

LAKEHEAD UNIVERSITY

THE EFFECT OF DISCONTINUING IRON SUPPLEMENTATION  
ON THE IRON STATUS OF WOMEN.

BY

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## ABSTRACT

Twenty four women were studied in order to examine the effect that discontinued iron supplementation has on the hematological values of serum ferritin, serum iron and hemoglobin.

Initially, 111 female volunteers were screened for iron deficiency by blood analysis (serum ferritin below 20  $\mu\text{g/L}$  and/or hemoglobin below 120 g/L). On the basis of ferritin and hemoglobin measurements, 44 women (39.6%) qualified as iron deficient. Twenty four of the selected women completed the 24 week study. Mean age was  $27.6 \pm 7.5$  years (range 18 to 40 years). Each of the subjects received the treatment of oral iron supplements (320 mg ferrous sulfate = 100 mg elemental iron taken as SLOW-Fe twice a day) for 12 weeks. Supplementation was then discontinued for an additional 12 weeks. Measurements of serum ferritin, serum iron and hemoglobin were taken at 0, 6, 12, 18 and 24 weeks of the study.

Statistical analysis using analysis of variance (ANOVA) indicated significant changes ( $p < .05$ ) in serum ferritin and serum iron values. No significant ( $p < .05$ ) change was found in hemoglobin values over the course of the study. Student-Newman-Keuls post hoc test was used to identify specific mean differences when a significant F ratio was observed. Serum ferritin values were significantly ( $p < .05$ ) increased following 6 ( $11.5 \pm 5.3$  to  $26.4 \pm 14.3 \mu\text{g/L}$ ) and 12 ( $11.5 \pm 5.3$  to  $30.6 \pm 14.9 \mu\text{g/L}$ ) weeks of oral iron supplementation. Twelve weeks of discontinued

supplementation did not significantly ( $p < .05$ ) alter serum ferritin values as noted by mean values of  $29.0 \pm 15.0 \mu\text{g/L}$  and  $28.2 \pm 17.1 \mu\text{g/L}$  for weeks 18 and 24, respectively. Serum iron values were significantly ( $p < .05$ ) increased ( $15.9 \pm 6.7$  to  $25.4 \pm 15.6 \mu\text{mol/L}$ ) after 6 weeks of oral iron supplementation. Serum iron values at 12, 18 and 24 weeks of the study were not significantly ( $p < .05$ ) different from the values at week 0 and week 6.

It can be concluded that oral iron supplementation (320 mg ferrous sulfate = 100 mg elemental iron taken as Slow Fe twice a day for 12 weeks) was successful in raising serum ferritin values (from  $11.5 \pm 5.3$  to  $30.6 \pm 14.9 \mu\text{g/L}$ ) in iron deficient women. Within the limitations of this study, 12 weeks of discontinued oral iron supplementation does not pose a threat to the iron status of iron repleted women.

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**CHAPTER 1**  
**INTRODUCTION**

**Introduction**

Iron deficiency affects approximately 10 to 30% of the world population (Beck, 1985). In Western countries iron deficiency is the most common nutritional deficiency (Beck, 1985). It is more commonly seen in women than men, especially women of child-bearing age. Women tend to be at an increased risk of developing iron deficiency due to the loss of iron through menstruation and pregnancy (deGruchy, 1970). Women also tend to have low daily iron intakes which may add to the problem. In a 1973 survey by Nutrition Canada, 76% of Canadian women had iron intakes below the recommended levels for the general population (Health & Welfare Canada, 1975).

To improve the iron status of iron deficient women a variety of supplementation programmes have been developed (Haymes, 1980). Whether it be through oral or parenteral administration many women use some type of a supplementation programme on a regular basis. Within the United States it has been estimated that 37% of the general adult population use nutrient supplements, and approximately 58% of these individuals use a multivitamin containing iron and/or other minerals (The Gallop Organization, 1982).

The effect of oral iron supplementation on the iron status (i.e., serum ferritin, serum iron and hemoglobin) of women has been well documented. Many studies have shown an improvement in the iron status of women taking iron supplements (Plowman & McSwegin, 1981; Schoene, Escourrov, Robertson, Nilson, Parsons, & Smith, 1983; Charoenlarp et al., 1988; Newhouse, Clement, Taunton, & McKenzie, 1989) and today iron supplementation is well recognized as a therapeutic remedy for iron deficiency. However, such studies have not addressed the possibility that the iron stores may have diminished shortly after the discontinuation of the supplementation programme. Within the clinical setting iron supplementation, in many cases, is continued only until iron stores have been replenished. But what happens to the iron stores once the supplementation programme has been discontinued?

A study by Hercberg, Galan, Soustre, Dop, Devanlay, and Dupin (1985) examined the iron status of 54 women after 30 days of iron supplementation (105 mg/day of elemental iron) and 30 days of subsequent discontinuation. Mean ferritin levels were significantly increased with supplementation, while subsequent discontinuation significantly ( $p < .05$ ) decreased ferritin levels from  $46.4 \pm 30.3$  to  $40.6 \pm 23.4$   $\mu\text{g/L}$ . Although Hercberg et al.'s (1985) study did not focus specifically on the effect of discontinued iron supplementation it does suggest that some women may be at a risk of developing iron deficiency soon after a successful supplementation programme.

### **Purpose of Study**

This study was designed for the primary purpose of examining the effects of discontinuing iron supplementation on the hematological values of serum ferritin, serum iron and hemoglobin in a group of iron replete women who were previously iron deficient. The study was also designed to: (a) establish the prevalency of iron deficiency in a group of women and, (b) to examine the effects of iron supplementation on the hematological values of serum ferritin, serum iron and hemoglobin in a group of iron deficient women.

### **Research Hypothesis**

Testing of the following null hypotheses was proposed to meet the purpose of the study.

1. There are no differences in pre-discontinued and post-discontinued values of serum ferritin, serum iron and hemoglobin following the discontinuation of iron supplements.
2. There are no differences in pre-supplement and post-supplement values of serum ferritin, serum iron and hemoglobin following iron supplementation.

### Significance of Study

Possible outcomes of the study included: (a) iron status of the group of women remained elevated for the entire 12 weeks following the discontinuation of iron supplements or, (b) the iron status of the group of women returned to an iron deficient state within the 12 weeks following the discontinuation of iron supplements.

The first outcome would suggest that the need for supplementing iron deficient patients for prolonged periods of time is unnecessary. This would support the current clinical practice of prescribing oral iron supplements for 2 to 3 months. Taking this a step further, some patients may find that by taking oral iron supplements for shorter periods of time or on a less frequent basis improves their iron status to the same degree as with a daily extended supplementation programme. A recent study by Wright and Southon (1990) suggests that less frequent iron supplementation, such as every 2nd or 3rd day, may be equally as beneficial to iron deficient subjects as routine daily supplementation. Such a regimen may minimize unpleasant side-effects of oral iron therapy, decrease the risk of adverse effects of iron on the absorption of other essential minerals and substantially cut the cost of supplementation programmes.

The second outcome may imply that iron supplementation is somewhat futile unless administered on an on-going basis or for

longer periods of time. Either way, this is a project that will have an impact on the well being of a significant number of females in the population.

### **Limitations**

- 1) Training, diet, menstrual blood loss and other physical factors which may have affected the testing were beyond the control of the investigator.
- 2) Subjects rate of iron depletion were slightly accelerated by the withdrawal of blood samples.
- 3) Exclusion criteria employed cutoff points that arbitrarily established levels of normality or deficiency. This is particularly important considering the normal variability of each iron variable.
- 4) The subjects willingness to follow instructions concerning personal nutritional habits and activities.
- 5) An alpha level of .05 was established as the level of significance for statistical tests.

### **Delimitations**

- 1) This study was delimited to females, age 18 to 40, with iron deficiency who resided in the vicinity of Thunder Bay, Ontario.
- 2) The only blood parameters that were measured included serum ferritin, serum iron and hemoglobin.
- 3) Due to ethical concerns, compounding blood loss effects and

money constraints, blood analysis occurred only at 6 week intervals during the 24 week study period. Significant changes in iron status that could possibly occur over a shorter time span were not investigated.

## Definitions

### Iron Status:

**Serum Ferritin:** a sensitive index of the earliest stage of iron deficiency-depletion of body iron reserves (Valberg, 1980). It is estimated that one ug/L of serum ferritin is equivalent to 8 mg of storage iron (Walters, Miller & Worwood, 1973). Levels below 20 ug/L are associated with absent bone marrow iron (Pakarinen, 1980). Levels up to 64 ug/L may still represent an iron deficient state (Heinrich, Bruggemann, Gabbe, & Glaser, 1977).

**Serum Iron:** (also called plasma iron and total iron); useful in the clinical evaluation of iron deficient erythropoiesis. Exhaustion of the iron stores affects the supply of iron to the plasma pool and hence to the erythroid bone marrow (Bothwell, Charlton, Cook & Finch, 1979).

**Hemoglobin:** an iron containing conjugated protein found in red blood cells and capable of transporting oxygen and carbon dioxide to and from the working tissues.



**Iron Deficiency:**

A state which develops if the loss of iron is greater than the amount obtained (Pakarinen, 1980). Iron deficiency is commonly divided into three stages: (a) prelatent, (b) latent, and (c) manifest (Clement & Sawchuk, 1984).

- a) Prelatent iron deficiency:** the earliest stage of iron deficiency characterized only by a decrease in storage iron (and decreased serum ferritin) and increased intestinal absorption (Strauzneberg, Kassner, Bohm & Schneider, 1981).
- b) Latent iron deficiency:** in addition to the indices of prelatent iron deficiency, the level of total serum iron is decreased (Strauzenberg et al., 1981). The criterion for prelatent/latent iron deficiency in this study is serum ferritin levels below 20  $\mu\text{g/L}$ . Levels below this are associated with absent bone marrow iron (Pakarinen, 1983; cited in Clement & Sawchuk, 1984).
- c) Iron deficiency anemia:** refers to an advanced stage of iron deficiency characterized by a drop in hemoglobin levels (Clement & Sawchuk, 1984). In this study the criterion will be levels below 120 g/L (Williamson, 1981).

## CHAPTER 2

### REVIEW OF LITERATURE

#### Introduction

Due to the lack of literature pertaining to the discontinuation of iron supplementation, the general theory of the metabolism, requirements, status, and supplementation of iron will be discussed.

#### Iron metabolism

Iron, a metallic element, is found in two forms; ferrous, a divalent form ( $\text{Fe}^{++}$ ) of iron, and ferric, a trivalent form ( $\text{Fe}^{+++}$ ) of iron. During intestinal absorption iron must be in an ionic state and almost exclusively in the ferrous form (deGruchy, 1970). Most foods, however, contain iron in its ferric form bound to protein (Sonnenwirth & Jarett, 1980). Therefore prior to absorption iron must be split from the protein and reduced to its ferrous form within the stomach (Sonnenwirth & Jarett, 1980). Within the stomach acids such as hydrochloric and organic acids, release ferric iron from protein (deGruchy, 1970). The released free ferric ions are then reduced to the ferrous form at an acid pH by reducing agents in the food such as ascorbic acid (deGruchy, 1970). These free ferrous ions are mainly absorbed in the duodenum and upper small intestine, though some absorption occurs in the stomach, ileum, and colon (Winick, 1981). The absorptive mechanism which is regulated by the intestinal mucosal

cells, occurs in two phases. First, evidence suggests that a protein called mucosal transferrin binds with iron in the lumen of the gut and transports it across the border of the intestinal mucosa (Finch & Hueber, 1982). Second, iron within the intestinal mucosal cells may be bound either to apotransferrin and absorbed into the bloodstream as transferrin, or to apoferritin and deposited as ferritin within the mucosal cell itself (Winick, 1981).

### Iron absorption

According to Winick (1981) the two most important internal factors regulating the amount of iron absorbed are the size of internal iron stores and the rate of erythropoiesis. Experiments have shown that a decrease of iron stores increases absorption while an increase in iron stores decreases absorption (deGruchy, 1970). For example, in humans with iron deficiency the amount of food iron absorbed is increased from the usual 5 to 10 per cent to between 10 and 20 per cent (deGruchy, 1970). Other experiments have shown the effect of the rate of erythropoiesis on iron absorption. For example, the stimulation of erythropoiesis by the induction of hemolysis or by hemorrhage increases iron absorption while transfusion-induced polycythemia reduces the absorption of iron (deGruchy, 1970).

However, it is not understood how the bone marrow or iron stores inform the small intestine of their needs, or how the intestinal wall regulates the degree of absorption. Originally, it was thought that the degree of saturation of the iron acceptor protein, apoferritin, in the mucosal cells played the major role in controlling absorption of iron (deGruchy, 1970). This theory, known as the mucosal block theory, postulated that when all the apoferritin in the mucosal cells was converted to ferritin absorption of iron ceased (deGruchy, 1970). Although this theory has since been refuted it does appear that the mucosal cells do play a controlling role in determining the amount of iron absorbed. Iron transfer across the mucosal cell is part of an active metabolic process. In this process a proportion of the iron taken up by the mucosal cells is delivered to the transferrin of the plasma while the remainder is deposited as ferritin in the mucosal cells (deGruchy, 1970). It appears that both transferrin and ferritin may play an important role in determining the amount of iron absorbed (Finch & Hueber, 1982).

Other factors which are claimed to affect iron absorption but appear to be of much less importance include the amount of iron in diet, form of iron in diet (bioavailability), pancreatic secretions, gastroferrin, hydrochloric acid, and chelation (Beck, 1985).

### Iron balance

In healthy individuals, body iron is repeatedly reutilized; hence, daily absorption and loss of iron tends to be small (Beck, 1985). However, despite the minuteness of daily iron turnover, iron balance within the body is important. Generally, iron balance is maintained by physiological adjustment of the absorption mechanism so that the amount of iron crossing the small intestinal epithelial barrier is related to the internal iron status, type and amount of dietary iron, and the rate of erythropoiesis (Parr, Bachman, & Moss, 1984).

Typically, Western diets contain about 7 mg of iron per 1000 calories (U.S. Department of Health, Education, and Welfare, 1972). According to Hallberg (1984) only a small portion of the iron in food is absorbed, and this amount varies greatly, depending on the composition of the diet. A normal individual absorbs about 4-10% of the total iron ingested (Beck, 1985). Such percentages vary depending on the type of food ingested. For example, eggs, liver and leafy vegetables have low absorption values, whereas muscle, fish and soybeans have higher absorption values (Beck, 1985).

Dietary iron, which enters a common pool within the intestinal lumen, exists as either heme iron or non-heme (ionic) iron. Within foods each of these irons are bound to other constituents that either interfere with or promote iron

absorption. Heme iron is bound to porphyrin in hemoglobin and myoglobin and is not affected by other dietary components allowing up to 35% of the iron to be absorbed by iron-deficient subjects (Winick, 1981). For non-heme, or ionic, iron the case is much different. Ionic iron is present in foods as part of a ferric hydroxide complex. Various components of an individual's diet such as, phytates and phosphates, will combine with ionic iron to form insoluble salts making the iron less available for absorption. Therefore, certain good sources of iron, such as corn (high in phytates) and eggs (high in phosphates), do not supply the amount of usable iron one would think. For example, the absorption of iron from grains and vegetables alone would be less than 5% (Finch & Hueber, 1982). In contrast, foods containing reducing substances like meat and ascorbic acid will combine with ionic iron to favour absorption (Winick, 1981).

### Body iron

Total body iron is estimated to be between 2 and 6 gm or an average of 35 mg per kg of body weight (Parr et al., 1984). Of this total, 60% to 70% is classified as essential or functional iron, and is incorporated into hemoglobin, myoglobin, and certain respiratory enzymes (Clement & Sawchuk, 1984). The remaining 30% to 40% of the total body iron is classified as storage iron and is reserve for any iron imbalances (Clement & Sawchuk, 1984). It is estimated that, in females, total body iron is distributed as

follows: 60% to 70% (2.5 g) in hemoglobin; 10% to 12% (0.3 g) in myoglobin and enzymes; and 29% (0.3 g) as ferritin and hemosiderin, stored in the liver, spleen, and bone marrow (Winick, 1981).

Of the storage iron, 65% is in the form of ferritin and the remaining 35% as hemosiderin (Sonnenwirth & Jarett, 1980). Studies have indicated that in healthy individuals serum ferritin measurements provide an accurate estimation of body iron stores (Jacobs, Millen, Worwood, Beamish, & Wardrop, 1972; Lipschitz, Cook, & Finch, 1974; Walters et al., 1973). It is estimated that 1  $\mu\text{g/L}$  of ferritin is equivalent to 8 mg of storage iron (Walters et al., 1973). Certain conditions such as infection, inflammation, or malignancy are known to elevate serum ferritin measurements (O'Toole, Iwane, Douglas, Applegate & Hiller, 1989). Other studies have shown that serum ferritin values may be elevated following strenuous training bouts (Dickson, Wilkinson, & Noakes, 1982; Lampe, Slavin, & Apple, 1986; Taylor et al., 1987). However, according to Pakarinen (1980) the only condition in which serum ferritin concentration is decreased is iron deficiency.

Hemosiderin, which cannot be readily mobilized, is the main storage form of iron during periods of iron overload (Sonnenwirth & Jarett, 1980). Therefore, hemosiderin deposits within the

liver and bone marrow allow an estimate of the degree of iron overload (Sonnenwirth & Jarett, 1980).

### Iron excretion

Normally, 0.5 to 1 mg of iron is lost daily (Jacobs, 1985). The sources of such iron loss include the urine (0.1 mg/day), feces (shed cells and microscopic amounts of blood, 0.6 mg/day), and shed cutaneous cells and perspiration (0.1 mg/day) (Sonnenwirth & Jarett, 1980). In addition, females lose a significant yet variable loss of iron through menstrual blood loss. Estimates range from 8 mg to 38 mg per menstrual period (Jacobs, 1985; Parr et al., 1984). Therefore, menstrual blood loss can account for a daily iron loss of 0.7 to 2 mg/day (Beck, 1985). Estrogen containing oral contraceptives reduce menstrual blood loss by about one-half, while intra-uterine devices increase losses (Finch, 1980).

### Iron requirements

In a balanced situation iron requirements are largely determined by the amount lost from the body (Jacobs, 1985). A female of menstrual age requires approximately 1 mg to 2 mg of iron per day, while during pregnancy or lactation this increases to between 2 mg and 4 mg of iron per day (Beck, 1985). However, since only a fraction of the iron present in food is absorbed it is necessary to provide larger amounts of iron in the diet to be



certain that 1 mg to 4 mg will be absorbed. The Canadian recommended intake of iron is 14 mg/day (Health and Welfare Canada, 1983).

### Diagnostic methods

Measurement of body iron stores is the most reliable method of diagnosing iron deficiency (Worwood, 1977). There are only a few points at which the metabolic pathways of iron can be sampled, these include the blood, bone marrow, liver or urine. Hematological examinations are the most common diagnostic method used in the indication of iron deficiency (Pakarinen, 1980). The parameters of blood which tend to be the most useful in the diagnosis of iron deficiency are serum ferritin, serum iron, hemoglobin, % transferrin saturation, and unsaturated iron binding capacity.

Serum ferritin which is derived from intracellular non-erythroid iron storage compartments is generally recognized as a reliable indicator of body iron stores (Jacobs et al., 1972; Cook, Lipschitz, Miles, & Finch, 1974; Lipschitz et al., 1974; Siimes, Addiego, & Dallman, 1974). In most normal adults serum ferritin concentrations lie within the range 15-300  $\mu\text{g/L}$ , but concentrations are related to both age and gender (Jacobs, 1985). Generally men have higher serum ferritin concentrations than women, however in older individuals there may be very little gender difference (Jacobs, 1985). There may be some day-to-day

individual variation in serum ferritin concentrations but such variation is generally no greater than that which may arise from methodological variation (Dawkins, Cavill, Ricketts, & Worwood, 1979).

No condition except iron deficiency has been reported to produce low serum ferritin concentrations (Pakarinen, 1980; Valberg, 1980). The concentration which marks the lower limit of normal is hard to define yet many researchers use 15  $\mu\text{g/L}$  as a diagnostic indicator of iron deficiency (Jacobs et al., 1972; Lipschitz et al., 1974). At the upper end of the serum ferritin spectrum factors other than iron storage may affect serum ferritin values (Cavill, 1982). Conditions such as tissue necrosis, pancreatic, lung, or liver tumours, acute infection or inflammation, and/or certain anemias which create a shift of iron from erythroid cells to reticuloendothelial stores may account for high levels of serum ferritin (Cavill, 1982). If iron deficiency is associated with such pathological conditions serum ferritin concentrations may be greater than 15  $\mu\text{g/L}$  (Jacobs, 1985).

Measurements of serum iron are a reflection of the amount of iron in transit within the body at a particular moment in time (Sonnenwirth & Jarett, 1980). Serum iron levels are a result of such factors as hemoglobin destruction, iron absorption and the release of iron from iron stores (Sonnenwirth & Jarett, 1980).

Serum iron normally ranges from 10 to 25  $\mu\text{mol/L}$  for women (Statland, Winkel, & Bokelund, 1976). Concentrations below 10  $\mu\text{mol/L}$  are generally considered to be indicative of iron deficiency (Statland et al., 1976). However, the diagnostic basis of the serum iron concentration in predicting iron deficiency is limited. First, serum iron concentrations tend to have poor stability. Zilva and Patson (1966) have shown that serum iron is highest in the morning and decreases 30% to 50% by evening. A study by Statland et al. (1976) suggests that day-to-day variation in serum iron concentration may be considerable. In fact, some of the day-to-day variations noted in the study were such that on one day the serum iron concentration fell to a level associated with iron deficiency and on the next day rose to a level equated with iron overload. Second, a low serum iron concentration may be associated with a number of factors, only one of which is true iron deficiency. Acute infection, inflammation or minor injuries have been associated with a fall in the serum iron concentration (Cavill, 1982). Both chronic and malignant diseases, such as rheumatoid arthritis and Hodgkin's disease, are also associated with low serum iron concentrations (Cavill, 1982). Women tend to have lower serum iron concentrations than do men (Zilva & Patson, 1966) and menstruation in women may produce a fall in serum iron concentrations (Mardell & Zilva, 1967). On the other hand oral contraceptives are known to increase serum iron levels in women

(Burton, 1967). Therefore, it appears that results from serum iron measurements may be diagnostically misleading.

The largest and most readily assessed iron store is that in the hemoglobin of circulating red blood cells (Cavill, 1982). Since erythropoiesis is generally the dominant route for plasma iron turnover, red blood cell synthesis will be maintained until the supply of iron from body iron stores is exhausted. Therefore, a fall in hemoglobin concentration tends to be a reliable sign of storage iron depletion (Cavill, 1982). However, it should be noted that hemoglobin synthesis can be affected by other factors although iron supply tends to be the most common (Cavill, 1982). For example in patients suffering from any anemia which is not caused by blood loss iron is transferred from the red cell compartments to the reticuloendothelial stores resulting in a low hemoglobin concentration (Jacobs, 1985). A diurnal variation in hemoglobin concentration has also been reported by several authors (McCarthy & Van Slyke, 1939; Biggs & Allington, 1951; Stengle & Schade, 1957). As well, strenuous exercise has been shown to increase the concentration of hemoglobin due to a shift of fluid from the plasma to the interstitial fluid (Novosadova, 1972; Van Beaumont, Greenleaf & Juhos, 1972).

### Iron deficiency

Iron deficiency is a symptomatic state, which is always secondary to an underlying pathogenesis. Such underlying causes may be an increased demand of the body for iron, loss of blood by hemorrhage, and/or inadequate iron intake (deGruchy, 1970). When a negative iron balance occurs iron is mobilized from the tissue stores to supply iron for the formation of hemoglobin. The depletion of iron from tissue stores occurs in variable stages within the body. For this reason, iron deficiency exists in three stages, each of increasing severity but not necessarily independent of each other.

The depletion of iron stores in the liver, spleen, and bone marrow marks the first stage in the development of iron deficiency. This iron depletion stage, also referred to as prelatent iron deficiency, is characterized by a decrease in storage iron and increased intestinal absorption (Strauzenberg, Kassner, Bohm & Schneider, 1981). Prelatent iron deficiency can be detected by measuring serum ferritin levels which are considered to be proportional to the size of body iron stores (Jacobs et al., 1972). Serum ferritin levels below 20  $\mu\text{g/L}$  of blood are considered to be indicative of prelatent iron deficiency. Normal values of serum ferritin range from 25 to 59  $\mu\text{g/L}$  of blood for women (Parr et al., 1984).

Once iron stores are depleted, serum ferritin levels do not reflect more advanced degrees of iron depletion. The second stage of iron deficiency termed iron deficiency erythropoiesis, or latent iron deficiency, is detected by a decrease in serum iron levels (below 11  $\mu\text{mol/L}$ ), a decrease in transferrin saturation (less than 20% saturation), and/or an increase in total iron-binding capacity (greater than 400 g/L) (Parr et al., 1984).

It is only when tissue stores are exhausted that the supply of iron to the marrow for hemoglobin synthesis becomes inadequate, and iron deficiency anemia occurs (deGruchy, 1970). Therefore, hemoglobin concentrations serve as an appropriate indicator of this late stage. This stage of iron deficiency is generally termed manifest iron deficiency or iron deficiency anemia and is marked by hemoglobin concentrations below 120 g/L. Normal hemoglobin concentrations for women are between 120 and 160 g/L.

### Iron supplementation

Iron may be administered by two methods: (a) by oral ingestion, or (b) by parenteral injection, either intramuscular or intravenous (deGruchy, 1970). The majority of patients tend to respond satisfactorily to the cheaper and safer oral iron therapy. However, the more expensive parenteral iron, which may

be accompanied by severe side effects, is a very useful form of treatment in a small number of cases (deGruchy, 1970).

The success of iron supplementation may be measured in two ways: (a) Was there a significant improvement in iron status?, and (b) were there any negative side effects? (Newhouse, 1989). A potential side effect of oral iron therapy is the interference iron may play on other trace minerals, namely, copper and zinc (Newhouse, 1989). Other side effects such as nausea, constipation and abdominal pain have also been reported to affect a small percentage of the population (Newhouse, 1989).

Oral iron supplementation has been used in an attempt to reverse iron deficiency within the female population. Most of the early studies, however, failed to show significant group improvement following iron supplementation (Cooter & Mowbray, 1978; Pate, Maguire, & Van Wyk, 1979; Weswig & Winkler, 1974).

Weswig and Winkler (1974) gave one half of the Oregon State University men's varsity swimming team 325 mg of ferrous sulfate (65 mg of elemental iron) 6 times a week for 5 months. In addition, each individual received a multivitamin supplement which included Vitamin C. Neither hemoglobin, blood iron, nor plasma iron changed significantly over the treatment period.

Cooter and Mowbray (1978) investigated the effects of iron supplementation on serum iron, total iron binding capacity, hemoglobin and percent hemoglobin saturation with iron. After administering 18 mg of ferrous sulfate 5 times a week for 4 months to 5 female college basketball players no significant changes were observed in any of the experimental variables.

In another similar study Pate et al. (1979) studied the effects of iron supplementation in 26 female athletes from the University of South Carolina. The 26 athletes ingested a time release capsule which contained 167 mg of ferrous sulfate (33.4 mg of elemental iron) daily for five to nine weeks. No significant differences were found in hemoglobin, hematocrit, serum iron or % saturation of transferrin.

One of the first studies to show a significant increase in hemoglobin by dietary supplementation was done by Plowman and McSwegin (1981). In the study, eleven female cross country runners received a daily combination supplement of 1170 mg of ferrous sulfate (234 mg of elemental iron) and 450 mg of ascorbic acid for a minimum of 12 weeks. A significant increase in hemoglobin values pre and post supplementation was found.

According to Plowman and McSwegin (1981) there are several reasons which might explain why the results of their study differed from the previous ones. First, the amount of elemental



iron ingested in their study was considerably higher. Second, to insure maximal absorption the iron supplement was given along with Vitamin C which has been shown to increase the bioavailability of dietary iron. Third, a tablet form rather than a time release capsule was utilized. The tablet form may be a more efficient way to provide for iron absorption. Finally, Plowman and McSwegin (1981) controlled the sampling of blood in relation to the menstrual cycle. Zilva and Patson (1966) noted that serum iron was significantly reduced between the two days immediately prior to and the first 3 days of menstrual blood flow in contrast to the rest of cycle. Plowman and McSwegin (1981) avoided taking blood samples immediately prior to and during the days of menstrual blood flow thereby controlling for such variation in serum iron.

Valberg (1980) noted that oral iron treatment needs to be continued for 2 months to replenish iron stores after the hemoglobin concentration has reached normal values. It seems that hemoglobin is the first to be affected by an enhanced supply of iron. The body uses the increased supply of absorbed iron in the manufacture of hemoglobin before replenishing the storage forms (Plowman & McSwegin, 1981). An enhanced level of serum iron would occur next followed by the replenishment of storage iron (Plowman & McSwegin, 1981).

In a study by Newhouse, Clement, Taunton & McKenzie (1989) ferritin levels increased in one subject from 7 to 12  $\mu\text{g/L}$  over 8 weeks, while a different similarly iron-treated subject responded with an increase of 18 to 83  $\mu\text{g/L}$ . Newhouse et al. (1989) speculated that differences in absorption caused such variability, however the reason for the large absorption differences remains a question.

It appears that some iron deficient individuals do not respond to iron supplementation (Newhouse, 1989). The reason for such nonresponse is speculative but may involve malabsorption due to strenuous activity, other components of the diet, pathological conditions, and/or prior trace mineral status (Newhouse, 1989). Although a few recent studies involving iron supplementation were successful in improving the iron status of the majority of their subjects each study noted cases of nonresponse (Charoenlarp et al., 1988; Newhouse et al., 1989; & Schoene et al., 1983).

**CHAPTER 3**  
**METHODOLOGY**

**Subjects**

One hundred and eleven healthy females between the ages of 18 and 40 from the vicinity of Thunder Bay formed the population from which a sample was drawn. The risks of the study were described and the subjects signed informed consent forms (Appendix A). All procedures were approved by the Lakehead University Ethics Committee. The subjects were permitted to withdraw at any time during the study.

Forty-four subjects were iron deficient as defined by either a serum ferritin value below 20  $\mu\text{g/L}$  and/or a hemoglobin level below 120 g/L. Twenty-four of the subjects were able to complete the entire 24 week study and the physical characteristics of the subjects are shown in Table 1. The reason for drop outs was "leaving town" in 19 cases and lack of compliance in one.

Table 1

Physical Characteristics of the Subjects

Subjects	Age (yrs)	Height (cm)	Weight (kg)
1	36	169.0	64.5
2	22	172.0	68.0
3	20	185.4	72.0
4	31	172.0	67.0
5	32	165.0	57.5
6	21	164.0	54.0
7	19	171.0	59.0
8	38	153.0	52.7
9	18	177.0	65.8
10	21	173.0	64.5
11	30	165.0	55.5
12	37	163.8	63.6
13	22	170.0	67.0
14	39	160.0	55.5
15	20	175.0	59.0
16	36	175.3	65.0
17	34	163.0	52.0
18	20	152.4	52.3
19	27	165.5	57.0
20	26	168.0	63.0
21	32	171.0	71.0
22	21	165.0	58.0
23	21	165.1	70.4
24	40	167.6	66.0
Mean	27.6	167.8	61.7
SD	7.5	7.2	6.3

Exclusion criteria

Criteria for exclusion from the study were as follows:

- a) occult blood loss in the stool as determined from serial fecal specimen analysis during week one of the preliminary study (Hemocult, Smith-Kline Diagnostics, Inc.),

- b) blood loss in the urine as determined by color changes on a dip-and-read Chemstrip during week one of the preliminary study (Chemstrip, Boehringer Mannheim Canada Ltd.),
- c) failure to take 75% of the prescribed supplements (checked by pill counts at 6 week intervals),
- d) repeated ingestion of acetylsalicylic acid (aspirin) or medications which may cause acute or chronic blood loss from the gastrointestinal tract one week prior to the baseline test or during the 12 week supplementation programme or 12 week discontinuation period,
- e) pregnancy, blood donation or loss of more than 250 mL of blood through injury within one month of the baseline test or during the supplementation or discontinuation period,
- f) and finally, a fever within 2 weeks of any of the blood tests.

#### Hematological data

Blood analysis occurred at 0 ( $T_0$ ), 6 ( $T_1$ ), 12 ( $T_2$ ), 18 ( $T_3$ ) and 24 ( $T_4$ ) weeks of the study. Subjects reported to the physiology lab at the Lakehead University Fieldhouse in the morning having avoided physical activity in the preceding 24 hours. The sampling of blood was scheduled to avoid the onset and duration of menstruation. No supplements, food or beverage were ingested by the subjects in the morning prior to the drawing of blood. Blood was drawn between 8:00 and 11:30 am, and each

subject reported at approximately the same time for each consecutive blood test to control for diurnal variation.

Phlebotomy was conducted by a qualified technician familiar with the drawing, storing and analysis of blood. Twenty-three milliliters of blood was collected in Vacutainer tubes by antecubital venipuncture technique from each subject during each of the five blood tests. Samples for hemoglobin assays were collected in 3 mL tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Blood for serum ferritin and serum iron were collected in 5 mL tubes containing a clot activator and serum separator and centrifuged at 3000 revolutions per minute (RPM) for 10 minutes. Each sample was drawn from the cubital vein while the women were prone.

Blood assays were performed at the Port Arthur General Hospital in Thunder Bay, Ontario. Serum ferritin concentrations were determined by the two-site Quantimmune Ferritin Immunoradiometric Assay (Bio-Rad Laboratories, Mississauga, Ontario). Serum iron was measured using Kodak Ektachem Clinical Chemistry Slides (Eastman Kodak Company, Rochester, New York). Hemoglobin assays were performed on the Coulter Counter instrument (Coulter Electronics, Hialeah, Florida).

### Experimental design

Forty-four of the original 111 subjects were iron deficient and were prescribed a normal therapeutic iron dose (320 mg ferrous sulfate or 100 mg elemental iron) taken as two SLOW-Fe tablets/day, in the morning and evening, for 12 weeks. Serving as their own controls, the subjects then discontinued iron supplementation for an additional 12 weeks. The response of serum ferritin, serum iron and hemoglobin was monitored with blood analysis at 6 week intervals. Twenty-four subjects completed the full 24 week treatment and these subjects formed the pool for statistical analysis.

Training, use of concomitant and study medication, and the magnitude of menstrual blood loss was monitored in a log book (Appendix B) by each of the subjects for the duration of the study. No fitness or regular training criteria were imposed on the subjects, however, each subject was asked to record the type, duration (volume) and intensity of each training session. Training volumes for  $T_0$ ,  $T_2$  and  $T_4$  were determined by averaging the weekly training volumes preceding these blood collection dates. The magnitude of menstrual blood loss was based on two measurements: (a) duration of menses (# of days), and (b) percent saturation of each tampon/pad used per cycle. The percent saturation of tampon/pads was a subjective measurement made by each subject. Prior to the collection of menstrual data, each

subject was instructed on the estimation of tampon/pad saturation. For example, a completely saturated tampon/pad would represent 100 % saturation, whereas a tampon/pad that was only spotted might represent 10% saturation. Subjects were asked to record percent saturation as a fraction (e.g., 10% = 0.10). The percent saturations of each tampon were summed to obtain a value which reflected volume of blood loss per cycle (e.g.,  $.75 + 1.00 + .60 + .50 = \underline{2.85}$ ). No units were applied to this measurement since it was not an objective measurement of volume. The magnitude of menstrual blood loss was determined for  $T_0$ ,  $T_2$ , and  $T_4$  and was calculated using data from the menstrual period which preceded each of the three blood collection dates. As well, diet was not controlled but subjects were asked to record food intake for a 3 day period (2 week days and 1 weekend day) at week 0 and week 24. A 3 day dietary analysis (Appendix C) using Moseby Diet Simple (N-Squared Computing, Salem, Oregon) helped to monitor the consistency of each subjects diet. Subjects were instructed to keep training and diet consistent throughout the study. Record of any dietary or training inconsistency will be used in the discussion to shed light on the pattern of results.



Table 2

Schedule of Events

Event	Base line (T <sub>0</sub> )	Iron Supplementation		No Supplementation	
		(T <sub>1</sub> )	(T <sub>2</sub> )	(T <sub>3</sub> )	(T <sub>4</sub> )
Patient consent	*			*	
Blood loss studies	*				
Diet analysis	*				*
Hematological tests	*	*	*	*	*
Dispense medication	*	*			
Collect medication			*		
Dispense diary	*		*		
Collect diary			*		*

Table 3

Design of the Study

	Base line (T <sub>0</sub> )	Iron Supplementation		No Supplementation	
		(T <sub>1</sub> )	(T <sub>2</sub> )	(T <sub>3</sub> )	(T <sub>4</sub> )
Serum Ferritin	*	*	*	*	*
Serum Iron	*	*	*	*	*
Hemoglobin	*	*	*	*	*

Statistical analysis

A 1 x 5 (treatment x time) analysis of variance with repeated measures on the second factor was utilized for each of the dependent measures. The independent variable was iron supplementation. The dependent variables were levels of serum ferritin, serum iron and hemoglobin. Student-Newman-Keuls post hoc test was used to identify specific mean differences when a significant F ratio was observed for the dependent measures. Change scores (i.e., T<sub>2</sub> value - T<sub>0</sub> value; T<sub>4</sub> value - T<sub>2</sub> value) for

each dependent variable, days of menses, percent saturation of tampon, and iron intakes were calculated. Pearson correlation coefficients were computed for serum ferritin versus days of menses, percent saturation of tampons, and iron intakes. Two-tailed significance levels were used for all correlations and the level of significance for all tests was set at  $p < .05$ . All data was analyzed using the Statistical Package for the Social Science (SPSS).

## CHAPTER 4

### RESULTS

#### Prevalence of Iron Deficiency

One hundred and eleven women (age 18-40 yr) underwent blood testing to select a sample population who fulfilled the criteria of iron deficiency. The results are presented in Table 4.

**Table 4**

Mean Iron Values of Normal vs. Iron Deficient Subjects at Initial Screening (T<sub>0</sub>)

Group	N	Serum ferritin ( $\mu\text{g/L}$ )	Serum iron ( $\mu\text{mol/L}$ )	Hemoglobin ( $\text{g/L}$ )
Normal *	67	37.1 $\pm$ 17.8	19.1 $\pm$ 7.5	138.8 $\pm$ 7.3
Iron deficient **	44	11.3 $\pm$ 5.8	14.6 $\pm$ 6.4	135.2 $\pm$ 10.0

\* Serum ferritin > 20  $\mu\text{g/L}$  and hemoglobin > 120  $\text{g/L}$ .

\*\* Serum ferritin < 20  $\mu\text{g/L}$  and/or hemoglobin < 120  $\text{g/L}$ .

The frequency distributions of serum ferritin levels for the original 111 subjects are displayed in Figure 1. The mean serum ferritin value was 27.9  $\pm$  21.0  $\mu\text{g/L}$ . Forty three (38.7%) of the 111 subjects had serum ferritin levels of 20  $\mu\text{g/L}$  or less, while 16 subjects (14.4%) had serum ferritin levels below 10  $\mu\text{g/L}$ , indicating extremely depleted iron stores.

The frequency distributions of hemoglobin levels for the original 111 subjects is displayed in Figure 2. The mean

hemoglobin value was  $137.4 \pm 8.6$  g/L. Two subjects had both low serum ferritin (less than  $20 \mu\text{g/L}$ ) and low hemoglobin (less than  $120$  g/L) while two other subjects had low hemoglobin with normal serum ferritin ( $20$  to  $160 \mu\text{g/L}$ ).

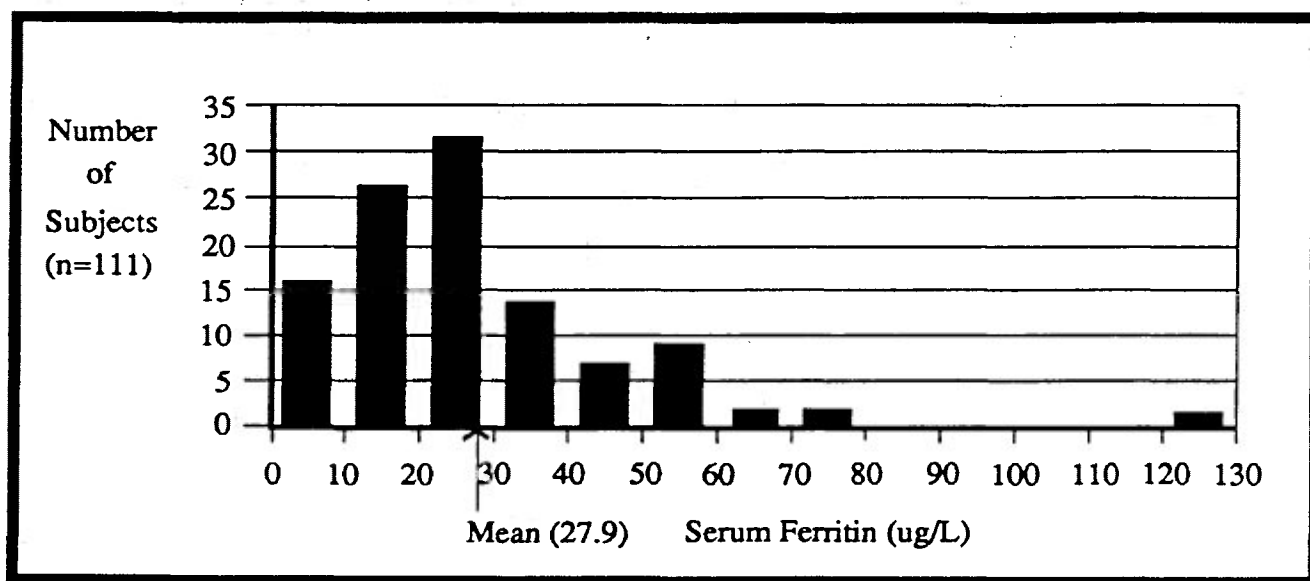


Figure 1. Distribution of serum ferritin levels for the subjects initially screened for inclusion in the study.

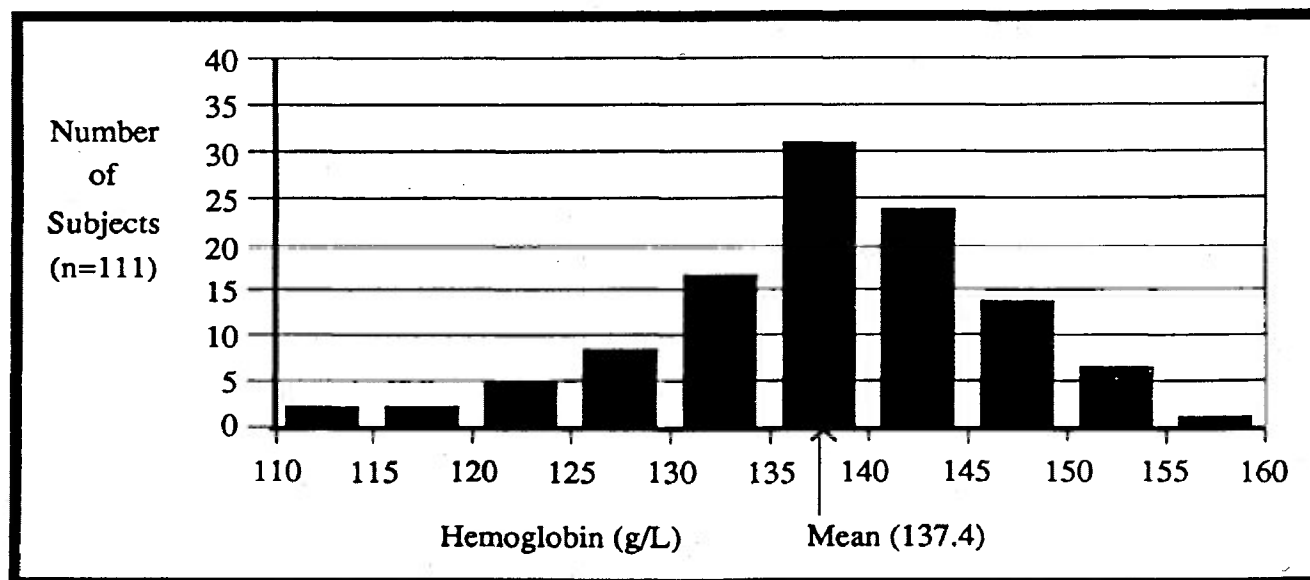


Figure 2. Distribution of hemoglobin levels for the subjects initially screened for inclusion in the study.

On the basis of ferritin and hemoglobin measurements, 44 women qualified to continue with the study. Twenty four of the selected women completed the 24 week study. The frequency distribution of serum ferritin levels and hemoglobin levels at  $T_0$  for the 24 subjects who completed the study are displayed in Figure 3 and 4, respectively.

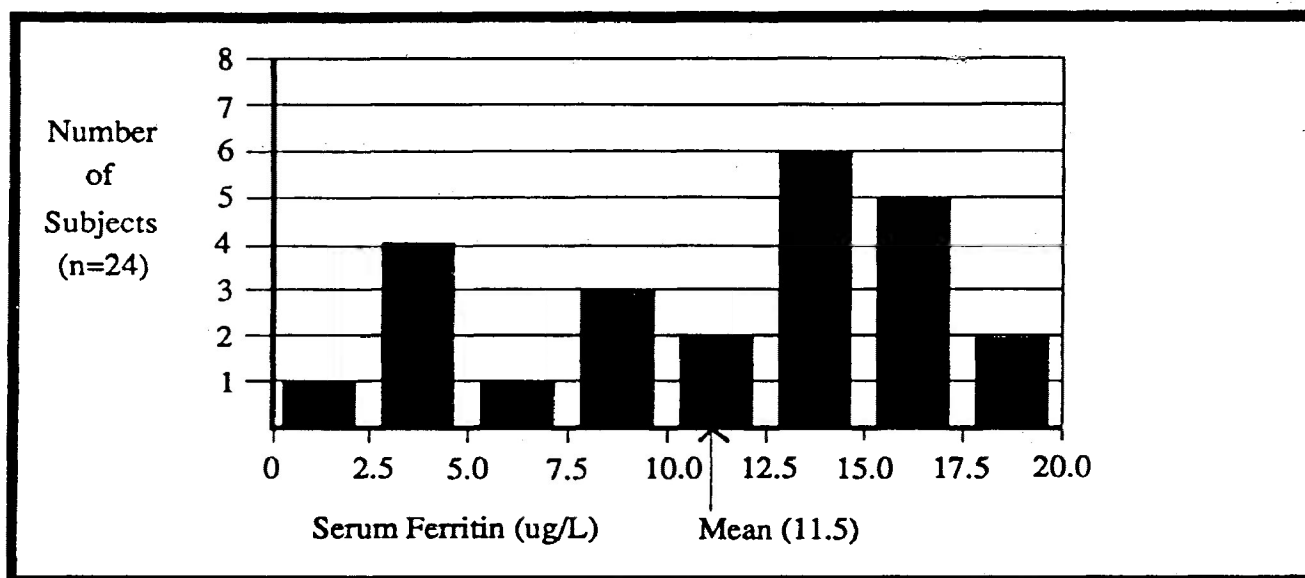


Figure 3. Distribution of serum ferritin levels at week 0 for the subjects who completed the study.

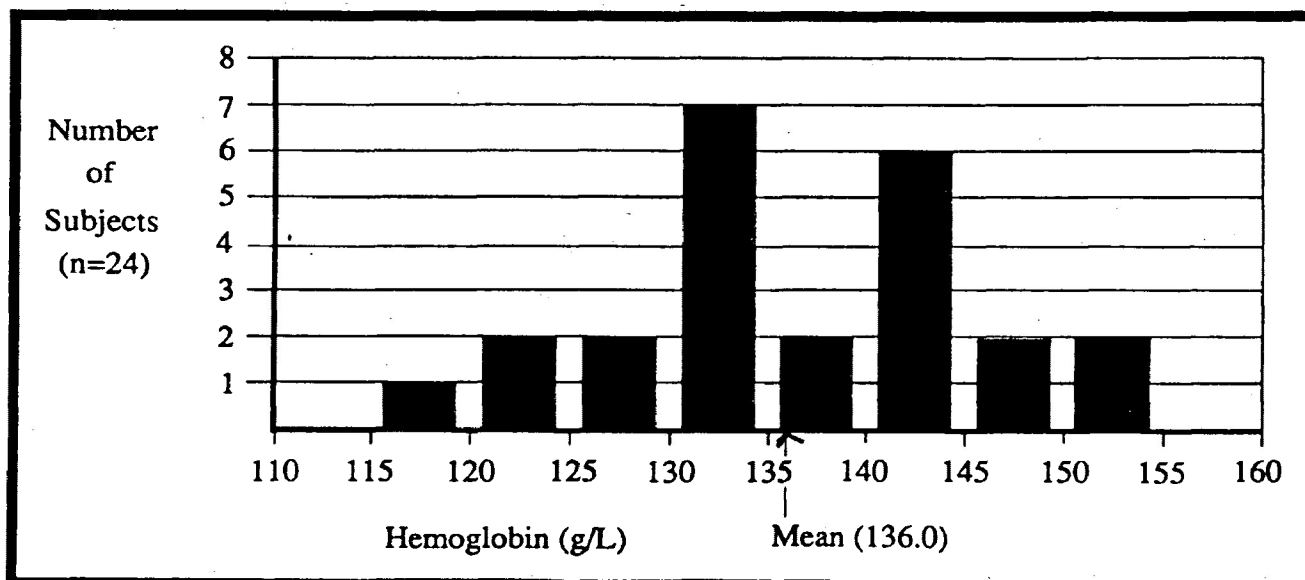


Figure 4. Distribution of hemoglobin levels at week 0 for the subjects who completed the study.

For the 24 women who completed the study only one subject (4.2%) was considered anemic (hemoglobin below 120 g/L) at  $T_0$ . As well, 9 subjects (37.5%) had serum ferritin levels below 10  $\mu\text{g/L}$  at  $T_0$ , indicating severely depleted iron stores.

### Effect of Iron Supplementation and Discontinuation

The effects of oral iron supplementation and subsequent discontinuation on each hematological variable for the 24 subjects are presented in Table 5. Significant changes were noted for serum ferritin and serum iron values while hemoglobin values were not significantly ( $p < .05$ ) altered during the 24 week period.

**Table 5**

### Changes in Mean Hematological Values following Supplementation and Discontinuation Periods

Variable	N		$T_0$	$T_1$	$T_2$	$T_3$	$T_4$	F Ratio
			baseline	supplementation		discontinuation		
S. Ferritin ( $\mu\text{g/L}$ )	24	x	11.5	26.4	30.6	29.0	28.2	13.84 *
		SD	5.3	14.3	14.9	15.0	17.1	
S. Iron ( $\mu\text{mol/L}$ )	24	x	15.9	25.4	24.3	21.9	17.9	3.87 *
		SD	6.7	15.6	15.4	9.8	8.3	
Hemoglobin (g/L)	24	x	136.0	137.9	137.5	135.0	134.2	1.70
		SD	8.9	9.6	9.3	7.0	6.9	

\* statistically significant ( $p < .05$ ). S: Serum.

The Student-Newman-Keuls post hoc test was used to determine pairs of measurements that were significantly different ( $p < .05$ ) for serum ferritin and serum iron (Figures 5 & 6). Serum ferritin levels were significantly ( $p < .05$ ) increased (11.5 to 30.6  $\mu\text{g/L}$ ) following twelve weeks of iron supplementation. A significant ( $p < .05$ ) increase (11.5 to 26.38  $\mu\text{g/L}$ ) was also noted after six weeks of iron supplementation. Twelve weeks of discontinued supplementation did not significantly ( $p > .05$ ) alter serum ferritin levels, as noted by mean values of  $29.0 \pm 15.0$   $\mu\text{g/L}$  and  $28.2 \pm 17.1$   $\mu\text{g/L}$  for  $T_3$  and  $T_4$ , respectively.

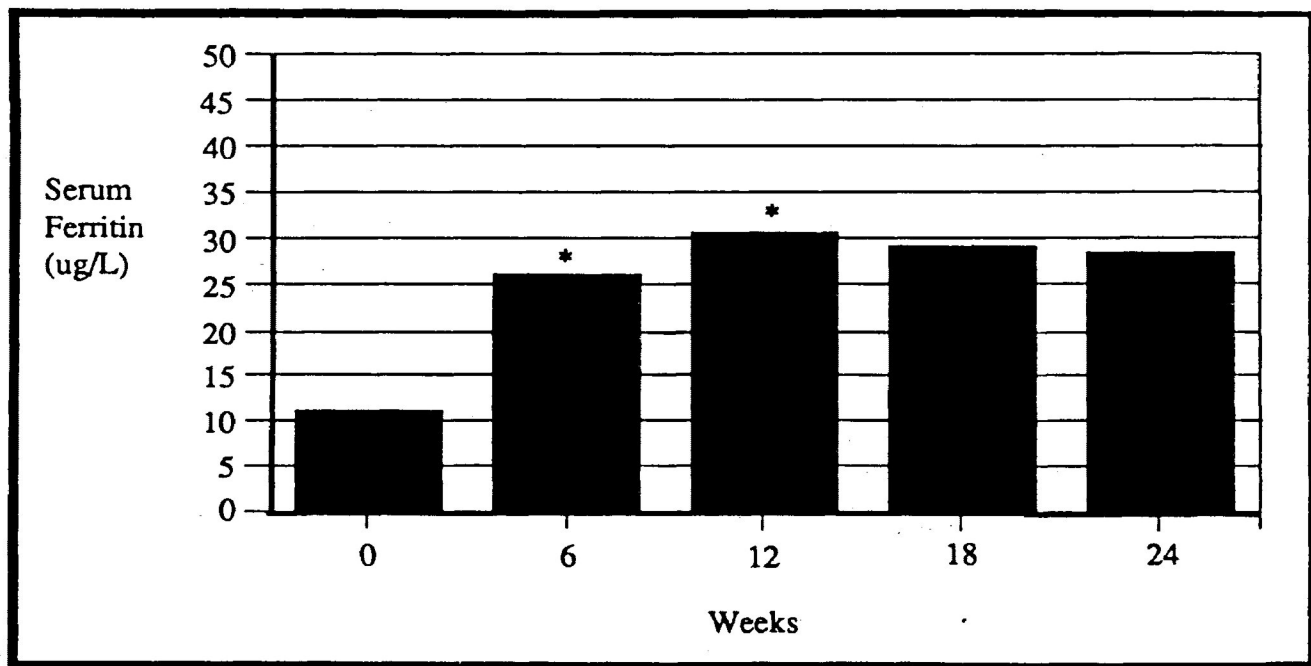


Figure 5. Mean serum ferritin levels at 0, 6, 12, 18 and 24 weeks of the study.  
(\* Significantly ( $p < .05$ ) different from week 0).

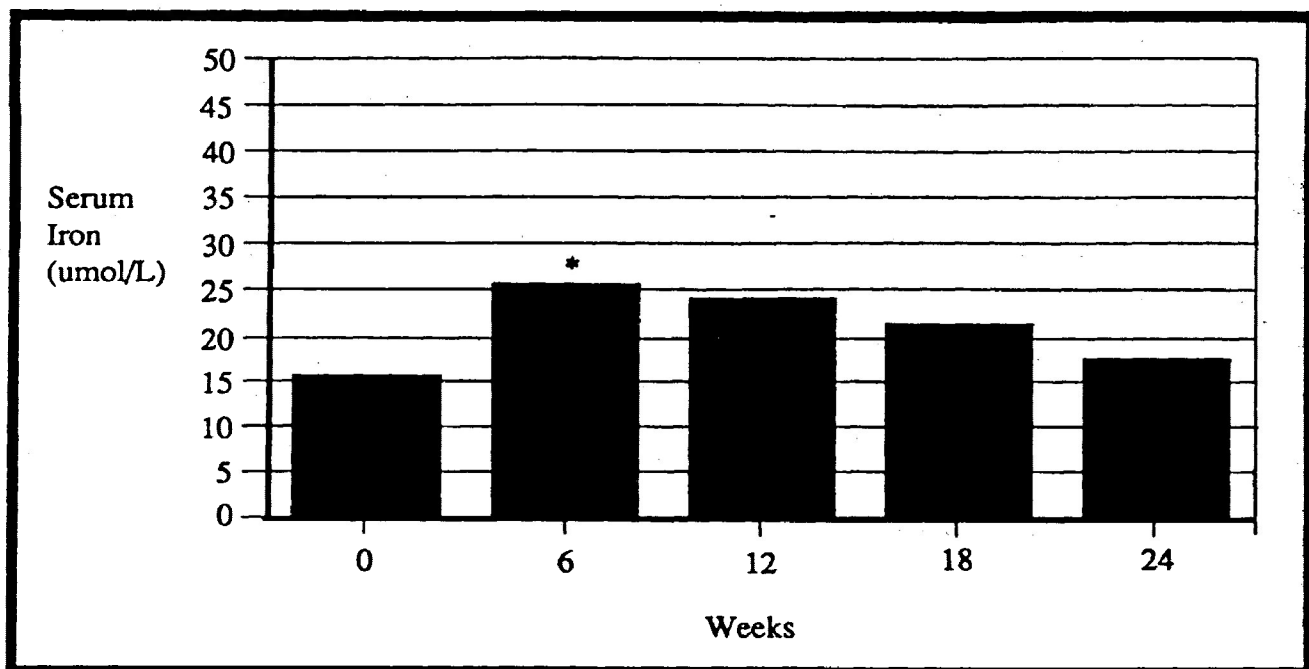


Figure 6. Mean serum iron levels at 0, 6, 12, 18 and 24 weeks of the study.  
 (\* Significantly ( $p < .05$ ) different from week 0).

Serum iron levels were significantly ( $p < .05$ ) increased (15.9 to 25.4  $\mu\text{mol/L}$ ) following six weeks of iron supplementation. However, serum iron levels at  $T_2$ ,  $T_3$  and  $T_4$  were not significantly different ( $p > .05$ ) from the  $T_0$  or  $T_1$  values (Figure 6). It is interesting, however, to note the gradual decrease in serum iron levels after week 6 ( $T_1$ ) of the study.

Mean hemoglobin levels for weeks 0 through 24 are presented in Figure 7. Hemoglobin levels remained fairly consistent and showed no significant ( $p > .05$ ) changes for the duration of the study. However, there was a slight increase in levels at week 6 followed by a gradual decrease in hemoglobin levels towards week 24.



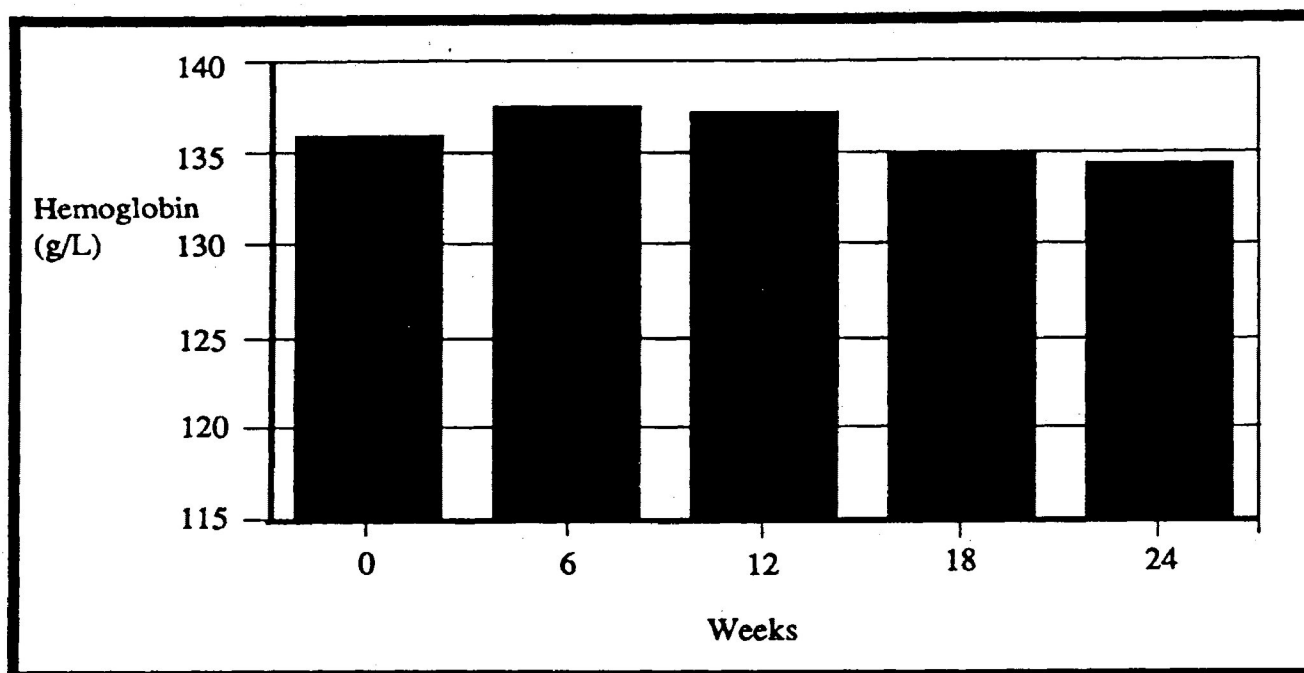


Figure 7. Mean hemoglobin levels at 0, 6, 12, 18 and 24 weeks of the study.  
(\* Significantly ( $p < .05$ ) different from week 0).

Change scores for each dependent variable were standardized (Figure 8) by dividing mean change by the average standard deviation of change. This is an effect size measurement which permits valid comparisons between the dependent variables. (Glass, McGaw, and Smith, 1981). The largest positive change after supplementation was found for serum ferritin levels, while serum iron and hemoglobin levels showed smaller positive changes. Subsequent discontinuation resulted in small negative changes in serum ferritin, with comparatively larger negative changes in serum iron and hemoglobin levels. The change in hemoglobin levels following discontinuation is noteworthy.

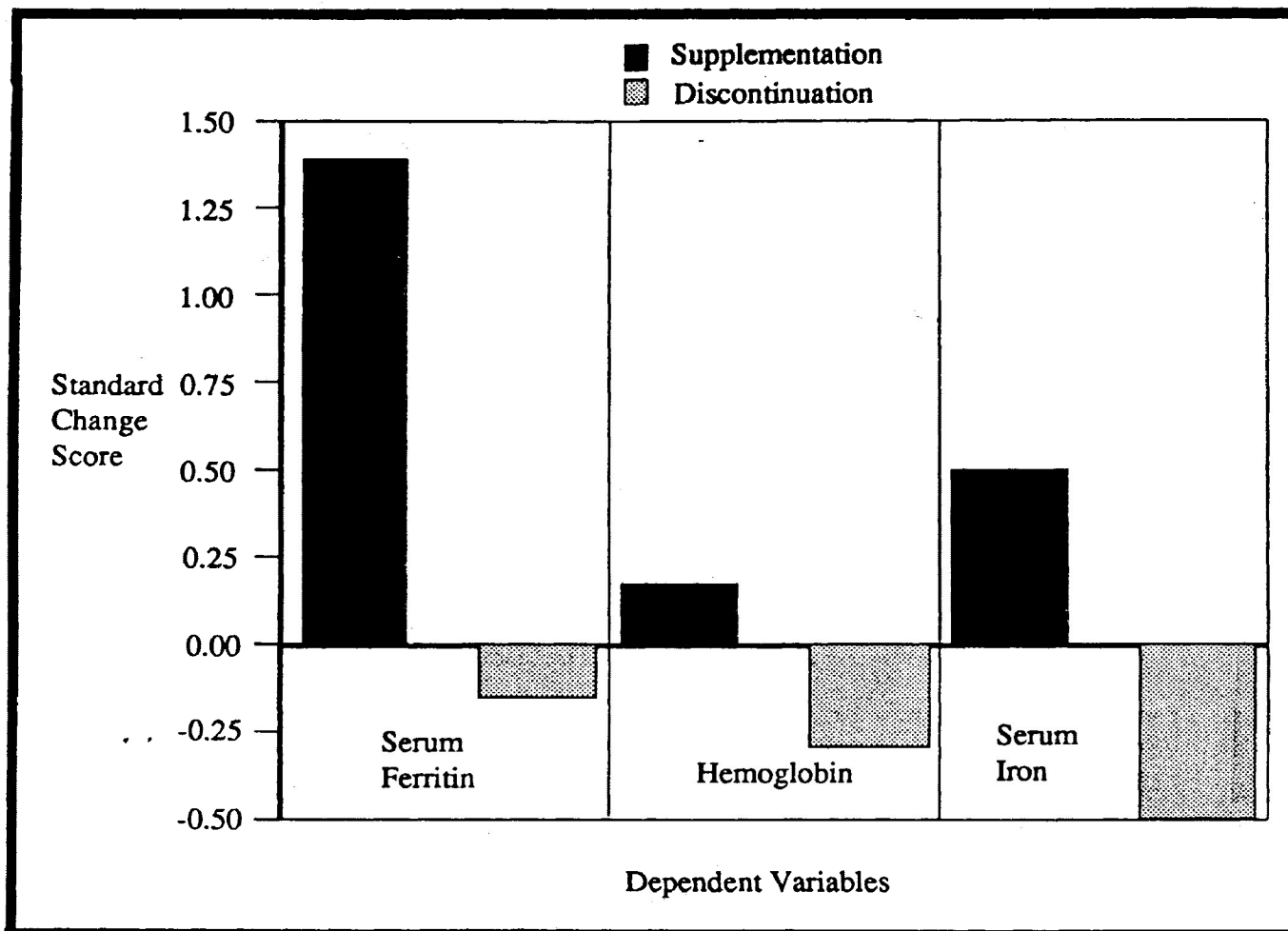


Figure 8. Supplementation vs. Discontinuation summary. (Standard change score = mean change score divided by standard deviation of change).

Mean training volumes, iron intakes, duration of menses (DOM) and percent saturation of tampon/pads (PST) for weeks 0, 12 and 24 are displayed in Table 6.

**Table 6**

Mean Training Volumes, Iron Intakes, DOM, and PST for Weeks 0, 12 and 24.

Variable	T <sub>0</sub>	T <sub>2</sub>	T <sub>4</sub>
Training volume (hrs/wk) (n=16)	5.0 ± 2.1	4.3 ± 1.8	4.4 ± 2.2
Iron intake (mg/day) (n=12)	15.2 ± 5.9	*	12.8 ± 3.3
DOM (# of days) (n=12)	4.8 ± 1.6	4.4 ± 1.3	4.5 ± 0.8
PST (n=11)	9.9 ± 6.7	8.0 ± 5.7	8.2 ± 5.2

\* No data collected. DOM: Duration of Menses. PST: Percent Saturation of tampons/pads.

Training was monitored by record of type, duration and intensity of exercise. The subjects were all active and participated in activities ranging from Varsity athletics (e.g., basketball, volleyball and track & field), jogging, swimming, cross-country skiing, to weight lifting. Mean training volumes remained consistent for the duration of the study ranging from 4.3 ± 1.8 to 5.0 ± 2.1 hours/week. Mean values for training intensities were not obtained due to poor compliance with filling out training logs. However, the majority of the subjects trained regularly at three quarters maximal effort.

Mean daily iron intakes for the subjects was 15.2 ± 5.9 mg/day at T<sub>0</sub> (n=19) and 12.8 ± 3.3 mg/day at T<sub>4</sub> (n=12).

The magnitude of menstrual blood loss was estimated by duration of menses and percent saturation of tampons. Mean durations of menses for  $T_0$ ,  $T_2$ , and  $T_4$  were  $4.9 \pm 1.4$ ,  $4.8 \pm 1.8$ , and  $4.7 \pm 0.9$  days. Percent saturation of tampons for  $T_0$ ,  $T_2$ , and  $T_4$  were  $9.3 \pm 6.0$ ,  $9.9 \pm 9.8$ , and  $9.7 \pm 6.7$ , respectively.

Correlations were computed for serum ferritin versus days of menses, percent saturation of tampons, and iron intake at  $T_0$ . Correlations using change scores were also computed for serum ferritin versus DOM, PST and iron intake. Correlations are presented in Table 7.

Table 7

Correlations for Serum Ferritin versus Days of Menses (DOM), Percent Saturation of Tampons (PST), and Iron Intakes

Correlations	n	r	p(probability)
Serum ferritin with iron intake ( $T_0$ )	19	.30	0.22
" " " DOM ( $T_0$ )	20	.41	0.07
" " " PST ( $T_0$ )	18	.51	0.03
<u>Change Scores</u>			
S. ferritin with iron intake ( $T_4 - T_0$ )	12	.32	0.32
" " " DOM ( $T_2 - T_0$ )	15	-.35	0.10
" " " DOM ( $T_4 - T_2$ )	14	-.40	0.08
" " " PST ( $T_2 - T_0$ )	13	.10	0.37
" " " PST ( $T_4 - T_2$ )	13	-.25	0.21

A significant ( $p < .05$ ) positive correlation was found between serum ferritin and percent saturation of tampons at  $T_0$ . This would suggest that subjects who had high serum ferritin levels at  $T_0$  also tended to have higher volumes of menstrual blood loss at  $T_0$  or vice versa. All other correlations were non-significant ( $p > .05$ ).

## CHAPTER 5

### DISCUSSION

#### Prevalence of Iron Deficiency

The mean serum ferritin from the 111 women involved in the initial screening was  $27.9 \pm 21.0$   $\mu\text{g/L}$ . Similar results were noted by Valberg, Sorbie, Ludwig, and Pelletier (1976) and Cook, Finch and Smith (1976) who found mean serum ferritin levels of 23  $\mu\text{g/L}$  in 95 Canadian and 25  $\mu\text{g/L}$  in 370 American menstruating women, respectively. Although such values fall within the reference levels (i.e., 18-300  $\mu\text{g/L}$ ) for menstruating women, they should be considered as 'common' values and not 'normal' values. As noted by Hercberg et al. (1985, p. 236), "values observed in most studies concern groups of 'all-comers' women, whose characteristic is to be, by definition, a population at risk, with an important proportion of iron deficient women."

Early studies by Scott and Pritchard (1967) and Monsen, Kuhn, and Finch (1967) which evaluated the iron status of healthy menstruating women, using bone marrow techniques for determining iron storage, found that more than 25% of the subjects in their studies were iron deficient. More recent studies have found an equal or even greater prevalency of iron deficiency in menstruating women. After screening 155 women (age 18-40), Newhouse et al. (1989) found that 61 of the subjects (40%) were

iron deficient (serum ferritin < 20  $\mu\text{g/L}$ ). This compares to the finding in the present study where 38.7% of the subjects were considered to be iron deficient (serum ferritin < 20  $\mu\text{g/L}$ ).

Bothwell et al. (1979) noted that the high prevalency of iron deficiency in menstruating women is largely a function of the limited availability of iron in the contemporary diet. In the present study iron intakes of the subjects averaged  $15.2 \pm 5.9$  mg/day at  $T_0$  and  $12.8 \pm 3.3$  mg/day at  $T_4$  (n=12).

Approximately 55% of the women had mean daily iron intakes below the Canadian recommended intake of 14 mg/day (Health and Welfare Canada, 1983). A study by Newhouse et al. (1989) which examined the effects of prelatent/latent iron deficiency on physical work capacity found that approximately 80% of the subjects had mean dietary iron intakes below the recommended daily allowance. The Nutrition Canada survey (1975) also indicated inadequate mean iron intakes of 11.1 mg/day for the females in the 20 - 39 age category (Health and Welfare Canada, 1975). These results reinforce the common finding that it is difficult for the typical Western diet to meet the iron demands of the menstruating women (Soustre, Dop, Galan, & Hercberg, 1986).

The mean serum iron level of the 111 women who were initially screened was 17.3  $\mu\text{mol/L}$ . This compares to the studies by Hercberg et al. (1985) and Soustre et al. (1986) who found mean serum iron levels in menstruating women of  $18.4 \pm 7.9$   $\mu\text{mol/L}$

and  $19.9 \pm 0.5 \mu\text{mol/L}$ , respectively. The normal range for serum iron levels is defined as  $11 - 29 \mu\text{mol/L}$ . Of the initially screened subjects, 21.6% (24 of 111) had below normal serum iron levels. Other studies (De Wijn et al., 1971; Strauzenberg et al., 1981) have reported similar findings. De Wijn et al. (1971) reported serum iron levels of less than  $12.5 \mu\text{mol/L}$  in 22.5% of the female Olympic athletes in their study. Strauzenberg et al. (1981) examined 1,416 female athletes and found that 20% of the women had reduced serum iron levels (less than  $11 \mu\text{mol/L}$ ). It should be noted that serum iron levels should be interpreted with a certain reserve due to the fact that diurnal and day-to-day variations of the serum iron measurement are relatively large and may reach  $5 \mu\text{mol/L}$  (Strauzenberg et al., 1981).

From the 111 women initially screened, it was found that the mean hemoglobin level was  $137.4 \pm 8.6 \text{ g/L}$ . The normal range is defined as 120 to 160 g/L. Similar results were noted by Newhouse et al. (1989) and Hercberg et al. (1985) who found mean hemoglobin levels of 130.0 g/L in 155 Canadian women and 134.0 g/L in 107 French women, respectively. Although the present research found a high prevalency of iron deficiency in its population group, the population was comparable to a non-anemic one for the distribution of hemoglobin. Only 4 subjects (3.6%) had hemoglobin levels below 120 g/L. Soustre et al. (1986), who examined 203 healthy menstruating women, found that 2.9% of the women had hemoglobin levels below 120 g/L.



### Effect of iron supplementation

Iron supplementation improved the iron levels of all subjects in the study. However, 7 subjects (29.2%) had ferritin levels less than or equal to 20  $\mu\text{g/L}$  after 12 weeks of supplementation. Of the 7 subjects, 5 had ferritin levels less than 5  $\mu\text{g/L}$  at baseline. Therefore, 12 weeks of oral iron supplementation was not sufficient in elevating iron stores to normal levels in severely iron depleted subjects within the present study. As well, it is interesting to note the large standard deviation of mean serum ferritin levels following supplementation in both Newhouse et al's (1989) study ( $37.7 \pm 19.7 \mu\text{g/L}$ ) and the present study ( $30.6 \pm 14.9 \mu\text{g/L}$ ). One individual in this study increased their ferritin from 4.3 to 11.5  $\mu\text{g/L}$  while another similarly iron treated subject responded with an increase from 6.7 to 47.0  $\mu\text{g/L}$  at  $T_4$ . Therefore, there appears to be a high degree of variability in how subjects respond to iron therapy. Differences in absorption are likely the largest contributing factor to this variability. According to Jacobs (1985), the absorptive mechanism, whereby the amount of iron crossing the small intestinal epithelial barrier is related to internal iron status, may be defective in some people. Such variability in absorption may be due to genetics and/or interrelationships that iron has with other nutrients.

Among other factors known to influence iron stores, magnitude of menstrual blood loss and daily iron intakes seem to be of particularly importance (Soustre et al., 1986). Of interest then, would be whether changes in serum ferritin were correlated to changes in DOM, PST, or iron intake during the study. None of the three correlations were significant ( $r = .41$ ,  $p > .07$ ) in the present study (Table 7). A low correlation suggests that only a small portion of the variance in one variable is predictable from the variance in the other. Therefore, the changes in DOM, PST, and iron intake values do not aid in the prediction of changes in serum ferritin values in this study. Such a lack of correlation was expected since DOM, PST and iron intakes remained fairly consistent throughout the study.

Pearson correlation coefficients were also calculated to determine if baseline values of serum ferritin were correlated to baseline DOM, PST or iron intake values (Table 7). Percent saturation of tampons showed a significant positive correlation ( $r = .51$ ,  $p < .05$ ) with serum ferritin. All other correlations were non-significant ( $p > .05$ ). Considering that both DOM and PST are reflections of the magnitude of menstrual blood loss, the positive correlation found in this study contradicts the negative correlation found in Soustre et al.'s (1986) study. Soustre et al.'s (1986) study examined 127 women and found that serum ferritin was negatively correlated with duration of menses ( $r = -0.23$ ,  $p = 0.01$ ). Simple explanations for this contradiction may

be related to the smaller n used in the present study and the fact that DOM and PST may have been poor indicators of the magnitude of menstrual blood loss in the present study.

The finding that serum ferritin and daily iron intake were not correlated supports the findings of Soustre et al.'s (1986) study. Only a few studies have noted a relationship between diet and iron status. According to Soustre et al. (1986) this is probably due to the difficulty of evaluating dietary intakes over a relevant period of time since the iron status of an individual is probably a resultant of intakes (and losses) of iron in the preceding weeks or months. According to Hallberg (1983), the absorption of iron from the diet is determined more by meal composition than by the amount of iron present in the diet. Therefore, classification of food items into those which enhance and those which inhibit iron absorption may have been more appropriate for determining the relationship between diet and iron status.

The present study found no significant ( $p > .05$ ) change in hemoglobin levels ( $136.0 \pm 8.9$  to  $137.5 \pm 9.3$  g/L) during the supplementation period. Similar results were found by Newhouse et al. (1989) and Pate et al. (1979) who found that oral iron supplements administered to non-anemic women athletes, had no statistically significant impact on hemoglobin levels. According to Plowman and McSwegin (1981) this result is to be expected

since only under anemic circumstances does the body normally use the increased supply of absorbed iron in the manufacture of hemoglobin before replenishing the storage forms of iron. Newhouse and Clement (1988) concluded that the major determinant of a hemoglobin response is likely to be the initial degree of iron deficiency.

#### Effect of supplement discontinuation

Mean serum ferritin levels were not significantly decreased after six (30.6 to 29.0  $\mu\text{g/L}$ ) or twelve weeks (29.0 to 28.2  $\mu\text{g/L}$ ) of discontinued supplementation. These results contradict the findings of Hercberg et al.'s (1985) study where ferritin levels were significantly decreased from  $46.4 \pm 30.3$  to  $40.6 \pm 23.4$   $\mu\text{g/L}$  following 30 days of discontinued supplementation. Such contradiction may have resulted from the different durations of iron treatment used in Hercberg et al.'s (1985) study compared with the present study (i.e., 4 and 12 weeks respectively). Valberg (1980) noted that oral iron supplementation needs to be continued for at least 2 months to replenish iron stores. It may be that some of the subjects in Hercberg et al.'s (1985) study were not sufficiently repleted prior to discontinuing supplementation. This may have placed the subjects in a vulnerable position for developing depleted iron stores during the one month discontinuation period. Another possible explanation may stem from the fact that not all of the subjects

were iron deficient at the beginning of Hercberg et al.'s (1985) study. Therefore, once treated with iron supplements these previously non-deficient subjects may have developed falsely elevated iron stores. Subsequent discontinuation of iron supplements may have caused the iron stores in these subjects to quickly return to their previous normal levels since elevated levels of serum ferritin were not needed.

Although mean serum ferritin levels were not significantly decreased with discontinued supplementation the large standard deviation at  $T_4$  ( $28.2 \pm 17.1 \mu\text{g/L}$ ) suggests that there is a great deal of variability in the response to discontinued iron supplementation. For example, one individual in this study showed a decrease in ferritin from 48.5 to 11.7  $\mu\text{g/L}$  while a different individual had an decrease in ferritin from 47.9 to 37.1  $\mu\text{g/L}$  during the 12 week discontinuation period. This would suggest that certain individuals may be prone to developing iron deficiency following a successful iron supplementation programme.

Although mean hemoglobin levels were not significantly ( $p > .05$ ) decreased following 12 weeks of discontinued iron supplementation the values did decrease from  $137.5 \pm 9.3 \mu\text{g/L}$  at  $T_2$  to  $134.2 \pm 6.9 \mu\text{g/L}$  at  $T_4$ . It is also interesting to note that the value at  $T_4$  was less than the baseline ( $T_0$ ) value of  $136.0 \pm 8.9 \text{ g/L}$ .

## CHAPTER 6

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

#### Summary

The primary purpose of this study was to determine the effects of discontinued iron supplementation on the hematological values of serum ferritin, serum iron and hemoglobin in a group of iron replete menstruating women who were previously iron deficient. Secondary purposes of the study included: (a) to establish the prevalency of iron deficiency in a group of menstruating women and, (b) to examine the effects of iron supplementation on the hematological values of serum ferritin, serum iron and hemoglobin in a group of iron deficient women. One hundred and eleven women (18-40 yr of age) were initially screened for iron deficiency (serum ferritin below 20  $\mu\text{g/L}$  and/or hemoglobin below 120 g/L). Forty four women (39.6%) qualified as iron deficient. Twenty four of the selected women completed the 24 week study. Mean age was  $27.6 \pm 7.5$  years (range 18 to 40 years). Analysis of the data indicated that iron supplementation significantly ( $p < .05$ ) improved serum ferritin levels while levels of serum iron and hemoglobin were not significantly increased after 12 weeks of iron supplementation. Subsequent discontinuation did not significantly alter serum ferritin, serum iron or hemoglobin values from their  $T_2$  levels.

### Conclusions

Oral iron supplementation (320 mg ferrous sulfate = 100 mg elemental iron taken as SLOW-Fe twice a day for 12 weeks) was successful in raising serum ferritin levels in iron deficient women.

Within the limitations of the study, 12 weeks of discontinued oral iron supplementation does not pose a threat to the iron status of iron repleted menstruating women.

### Recommendations

1. Use of the dietary history method for diet analysis may give a better indication of the relationship between iron status and dietary intakes.
2. A better indicator for the magnitude of menstrual blood loss is needed to accurately establish the correlation between menstrual blood loss and iron status.
3. Examination of a large group of severely iron depleted (serum ferritin below 12  $\mu\text{g/L}$ ) non-anemic women is needed to establish this specific group's response to discontinued oral iron supplementation.

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## Appendix A

### Lakehead University School of Physical Education and Athletics

#### Subject Consent Form

The purpose of this study is to:

A. examine the effects of discontinuing iron supplementation on the hematological values of serum ferritin, serum iron and hemoglobin.

B. examine the effects of oral iron supplementation on the hematological values of serum ferritin, serum iron and hemoglobin.

C. establish the prevalency of iron deficiency in a group of women.

Subjects will be blood tested for serum ferritin, serum iron and hemoglobin. Those meeting the criteria for iron deficiency (serum ferritin below 20  $\mu\text{g/L}$  and/or hemoglobin below 120 g/L) will continue in the study and undergo the following tests:

A. urinalysis: for hemocyturia (blood loss in urine)

B. stool analysis: for occult blood loss

C. 3-day dietary records: for nutrient intake analysis

You will be asked to record all training, an estimate of menstrual blood loss, and any other factors that may influence testing for the duration of the study. Training and diet should be kept consistent with previous habits.

Testing will be performed at 6 week intervals for a duration of 24 weeks. The treatment will be 12 weeks of iron supplementation [a daily dosage of 320 mg ferrous sulfate (100 mg elemental iron) taken as SLOW-Fe twice a day] followed by 12 weeks of no supplementation. Supplementation of dietary iron will not be permitted for the duration of the second 12 week period.

Side effects of iron supplementation have been reported to affect a small percentage of the population and these include nausea, constipation, and abdominal pain (Gomez & Gomez, 1969).

Blood sampling will be conducted by qualified technicians. The amount of blood drawn will be small and there will be little discomfort with the procedure. There may be slight bruising at the point of puncture. Iron status may return to a deficient state during the 12 week discontinuation period. However, blood analysis will monitor for any serious drops in iron levels.

Publication of results will not reveal subject identity as subjects will be referenced by number.

I have read and understand the above explanation of the purpose and procedures for this study and agree to participate. I also understand that I have the option to withdraw from the experiment at any time and/or omit any part of any test. I further consent to the use of information obtained from these tests by Lakehead University.

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Signature

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Witness

---

Date

## Appendix B

## Exercise Diary/Menstrual Blood Loss Record

Week #: \_\_\_\_\_ Name: \_\_\_\_\_

Monday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

Tuesday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

Wednesday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

Thursday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

Friday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

Saturday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

Sunday (Date) \_\_\_\_\_  
 Exercise: Type \_\_\_\_\_ Duration \_\_\_\_\_  
 Intensity \_\_\_\_\_ Comments \_\_\_\_\_  
 Menstrual Blood Loss: % saturation of tampon/pad #1 \_\_\_\_\_ #2 \_\_\_\_\_  
 #3 \_\_\_\_\_ #4 \_\_\_\_\_ #5 \_\_\_\_\_ #6 \_\_\_\_\_  
 Medications \_\_\_\_\_ Health Complaints \_\_\_\_\_

## Appendix C

### Dietary Analysis

Three days of dietary intake (two weekdays and one weekend day) and physical activity was recorded by each subject and analysed using Moseby Diet Simple (N-Squared Computing, Salem, Oregon). The instruction sheets and diet record forms, developed by the YMCA (Vancouver YMCA, British Columbia, 1983) were used by the subjects and are shown on the following pages (pp. 69-73).



## YMCA COMPUTERIZED NUTRITIONAL ANALYSIS SECTION B

### INSTRUCTIONS FOR COMPLETING FOOD RECORD (You May Keep This Portion)

1. Use a worksheet (see sample) to list **EVERYTHING YOU ATE OR DRANK** for EACH DAY to be analyzed. Be sure to include:

- a) **ALL FOOD AND DRINKS**, for example: snacks, sugar and cream in coffee, mayonnaise in a sandwich, sauce on vegetables, candies, soft drinks, wine, etc.
- b) **THE TIME OF DAY** these foods were consumed.
- c) **THE AMOUNT OF FOOD** that you ate (ounces, slices, cups, teaspoons, etc., in whole or decimal numbers).

TIME OF DAY?	HOW MUCH?	FOOD OR DRINK?

2. On the **WHITE COMPUTER SHEET**, enter the **NUMBER OF SERVINGS** of ALL foods and drinks **ACCORDING TO LISTED SERVING SIZE**.

a) **Break down all combination foods.**  
(For example, cheese omelette in food record above.)

b) **NOTE THE SERVING SIZE**

c) **WRITE** the number of servings you had in the **CORRECT TIME SLOT**

FOODS EATEN	Serving Size	NO. OF SERVING			CODE Office Use Only
		5 am - 11 am	11 am - 5 pm	5 pm - 5 am	
<b>MEAT, FISH, EGG OR POULTRY</b>					
EGG	1				10129
Cheese: Cheddar	1 oz				10009
Vegetable Oil	1 tbsp.				40518

### SECTION C: ACTIVITY RECORD

1. Use a worksheet (see sample) to **RECORD YOUR ACTIVITY FOR EACH DAY**, (that is, for 24 HOURS.) Activity levels explained below.

2. **TOTAL THE NUMBER OF HOURS SPENT AT EACH LEVEL.** The total must **EQUAL 24 hours.**

3. **WRITE THE TOTAL HOURS** spent at **EACH LEVEL** in the **ACTIVITY** section at the top of the **WHITE COMPUTER FORM.**

TIME OF DAY	LENGTH OF TIME	ACTIVITY	LEVEL*

ACTIVITY: Hours at each level 1. \_\_\_\_\_ 2. \_\_\_\_\_ 3. \_\_\_\_\_ 4. \_\_\_\_\_ 5. \_\_\_\_\_ \* 24.0 Hours  
Sleeping/Resting      Sitting/standing      Light Activity      Active      Very Active

**CHECK THAT YOU HAVE COMPLETED ALL SECTIONS OF THE GREEN PERSONAL FORM AND WHITE COMPUTER**

## EXAMPLES OF LEVELS OF ACTIVITY

### LEVEL 1

**SLEEPING**, resting

### LEVEL 2

Sitting, eating, watching T.V. **classwork**, **office work**, driving, playing quietly, playing a musical instrument, sailing, **STANDING**, personal toilet, teaching, laboratory work, **HOUSEWORK**, **SHOPPING**, cooking, bowling, pool

**LEVEL 3** (Count only the **TIME** that you are **ACTIVELY PARTICIPATING**)

**BRISK WALKING** (2-3 m.p.h.), **BICYCLING** (5.5 m.p.h.), farming, gardening, mechanical work, electrical trades, house-painting, restaurant trades, loading/stacking, carpentry, dancing, baseball, weight training, golf, **RECREATIONAL** swimming, table tennis, tennis, volleyball, canoeing or rowing

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**LEVELS 4 and 5:** Activities at these levels should cause you to raise your **HEART RATE**, to **BREATHE HARD**, to **PERSPIRE**, to use **ALL YOUR EFFORT**. Count only the **TIME** that you are **ACTIVELY PARTICIPATING**.

---

### LEVEL 4

**WALKING/JOGGING** (4.5-5 m.p.h.), hiking, **BICYCLING** (10 m.p.h.), digging, skipping, downhill skiing, recreational basketball, badminton or skating, canoeing (4 m.p.h.), competitive table tennis or tennis

### LEVEL 5

**RUNNING** (more than 5 m.p.h.), football, hockey, soccer, lacrosse, bicycling (13 m.p.h.), **COMPETITIVE** badminton, volleyball or basketball, racquet sports, vigorous skating or rowing, cross-country skiing, martial arts, swimming (45 yards/min. or more)





FOODS EATEN	Serving Size	NO. OF SERVINGS			CODE Office Use Only	FOODS EATEN	Serving Size	NO. OF SERVINGS			CODE Office Use Only
		5 am - 11 am	11 am - 5 pm	5 pm - 5 am				5 am - 11 am	11 am - 5 pm	5 pm - 5 am	
<b>VEGETABLES</b>					<b>DESSERTS, SWEETS, BAKED GOODS</b>						
COOKED: asparagus	4 sprs.				Jam, jelly, honey, syrup	1 tbsp.					
beans, green, yellow	1/2 cup				Molasses	1 tbsp.					
beets	1/2 cup				Sugar: white, brown	1 tsp.					
broccoli	1 cup				Sweet sauce, topping	2 tbsp.					
Brussels sprouts	8				CANDY: hard(6) caramels(3) marshmallows(4)	1 serv.					
cabbage	1/2 cup				chewing gum	1 piece					
carrots	1/2 cup				chocolate bar	1					
cauliflower	1 cup				chocolates	1					
chinese greens, bok choy	1 cup				CAKE: angel food	2 1/2 wedge					
corn, kernel (1/2 cup), cob (1 sm.)	1 serv.				fruit	2 1/2" x 1 1/2" x 1 1/2"					
eggplant	1/2 cup				plain, no icing	2 1/2" sq.					
mixed vegetables	1/2 cup				rich, with icing	2 1/2" sq.					
onions	1/2 cup				COOKIES: brownies, squares	1					
parsnips	1/2 cup				chocolate chip	1					
peas	1/2 cup				granola bars	2					
potatoes: baked, boiled, mashed	1 med.				oatmeal	1					
french fries, fried potatoes	1 cup				other, assorted	1					
scalloped	1/2 cup				Doughnut, Danish pastry, eclair	1					
sweet, yam	1 med.				Fruit crisp	1/2 cup					
spinach	1/2 cup				Jellied dessert, jello	1/2 cup					
squash: winter, yellow	1/2 cup				Pie (3 1/2" wedge), tart, turnover	1					
summer, zucchini	1/2 cup				Popsicle, ices	1					
tomatoes, baked, canned	1/2 cup				PUDDING: egg custard	1/2 cup					
turnips	1/2 cup				milk	1/2 cup					
RAW: salads: cabbage with mayonnaise dressing	1/2 cup				rice, tapioca	1/2 cup					
jellied vegetable	1/2 cup				Sherbet	1/2 cup					
potato, with dressing	1/2 cup				<b>BEVERAGES OR DRINKS</b>						
avocado	1/2				ALCOHOL: beer	12 oz					
carrots	1 med.				liqueur(1 oz), dessert wine(1 1/2 oz)	1					
celery	1 stalk				liquor	1 1/2 oz					
cucumber	7 slices				wine, table	4 oz					
green pepper, diced	1/4 cup				Carbonated, pop (non-diet)	10 oz					
lettuce, chopped	1 cup				Coffee	1 cup					
mushrooms	1/4 cup				Fruit flavoured drink (crystals)	1 cup					
onions, green	1				Hot chocolate (no milk)	1 cup					
radishes	2				Lemonade	1 cup					
sprouts: bean, alfalfa	1/4 cup				Postum, coffee substitutes	1 cup					
tomato	1 med.				Tea, (if iced, add 2 tsp. sugar)	1 cup					
JUICES: tomato, vegetable	1/2 cup				<b>SNACKS</b>						
					Corn chips or snacks, cheezies	1 sm. pkg.					
					Popcorn (with oil and salt)	1 cup					
					Potato chips	1 sm. pkg.					
					Pretzels	1 sm. pkg.					

FOODS EATEN	Serving Size	NO. OF SERVINGS			CODE Office Use Only	FOODS EATEN	Serving Size	NO. OF SERVINGS			CODE Office Use Only
		5 am - 11 am	11 am - 5 pm	5 pm - 5 am				5 am - 11 am	11 am - 5 pm	5 pm - 5 am	
<b>MEAT, FISH, EGG OR POULTRY</b>					<b>SOUPS, SAUCES, CONDIMENTS, OTHER</b>						
	1										
cod, halibut, sole; baked	3 oz				SOUPS: broth: consomme, plain	1 cup					
deep fried	6 oz				with vegetables	1 cup					
fish sticks	1				with noodles	1 cup					
fresh water fish	3 oz				chowder, clam (no milk)	1 cup					
salmon, tuna; baked	3 oz				cream soups, all (with milk)	1 cup					
canned	1/2 cup				lentil, split pea	1 cup					
shellfish: clams(5), oysters(9)	1 serv.				SAUCES: gravy	1/4 cup					
crab, lobster	3 oz				tomato	1/4 cup					
shrimp, scallops	3 oz				white, (if cheese, add 1/2 oz cheddar)	1/4 cup					
beef, veal; ground, hamburger pattie	3 oz				Mustard	1 tsp.					
roast	3 oz				Pickles (2 slices), relish (1 tbsp.)	1					
steak	6 oz				Soy sauce	1 tbsp					
stew meat	3 oz				Tomato ketchup	1 tbsp					
deli-style meats (1 slice)	1 oz				Lemon juice	1 tbsp					
lamb: roast, chop	3 oz				<b>COMBINATION DINNERS</b>						
liver, organ meats	3 oz				Baked beans with tomato sauce	1 cup					
liver pâté	1 oz				Chili with beans	1 cup					
luncheon meat, canned	1 oz				Chop suey with meat	1 cup					
pork: bacon (side)	2 slices				Macaroni and cheese	1 cup					
ham, back bacon	3 oz				Meat loaf	3 oz					
roast, chops (2 small)	3 oz				Meat pie (1 small, 3 1/2" wedge)	1					
sausages (4" link)	1				Pizza with cheese, tomato sauce	6" wedge					
spareribs	6 ribs				Spaghetti with cheese, tomato sauce	1 cup					
weiner (frankfurter)	1				Stew meat with vegetables	1 cup					
chicken; roast	3 oz				<b>FATS, CREAMS AND OILS</b>						
fried	3 oz				Butter, 1 pat	1 tsp.					
turkey: roast	3 oz				CREAM: coffee, 1 pkg.	1 tbsp.					
<b>BEAN ALTERNATES</b>											
beans, COOKED: kidney, red	1/2 cup				sour	1 tbsp.					
lima	1/2 cup				whipped	1/4 cup					
soy	1/2 cup				Coffee whitener, cream substitute	1 tsp.					
white	1/2 cup				Margarine	1 tsp.					
peas	1/2 cup				Vegetable oil	1 tbsp.					
beans: salted, roasted	1/4 cup				SALAD DRESSINGS: blue cheese	1 tbsp.					
unsalted, raw	1/4 cup				french, oil and vinegar	1 tbsp.					
nut butter	2 tbsp.				low fat, diet	1 tbsp.					
oils: sunflower, sesame	2 tbsp.				mayonnaise	1 tbsp.					
bean curd	2 oz				Thousand Island	1 tbsp.					

## Appendix D

Raw Data

Serum Ferritin Values ( $\mu\text{g/L}$ ) for each Subject at 0, 6, 12, 18,  
and 24 Weeks of the Study

Subject	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
#	(week 0)	(week 6)	(week 12)	(week 18)	(week 24)
1	14.7	24.5	31.5	36.3	27.3
2	10.7	24.9	27.6	9.8	24.0
3	4.8	17.4	20.0	15.5	28.1
4	17.4	78.8	34.6	62.0	39.7
5	14.9	35.9	47.9	28.2	37.1
6	16.8	19.9	25.8	18.1	21.8
7	2.1	5.0	12.2	7.8	7.7
8	7.5	18.5	28.4	24.0	18.5
9	18.8	34.7	22.6	38.6	52.6
10	10.0	39.2	54.6	42.5	39.2
11	3.8	25.2	18.1	16.4	8.5
12	18.7	29.1	41.3	42.1	57.6
13	14.2	25.9	17.1	44.3	59.3
14	6.7	32.9	47.0	36.5	23.6
15	12.7	19.1	32.0	27.6	30.1
16	7.8	15.7	22.2	20.1	39.4
17	17.4	43.8	41.7	46.3	12.0
18	16.7	26.6	48.5	28.5	11.7
19	13.1	29.9	70.1	44.4	61.0
20	4.0	12.2	15.0	12.7	11.0
21	16.2	14.9	22.2	17.2	11.6
22	4.3	17.9	11.5	12.0	6.5
23	9.1	20.3	24.0	50.2	33.6
24	12.9	20.9	18.0	14.8	14.6
Mean	11.5	26.4	30.6	29.0	28.2
SD	5.3	14.3	14.9	15.0	17.1

Serum Iron Values ( $\mu\text{mol/L}$ ) for each Subject at 0, 6, 12, 18 and 24 Weeks of the Study

Subject #	T <sub>0</sub> (week 0)	T <sub>1</sub> (week 6)	T <sub>2</sub> (week 12)	T <sub>3</sub> (week 18)	T <sub>4</sub> (week 24)
1	17.9	14.5	15.5	17.6	11.2
2	30.1	12.5	18.9	7.4	7.7
3	5.1	22.0	15.6	29.2	13.2
4	16.9	16.6	13.3	13.1	21.6
5	16.0	22.6	13.6	18.8	22.8
6	20.6	22.6	13.7	20.6	7.6
7	9.0	85.9	79.7	24.0	36.0
8	13.9	18.7	20.0	24.6	12.6
9	11.9	21.0	18.8	16.7	24.4
10	17.3	22.5	36.0	53.0	17.8
11	10.0	8.5	17.9	18.8	10.9
12	16.2	27.0	8.9	27.1	6.3
13	13.0	28.8	35.7	22.4	23.9
14	23.0	16.7	43.8	25.9	14.9
15	22.0	12.1	13.0	12.3	16.6
16	4.9	12.2	17.0	23.5	29.3
17	14.2	20.6	11.8	17.0	12.0
18	10.2	28.5	22.5	18.3	18.9
19	23.9	32.3	43.8	30.7	35.0
20	22.2	40.4	19.2	12.2	24.6
21	13.2	40.3	32.9	9.6	9.7
22	8.8	38.9	27.7	33.9	14.9
23	29.0	21.4	27.6	32.7	24.8
24	13.5	22.6	16.0	15.3	12.6
Mean	15.9	25.4	24.3	21.9	17.9
SD	6.7	15.6	15.4	9.8	8.3

Hemoglobin Values (g/L) for each Subject at 0, 6, 12, 18 and  
24 Weeks of the Study

Subject #	T <sub>0</sub> (week 0)	T <sub>1</sub> (week 6)	T <sub>2</sub> (week 12)	T <sub>3</sub> (week 18)	T <sub>4</sub> (week 24)
1	130	123	128	120	132
2	141	139	139	138	138
3	138	137	132	143	132
4	131	120	129	132	123
5	143	145	131	139	140
6	140	144	148	139	134
7	132	136	148	148	136
8	128	135	139	128	132
9	150	140	156	149	143
10	147	151	147	139	140
11	134	134	141	136	127
12	141	148	134	132	147
13	129	130	128	124	118
14	141	130	149	135	132
15	130	135	138	130	134
16	124	127	123	130	133
17	133	134	123	136	132
18	148	153	134	136	145
19	153	147	149	141	142
20	134	156	146	142	134
21	137	150	135	133	128
22	117	136	143	132	128
23	140	134	136	130	131
24	123	127	124	128	140
Mean	136.0	137.9	137.5	135.0	134.2
SD	8.9	9.6	9.3	7.0	6.9

Magnitude of Menstrual Blood Loss

Subject #	Saturation of Tampons/Pads			Duration of Menses (Days)		
	T <sub>0</sub>	T <sub>2</sub>	T <sub>4</sub>	T <sub>0</sub>	T <sub>2</sub>	T <sub>4</sub>
1	4.5	5.3	6.8	6	5	5
2	14.5	*	*	5	*	*
3	*	5.4	7.7	*	3	4
4	16.2	11.6	14.5	6	4	5
5	*	*	*	6	6	*
6	6.4	*	*	6	*	*
7	*	*	*	*	*	*
8	*	40.1	25.3	*	9	7
9	9.6	8.6	9.8	5	4	5
10	*	*	*	3	3	4
11	10.0	19.4	14.8	6	7	5
12	24.7	15.5	15.5	6	6	6
13	5.4	*	*	6	*	*
14	6.3	6.6	7.2	3	4	4
15	7.6	1.3	2.2	7	3	4
16	0.8	0.5	0.6	3	3	3
17	17.1	8.8	8.5	6	6	5
18	12.7	*	*	4	*	*
19	2.5	1.5	0.6	2	3	4
20	2.3	*	*	3	*	*
21	9.4	8.5	9.8	5	5	4
22	*	*	12.8	*	*	5
23	8.6	9.2	*	6	7	*
24	8.2	6.5	*	5	4	*
Mean	9.3	9.9	9.7	4.9	4.8	4.7
SD	6.0	9.8	6.7	1.4	1.8	0.9

\* = missing value.

Total Daily Dietary Iron Intakes (mg/day)

Subjects	T <sub>0</sub>	T <sub>4</sub>
1	15.3	11.3
2	11.3	*
3	*	*
4	14.4	14.8
5	14.2	13.3
6	*	*
7	13.8	*
8	14.2	15.9
9	9.3	12.8
10	10.4	7.3
11	15.0	*
12	15.7	*
13	30.1	19.0
14	10.7	11.2
15	14.2	13.0
16	17.5	*
17	24.9	9.9
18	*	*
19	12.8	15.4
20	8.5	*
21	13.8	*
22	12.3	9.1
23	*	*
24	*	*
Mean	14.7	12.8
SD	5.1	3.3

\* = missing value.