million Americans considered themselves to be vegetarian and concluded that teenagers were the fastest growing segment of that population. A poll in 2000, estimated that approximately 2% of 16-17 year olds in the United States were vegetarians and these individuals were more likely to be female (Vegetarian Resource Group, 2000). Vegetarianism is not a health risk per se, but coupled with a weight and body conscious teenage girl, it may result in an unhealthy dietary practice (Perry, McGuire, Neumark-Sztainer, & Story, 2001). Jacobs-Starkey, Johnson-Down, & Gray-Donald (2001), indicated that approximately 57% of adolescent females are not meeting the recommended number of servings from the Meat and Alternatives food group in the *Canada's Food Guide to Healthy Eating*. The concern is that nutrient bioavailability becomes compromised with a vegetarian diet and the risk of nutritional deficiencies is increased (Venti & Johnston, 2002). The non-heme iron found in plant sources is less bioavailable than heme sources and removing meat from the diet can reduce the absorption of this non-heme source by up to 70%.

E. Attention

Attention is considered one of the cognitive processes that serve as the foundation on which the development of the higher intellectual skills of cognition rely (Gilandas, Touyz, Beaumont, & Greenberg, 1984). Cognition refers to a process or mental act that uses thinking, problem solving, perception, intuition, and attention in order to acquire knowledge (Strub & Black, 1977). Although all of these are important aspects of

cognition, an individual must be able to establish some level of ability to sustain attention over a period of time before the more complex aspects of cognition such as abstract thinking, reasoning, comprehension and problem solving can occur. Functional magnetic resonance imaging (fMRI) studies have frequently observed increased electrical activations as well as enhanced hemodynamic changes in the frontal cortices, parietal cortices, and the cingulate cortex during examination of spatial attention (Han et al., 2004). More specifically, these responses have also been observed in a variety of tasks that are associated with covert visual orienting (Gitelman, Nobre, Parrish, LaBar et al., 1999).

Research in recent years has made it possible to consider attention as a separate organ system with its own anatomy, circuitry, and set of functions (Fan & Posner, 2004). Posner and Petersen (1990) have suggested that different sources of attention employ separate brain mechanisms that can be classified into 3 separate but interrelated attentional networks and defined in their anatomical and functional terms. The functions of these networks involve maintaining an alert state, executive control, and orienting to sensory input. The alerting network involves the ability to increase vigilance and has been linked to the thalamic, frontal and parietal regions of the brain (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005). Fan & Posner (2004), also note that this network is influenced by the norepinephrine system which itself has been connected to maintaining an alert state. Neuroimaging studies have further shown a right hemisphere bias for norepinephrine and sustained attention. The executive control, involves the anterior cingulate cortex and lateral prefrontal regions, which are targeted areas of the ventral tegmental dopamine system (Fan et al., 2002). The functions of this network

involve more complex processes that involve conflict resolution. It has been proposed that the anterior cingulate cortex may be more involved with conflict monitoring whereas the dorsolateral prefrontal cortex is more involved in resolving the conflict. This network becomes heightened in situations that involve planning, decision making, error detection, novel or not well-learned responses, decisions with difficult or dangerous conditions, and in overcoming habitual actions (Fan & Posner, 2004). The third network, the orienting system, is involved in selecting specific information within substantial amounts of sensory input and it has also been associated with the frontal and parietal lobes and with the frontal eye fields in the prefrontal areas (Fan, McCandliss, Sommer, Raz, & Posner, 2002). More specifically, fMRI studies have identified the involvement of the superior parietal lobe following the presentation of the cue and the temporal-parietal junction when disengagement occurs following a target presented at an uncued location (Corbetta, Kincade, Ollinger, McAvoy & Shulman, 2000).

Orienting can be reflexive or voluntary. Reflexive orienting refers to attention that is directed to a target's location by the sudden presentation of a stimulus, or it can be voluntary, in which the person directs attention and searches their visual field to look for a target (Fan et al., 2002). The orienting of attention can be done so with or without the use of eye movements. Orienting that includes the use of head or eye movements is termed overt orienting and orienting that does not involve head or eye movements is termed covert orienting of attention (Posner, 1980). The neurotransmitter relation of this network has been identified as acetylcholine, which arises from the cholinergic systems (Fan & Posner, 2004).

I. Covert Orienting of Attention

The research will not dispute that there is a large connection between the orienting or shifting of attention with movement of the eyes, but researchers have also noted that both overt and covert orienting to positions in space are very closely coupled in daily life (eg., Posner, 1980). This is possibly the result of these two mechanisms having very similar regions of modulation. Peterson, Corbetta, Miezin, and Shulman (1994), found that regions of the superior parietal cortex were activated when attention was shifted to a peripheral location, whether the shift was the result of an endogenous trigger or an exogenous one.

With research in this area, there has always been some speculation that one can shift attention to a location in space independent of eye movements and with past research, successful reports of attentional shifts without eye movements have been frequent (Posner, Nissen, and Ogden, 1978; Klein, 1979). The significance may be noted in more efficient visual searches since the visual system cannot fully process all the inputs it receives (Wolfe, 1994). In order to ensure a more efficient search system, one would have to avoid having attention return to a location that was previously attended to. Posner and Cohen (1984) had noticed that if attention was captured at a cued peripheral location and then shifted away from this area; the time to detect the presence of a target in the previously cued location would be delayed. This result is referred to as inhibition of return (IOR) which infers that attention is biased against returning to a location in the visual environment that was previously searched and thus advocating orienting to novel locations (Klein, 2000). The superior colliculus has been greatly involved in this process but the extent of which is still unclear. Klein (2000) suggests that IOR may be more

likely the result of decreased inputs to the superior colliculus from the parietal cortex rather than due to inhibition in the superior colliculus itself and that the superior colliculus would more likely act as the endpoint in IOR. He further suggests that this may be due to the parietal cortex's role in reflexive orienting of attention and to the connection with spatial working memory.

F. Memory

James Olds began studying the relationship between brain function and behaviour in the 1950's and particularly with the pleasure centers of the brain. Olds and Milner, in the 1970's discovered a phenomenon that they referred to as brain reward. This phenomenon implicated certain nerve cells and fibres that could be affected by drugs that interact with the substances that those nerve cells secreted (Routtenberg, 1978). The areas were primarily the frontal cortex, the hypothalamus, and deep within the brain stem and other extensions were found within the midbrain, which houses the superior and inferior colliculi, and to the hindbrain, which includes the cerebellum (Nauta & Freitag, 1979). These same regions are closely associated with the catecholamine neurotransmitter pathways of dopamine and norepinephrine (Fan & Posner, 2004; Klein, 2005; Dreyer, 2006). Dopamine pathways have been found in a portion of the cortex in the temporal lobe and fibres from this region extend to the hippocampus. The hippocampus has been shown to be involved with the formation of memory and with memory of spatial relations. Routtenberg (1978) suggested that activity in the brain reward pathways, might actually facilitate the formation of memory, but noticed that this might only be the case in

the instance that something has previously been learned and may actually inhibit memory if stimulated during the learning process.

Memories are said to originate as sensory impressions. Mishkin and Appenzeller (1987) described the pathways of the visual system as being the starting point of processing sensory information into memory. They agreed that the central visual system begins at the primary visual cortex, which is an area at the back of the brain, called the striate cortex. The striate cortex receives information from the retina via the optic nerve and the lateral geniculate body. It is more strongly activated to stimuli that are simple and appear in specific, more central, locations in the visual field. The superior and inferior colliculi are more involved in continuation of that visual pathway to the temporal lobe for processing of more complex stimuli within larger segments of the visual field. The crucial areas of storing these impressions into memory have been identified as the hippocampus and the amygdala, two structures that are located on the inner surface of the temporal lobe. Damage to these areas has resulted in global amnesia along with other areas such as the thalamus and hypothalamus, two divisions of the diencephalon (Mishkin and Appenzeller, 1987). The diencephalon receives inputs from the hippocampus and the amygdala.

Memory of spatial relations takes a slightly different path. Objects are processed in regions of the temporal cortex. Processing of spatial locations travels up a different pathway to the posterior parietal cortex where the now perceived spatial perceptions activate the subcortical memory system (Mishkin and Appenzeller, 1987). The hippocampus is again largely involved in the memory of spatial relations.

Memory can be broken down into three basic distinctions. These are encoding, storage, and retrieval. Researchers have also included a distinction between what is now termed working memory and long-term memory. Working memory (WM) refers to the ability to maintain and manipulate information over a brief period of time; whereas long-term memory can retain information for years (Schacter, 2004). There are also subdivisions of long-term memory: an explicit or declarative memory that involves previous experiences and acquired facts that are consciously called to mind; and an implicit or nondeclarative memory, that involves changes in behaviour or performance due to past experiences, whether or not they can actually be remembered. Within these 2 subdivisions, several forms of memory such as episodic, semantic, priming, and procedural memory, are well recognized.

Although there is much interest with long-term memory, working memory has consistently been implicated in cognitive functions that relate to iron and more specifically, attention. In fact, several researchers have found that working memory is a necessary component required for the proper functioning of certain mechanisms involved in the attentional processes (Hester & Garavan, 2005; Castel, Pratt and Craik, 2003; Espy & Bull, 2005). Hester and Garavan (2005), in their 3 experiments found that increasing WM load resulted in decreasing the executive control of attention. They were based on Kane, Bleckley, Conway, & Engle's (2001) suggestion that the ability to keep relevant information active and easily accessible would be a reflection of one's ability to control attention. They believed this because "coherent and goal-oriented behaviour in interference-rich conditions requires both the active maintenance of relevant information and the blocking or inhibiting of irrelevant information" (p. 170).

With the close connection between the executive control of attention and working memory, Espy and Bull's (2005) study was conducted to compare one group with high digit spans and one with low digit spans against performance on 4 different executive tasks that varied in their nature. The only results that differed between the groups were their performance on tasks of inhibitory attentional control. The findings suggested that with the inhibitory processes of visual attention, there was at least some sort of dependence on short-term memory processes.

Inhibition of return (IOR), a mechanism that is thought to make visual search more effective by inhibiting the return of attention to previously attended locations in favour of novel locations, has also been studied in connection with working memory. It has been found that IOR is present when cued objects have moved to novel locations (Tipper, Driver, & Weaver, 1991) and also present at multiple locations at the same time (Ogawa, Takeda, & Yagi, 2000). This robust mechanism has also been found to last as long as 3,000 ms (Vaughan, 1984). These findings lead to the belief that there would have to be some memory process that would 'tag' cued locations and also be able to maintain and update information over a long period of time (Castel, Pratt, & Craik, 2003).

The current research acknowledges a relationship between memory and attention; however, the effects of iron status within these processes are still unclear.

G. Iron Supplementation

There are many factors that could contribute to cognitive deficits in an individual but nutrition is one factor that can be easily modified. Iron supplementation is indicated

when an iron deficiency is diagnosed and restoration of the body's iron stores to normal levels is required (Food and Nutrition Board, 2002). It has been documented in many articles that supplementing with iron improves iron status both in the circulatory system (Groner et al., 1986, Bruner et al. 1996, Viteri, 1997, Lynn & Harland, 1998, Seshadri & Gopaldas, 1989) and in the brain (Youdim, 2000, Beard, 2003). The results from the study by Groner et al. (1986) indicated a beneficial effect of iron therapy and cognitive test scores in some measures of attention span and short-term memory. Bruner et al. (1996) found that attention and memory, and also mood and energy improved after receiving iron treatments and also that these results were apparent even before there was any improvement in the hemoglobin levels. This would imply that even those with mild iron deficiency might benefit from iron treatment. What it also suggests is that there may be cognitive impairments that precede any clinical signs of anemia (Haltermann et al., 2001).

Consideration into the type of iron supplement, the dose required for improving iron status, and the length of time it takes to reach that state is necessary when using iron supplements as a therapeutic intervention. There are many different forms of iron supplements that are available: ferrous sulfate; ferrous fumarate; ferrous citrate; ferrous succinate; and ferrous gluconate (DiSilvestro, 2004). These compounds are more readily absorbable than other iron forms containing ferric compounds. Ferrous gluconate may be a more acceptable choice if administering to a young adolescent population as it has a greater bioavailability compared to other forms but is also known to have fewer adverse side effects because it is milder on the stomach (Tsang, 2004). However, iron preparations contain different amounts of elemental iron. Ferrous gluconate contains

approximately 12% elemental iron whereas ferrous fumarate contains 33% and ferrous sulfate contains 20% (Allen, 2002). There may be a tradeoff between potential side effects and effectiveness but previous studies using ferrous gluconate to improve iron status have resulted in significant improvements (e.g., Cantagallo, Perini, & Cantagallo, 1997).

The importance of balancing iron levels in body increases as iron is lost everyday within the GI tract, urine, skin, and also due to menstruation in females (DiSilvestro, 2004). The Recommended Daily Allowance (RDA) is based on iron balance. Iron balance is the difference between iron intake and retention and the body's iron requirements (Food and Nutrition Board, 2002). The RDA for adolescent girls is approximately 15-18 mg (Quilici-Timmcke, 2004). The RDA is determined with the assumption that 18% of the iron consumed will be absorbed and a portion of that will be heme iron. An ideal dose of iron used for supplementation purposes may vary, but have been as high as 200 – 300 mg/day. Other studies have used 100 mg/day and found a significant improvement in iron status (Newhouse et al., 1993). A larger quantity would be justified as a therapeutic intervention to purposely affect iron status more quickly. Considering iron supplements are classified as a non-heme source of iron and may only have a 3-6% absorption rate, DiSilvestro (2004), states that a "supplement must greatly exceed the RDA to have the same impact as an RDA dose of iron from a meat-containing diet" (heme iron).

Previous researchers have used varying lengths of supplementation trials with positive results. A 16-week study by Lynn and Harland (1998), an 8-week study by Bruner et al. (1996), and a 4-week study by Groner et al. (1986), all resulted not only in

an improvement in iron status but also showed an improvement in numerous cognitive tests that were administered.

The overall indications for supplement use has been well established and documented as being an effective intervention in the treatment of iron deficiency. These studies have also shown its' effectiveness in improving the cognitive abilities, and more importantly the attention and memory aspects of cognition, in iron replete, adolescent individuals.

CHAPTER THREE
METHODOLOGY

A. Ethical Approval

Approval to conduct this study was granted by the Research Ethics Board of Lakehead University in Thunder Bay, Ontario. It was granted under the supervision of Dr. Ian Newhouse, Dean of Professional Schools, Dr. Jim McAuliffe, School of Kinesiology, Dr. Michel Bedard, Department of Public Health, Dr. Chris Lai, M.D., Thunder Bay Regional Health Sciences Centre, and Donna Newhouse, Northern Ontario School of Medicine. Approval was also granted by the Lakehead Public School Board and by each high school principal. Signed consent forms by each participant and one of their legal guardians, were accepted as approval to participate in this study.

B. Participants

Seventy-one, asymptomatic, Grade 10 adolescent females (ages 14-16), from 6 different high schools within the Thunder Bay area, volunteered to participate in the initial assessment of iron status and cognitive performance. Their iron status was determined using the parameters in the following table.

Table 2: Parameters for Determining Iron Status

Iron Sufficient	**Iron Deficient**	Iron Deficient Anemic
SF > 20 µg/l	**SF ≤ 20 µg/l**	SF ≤ 12 µg/l
HB ≥ 120 g/l	**HB ≥ 120 g/l**	HB < 120 g/l

The same parameters were used in previous studies conducted by Dr. Newhouse and colleagues (1989, 1993).

C. Procedure

The testing was conducted on the site of each school on separate days. Participants were asked to arrive at the site of testing in an overnight fasted state. Blood work was completed between the hours of 9:00 and 11:00 a.m. On test day, the participants were informed on how the testing was going to proceed and then asked to fill out the health questionnaire and additional question sheet if they had not previously done so. The participants were then brought into a separate enclosed area to have a blood sample drawn while the others waited outside. A certified phlebotomist collected the blood samples in two, 3 ml vacutainer tubes (green and lavender). The participants were then advised to remain seated for a few minutes in case of any hematological effects on the body. After the blood samples were drawn, the participants were given a time card (see Appendix B(D)) to return for the cognitive tests and in the meantime could return to their scheduled classes. The cognitive tests would be completed in the same order starting with the Motor – Free Visual Perception Test – Third Edition (MVPT-III), followed by the Digit Span, the Covert Orienting of Visual Attention Task (COVAT), and then parts A and B of the Trail Making Test (TMTA, TMTB). The order of the tests was predetermined by a pilot study conducted at a lab in C.J. Saunders Fieldhouse. They would run from the longest to the shortest length of time. The tests were to be completed in 5 stations with 4 different examiners. Each setting was somewhat different due to the different areas allotted by each school, but careful consideration was taken to make each

station as similar as possible. The MVPT-III was divided into two separate stations to equal out the time frame to complete each station. Approximately every 10 minutes the next participant could start at station 1. The total time required to complete the tests was approximately 40 minutes. The COVAT and the TMT were conducted in a separate room by one examiner. The room required adjustable lighting, as the COVAT required a dimly lit room in order to properly view the computer screen. A small lamp that was placed under the testing table was used for each participant to try to maintain a similar environment. The lights were then turned on in order for the participant to complete the TMT.

After the testing was complete the blood samples were taken to the Thunder Bay Regional Health Sciences Centre to be analyzed for levels of serum ferritin and hemoglobin, which were used to determine iron status. Vitamin B12, folate and albumin were also going to be analyzed as possible confounding variables. A physician at the Thunder Bay Health Sciences Centre examined the lab results and determined the iron status of each participant. Folate was originally going to be analyzed as a possible confounding variable in conjunction with vitamin B12; however, as a result of the hematocrit and B12 levels being within normal ranges, the analysis was not necessary.

D. Cognitive Tests

For a detailed description of each test refer to Appendix B(A, I-IV).

I. Motor-Free Visual Perception Test –Third Edition

The MVPT-III was the first test completed. It took approximately 20 minutes to complete. The participant is seated at a small table directly across from and facing the examiner. The test plates are bound in a stand up easel cover that is placed in between the

examiner and examinee. Each test item is made up of black and white line drawings for both the stimulus test plates and the answer choices. The test is administered much like a multiple-choice test with the 4 answer choices arranged horizontally across the plate. Most of the stimulus items are on the same page with the answer except for those test items that involve a memory portion. In this case, the stimulus is taken away after a short time.

Instructions for the participants are found in Appendix B (B-I). The examiner has the instructions for each plate on the back of the practice plates for each of the seven sections. They are to be read exactly as written in order to be sure that each participant receives the same instructions. Additional reminder instructions for each test plate are also written on the back of the previous plate and are to be read if participant needs to recall the instructions for that section. The examiner has to tell the participant that a test item is an example when they come to it. If the example is answered incorrectly, then the examiner must explain why by pointing to the part on the test item that they interpreted incorrectly and then go to the second example if there is one provided. The examiner has to make sure that the participant fully understands the instructions before continuing with the test. If the participant doesn't know the answer to any one of the test items, the examiner is to encourage the participant to make an educated guess.

II. Digit Span

This was the second test in the circuit. It took approximately 10 minutes to complete. For the purpose of this study, a visual stimulus format rather than an auditory one was used. The researchers agreed that a computer program would be more precise

with the timing of the numbers in the sequence than the researchers would be with a verbal stimulus.

The program was set up as a Power Point presentation. The different series of numbers was taken directly from the manual. The font colour of the numbers was black and presented on a white background. Times New Roman was the type of font used and font size was 60. The sequences were centered in the middle of the page. Timing and ordering of the numbers appearing on the screen was automatic for one second in duration and one second after the previous event. Timing between a series of numbers was ten seconds and after two seconds, the word 'recall' appeared to signal the examinee to recall the sequence of numbers they had just viewed. It was presented in the Times New Roman font with a font size of 40. Two separate presentations were made for each of the forward and backward segments.

Instructions for the participant are found in Appendix B (B-II). When the participant recalls a series of numbers correctly, a '1' is placed in the appropriate space on the score sheet. If the examinee does not recall the sequence correctly the score is a '0' and a second chance is given. The participant has to recall a sequence correctly at least once out of the two attempts. If both attempts are recalled correctly, a '1' is given for each sequence and placed in the corresponding space on the score sheet. A total score of '2' is given for that series. The test ends when the participant fails to correctly recall both attempts in the same series. The total number of correctly recalled sequences for both the forward and backward sections is the total score for the participant.

III. Covert Orienting of Visual Attention Task (COVAT)

This was the third test in the circuit. It took approximately 5 minutes to complete this task. The paradigm that was used was similar to the Posner and Cohen (1984) paradigm (see Appendix B(E) for trial sequence). The initial display consisted of a central fixation dot with a 1° squared placeholder box 5.5° to the left and to the right. They were presented in white on a black background. The task was 100 trials with 2 SOA's. There were forty 100 ms and forty 800 ms trials and 20 catch trials that were randomly presented. The catch trials are those that would not present with a target in order to avoid speculation. The cue was presented as a slight enlargement and brightening of the placeholder box. The target was a filled in square that was 0.5° square, which would appear in either one of the placeholder boxes. The participant was to sit in at a table in a dimly lit room with a monitor approximately 40cm away. A portable grey wall was placed behind the monitor in order to make the setting similar in each school.

Instructions for the participant can be found in Appendix B (B-III). The examiner is to give a verbal description of the task to the participant. The examiner is to point out that catch trials are not to be responded to and a beep will sound when a mistake is made but to carry on. The examiner also places an emphasis on the participants not moving their eyes around the screen and having a continuous gaze on the central fixation dot.

A computer program designed for the task, analyzes the data for each participant. A mean is derived for each of the cued and uncued responses for each SOA. The cued data minus the uncued data for the 100 ms SOA will give the result of the cueing effects, or facilitation. The cued data minus the uncued data for the 800 ms SOA would indicate the inhibitory effects, or IOR.

IV. Trail Making Test – (TMTA, TMTB)

The TMT was placed right after the COVAT. It takes approximately five minutes to complete along with the COVAT and was conducted by the same examiner to equal a ten-minute station. The participant was seated at a computer desk in the same room or a room directly adjacent to the COVAT room (depending on the space provided by the schools).

Instructions for the participant can be found in Appendix B (B-IV). One 8½ x 11 piece of paper, containing the test for Part A, is placed face down on the desk along with a pencil. The side that is face up contains an example of Part A. The participant reads the written instructions after which the examiner will give a verbal explanation. Once the participant feels that she understands the instructions and has completed the example. Then the test for Part A can be administered. When the examiner says "Are you ready? Go!" the examiner starts the stopwatch and the participant can pick up the pencil and complete the test as quickly as possible. Once finished, the participant places the pencil on the table, which signals the examiner to stop the watch. The same procedure will then follow with Part B of the Test.

If the participant makes a mistake, the examiner stops the participant as soon as the mistake is made and brings it to her attention immediately. The participant is then instructed to return to the last correct position and continue from that point. This has to be done as quickly as possible as the time on the stopwatch continues to run. The consequence for errors would be an increase in the time to complete the test.

E. Administration of Supplements

Participants and the parents/guardians were invited to attend an information session outlining details of the study and to answer any questions they had about the entirety of the study and the supplements. The supplements/placebos were handed out at this session. The administration of the supplements/placebos was handed out in a double-blind fashion. The full 8-week supply was given in one bottle that was numbered. The participants lined up single file (in no particular order) and the supplements/placebos were handed in numerical order. The envelope containing the code (developed by Jamieson Laboratories) to determine which bottle was either supplement or placebo was kept by the supervisor and only revealed upon completion of the data collection.

F. Posttest Procedure

Procedures for the post supplementation testing were conducted exactly as the pretest procedures. The posttest was conducted in the School of Kinesiology Research Centre at Lakehead University. The procedural format of the study is outlined below in Figure One.

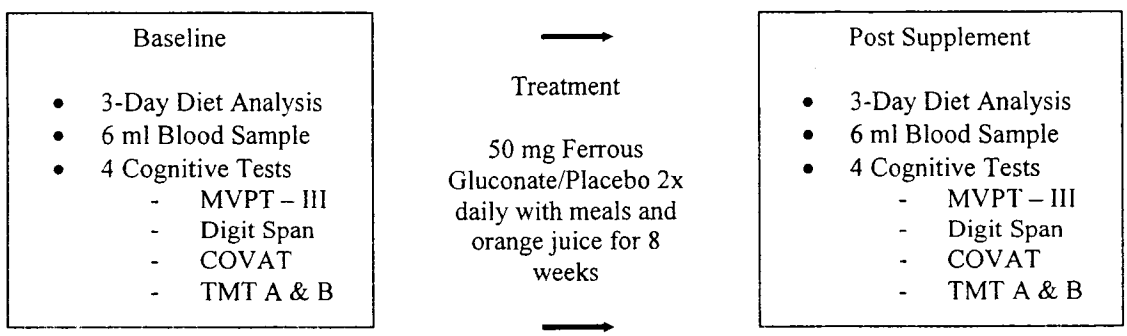

Figure 1: Schematic of the Testing Protocol – *ml* = milliliter; *mg* = milligrams; *MVPT-III* = Motor-Free Visual Perception Test – Third Edition; *COVAT* = Covert Orienting of Visual Attention Task; *TMT* = Trail Making Test.

G. Analysis of Data

I. Participants/Iron Status

The participants were identified as iron deficient as set out by the parameters in Table 4 (p. 44) at the Thunder Bay Regional Health Sciences Centre. A summary table of means will present the data for the pre and post supplement hematologic values.

II. Cognitive Tests

The cognitive tasks were analyzed with a 2 (treatment: active / placebo) x 2 (time: pre / post supplementation) analysis of variance (ANOVA) for each test. The COVAT was analyzed by separating the resulting facilitory effects (100ms SOA) and the inhibitory effects (800ms SOA) using two separate ANOVA's: 2 (time: pre/post) x 2(treatment: active/placebo) x 2 (trial type: cued/uncued). Pearson-r Correlations were also used to examine any relationship between the change value of ferritin levels and the change in cognitive test scores. Statistica version 5.0 by StatSoft, Inc., 1984-1996 was used to conduct the analyses. The level of significance was set at .05 for all analyses conducted.

III. Dietary Analysis

Nutribase 5 Plus version 5.12 by Cybersoft, Inc., 1986-2004 was used to analyze the 3-day dietary intakes of the participants, pre and post supplementation. A 2(treatment: active/placebo) x 2(time: pre/post) repeated measures analysis of variance was used for each variable. A 2(treatment: active/placebo) x 4(intake: dietary iron/vitamin C/calcium/dietary fibre) x 2(time: pre/post) repeated measures analysis of variance was used to determine interactions between the variables. An analysis of covariance was conducted to show adjusted means of the amount of change in the dietary intake of iron.

The results are based on 21 participants pre supplementation and 20 participants post supplementation.

IV. Supplementary Analyses

A subjective questionnaire completed by the participants identified the number of days per week of physical activity they participated in and the intensity of exercise bouts before the testing was conducted. They were each divided into 3 groups: rare or never; 1 or 2 days a week; 3 or more days a week; and light; moderate; or high intensity. Pearson-r Correlations were used to determine possible relationships between the above variables and the change in ferritin levels of the participants.

A Pearson-r Correlation was also conducted to determine any relationship between which week the testing was conducted during the participants' menstrual cycle and the change in ferritin levels from pre to post supplementation. The participants were asked to write down the date of the start of their last cycle. From the responses, 6 groups were formed: Week 1 – 1-7 days; Week 2 – 8-14 days; Week 3 – 15-21 days; Week 4 – 22-28 days; Week 5 – 29-35 days; and, Week 6 – 36-42 days.

V. Compliance

A pill count that had to be within a minimum 75% (or 6-week supply consumed) would be the cutoff to remain in the study. At least 90 pills had to be consumed and no more than 30 pills were to be left over at the end of the supplementation trial. A Pearson-r Correlation was used to examine any relationship between the amount of pills taken and the change in ferritin levels from pre to post supplementation.

CHAPTER FOUR
RESULTS

A. Participants

Seventy-one participants were initially tested to determine iron status. Forty-two participants (59%) were identified as being iron deficient (ID) as set out by the parameters of this study (serum ferritin ≤ 20 μg/l, hemoglobin ≥ 120 g/l) and no participants were identified as iron deficient anemic (hemoglobin < 120 g/l). Of the 42 ID individuals, eight of them declined enrolment into the supplementation study for reasons unknown. The 34 remaining ID individuals and their guardians were then given instructions and upon receiving a signed consent form, were enrolled in the 8-week supplementation program. After initial enrolment in the supplementation trial there were 13 participants who withdrew. The reasons were either: non-compliance; some gastrointestinal discomfort; were on other medication and physician had advised them to withdraw; family vacation at time of testing; serious illness within family and couldn't make test dates; or, didn't show up on test dates. As a result, 21 participants were included in the final analysis. Following the posttest, the number of participants, either active or placebo was decided by the code that was developed by Jamieson Laboratories.

B. Results

Table 5 below outlines the descriptive statistics for the parameters tested. The values represent the mean and the (standard error).

Table 3: Summary Table of Means for Parameters Tested

ID Adolescent Females Ages 14–16 yrs (SF ≤ 20 µg/l, Hb ≥ 120 g/l)		Normal Values	Active Group (N=12) Pre	Active Group (N=12) Post	Placebo Group (N=9) Pre	Placebo Group (N=9) Post
Hematologic Parameters	Ferritin (µg/L)	20–160	**12.03** (1.63)	**22.30** (2.63)	**12.69** (1.63)	**16.96** (2.07)
	Hemoglobin (g/L)	120-160	**133.92** (1.81)	**137.50** (2.14)	**135.11** (2.35)	**136.56** (2.51)
	Vitamin B12 (pmol/L)	100–700	**308.17** (26.80)	**310.67** (25.87)	**428.22** (34.45)	**416.55** (37.82)
Cognitive Tests	TMT*A* (seconds)	0 -26	**13.24** (1.23)	**13.07** (1.53)	**15.72** (1.49)	**11.92** (.79)
	TMT*B* (seconds)	0 - 65	**51.00** (3.81)	**49.45** (4.40)	**46.65** (4.02)	**45.85** (1.60)
	MVPT-III (number correct)	51 - 56	**57.50** (1.00)	**58.25** (1.17)	**56.89** (1.14)	**58.56** (1.23)
	Digit Span (fwd & bkwd)	13 +/- 4	**15.92** (.50)	**18.25** (.86)	**18.67** (1.22)	**19.78** (1.23)
COVAT	100 ms SOA (cued reaction ms)	n/a	**400** (20)	**378** (18)	**428** (21)	**384** (21)
	100 ms SOA (uncued reaction ms)	n/a	**415** (23)	**384** (17)	**433** (18)	**395** (22)
	800 ms SOA (cued reaction ms)	n/a	**392** (11)	**378** (16)	**429** (20)	**410** (26)
	800 ms SOA (uncued reaction ms)	n/a	**357** (13)	**338** (19)	**373** (18)	**361** (23)
	Facilitation (100 ms SOA)	negative number	**-15** (9)	**-6** (9)	**-5** (10)	**-11** (12)
	Inhibition (800 ms SOA)	positive number	**35** (10)	**40** 11)	**56** (11)	**49** (12)
3-Day Dietary Intake	Dietary Iron (mg)	54	**45.25** (4.32)	**41.83** (6.14)	**38.67** (3.80)	**41.75** (6.31)
	Vitamin C (mg)	180	**299.08** (54.58)	**297.25** (59.24)	**572.67** (142.43)	**282.13** (67.40)
	Calcium (mg)	3600	**2529.17** (306.90)	**2458.92** (418.78)	**2260.22** (310.51)	**2801.50** (423.63)
	Dietary Fibre (g)	75	**38.83** (3.13)	**41.42** (4.46)	**35.44** (5.03)	**33.38** (4.13)

Values in brackets represent the standard error of the mean.
ms=milliseconds, mg=milligrams, g=grams

The results of the ANOVA's for the cognitive tests are listed below.

Trail Making Test Part A (TMTA) – The main effects for treatment ($F(1,19) = 0.17$, $MSe = 26.60$, $p = .68$) and time ($F(1,19) = 3.65$, $MSe = 11.11$, $p = .07$) were not significant. The interaction between treatment and time was not significant ($F(1,19) = 3.06$, $MSe = 11.11$, $p = .10$).

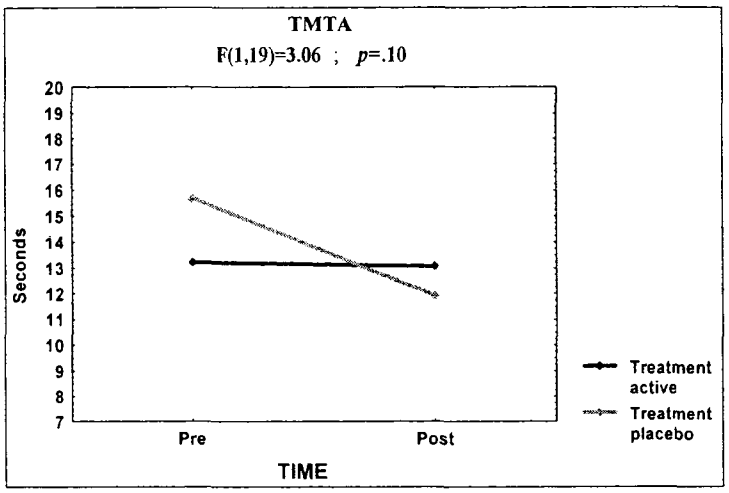

Figure 2: Interaction Effects of the Trail Making Test Part A

Trail Making Test Part B (TMTB) – The main effects for treatment ($F(1,19) = 0.73$, $MSe = 223.52$, $p = .40$) and time ($F(1,19) = 0.17$, $MSe = 82.61$, $p = .68$) were also not significant. The interaction between treatment and time was not significant ($F(1,19) = 0.02$, $MSe = 82.61$, $p = .90$).

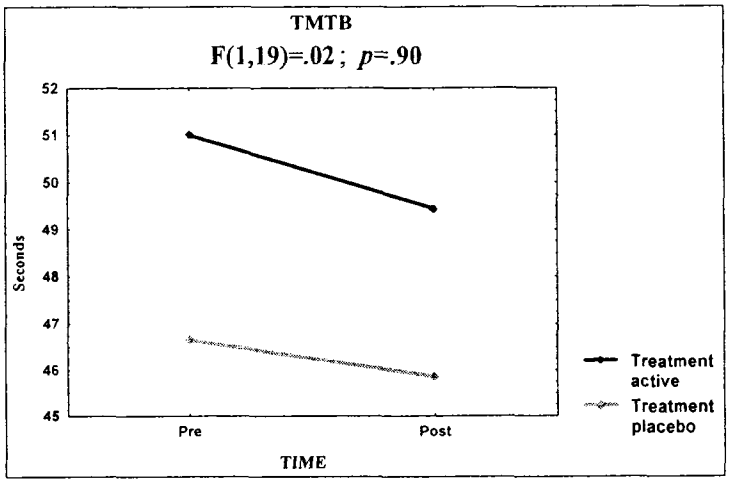

Figure 3: Interaction Effects of the Trail Making Test Part B

Motor-Free Visual Perception Test – 3rd Edition (MVPT-III) – Treatment ($F(1,19) = 0.01$, $MSe = 23.28$, $p = .92$) and time ($F(1,19) = 3.96$, $MSe = 3.80$, $p = .06$) main effects were not significant. The interaction effect between treatment and time ($F(1,19) = 0.57$, $MSe = 3.80$, $p = .46$) was also not significant.

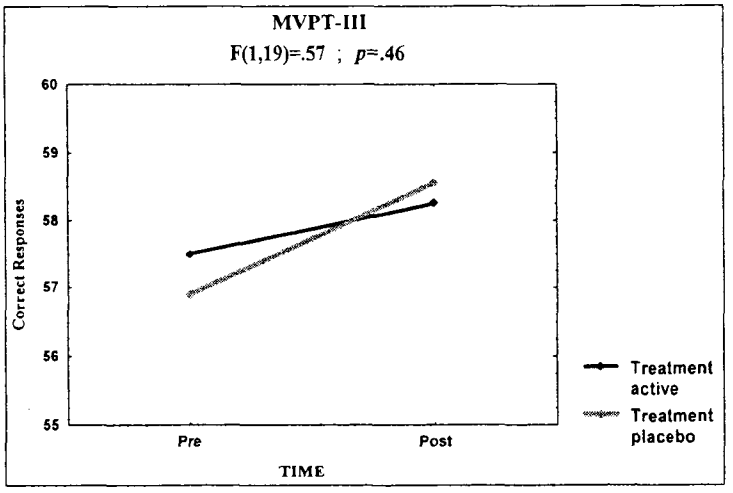

Figure 4: Interaction Effects of the Motor-Free Visual Perception Test

Digit Span (DS) – The main effect for treatment ($F(1,19) = 2.91$, $MSe = 16.15$, $p = .10$) was not significant, however the main effect for time ($F(1,19) = 13.87$, $MSe = 2.2$, $p = .001$) was significant for this test. The interaction between treatment and time ($F(1,19) = 1.75$, $MSe = 2.20$, $p = .20$) was not significant.

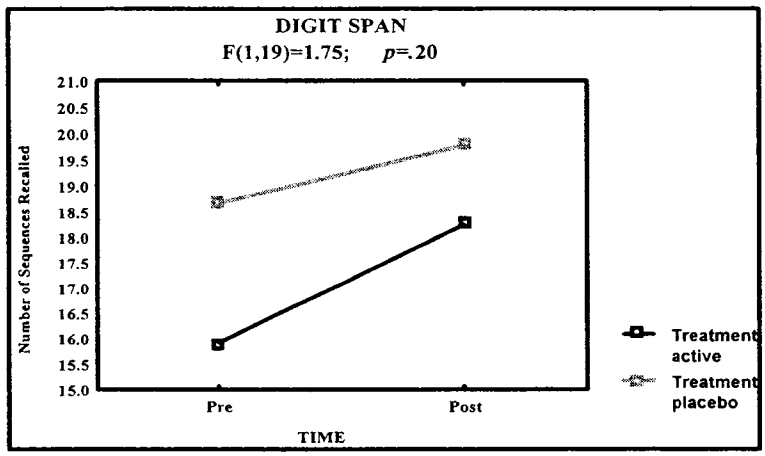

Figure 5: Interaction Effects of the Digit Span

Covert Orienting of Visual Attention Task (COVAT)

100 ms SOA – The main effects for treatment ($F(1,19) = .33$, $MSe = 14838.87$, $p = .57$) and trial type ($F(1,19) = 3.02$, $MSe = 556.62$, $p = .10$) were not significant but there was a significant main effect for time ($F(1,19) = 15.08$, $MSe = 1561.66$, $p = .001$). None of the 2-way interactions (F's $< .71$, p's $> .41$) were significant. The interaction effect between treatment, trial type, and time ($F(1,19) = 1.19$, $MSe = 271.18$, $p = .29$) was not significant.

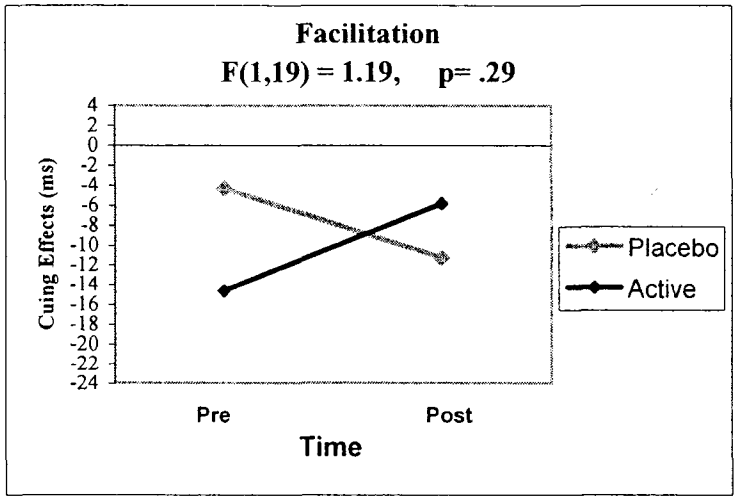

Figure 6: Interaction Effects of the 100 ms SOA

800 ms SOA – The main effects for treatment ($F(1,19) = 1.32$, $MSe = 10994.84$, $p = .26$) and time ($F(1,19) = 3.75$, $MSe = 1431.90$, $p = .07$) were not significant, however, the main effect for trial type ($F(1,19) = 56.00$, $MSe = 744.38$, $p < .01$) was significant. None of the 2-way interactions (F's < 1.67, p's $> .21$) were significant. The interaction between treatment, trial type, and time ($F(1,19) = .41$, $MSe = 502.64$, $p = .53$) was not significant.

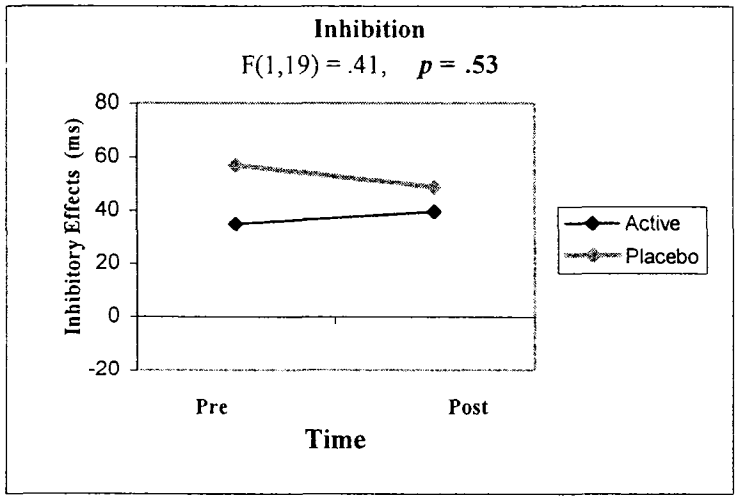

Figure 7: Interaction Effects of the 800 ms SOA

The results of the ANOVA's indicate that performance on the tests were not significantly different between the active and the placebo group after the supplementation trial.

C. Iron Status

Twelve of the 21 participants were given an active supplement and nine had been given a placebo. Figures 1 and 2 graph the means of their hematologic analysis for ferritin and hemoglobin respectively.

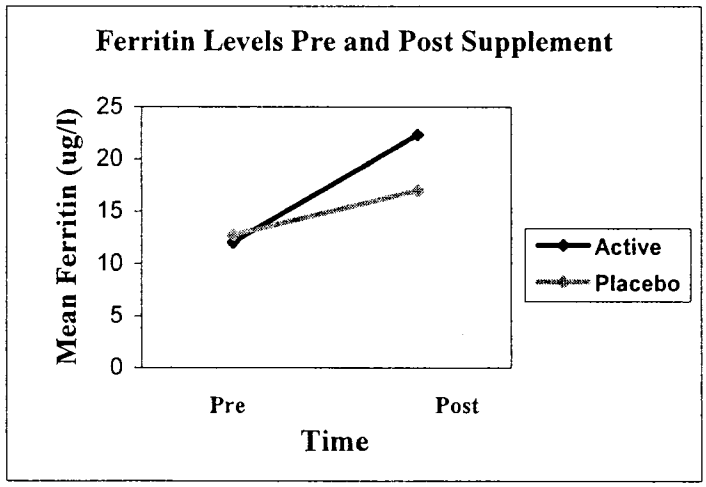

Figure 8: Mean Ferritin Levels

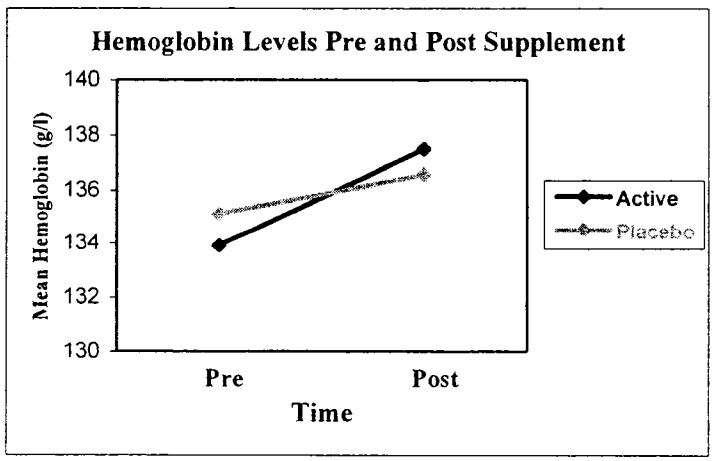

Figure 9: Mean Hemoglobin Levels

The analyses of the ANOVA's indicated that there were no main effects for treatment between the groups; however, the ferritin levels in both groups increased after the supplementation trial. The figures below represent how much the ferritin levels had changed after the supplementation trial. Figures 10, 11, & 12 present the distribution of change in ferritin levels post treatment for both the active and the placebo groups and the mean change in ferritin levels between groups, respectively. An independent *t*-test is used to test the difference between the active and placebo groups on the change in their ferritin levels.

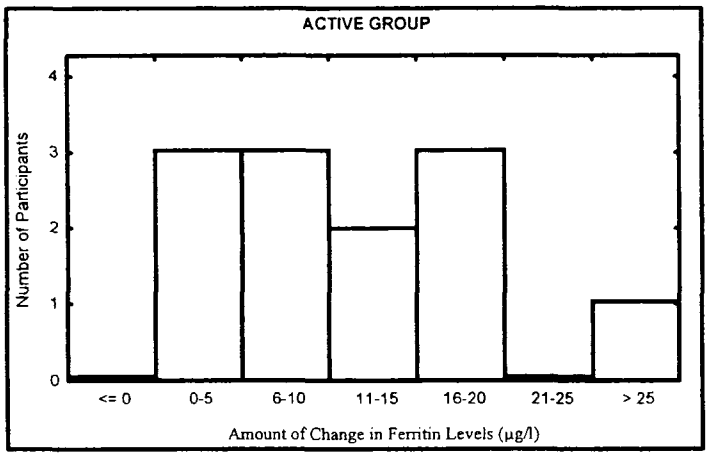

Figure 10: Change in Ferritin Levels – Active Group

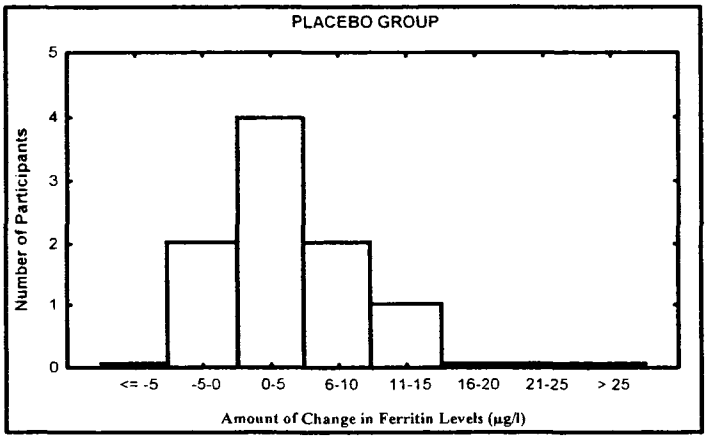

Figure 11: Change in Ferritin Levels – Placebo Group

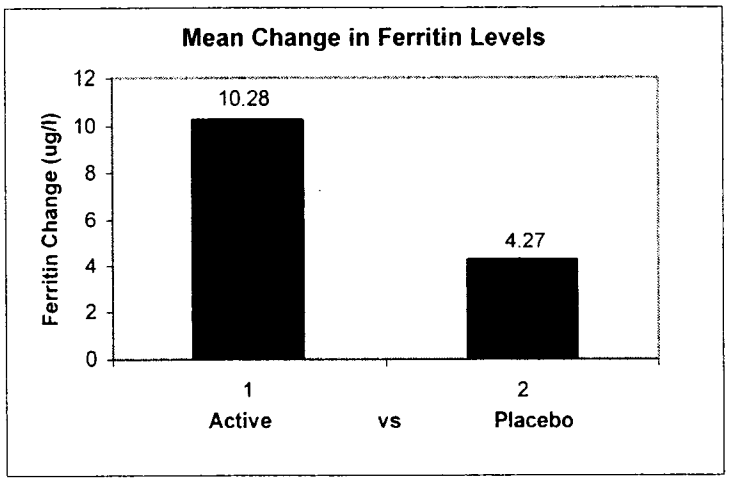

Figure 12: Mean Change in Ferritin Levels Between Groups

The analysis of the change in ferritin ($t(19) = 2.09, p = .051$) does not reach significance at the .05 level; however, the results indicate a trend toward a positive direction in affecting the ferritin levels. The difference in the mean change for hemoglobin ($t(19) = -.78, p = .44$), vitamin B12 ($t(19) = -.43, p = .67$), and albumin ($t(19) = .04, p = .97$) was not significant.

Even though instructions to all participants were to avoid changing their current dietary habits throughout the supplementation trial, the mean change in the placebo group still increased 4.27μg/l. The ANOVA result for the dietary intake of iron ($F(1,18) = .67$, $MSe = 184.34, p = .42$) was not significant though. Further analysis of the interaction between dietary intake of iron and three other variables measured was conducted with a 2(treatment: active/placebo) x 4(intake: dietary iron/vitamin C/calcium/dietary fibre) x 2(time: pre/post). There was a significant main effect for intake ($F(3,54) = 106.96$, $MSe = 510503, p = .001$) but the main effects for treatment ($F(1,18) = .051$, $MSe = 607089, p = .82$) and time ($F(1,18) = .151$, $MSe = 281489, p = .70$) were not significant. The 2-way interactions were not significant (F 's < 1.03, p 's $> .39$). The 3-way interaction ($F(3,54)$

= 1.53, *MSe* = 208229, $p = .22$) was not significant. This would suggest that the variables that may enhance or inhibit iron absorption did not influence the resulting ferritin levels between groups.

The amount of change in the dietary intake of iron within the groups was then examined. Table six outlines the results of an analysis of covariance (ANCOVA) for the change in dietary iron using the change in ferritin values as the covariate. The result of the ANCOVA analysis ($F(1,17) = 1.11$, *MSe* = 376.62, $p = .31$) was not significant. The adjusted means of the change in dietary iron are presented in Table 6.

Table 4: ANCOVA Results of the Change in Dietary Iron

	Change in Ferritin Levels	Change in Dietary Iron (mg)	
Group	Means	Means	Adjusted Means
Active	10.28	-3.42	**-4.91**
Placebo	4.76	3.75	**5.25**

D. Cognitive Test Correlations

Pearson Product-Moment Correlations were also conducted to examine if there was any relationship between the change in ferritin values and the amount of change in the cognitive test scores from pre to post supplement. The results indicate that any relationship that may exist between the change in ferritin and the change in cognitive tests is very weak and not significant (r's < .29, p's > .15).

E. Supplementary Findings

Normal daily GI losses were expected to be equal across the two groups but intense or strenuous physical activity has also been indicated in GI blood loss (Risser and Risser, 1990). A subjective questionnaire was given to the participants before and after supplementation regarding their exercise habits and intensity of exercise bouts. Pearson Product-Moment Correlations were used to determine probable relationships between the change in ferritin levels and the amount of physical activity and the level of intensity of exercise. The analysis showed that any relationship between the change in ferritin levels and the amount of physical activity in either the active or placebo group was weak and not significant (r's < .25, p's > .18). The analysis between the change in ferritin levels and the intensity of exercise bouts for either group was also not significant (r's < .39, p's > .3). It is not expected that the amount of physical activity or intensity of exercise would have affected the change in ferritin levels of these individuals.

The analysis for menstrual blood loss was also conducted using a Pearson Product-Moment Correlation to examine any relationship between the participants' change in ferritin levels and their menstrual cycle. The results indicated that the relationship between the variables in either group was weak and not significant (r's < .24, p's > .45) therefore suggesting that the change in ferritin levels was not likely affected by where the date of testing fell within the participants' menstrual cycle.

F. Compliance

Table Seven outlines the number of participants and the amount of pills taken over the 8-week trial. A total of 120 pills were supplied.

Table 5: Frequency Table for the Amount of Supplements Taken

Amount Taken (Max = 120)	**120**	**119-115**	**114-110**	**109-105**	**104-100**	**99-95**	**94-90**
Active (N=12)	7	0	0	2	0	2	1
Placebo (N=9)	6	1	0	2	0	0	0

A Pearson Product-Moment Correlation was used to examine any relationship between the amount of supplement pills that were taken and the change in ferritin levels after the 8-week trial. The results for the active group ($r = -.10, p = .76$) and the placebo group ($r = .21, p = .58$) indicate that any relationship between the amount of supplements taken and the change in ferritin levels is weak and not significant. Further analysis was conducted to determine if any relationship existed between those in the active group who did not consume the total amount of pills supplied and the change in ferritin levels. The analysis indicated that the relationship between the two variables was not likely to have any effect on the amount of how much the ferritin levels changed ($r = .17, p = .78$).

CHAPTER FIVE
DISCUSSION

It is recognized that iron status can have negative effects on cognition and brain function (Beard, 2003; Bryan et al., 2004). The relationship is more apparent in iron deficient anemic infants and young children but there is indication that individuals with low iron stores may also be affected (Groner et al., 1986). Adolescent females have an increased risk for being iron deficient yet the research in this area is limited (Halterman et al., 2001). Alterations in catecholamine neurotransmitter metabolism and nerve myelination continually demonstrate a possible role in cognitive deficits and these changes have been seen even before any drop in hemoglobin levels (Beard & Connor, 2003). Cognitive deficits have been reduced with iron supplementation but this is largely dependent on the stage of development and older individuals appear to have a greater chance for improvement.

The goal of this study was to determine the effects of iron supplementation on cognitive performance in adolescent females who were deficient in their iron stores. The hypotheses were that their low iron status would be reflected as impaired cognitive performance. The supplementation trial would then improve the iron status of iron deficient adolescent females and in turn may improve cognitive performance. Findings from the present study indicate that the participants' performance on the selected cognitive tasks was not impaired at pre supplementation in either group and the post supplementation results were not significantly improved in the active group when compared to the placebo group. However, ferritin levels improved only modestly in the

active group and this was not significantly different than the improvement noted in the placebo group.

Cognitive deficits have been apparent in iron deficient non-anemic individuals (Bruner et al, 1996; Pollitt, Hathirat, Kotchabhakdi, Missel, & Valyasevi, 1989). The present study does not support these findings. Performance levels were either well within the average/normal range, or in some cases, even found to be above average prior to the supplementation trial. Several issues may account for these results. Youdim, Hernandez-Rodriguez, Giordano, & Rios, (2001) had established that a deficiency of storage iron in the liver up to 90% may be witnessed while only a 35% - 40% decrease is noticed in the brain. This would suggest that the systemic iron status that was reported in the present study might not accurately reflect the iron status in the brain at the same time. Tucker, Sandstead, Penland, Dawson & Milne (1984) found evidence in their study though, that iron status, measured by serum ferritin and serum iron, was consistent with the electroencephalogram (EEG) recordings observed during the selected cognitive tasks. Their conclusion was that body iron stores relate to neurocognitive processes and ability, as the processes were associated with higher iron status; however, few of their participants were iron deficient. Although it is possible that in a larger sample of iron-deficient individuals who were then supplemented to improve their iron status would show a cognitive impairment and then improvement that would be reflected in the EEG readings. Further research is necessary to support this suggestion.

The time in which the iron deficient state was acquired with respect to the time the testing was conducted could also be a factor. It is unclear whether acute iron deficiency differs from chronic iron deficiency with respect to cognitive performance.

Youdim (2000) notes that the differences between systemic iron and brain iron is based on the slower turnover rate in the brain. Although a state of iron deficiency can be reached within 2-3 weeks of a diet short of the daily requirement of iron systemically, brain iron is rather resistant to systemic iron deficiency and its effects on brain functions may take several months (Shoham & Youdim, 2002; Ben-Shachar, Ashkenazi, & Youdim, 1986). Differences also exist between body and brain iron because uptake of iron into the brain actually increases when body iron status is low (Beard & Connor, 2003). The severity of the deficiency along with a longer duration of time being iron deficient is more likely going to result in a measurable cognitive deficit and thus be a better indicator than the hematologic state at any one particular time. In light of this, the participants in the present study who were identified as iron deficient by their hematologic indices may not have been brain iron deficient and therefore may provide an explanation for their performance being within normal ranges.

Hematological indices used to assess iron status can vary. Borel, Smith, Derr, and Beard (1991) found that day-to-day biological variation is a major component in the variability of the parameters used in assessing iron status. Even though ferritin is considered the most sensitive indicator of iron deficiency (Food and Nutrition Board, 2002), their study reported that ferritin had the greatest variability among the 4 different indices used and that it was greatest among their female participants. The hematology results of this study are based on a one-time measurement of iron status. It is possible that the hematological findings in the present study may be the result of this day-to-day variation.

Significant differences in iron status have commonly been reported in many supplementation experiments (Groner et al., 1986; Newhouse et al., 1993; Olivares, Pizzaro, Walter, Arredondo, & Hertrampf, 1999). This was not apparent in the present study. It is possible that difference is due to the type of supplement used. Ferrous gluconate is believed to be a better choice for a young population because it has a greater bioavailability when compared to the ferric compounds and may cause fewer adverse side effects because it is milder on the stomach when compared to the other ferrous compounds (Tsang, 2004). However, iron preparations contain different amounts of elemental iron. Ferrous gluconate contains approximately 12% elemental iron whereas ferrous fumarate contains 33% and ferrous sulfate contains 20% (Allen, 2002). Thus, the findings in the present study may have been the result of this difference.

Compliance with adhering to the supplement trial portion of the study was not a factor that could have affected the results. The participants had to have consumed at least 75% of the pills that were supplied in order to remain in the study. A frequency analysis indicated that 62% (13 of 21) of the participants had consumed all of the pills supplied. The analysis of those in the active group who did not consume all of their pills indicated that any relationship between the number of pills taken and the change in ferritin levels ($r = .17, p = .78$) was not considered a factor.

The duration of the supplement trial in this study was consistent with other supplementation trials and was therefore not expected to be a confounding factor in the results. Newhouse et al. (1993) and Bruner et al.(1996) reported significant improvements in iron status in their 8-week supplementation trials. A 4-week supplementation trial by Groner et al. (1986) also reported significant improvements in

iron status. Although a strict regimen for following the instructions for taking the supplements (2 x 50 mg daily) was not monitored or analyzed, it was not considered a confounding factor in the results. Studies examining the efficacy of weekly, twice weekly, or daily supplementation had reported that there was no significant difference between a twice weekly versus daily schedule (Shobha & Sharada, 2003) or a weekly versus daily schedule (Olivares et al, 1999) in improving iron status.

The intake of dietary iron offers an additional explanation for the non-significant findings. It was noticed that the placebo group had a greater positive change in their intake of dietary iron over the active group post supplement. Although instructions were given to both the participants and parents (guardians) to maintain their eating habits for the duration of the study, it was understood by the researchers that an increase in dietary intake of iron might result. It was expected though that the increase would be apparent across the sample. What was found by the study, according to the raw data and seemingly by chance, was that 63% of the participants in the placebo group improved their dietary intake of iron while 67% of the participants in the active group decreased their intake. Since iron supplements are classified as a non-heme source of iron, which is less bioavailable than the heme iron that is acquired from dietary sources, the results of the lab analysis on ferritin levels may be explained by this fact.

Exercise and menstrual blood loss were other factors that could affect iron status (Beard & Tobin, 2000; Johnson, 2006; Kim, Yetley, and Calvo, 1993). Beard and Tobin (2000) mentioned that the prevalence for iron deficiency would likely be higher in individuals within an athletic population and especially those who were younger females. Aside from the poor dietary intakes within this population, the iron deficient state may be

attributed to the increased rates of red cell and whole body iron turnover and the increased activity of iron-dependent oxidative enzymes. Kim et al. (1993) found that differences in iron status indices exist during the menstrual cycle. Their results indicate that the prevalence of an impaired iron status was significantly greater when the analysis was conducted during the menstrual phase as opposed to the luteal or late luteal phase. Information was collected subjectively pre and post supplementation about the amount and the level of intensity of exercise bouts and which week the date of testing fell within the participants' menstrual cycle. The results in this study indicated that there was no significant difference between the groups and that these variables were unlikely to have affected iron status in these particular participants.

Iron has been implicated in the production of the neurochemical modulators that mediate activity in the attentional and memory processes. Dopamine is the primary neurotransmitter within the frontal lobes that support the functions of executive control of attention (Fan & Posner, 2004). Executive control encompasses situations that involve planning and decision-making, conflict resolution, and other situations that involve novel responses and overcoming habitual actions (Posner and Dehaene, 1994). Norepinephrine is largely responsible for arousal levels and functions in the alerting network of attention. Being alert involves an internal change in preparation for perception of an upcoming stimulus and is critical for optimal performance in the higher cognitive tasks (Raz, 2004). The exact role of iron, either dietary or in supplement form, in the uptake of iron into the brain and how performance in cognitive tasks is affected by this, is still not fully elucidated by the current state of the literature.

An important finding from this study was that the prevalence of iron deficiency exceeded what was expected. Similar research within North America, reports that approximately 15-25% of a female adolescent population would be iron deficient with possible observations rising as high as 40% in certain regions (Bruner et al., 1996). The present study found that 59% of the initial sample tested was identified as iron deficient (ferritin levels ≤ 20 μg/l), a finding that greatly emphasizes the public health concern for this particular population. What is more of a concern is that 40% of these iron deficient individuals had ferritin levels that were at or below 12 μg/l. Levels below this value have indicated total depletion of iron stores that will ultimately limit the supply of iron and cause a measurable decline in the production of red blood cells (Food and Nutrition Board, 2002). Other research has indicated that 4-6% of samples tested may be identified as iron deficient anemic (Newhouse et al., 1993; Zlotkin, 2003). The present study did not however, identify any of the participants as having acquired a state of anemia (hemoglobin < 120 g/l).

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions

Previous studies have demonstrated that iron deficiency can impair performance on cognitive tasks and by improving iron status it would consequently improve cognitive performance. The present study did not have similar results. It is possible that although the participants were iron deficient systemically, they might not have been iron deficient in the brain at that same moment. This may be the difference between an acute and a chronic state of iron deficiency. Also, iron status was not significantly different between the groups post supplementation. This may have been the result of the type of iron supplement used and the difference in dietary iron intake between groups. The effect of iron supplementation may have been masked by the bioavailability advantage of the larger dietary source of iron consumed by the placebo group.

Overall, it cannot be concluded that iron deficiency does not have an effect on cognition. What is considered is that the brain has a mechanism that will protect itself from adversity in the body but a prolonged state of iron deficiency may compromise this protection and impairment in brain functions may result. Our findings clearly demonstrate that iron status was compromised in a large percentage of the adolescent female population in this sample and identification of iron deficiency in its early stages is critical if adverse consequences are to be prevented. A prolonged iron deficient state will not only lead to severe health conditions, it may also result in cognitive impairments that might not be completely reversible. Since, the present study could not support the

findings of previous research and the fact that the question still exists, further research in this area is warranted.

B. Recommendations for Future Research

Measuring ferritin levels has been widely used and well documented as an acceptable determinant of iron status in the body. Also, it is the most effective way of monitoring the effects of supplementation (Mei et al., 2005). Using it alone though may not be as effective for determining what is happening in the brain. A mean ferritin level from samples taken over several weeks may provide a more accurate indication of iron status in the brain because the turnover rate of iron is much slower in the brain than it is in the body. The inclusion of using the transferrin saturation rate as another indicator would be useful in identifying the lower limits or more severe cases of iron deficiency which may in turn, reveal larger cognitive deficits. These parameters along with functional magnetic resonance imaging techniques would greatly enhance the understanding of the connection between systemic iron status, brain iron status, and cognitive performance. Also, a much larger sample size would be required in order to accommodate the large amount of variance between participants.

Another recommendation to future studies in this area comes from the shortcomings of this study of having any prior history of iron status prior to enrollment. Previous research indicates that iron deficient infants are more susceptible to irreversible cognitive deficits (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000; Oski, Honig, Helu, & Howanitz, 1983). Knowing prior history would allow the researcher to highlight the adolescent stage of development and be able to determine whether any cognitive deficit

was related to the effects of iron deficiency within this time period. The researcher would then be able to determine if adolescent iron deficiency would result in cognitive impairment and if iron supplementation would reverse this effect.

Beard & Connor (2003) mention that the majority of studies they cited acknowledged that iron deficiency can have negative cognitive effects that were remedied by iron intervention. However, there were other studies they cited that did not. What they suggested was that the lack of identical conclusions was the result of test specificity and that the use of much more refined behavioural and cognitive measures would be necessary to identify actual cause and effect. Fan, Posner and colleagues (Fan & Posner, 2004; Fan et al., 2002; Posner & Dehaene, 1994) have begun to conceptualize the nature of attention and are now able to delineate attention into distinct functional components with its own anatomy and neural systems. In doing so, they have also been able to determine the neurochemical modulators that control activity in these components. The Attentional Network Test, developed by Fan and colleagues has been used in many different clinical conditions including Attention Deficit Hyperactivity Disorder, Schizophrenia, Stroke, and other brain injuries that have presented with impairments in executive control and has been effective in evaluating the attentional abnormalities within such conditions. Although the involvements of the neurotransmitters that have also been associated with iron are apparent in this test, no research (to the knowledge of the present researchers) has been conducted with the effect of iron status and performance on this test. This test may be another way to assess attentional abnormalities in not only iron deficient adolescent females, but also within other stages of development.

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APPENDIX A
Participant Data

A. Raw Data

I. Hematological Parameters

Part. #	Ferritin (μg/l)		Hemoglobin (g/l)		Vitamin B12 (pmol/l)		Albumin (pmol/l)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Active Group								
1-015	20.00	36.00	137	142	286	330	45.50	42.50
2-019	12.00	14.70	140	135	328	320	44.10	38.10
3-029	5.03	15.80	124	121	307	271	45.00	41.60
4-033	4.30	13.50	131	137	182	261	43.80	38.90
5-034	19.40	34.70	139	138	282	266	48.70	43.60
6-035	13.90	15.30	128	133	204	200	44.30	40.90
7-036	16.80	28.20	140	144	404	507	44.60	43.50
8-037	7.26	12.30	129	149	536	431	44.40	46.00
9-043	9.61	35.80	141	143	285	282	45.20	42.10
10-046	16.50	17.40	134	142	348	314	39.30	35.70
11-062	5.30	20.50	139	136	263	226	43.20	39.80
12-068	14.20	23.40	125	130	273	290	40.80	44.10
Placebo Group								
13-045	7.69	22.00	139	140	517	420	43.20	39.40
14-001	9.63	11.10	142	141	490	541	37.40	34.90
15-010	6.94	11.80	135	140	462	458	44.80	40.40
16-027	13.40	13.20	144	139	459	367	44.50	39.70
17-049	18.70	19.00	139	147	249	276	45.70	45.10
18-051	10.50	19.40	129	123	290	222	43.80	43.10
19-056	19.10	27.20	121	129	356	565	42.60	42.00
20-058	18.50	20.80	134	140	515	423	42.10	42.10
21-063	9.79	8.16	133	130	516	477	45.20	38.90

II. Cognitive Test Scores

Part. #	MVPT-III (raw score)		Digits Fwd / Bkwd / Total (value)						TMTA (sec)		TMTB (sec)	
	Pre	Post	Pre	Post / Pre	Post / Pre	Post	Pre	Post	Pre	Post	Pre	Post
Active Group												
1-015	54	54	8	11	9	8	17	19	8.06	10.21	52.56	78.84
2-019	62	64	8	10	7	9	15	19	6.84	10.83	33.78	38.18
3-029	54	59	7	9	7	6	14	15	17.83	13.26	60.39	52.83
4-033	61	62	11	12	6	8	17	20	12.73	11.41	47.57	42.73
5-034	53	55	11	11	7	10	18	21	19.42	11.76	46.20	36.48
6-035	55	52	11	10	5	9	16	19	13.45	15.44	37.38	53.51
7-036	55	52	10	14	8	8	18	22	16.42	12.76	44.66	38.25
8-037	61	60	9	12	6	10	15	22	12.11	11.51	65.86	37.47
9-043	55	58	8	8	5	5	13	13	14.23	12.94	45.63	57.13
10-046	59	61	8	9	8	8	16	17	9.62	8.30	54.43	43.06
11-062	59	61	10	10	8	8	18	18	9.27	9.52	42.12	37.12
12-068	62	61	8	8	6	6	14	14	18.85	28.87	81.43	77.78
Placebo Group												
13-045	61	61	8	12	8	6	16	18	14.35	16.99	63.36	54.91
14-001	56	59	9	10	8	11	17	21	16.96	10.29	54.22	40.34
15-010	59	54	14	16	8	8	22	24	26.41	12.29	57.00	48.71
16-027	54	61	10	10	8	8	18	18	14.16	11.62	30.55	45.50
17-049	60	63	12	9	8	8	20	17	17.57	10.69	34.54	41.39
18-051	57	59	12	11	7	8	19	19	15.06	10.94	35.19	40.54
19-056	60	62	9	11	10	8	19	19	11.47	7.72	38.29	48.17
20-058	51	53	12	15	13	12	25	27	13.42	13.80	56.65	44.38
21-063	54	55	7	8	5	7	12	15	12.12	11.95	50.07	48.75

III. COVAT

Part. #	100ms cued		100ms uncd		800ms cued		800ms uncd		Facilitation		IOR	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Active Group												
1-015	407	372	372	314	395	368	309	277	35	58	86	91
2-019	595	530	643	536	484	501	462	521	-48	-6	22	-20
3-029	445	427	466	427	417	390	364	334	-21	0	53	56
4-033	393	384	412	368	347	387	390	325	-19	16	-43	62
5-034	366	366	405	355	404	361	368	351	-39	11	36	10
6-035	329	313	345	326	390	393	349	346	-16	-13	41	47
7-036	367	346	380	399	367	347	364	363	-13	-53	3	-16
8-037	352	284	352	341	351	320	283	282	0	-57	68	38
9-043	370	420	405	396	418	447	340	348	-35	24	78	99
10-046	408	392	445	419	391	385	375	340	-37	-27	16	45
11-062	405	334	355	340	354	296	313	272	50	-6	41	24
12-068	368	372	401	389	390	341	372	302	-33	-17	18	39
Placebo Group												
13-045	440	350	476	376	472	397	395	369	-36	-26	77	28
14-001	461	383	451	380	518	444	438	355	10	3	80	89
15-010	363	370	361	351	386	415	307	427	2	19	79	88
16-027	382	324	365	325	371	278	301	292	17	-1	70	-14
17-049	342	334	401	374	355	352	330	330	-59	-40	25	22
18-051	448	439	471	480	411	518	424	438	-23	-41	-13	80
19-056	397	399	393	395	409	387	345	342	4	4	64	45
20-058	536	522	501	526	518	526	441	503	35	-4	77	23
21-063	486	332	475	348	424	370	372	292	11	-16	52	78

IV. 3-Day Dietary Analysis

Part. #	Iron (mg)		Vitamin C (mg)		Calcium (mg)		Dietary Fibre (g)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Active Group								
1-015	49	33	607	397	4232	4550	38	62
2-019	39	25	286	444	2120	4844	38	38
3-029	17	10	297	18	1931	373	22	13
4-033	35	44	374	611	1718	1899	35	34
5-034	54	70	326	508	4784	4050	34	69
6-035	38	23	666	447	2060	1827	55	54
7-036	62	40	258	159	2986	2533	52	37
8-037	68	56	57	457	2447	3463	57	45
9-043	31	23	134	257	1416	1692	26	25
10-046	36	34	96	94	2199	1009	37	34
11-062	60	70	350	180	1422	2095	39	48
12-068	54	74	138	25	3035	1172	33	38
Placebo Group								
13-045	19	35	259	633	574	1140	7	12
14-001	48	30	630	176	2552	3128	35	34
15-010	27	81	475	176	2775	3840	47	50
16-027	35	39	304	423	1592	2020	44	27
17-049	44	n/a	1122	n/a	2381	n/a	59	n/a
18-051	48	24	590	105	2423	2393	32	34
19-056	33	51	204	75	2502	2449	25	46
20-058	39	32	173	267	1614	5046	27	35
21-063	55	42	1397	402	3929	2396	43	29

APPENDIX B
Cognitive Tests: Description and Procedure

A. Cognitive Tests

I. Motor-free Visual Perception Test – Third Edition (MVPT-III)

The MVPT-III is a test designed to be a quick and reliable source that evaluates the overall visual processing abilities of participants ranging in ages from 4 years to 95 years and older. There is no motor involvement necessary in this test that may influence test results, specifically in those individuals with motor deficits who may not have a visual perceptual problem. Current theories of perception have recognized that visual perception both influences and is influenced by cognition (Colarusso and Hammill, 2003). Therefore, perception is not an independent process but rather one that includes many interrelated processes such as recognition, interpretation, and comprehension that are used simultaneously. Although there have been many subareas of visual perception processes that have been identified, the visual perception tasks used in this test include visuospatial relationships, visual discrimination, visual closure, figure ground, and memory which are considered foremost in all theoretical constructs of visual perception. The manual gives a brief description of each of these areas:

- ***Visuospatial Relationships*** *– involved with this skill are the abilities to orient one's body in space and to perceive the positions of objects in relation to oneself and to other objects. An example of a spatial relationship task would be the perception of pictures, figures, or patterns that are disoriented in relation to each other (for example, figure reversals or figural rotations).*

- ***Visual Discrimination*** *- this type of visual perception involves the ability to discriminate dominant features of different objects, for example, the ability to discriminate position, shapes, forms, colors and letter-like forms.*

- ***Figure Ground*** *– this is a form of visual discrimination that involves the ability to distinguish an object from background (or surrounding) objects.*
- ***Visual Closure*** *– this is also a form of visual discrimination and involves the ability to perceive a whole figure when only fragments are presented.*
- ***Visual Memory*** *– this requires the ability to recognize one stimulus item after a very brief interval.*

The test is administered much like a multiple-choice test with the 4 answer choices arranged horizontally across the page/plate. The test plates are bound in a stand up easel cover that is placed in between the examiner and examinee. Each test item is made up of black and white line drawings for both the stimulus test plates and the answer choices. Most of the stimulus items are on the same page with the answer except for the test items that involve a memory portion. There are seven sections and each section has either one or two practice plates. Colarusso and Hammill (2003) caution that the seven sections are not to be interpreted singly as there are not enough items in each section to determine performance. They also acknowledge that real world perceptual tasks are comprised of numerous processes cooperatively.

A single score that will represent an overall adequacy of the participants' visual perceptual ability will evaluate performance in the above areas. The raw score is then converted to a standard score for interpretation. Percentile ranks are used only to give an indication where in the population each participant falls. The table below is taken directly from the manual (Colarusso and Hammill, 2003) and used to give a verbal description of what their scores suggest.

Table 1: Description of MVPT-III Scores

Verbal Description	Raw Scores	Range of Standard Scores	Range of Percentile Ranks	Percentage in Population
Very Superior	61 - 65	130 and above	98 and above	2
Superior	59 - 60	120 - 129	91 - 97	7
High Average	57 - 58	110 - 119	75 - 90	16
Average	52 - 56	90 - 109	25 - 74	50
Low Average	47 - 51	80 - 89	9 - 24	16
Low	42 - 46	70 - 79	3 - 8	7
Very Low	42 and below	69 and below	2 and below	2

II. Digit Span

This Digit Span is a subtest of the Wechsler Adult Intelligence Scale, third edition and the Wechsler Memory Scales, third edition and is used to examine short-term/working memory abilities. Short-term memory was later renamed 'working memory' as it was found to have a more active degree of processing ability. The original view was that the information in short-term memory was either encoded into long-term memory or it was forgotten. It now encompasses the view that this is a place where information is stored temporarily and also a place where the information can be manipulated or calculated (WAIS – III, 1997). It is divided into two separate portions, the Digits Forward and the Digits Backward. The Digits Forward involves remembering and recalling a series of numbers in order, a simple span task that involves increasing the capacity of the storage compartment. The Digits Backward part also involves recalling a series of numbers, but this time in reverse order. The backward portion not only involves increasing the capacity of the storage compartment but also involves reordering the sequence of items, a complex span task that requires an additional burden on the mental

processing ability of the brain. The series of numbers increases with the addition of another number after a correct recall of the previous series.

The WAIS – III Manual identifies that there are generally two attentional mechanisms. One is auditory and the other visual. Even though research has suggested that there are differences in performance between the two mechanisms, the normative data in the manual does not discriminate between the two. The raw score is the total number of sequences recalled correctly in both the forward and backward sections. The raw score is then converted to age corrected scaled scores with a mean of 10 and standard deviation of 3. A score of ten is considered average. A score of 7 or 13 would then correspond with 1 SD below or above the mean. The WAIS – III technical manual (1997) notes previous research has found that in normally functioning adults, the ability to recall a verbal or visual stimulus in the order it was presented in, has been considered a measure of concentration, and the average performance is 7 digits recalled plus or minus 2. The average performance in the Digits Backward is typically one less (6 +/- 2) due to the increased demand of executive functioning.

III. Covert Orienting of Visual Attention Task

Posner and Cohen (1984) examined the covert orienting of visual attention. This involves aligning attention with a source of sensory signals that occurs without eye movements (Fossella, Posner, Fan, Swanson, & Pfaff, 2002). It was noticed that a biphasic pattern of reaction times occurs when using a peripheral exogenous cuing paradigm for the examination. Posner and Cohen also noticed that these reaction times were dependent on the time interval between the onset of the cue and presentation of the

target, termed stimulus onset asynchrony (SOA). What they found was that reaction times were faster when targets were in a cued location if the SOA's were short, under 200 ms. They suggested that this effect was the result of the presentation of the cue facilitating the movement of attention to the cued location. In longer SOA's, typically over 300 ms, they found that reaction times were slower in responding to targets in cued locations. This effect suggested that attention was inhibited from returning to previously cued locations and was a result of attention being biased to novel locations which became known as 'inhibition of return' or IOR.

The basic paradigm that Posner and Cohen (1984) initially devised was set out as 3 empty placeholder boxes situated horizontally on the computer screen. The subject was told to fixate their eyes on the center box. Then one of the peripheral boxes would be illuminated, representing the cue, and a target would follow after the short or longer time period in either of the peripheral boxes. The cue was thought to capture attention to that area and if the target appeared on that same side shortly after the cue then reaction time was quick to respond. If the length of time between the cue and target was delayed, it was thought that attention started to shift away from the cued area and momentum towards the uncued side would make reaction time to a target that appeared on that side faster.

The view held by Posner and Cohen (1984) is that facilitation is meant to improve the efficiency of target or stimulus detection and if no eye movements occur, then processing that area would be more efficient than other areas in the visual field. Their view on the inhibitory effect is that it encourages sampling of the environment by favouring new locations in the visual field and in doing so, releases attention from a single position so that concentration in that one area is not prolonged. They therefore

advocate that the orienting system is a crucial process in the awareness of visual signals as both facilitation and inhibition represent functional adaptations to changes in the visual world.

IV. Trail Making Test (Parts A & B)

This test is a subtest of the Halstead-Reitan Test Battery and was originally a part of the Army Individual Test Battery. The test is one of the most frequently used cognitive tests designed to detect cognitive impairment in many different neuropsychological conditions (Mitrushina, Boone, Razani, D'Elia, 2005). It is a measure of visual attention, simple motor and spatial skills, sequencing and executive function (Martin, Hoffman, Donders, 2003). The Trail Making Test immediately requires recognizing the symbolic significance of numbers and letters, an ability to scan the page continuously to identify the next number or letter in sequence, a flexibility in integrating the numerical and alphabetical series, and completion of these requirements under the pressure of time (Reitan, 1992). Time differences between Part A and Part B of the test have been attributed to the nature of each test. Part A has the participant connecting numbers in order whereas Part B has the participant connecting numbers and letters alternately. This would result in an additional demand of executive function and therefore take a longer time to complete than Part A. Mitrushina et al. (2005), also attributes the longer time to a more complex layout and increased attentional factor. The ability to deal with the numerical and language symbols is accomplished by the left cerebral hemisphere, the visual scanning task necessary to perceive the spatial distribution of the material is represented by the right cerebral hemisphere, and the speed and efficiency of

performance may be associated with an overall proficiency of brain function. The manual therefore suggests that the Trail Making Test is one of the best measures of general brain function (Reitan, 1992).

Reitan and Wolfson (1993), determined score ranges that would correspond to the adequacy of brain function rather than using percentile ranks. They devised 4 categories that would relate to the degree of impairment and is presented in Table 3 below.

Table 2: Categories of Results for the Trail Making Test

	0	1	2	3
TMT Part A	0 – 26 sec	27 – 39 sec	40 – 41 sec	52 + sec
TMT Part B	0 – 65 sec	66 – 85 sec	86 – 120 sec	121 + sec

They used the numbered categories to represent the following score ratings: 0 = excellent scores; 1 = normal but not perfect scores; 2 = mild to moderate impairment; and 3 = significant and severe impairment.

B. Participant Test Instructions

I. *Motor-Free Visual Perception Test - 3rd Edition* (MVPT-III)

- The object of this test is to choose the correct answer out of 4 possible answers by either saying the correct letter or pointing to it. The answers will be either A, B, C, or D.
- There are 7 sections with a total of 52 questions. There will be examples you can try at the beginning of each section.
- Please respond to each question. If you don't know the answer, make your best possible guess.
- You will not be timed. For the memory section, you will be given 5 seconds to look at the picture. You can respond whenever you are ready.

II. *Digit Span*

Forward

- Sit comfortably. The object of this test is to correctly repeat a series of digits/numbers that will be presented to you.
- A series of two numbers will appear on the screen at one-second intervals, after which the word 'recall' will appear. You have 10 seconds in which to verbally repeat them in the exact same order.
- Three digit series will be next (at one-second intervals). The word 'recall'. Repeat them in the exact order.
- Four digits will appear, 5 digits, and so on.

- You will continue to increase the digits until you make a mistake. You have two chances at each level to correctly recall the digit series. If you recall the series correctly, you move up to the next level. The test is over when you cannot repeat the series in any of the two trials.

Backward

- The object is to correctly repeat a series of digits/numbers that will be presented to you only this time you have to recall the series backwards (2-6-4 will be recalled as 4-6-2).
- You will first be presented with a series of two numbers at one-second intervals.
- You will continue to increase the digits until you make a mistake. Once a mistake is made you will have a second chance at that level. If you recall the series correctly, you move up to the next level. The test is over when you cannot repeat the series in one of the two trials backwards.

III. *Covet Orienting of Visual Attention Task*

- Sit comfortably. Your chin should not go any further forward than the edge of the table.
- The object is to test your reaction time to a target that will appear on the screen.
- Place your most preferred hand on the spacebar and focus on the dot in the center of the screen. Do not move your eyes away from this dot.

- You will see an outline of a square that will flash – this is called a cue. Do not respond to the cue. A target that looks like a filled square will follow the cue. Press the spacebar as fast as you can when you see the target (the filled square).
- Sometimes there will be a cue without a target. These are called catch trials. If you do not see the target do not respond.
- Remember – Do not move your eyes away from the dot in the center of the screen.

IV. *Trail Making Test*

Part A

- Make sure you are sitting comfortably. You will be given a sample test to try it first.
- The object of this test is to connect the numbers in order from 1 – 15 as fast as you can by drawing a line through each number with the pencil provided (1-2-3-4-5-…15).
- Start at number 1, where it says 'begin' and finish at number 15, where it says 'end'.
- Do not pick the pencil up from the sheet between numbers once you start. If you make a mistake, go back to the last number you got in the correct order and continue from there.
- When you are finished drop your pencil.
- You can start when the examiner says "Are you ready… OK…Go!"

Part B

- The object of this test is similar to the first one except this time you have to connect one number and then one letter in numerical and alphabetical order. There is a sample test for you to try first.

- There are 13 numbers (1 –13) and 12 letters (A- L).
- Start with the number 1, where it says 'begin' and draw a line to the letter A, then continue to the number 2, and next to the letter B, and so on until you get to the number 13, where it says 'end' (1-A-2-B-3-C…12-L-13).
- If you make a mistake, go back to the correct letter or number you had in order and continue from there.
- Drop your pencil when you are finished.

C. Circuit Timing Sequence

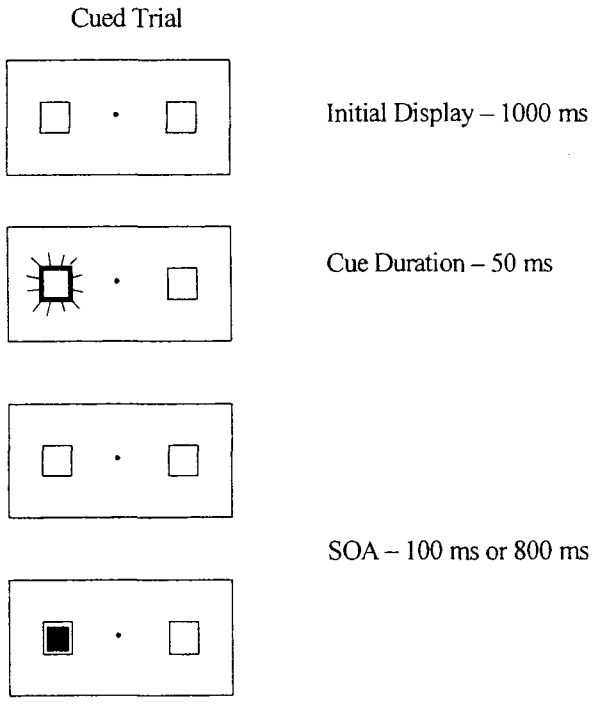
	1	2	3	4	5	6	7	8	9	10
MVPT1 10"	10:00	10:10	10:20	10:30	10:40	10:50	11:00	11:10	11:20	11:30
MVPT2 10"	10:10	10:20	10:30	10:40	10:50	11:00	11:10	11:20	11:30	11:40
COVAT 10"	10:20	10:30	10:40	10:50	11:00	11:10	11:20	11:30	11:40	11:50
TMT 10"	10:25	10:35	10:45	10:55	11:05	11:15	11:25	11:35	11:45	11:55
DIGIT SPAN 10"	10:30	10:40	10:50	11:00	11:10	11:20	11:30	11:40	11:50	12:00
TIME OUT 10"	10:40	10:50	11:00	11:10	11:20	11:30	11:40	11:50	12:00	12:10
MVPT1 15"	10:00	10:15	10:30	10:45	11:00	11:15	11:30	11:45	12:00	12:15
MVPT2 15"	10:15	10:30	10:45	11:00	11:15	11:30	11:45	12:00	12:15	12:30
COVAT 15"	10:25	10:40	11:55	11:10	11:25	11:40	11:55	12:10	12:25	12:40
TMT 15"	10:30	10:45	12:00	11:15	11:30	11:45	12:00	12:15	12:30	12:45
DIGIT SPAN 15"	10:35	10:50	12:05	11:20	11:35	11:50	12:05	12:20	12:35	12:50
TIME OUT 15"	10:45	11:00	12:15	11:30	11:45	12:00	12:15	12:30	12:45	1:00

With the circuit taking a total time of 40 minutes per person, 10 students will be finished in 2 hrs and 10 minutes. 20 students would therefore be through in 4 hrs and 20 minutes. If we started by 10:00, 20 students would be finished by 2:20. This is calculated based on a continual flow of students every ten minutes.

D. Sample Time Cards For Test Times

YOUR TIME IS 10:00	YOUR TIME IS 10:10
YOUR TIME IS 10:20	YOUR TIME IS 10:30

E. Trial Sequence for the COVAT



APPENDIX C
Research Ethics Board Material

A. Consent Form

"The Effects of Iron Supplementation on Visual Attention in Iron Deficient Adolescent Females"

In signing this consent form, I agree to participate in this study that will determine the effects, if any, of what iron supplementation has on certain tasks of visual attention.

I have been clearly and concisely informed of the exact nature of the study and explained and understand all benefits and potential risks that may be associated with participation in this study.

I acknowledge that the information that I have provided on the health questionnaire is true to the best of my knowledge.

I understand that any information collected about me will be kept strictly confidential and if results are published, I will not be identified in any way unless agreed upon. I also understand the results will be made available to me after completion of the study upon request.

I understand that because I am under the age of 18 and therefore not of legal consent age, the signature of a parent/legal guardian is required in order to participate in this study.

As a volunteer, I understand that I have the right to withdraw from the study at any time even after signing this form.

Signature of Participant *Date*

Signature of Parent/Legal Guardian *Date*

I have explained the nature of the study and believe that the participant has understood it well enough to provide an informed choice to participate.

Signature of Researcher *Date*

B. Cover Letter

Dear Potential Participant and Legal Guardian:

Thank you for volunteering to participate in a study on iron deficiency, supplementation, and their effects on visual attention and memory.

Tracey Larocque B.P.E., R.M.T., Dr. Ian Newhouse, Ph.D., Dean of Professional Schools, and principal investigator, in collaboration with Dr. Jim McAuliffe, Ph.D., Dr. Michel Bedard, Ph.D., and Donna Newhouse, Ph.D.(c) of Lakehead University along with Dr. Chris Lai, M.D. are conducting a study entitled "The Effects of Iron Supplementation on Visual Attention and Memory in Iron Deficient Adolescent Females".

The purpose of this study is to address the question of what effects that supplementing an iron deficient, 14-16 year old teenage girl, will have on certain tasks of visual attention. Iron deficiency is a blood condition indicating low levels of iron. This condition has been shown to have adverse effects on tasks that require attention. Through your participation, you will not only help to answer this question but also help to indicate the prevalence of iron deficiency in the female adolescent population in Thunder Bay. It will also be a great chance to get an idea of your health status.

As a participant, you will be required to:

- Fill out a brief health questionnaire.
- Complete a three-day dietary recall (write down what you've consumed in 3 days) before and after the supplementation program to get a general indication of nutritional intake.
- Provide a 6 ml blood sample to measure serum ferritin & hemoglobin levels to determine iron status, and albumin, folate, and vitamin B12 to indicate overall health status and act as controls, before and after supplementation program.
- Complete 4 tasks of attention and memory. These simple cognitive tests will be done on a computer or on paper. Total time is approximately 30-45 minutes, completed before and after supplementation program.

Each participant will then be randomly given either iron supplements (in pill form) or a placebo (sugar pill). The pill bottles will only be marked with an identification number. Knowledge of the contents of the bottles will go unknown by both the researchers and the participants until completion of the study. Those participants who did not receive supplements for purposes of the study, will be given the option to receive the same amount, if they so wish, at the conclusion of the study.

Skilled and trained professionals who will be monitoring the study will conduct all testing procedures and administration of the supplements. Any discomfort from the blood test is minimal but slight bruising at puncture site may occur. Any discomfort from the supplements is also minimal if taken as directed. The dosage is low enough that this should not be a problem, otherwise there is very little risk involved with participation in this study. Results of the tests during the study will be strictly confidential and be locked in the investigator's office. After completion, the data will be securely stored at Lakehead University for seven years. The results will be made available to the participants after completion of the study upon request.
As a volunteer, you or your legal guardian, have the right to withdraw from this study at any time. If you have any questions or concerns with the study you can reach Dr. Ian Newhouse at 343-8074 or you can call me, Tracey Larocque (Graduate Student) at 473-8446.

C. Health Information/Physical Activity Participation Survey

Name:_____ **Grade:**_____ **Age:**_____

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

	Yes	No
Are you currently pregnant?	____	____
Are you presently taking any contraceptives (Birth control medications)?	____	____

At what age did you begin to menstruate (have periods)? _____

When was the start date of your last period? _____

If you are on any medications can you please list them.

_____

If you are physically active can you describe the type of exercise you perform? i.e. running.

_____

Have you ever been diagnosed as anemic? ____ ____

Do you have any other known health condition? _____

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 heartbeat?

- o At least three times
- o Normally once or twice
- o Rarely or never

Intensity:

- o 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

D. Handout for Participants and Parents

Lakehead University Research Studies

"The effects of iron supplementation on visual attention and memory in iron deficient adolescent females"

What is iron?

Iron is one of the most abundant metals on Earth and is essential to normal human physiology. Most of the iron in the body is found in hemoglobin, a protein in red blood cells responsible for the transportation of oxygen to the tissues. It is also found in myoglobin, a protein that transports oxygen to the muscles. Other proteins allow for iron storage and transport throughout the blood. Iron is also involved in many enzymes that assist in biochemical reactions and is involved in the production of neurotransmitters and the myelin sheath.

There are two forms of dietary iron. Heme iron is found in foods that originally contained hemoglobin such as red meats, fish, and poultry. Nonheme iron is found in plant foods such as beans and lentils and is the type of iron added to iron-enriched and iron-fortified foods and in iron supplements. The bioavailability of these two forms is very different. Heme iron is more absorbable than nonheme iron – 15%-35% as compared to 2%-10%.

What is iron deficiency?

The World Health Organization considers iron deficiency to be the number one nutritional disorder in the world with as much as 80% of the people worldwide being affected. In the U.S., iron deficiency has been estimated at approximately 20% for women and approximately 14% of adolescent females, though this number can be as high as 40%.

Iron deficiency develops gradually and usually begins with a negative iron balance. Iron balance is the difference between iron intake and retention and the body's daily requirements. Iron deficiency is commonly categorized in 3 stages. In the first stage, there is a depletion of iron stores and an increase in intestinal absorption; the second stage is where the stores decrease enough to affect the supply of iron to the bone marrow for erythropoesis; the third stage is the more advanced stage in which there is a drop in hemoglobin levels indicating anemia.

Normal levels for hemoglobin are 120-160 g/l and normal serum ferritin levels are 20-160 ug/l.

What Causes Iron Deficiency?

The cause of iron deficiency is generally regarded as being due to a low dietary intake of iron. Other causes may be excessive blood loss or poor absorption of iron. Excessive blood loss is considered that from trauma, gastrointestinal diseases (such as cancer), and may include those females with menorrhagia and dysmenhorrhea (heavy, painful periods). Poor absorption of iron would include congenital disorders that impede absorption rate. The most likely cause of ID in the adolescent age group is related to dietary intake, the bioavailability of that iron, and the amount of iron losses.

Who is at risk?

Those most commonly at risk for being iron deficient are: women of childbearing age, pregnant women, preterm infants, toddlers, children and teenage girls. Teenage girls are at an increased risk due to the pubertal growth spurt that would increase the requirement of iron, continual menstrual blood losses that decrease iron levels, and social pressures that increase the prevalence of eating disorders and dietary fads that lead to a low dietary intake of iron.

What are the signs?

In the circulatory system, common signs and symptoms have been fatigue, irritability, dizziness, decreased immune function and poor work capacity. In the nervous system, noted signs and symptoms have been a lack of concentration, lack of focus, learning disabilities, and disturbances in attention, perception, and memory.

Iron Supplements

Supplements are used in order to improve the iron status of those individuals who have been identified as iron deficient. The quantity and type will vary. There are two types of iron supplements: ferrous compounds and ferric compounds. Ferrous compounds are the best absorbed with fewer side effects and include ferrous gluconate, ferrous sulfate, and ferrous fumarate. We will be using ferrous gluconate equaling the amount of 100 mg of elemental iron. This dosage is low enough that side effects are minimal but still high enough to be effective in raising the iron status of your daughter.

Previous studies with relatively the same quantity and for the same duration have shown to improve ferritin levels and positively affect scores on tasks that measure attention and memory. The supplements are to be taken twice daily with meals and a glass of orange juice (vitamin C helps to absorb the iron).

About this Study

The purpose of this study is to examine the effects of iron supplements on attention and memory tasks in adolescent females who were identified as being iron deficient in accordance to the parameters that we have set out for this study.

This study is a double-blind study in which half of the participants will be given the supplement and half will be given placebos. This is the only true way we can tell if it is the supplements or some other intervening factor that would be beyond the control of the researchers. The division is unknown to the researchers until the conclusion of the study. Those who receive the placebos will be given supplements at the end of the study if they so wish.

Your Role

3 day dietary analysis – pre and post supplementation
Maintain current eating habits and exercise level
Take supplements as instructed
Pill count at half duration – so please maintain compliance
Retesting at conclusion of supplements (end of Aug. to beginning of Sept.)