THE EFFECTS OF EXERCISE AND DIETARY IRON ON IRON STATUS IN 19 MONTH OLD ADULT FEMALE RATS

by

Charusheela M Chitale

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

HUMAN NUTRITION AND FOODS

APPROVED:

Charlotte Pratt

C.A. Pratt, Ph.D., Chairperson

Charles D Schlenker

E.D.Schlenker, Ph.D.

Mahmood A. Khan

M.A. Khan, Ph.D.

May, 1995
Blacksburg, Virginia

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(ABSTRACT)

This study investigated the effects of exercise and dietary iron on iron status in 19-month-old female rats. Fifty-seven female Sprague-Dawley rats were randomly assigned to one of five experimental groups: baseline (BL); iron sufficient-exercise (HE); iron sufficient-sedentary (HS); moderate iron deficient- exercise (LE) and moderate iron deficient-sedentary (LS). The six-week exercise protocol involved swimming 5 days/week beginning at 10 minutes/day and ending at 1 hour/day by the fifth and sixth week. The results indicated a significant effect of exercise on food intake and on body weight, with higher levels of intake and body weight in the LS group compared to the exercised groups. Soleus muscle weight was significantly lower than BL in the HS, LE and LS groups. Gastrocnemius muscle weight was significantly higher in the HS than in the LE group. Cardiac weights were comparable in the LE, LS and HE groups but significantly higher than the HS group. Cardiac citrate synthase activities were not significantly different among the groups. No significant effects of diet, activity or an interaction effect were noted on hemoglobin, serum iron and total iron binding capacity.

Hematocrit concentrations demonstrated a significant effect of activity, with elevated levels in the exercised groups compared with baseline. Serum iron levels were significantly lower in the LE group than the iron sufficient groups. The TIBC levels were significantly lower in the LE group than in the baseline. Liver iron concentrations showed a significant effect of diet, with higher concentrations in the iron sufficient groups compared to the LE group. Iron concentrations in the spleen and soleus muscle were unchanged however spleen iron concentrations were significantly higher in BL than in the experimental groups. Exercise had a significant effect on the gastrocnemius muscle with lower iron concentrations in the sedentary groups than in the exercised groups.

These results suggest a trend towards redistribution of iron stores as a result of exercise as evidenced by alterations in iron concentrations in some tissues and a trend towards elevated hemoglobin and hematocrit levels. Thus, dietary iron deficiency and exercise adversely affected various components of iron metabolism in the aged female rat although it was not manifested as iron deficiency.

ACKNOWLEDGEMENTS

I would like to express a special thanks to my parents, my family and especially Chitra , Rangu and Arun for their love, encouragement and support in making this possible.

My sincere gratitude and thanks to my advisor, Dr. C.A. Pratt for her advice, guidance and patience that allowed me to complete this project.

I also wish to express my appreciation and thanks to the members of my advisory committee, Dr. E.D. Schlenker and Dr. M.A.Khan, for their valuable input.

A sincere thanks to Kathy Reynolds for her guidance, support and patience which saw me through the entire research. And to Satya, thanks for your suggestions and support.

A heartfelt thanks to some very special people, Colette, JoAnn and Bob, Bruce, Ritu, Christina, Debbie and Doris Fields for their friendship, support and in the preparation of this document.

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INTRODUCTION

Within the past several years there has been increasing interest especially among older adults, in physical exercise (Anderson, 1985). Vigorous exercise has been promoted by various organizations such as the American Heart Association, the President's Council on Physical Fitness and by physicians and medical scientists for improved overall health (Holloszy, 1983). The recent report of the United States (U.S.) Department of Health and Human Services, "Healthy People 2000: National Health Promotion and Disease Prevention Objectives", indicates prioritizing maintenance of health and functional independence of older persons and includes increased physical activity as one of the specific objectives to improve the health status of the elderly (ADA report, 1993).

Characterizing the changing nature of the older adult population, the U.S. Census Bureau now uses three classifications instead of one: the young old, ages 65 through 74; the old old, ages 75-84; and the oldest old, ages 85 and beyond (Williams and Anderson, 1993). Projected demographic changes indicate that the percentage of people older than 65 years will increase to 21% of the population by the year 2030. A shift in the age of the population has occurred with persons living 85 years or longer making up the fastest growing segment of the elderly population (U.S Dept. of Health and Human Services, 1988; Chernoff, 1990). Overall, 5% of the elderly suffer from some form of nutritional anemias (Dwyer et al., 1990). Females age more successfully, and out survive the males

by 6.9 years (Morley, 1993). As the number of elderly females increases, the public health implications of exercise become extremely important. The American Dietetic Association (ADA) affirms that research on nutrition and aging has been neglected. (ADA report, 1993). Nationally, there has been a concern for exploring the changes in nutrient requirements with advancing age (ADA Report, 1993; Holloszy, 1993). A need for more studies on the long term effects of exercise on nutrient requirements as well as differences in adaptations to exercise training in the elderly has been expressed by various researchers (Aiken,1985; Munro, 1989). Thus, although the beneficial effects of exercise have been documented, the role of trace mineral deficiency and exercise on older persons needs further exploration.

Iron deficiency is the most common nutrient deficiency among all populations, particularly females, worldwide. Iron is also one of the important trace minerals that has come to the forefront in sports nutrition. Studies on the effect of exercise and iron intake in the older female are minuscule. Dietary analyses of women athletes have revealed iron intakes to be below the recommended daily allowances (RDA) (Jensen et al., 1991; Lloyd et al., 1992; Powell and Tucker, 1991). Exercise as a factor that may further aggravate iron status is now being considered, especially in females with reduced iron stores secondary to blood losses through menstruation, pregnancy and insufficient intake (Jensen et al., 1991; Lyle, 1992).

Iron is present in every living cell and it plays a role in many diverse biological processes. The primary function of iron is the transport of oxygen. As iron is an essential

component of hemoglobin (Hb) in the red blood cells (RBC), myoglobin (Mb) in the muscles, and various enzymes required for energy production, the effect of exercise on iron metabolism is of prime importance. Anemia associated with intensive training has been frequently described in the Japanese literature (Yoshimura, 1970). The term "Sports Anemia" was coined to define the drop in hemoglobin that occurs in response to acute strenuous exercise. Female athletes in particular, frequently exhibit subnormal levels of hemoglobin and sports anemia (Sherman and Kramer, 1990). Iron depletion in athletes has been attributed to a low dietary intake of iron and iron losses through feces, sweat or menses, increased hemolysis and hematuria, and blood loss from the gastrointestinal system (Clement and Asmundson,1982; Magnusson et al., 1984 a). Iron deficiency anemia decreases work capacity through combined effects on maximal oxygen uptake and muscle metabolism. Diminished function is proportional to the degree of anemia (Newhouse and Clement, 1988). Iron deficiency, with or without anemia, has been associated with reduced performance, fatigue and a compromised iron status (Clement and Asmundson, 1982; Clement and Sawchuk, 1984; Jensen et al., 1991). A study by Borel et al. (1991) suggested the adverse manifestations of iron deficiency anemia in which mild to moderate anemia was observed. Several investigators have reported iron deficiency and/or depletion in athletes. Reduced hemoglobin, hematocrit, serum ferritin, transferrin saturation and total iron binding capacity (TIBC) were reported in athletes by Clement and Asmundson (1982), Frederickson et al. (1983), Magnusson et al. (1984), Parr et al. (1984) and Haymes (1987).

Animal models have provided evidence of iron depletion with exercise training. In exercising rats fed adequate amounts of iron, a reduction in iron stores in the liver, spleen and cardiac muscles were observed in comparison with their sedentary counterparts (Strause et al., 1983; Ruckman and Sherman, 1981). Gagne et al. (1994), demonstrated the effects of exercise on iron metabolism using the adult female Sprague-Dawley rat model. The study, using 6-8 month old female rats suggested that exercise affects various parameters of iron metabolism with alterations in distribution of iron stores. Exercise training combined with low iron intake appeared to enhance early characterization of a latent iron deficiency. Prasad and Pratt (1990) used the same model in 13-month-old female rats and observed low iron stores in the liver, spleen, soleus and gastrocnemius muscles of the exercising female rats. Their results suggest that there may be an iron cost associated with physical training. Most of the studies documenting effects of physical training on iron status have involved young male animals and athletes. Research on tissue iron stores during moderate physical activity is minimal. It is unknown whether a reduction in iron intake alone or with exercise training affects various components of iron metabolism in the mature animal model. The objective of this study was to determine the effects of exercise training and iron intake on the hematological and tissue iron parameters in the 18-20 month old adult female rat. Additionally, changes in the activity of the respiratory enzyme, citrate synthase, were examined.

This research builds on earlier work of Gagne et al. (1994) and Prasad and Pratt (1990), by examining older mature animals. The emphasis on the categorization of the

population into the young old, old old and oldest old, calls for research in all the categories of older adults. This research will also broaden our knowledge of the effects of moderate exercise on the tissue stores of one of the most important trace minerals, iron. The following hypotheses were examined: (1) 19-month-old female rats fed an iron sufficient diet (42 ppm) will demonstrate higher tissue iron stores and hematological parameters than those fed an moderate iron deficient diet (7 ppm). (2) Exercising (19-month-old) rats fed either an iron sufficient or a moderate iron deficient diet will have lower tissue iron stores and hematological parameters than the sedentary rats fed either an iron sufficient or a moderate iron deficient diet. (3) Citrate synthase, a marker enzyme of the Krebs cycle will be elevated in the cardiac muscle of the exercised rats than in the sedentary rats.

REVIEW OF LITERATURE

OVERVIEW OF IRON METABOLISM

Iron, an essential trace element was first recognized as a constituent of body tissues in 1713 and a vital nutrient for animals in the 1860's (Czajka-Narins,1984; Guthrie,1986). Present in all cells of the body, it is a key component of several enzymes responsible for electron transport (cytochromes) and plays an important role in the transport (as hemoglobin, myoglobin) and activation of oxygen (as oxidase and oxygenase) (Hallberg, 1984). Iron deficiency can therefore affect several metabolic functions related to the production of energy (Grandjean and Ruud,1990).

The newborn infant at term has an iron (fe) content of about 250-300 mg (75 mg/kg body weight) which increases to about 3-5 gm in a normal (70 kg) adult man (about 40-50 mg of fe/kg body weight and about 35-50 mg for the female) (Czajka-Narins, 1984; Hallberg, 1984; Mahan and Arlin, 1992; Williams and Anderson, 1993). Age, sex, size, nutritional status, general health and the size and amount of iron stores affects the total iron content of the body (Guthrie, 1986). Most of the body iron exists in complex forms bound to protein either as porphyrin or heme compounds or as ferritin and transferrin. Free inorganic iron occurs in the body only in very small amounts (Swaminathan, 1985). Iron stores in men and women amount to about 1000 mg and 300-

400 mg respectively (Guthrie, 1986; Haymes, 1987). Approximately 90% of iron is recovered and reused by the body (Mahan and Arlin, 1992).

DISTRIBUTION AND FUNCTIONS OF IRON IN THE BODY

Body iron, the distribution of which is presented in Table 1 can be divided into two components:

(i) essential or functional iron including the transport iron which comprises two-thirds of iron in body and

(ii) storage iron which comprises one-third of the remaining body iron (Goodhart and Shils, 1980; Guthrie, 1986).

Iron status is best characterized in relation to the amount of iron contained in the storage and functional compartments (Bothwell et al., 1979). Hemoglobin, the main component of red blood cells (RBC), is a conjugate of a protein (globin) and four molecules of iron and accounts for over two-thirds (60-70%) of the total body iron (Gibson, 1990). About 3-4 mg of iron is present in the plasma bound to the carrier protein transferrin for transport between sites of absorption, storage and utilization. Transferrin turnover is rapid and up to ten times the 4 mg amount is exchanged each day (Guthrie,1986; Hallberg, 1984).

TABLE 1. DISTRIBUTION OF IRON IN THE BODY

	Approximate amount(mg)		
	Total (%)	Men	Women
Hemoglobin	60-75	2100	1750
Myoglobin	3	10	100
Storage iron (liver, spleen, bone marrow)	0-30	1000	400
Tissue iron (enzymes)	5-15	350	300
Transport iron (transferrin)	1	4	4
Serum ferritin	1	0.3	0.1
TOTAL		3554.3	2554.1

Source : Guthrie (1986).

Myoglobin, which serves as a reservoir of oxygen for muscle metabolism comprises another 5% of total body iron. Structurally similar to Hb, it has one molecule bound to protein instead of four. The remainder of the total body iron is distributed throughout the cells as a major component of the intracellular heme enzymes or as a cofactor in enzymes. These enzymes are involved in energy metabolism and in the process of cellular respiration through the transfer of electrons in the electron transport system (Czajka-Narins, 1984; Goodhart and Shils, 1980; Williams and Anderson, 1993).

Storage iron, located primarily in the parenchymal cells of the liver and in smaller amounts in the reticuloendothelial cells of the bone marrow, liver and spleen, makes up the remaining 25% (about 200-1500 mg) of iron (Bothwell et al., 1989; Czajka-Narins, 1984). Designed for the reversible storage of iron, it functions as an iron reserve to replenish deficits in functional iron (Cook and Skikne, 1989; Hallberg, 1992). Approximately, two-thirds of the storage iron consists of ferritin, a soluble iron protein complex that is one-fifth iron whereas hemosiderin, an insoluble iron protein complex which is one-third iron, makes up the remainder. The ferritin to hemosiderin ratio in the liver varies according to the total amount of iron stored. A small amount of ferritin is present in the blood in amounts paralleling storage iron (Czajka-Narins, 1984; Gibson, 1990; Guthrie, 1986).

IRON METABOLISM IN HUMANS

Iron metabolism can be described as two loops; an internal loop represented by formation and destruction of red cells and an external loop represented by the losses of iron from the body and the absorption of dietary iron. Internal iron metabolism is mainly involved with a recycling of iron from the catabolized red cell mass (Hallberg, 1984). The released heme iron is transported by the carrier protein, transferrin, to the bone marrow for formation of Hb in the new RBC. Iron balance in the body is maintained by the consistent reuse of internal iron stores from the catabolized red cells, through access to the storage protein, ferritin, and through regulation of the absorbed iron from the intestines (Hallberg, 1992; Rucker, 1991). Body iron follows a unique system of interrelated absorption, transport, storage and excretion. An overview of iron metabolism is presented in Fig 1.

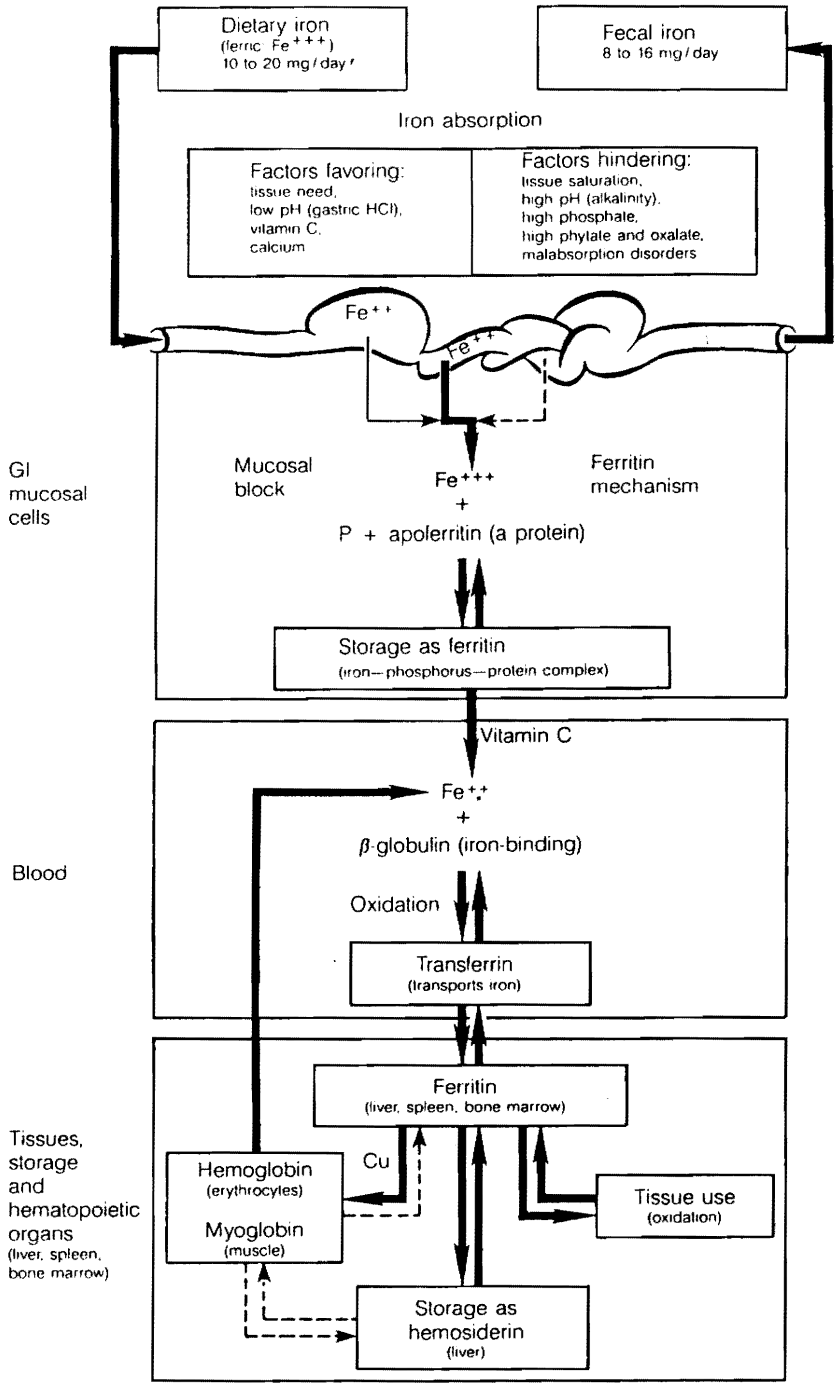


FIG.1: SCHEMATIC OUTLINE OF IRON METABOLISM IN ADULTS

SOURCE : Williams and Anderson (1993).

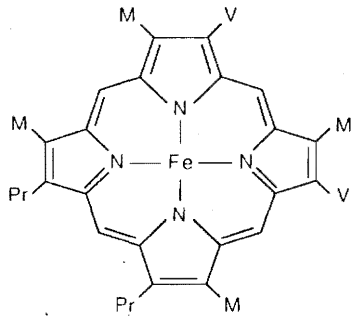
IRON ABSORPTION

Dietary iron is digested and processed in the intestinal lumen and taken up across the mucosal brush border membrane. It is then transported through the intestinal absorptive cells and across the basolateral membrane to the sites of iron metabolism and storage within the body. However, details regarding the regulatory mechanisms controlling iron absorption still remain unclear (Flanagan,1989). Mucosal transferrin excreted in the bile acts as a shuttle protein in facilitating iron absorption. It picks up iron in the intestinal lumen and takes it to the surface of the intestinal cells where it binds to the transferrin receptor, releases the iron into the cell, and returns to the lumen for more iron. Within the mucosal cell the iron may combine with apoferritin to form ferritin for temporary storage (Mahan and Arlin, 1992; Monsen, 1988). Dietary iron absorption depends on the meal composition and iron content, the ability of the upper small bowel to absorb iron as well as the iron status of a subject- with increased absorption in the iron deficient (Bothwell et al., 1989; Hallberg, 1992). A daily absorption of 0.9 mg of iron is sufficient for the average adult (Monsen, 1988).

Food iron occurs primarily in the oxidized form as ferric (Fe+++) iron or as the reduced ferrous (Fe++) iron. The reduced form, ferrous iron is better absorbed. Iron can further be classified as heme iron (from cellular animal tissue) or nonheme iron (from plant sources). On absorption into the mucosal cell the two pools of dietary iron (heme and nonheme) form one common pool of biological iron (Flanagan, 1989; Mahan and Arlin,

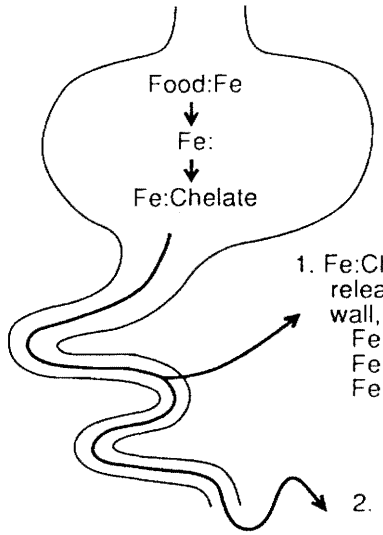
1992; Monsen, 1988). Both forms as shown in Fig 2 below have different mechanisms of absorption. Nonheme iron absorption ranges from 2-20% and is influenced by bioavailability and the interplay of promoters and inhibitors in the diet. Ingestion of enhancing factors along with a meal of low iron availability can quadruple the absorption of nonheme iron (Monsen and Balintfy, 1982). Major enhancers of nonheme iron absorption from the common pool are meat and organic acid including citric acid, lactic acid and particularly ascorbic acid. Inhibitors of nonheme iron absorption include tannic acids, tea, certain salts such as EDTA and large quantities of calcium phosphate, phosvitin in egg yolk, antacids, phytates, polyphenols, dietary fiber complex, calcium and phosphorus (Bothwell et al., 1989; Monsen and Balintfy, 1982). However, results of a study by Cook et al. (1991) indicate that with the varied western diet, the significance of nonheme iron bioavailability in influencing iron status becomes less important. Heme iron, being highly bioavailable, has an absorption range of 15-35% which is inversely related to the quantity of iron stores, and influenced only minimally by intraluminal factors or meal composition (Bothwell et al., 1989; Mahan and Arlin, 1992; Monsen, 1988). Iron from heme is well absorbed with meat or soy protein (Bothwell et al., 1989). In a reference individual, about 23% of heme iron is absorbed while the nonheme iron absorption ranges from 3-8% (Monsen, 1988).

I. HEME IRON



Absorbed as intact protoporphyrin molecule

II. NONHEME IRON



Food Fe is released and rapidly chelated

1. Fe:Chelates which release Fe at gut wall, e.g.
   Fe:Ascorbic Acid
   Fe:Amino Acid
   Fe:Intermediary products of digestion

2. Fe:Chelates which remain chelated & are excreted, e.g.
   Fe:Ca:PO4
   Fe:Tannate
   Fe:Intermediary products of digestion

FIG. 2: HEME AND NONHEME IRON ABSORPTION

SOURCE: Monsen, E.R. (1988).

IRON EXCRETION

The excretory capacity of the body for iron is very limited and is regulated through the process of absorption with only minute losses through renal excretion (Williams and Anderson, 1993). The balance of iron nutrition is determined by the amount of iron excreted and the amount of iron absorbed (Monsen, 1988). Iron is excreted from the body via the feces, sweat, the exfoliation of hair and skin, and through the normal gastrointestinal (GI) and menstrual blood loss. Iron excretion in the urine is minimal (Mahan and Arlin, 1992; Williams and Anderson, 1993). Iron excreted in the feces consists of unabsorbed food iron, iron from blood lost into the alimentary canal, unabsorbed biliary iron, and iron from desquamated mucosal cells (Goodhart and Shils, 1980).

IRON LOSSES AND REQUIREMENTS

Basal physiological losses of iron from the body through the skin, GI and urinary tract in a healthy adult are estimated to be 1 mg/d (14 ug/ kg/d) (Bothwell et al., 1989; Green et al., 1968; Hallberg, 1992). Losses in the urine, perspiration and exfoliation of cells vary from 0.2-0.5 mg/d, with 0.7 mg/d lost through the feces (Guthrie, 1986). Iron losses through sweat have been found to be negligible (Brune et al., 1986). Pathological losses of iron through bleeding may also increase the iron requirements of an individual

(Bothwell et al., 1989). Losses in infants and children may be proportionately greater than in adults because of the higher surface area of the GI tract and skin relative to body weight (Bothwell et al., 1989). Iron requirements for a child between 1-2 years of age are 0.4 and 0.3 mg/day, respectively, with increased requirements of 0.5-0.8 mg/day during the remainder of the childhood. The growth spurt of adolescence with increased Hb concentration and obligatory iron losses results in a daily iron requirement of about 1.6 mg (Bothwell et al., 1989; Hallberg, 1992). To meet the physiological requirements for iron, the daily recommended dietary intakes for infants and children have been set at 6-10 mg/day and 10-12 mg/day, respectively, while the recommended daily allowances (RDA) for adult males is 10 mg/d (NRC, 1989; Williams and Anderson, 1993). Table 2 presents RDA for iron.

TABLE 2: RECOMMENDED DIETARY ALLOWANCES FOR IRON

Category	Age (years)	Iron (mg)
Infants	0.0-0.5	6
	0.5-1.0	10
Children	1-7	10
	7-10	10
Males	11-18	12
	19-50	10
	51+	10
Females	11-18	15
	19-50	15
	51+	10
Pregnant		30
Lactating	1st 6 months	15
	2nd 6 months	15

Source: National Academy of Sciences, Washington, D.C. (1989).

IRON LOSSES IN WOMEN

Iron losses through menstruation along with the obligatory iron losses of 0.8 mg/day in a 55 kg adult female account for an additional 0.6 mg/day. Losses of greater than 1.4 mg/d have been reported in approximately 5-10% of women (Bothwell et al., 1989; Hallberg, 1984; Mahan and Arlin, 1992). Total iron losses in an adult female therefore may amount to around 2 mg/d (Bothwell et al., 1989; Hallberg, 1984). Oral contraceptives decrease menstruation losses by 50% while intrauterine devices increase losses by 100% (Cole et al., 1971). The RDA for female adolescents is 15 mg/day. In addition to the normal requirements during pregnancy, an expansion in the hemoglobin mass requires 0.5-1.5 mg of absorbed iron per day. Formation of the placenta, cord and fetus requires another 400 mg of total iron over the course of the pregnancy. Blood loss at delivery (usually 500-600 ml) requires an additional 300-350 mg of total iron. Thus the total cost of pregnancy, in excess of the normal basal requirement of 1 to 2 mg of absorbed iron per day, is equal to approximately 4 mg/day (Rucker, 1991). Iron losses during lactation amount to 0.15-0.3 mg/day. Since the increased requirements of pregnancy cannot be met by diet alone, a daily iron supplement of 30 mg elemental iron is recommended. The RDA for pregnancy and lactation is therefore set at 30 mg/day and 15 mg/day respectively (NRC, 1989).

IRON DEFICIENCY

Iron deficiency is one of the most common nutritional deficiencies in both developing and developed countries. Causes of iron deficiency have been ascribed to a variety of factors such as a deficient diet, periods of accelerated demands, increased iron losses or inadequate absorption. It has been defined as the state in which body iron stores are completely exhausted, providing less iron than is required for the normal formation of Hb, iron enzymes and other functioning iron compounds (Beutler, 1988; Cook and Skikne, 1989). Severe iron deficiency is characterized by microcytosis while mild iron deficiency is often normocytic (Beutler, 1988). Iron deficiency without anemia is a condition that precedes iron deficiency anemia. The criterion for the diagnosis of iron deficiency anemia is a Hb concentration more than 2 SD below the mean (12 gm/100ml for nonpregnant women and 13 gm/100 ml for men) as well as evidence of iron deficient erythropoiesis and iron depletion (Haymes, 1987). However a decreased Hb concentration alone cannot be classified as iron deficiency anemia (Haymes, 1987). Abnormalities of iron deficiency have been identified in population groups such as pregnant women, infants and children, and women of child bearing age. Such abnormalities contribute to a decreased work capacity and less efficient response to exercise (Dallman, 1989). Small functional iron deficits have also been associated with liabilities relating to a decrease in hematological symptoms, impaired attention span, poor growth, alteration of temperature regulation, immune function and a reduced work

capacity and other fundamental metabolic activities (Cook and Skikne, 1989; Hercberg and Galan, 1989).

Onset of iron deficiency anemia follows a progression of three stages. Depletion of the body iron stores characterize the first stage known as iron depletion. A linear relationship exists between the ferritin level and total iron stores (Beutler, 1988). Herbert (1993) further splits this Stage I of negative iron balance/iron depletion into two stages: inadequate absorption may produce a moderate depletion of iron reserves (stage I) and/or a severe depletion of iron reserves, with no dysfunction (stage II). Stage II occurs when iron depletion progresses to iron deficiency without anemia and is known as iron deficient erythropoiesis. This deficiency may be recognized by an assay of plasma iron supply to erythropoietic cells (reflected by decreased transferrin saturation) and of iron availability for Hb synthesis (reflected by increased erythrocyte protoporphyrin - the base compound to which Fe is attached to make heme) (Expert Scientific Group, 1985). With continuous iron deficiency, erythrocyte Hb falls, characterizing the third and final stage known as iron deficiency anemia and identified by a decrease in Hb or hematocrit (Hct) concentration and a decrease in mean corpuscular volume (MCV) (Haymes, 1987; Expert Scientific Group, 1985). Evaluation of the second National Health and Nutrition Examination survey (NHANES II) (1976-1980) by the Expert Scientific Work Group (1985) reported the highest incidence of iron deficiency in 11-14 year old boys (12.1%) and 15-19 year old girls (14.2%). Fig 3 presents the stages of iron status from normal through the stages of negative and positive iron balance for an adult man.

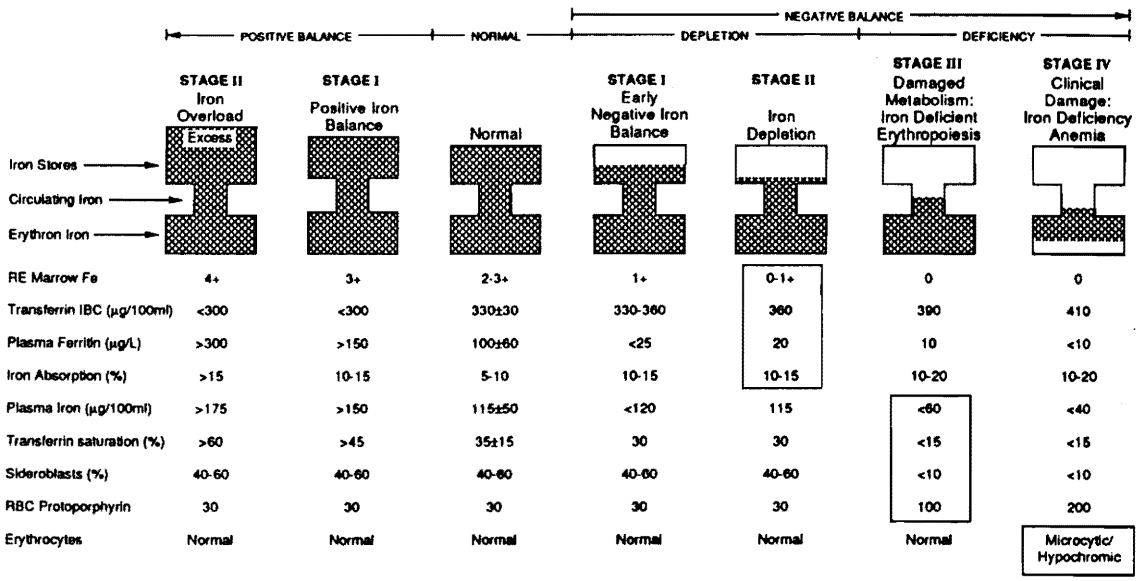


FIG. 3 SEQUENTIAL STAGES OF IRON STATUS

Source : Herbert (1992).

INDICATORS FOR EVALUATION OF IRON STATUS

Iron measurements are classified as those that indicate either residual iron in the storage compartment or depleted iron in the functional compartment. Table 3 presents the laboratory measurements of iron deficiency. Measurement of the concentration of Hb in whole blood is the most widely used screening test for iron deficiency anemia. Hematocrit, defined as the volume fraction of packed red cells, is reduced along with Hb in severe iron deficiency anemia. Red cell indices such as MCV, mean cell hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH) derived from measures of Hb, Hct and RBC count provide additional information in the diagnosis of anemia (Gibson, 1990). One of the most important indices in the diagnosis of iron deficiency is the free erythrocyte protoporphyrin (FEP) concentration. It is elevated when there is insufficient iron to complex with all the protoporphyrin synthesized in the red blood cell precursor (Beutler, 1988). A reduction in MCV below 80 fl has the same diagnostic specificity as elevated FEP levels (Cook and Skikne, 1989). Determination of the plasma iron level and iron binding capacity is one of the oldest and most useful methods for evaluation of body iron stores because the bone marrow examination, though the best way to diagnose iron deficiency, is both an uncomfortable and costly method (Beutler, 1988).

TABLE : 3. LABORATORY MEASURES OF IRON DEFICIENCY

	Measurements	Diagnostic range
a)	Storage Iron	
	Bone marrow examination	Absent
	TIBC	> 400 ug/dl
	Serum ferritin	< 12 ug/l
b)	Functional Iron	
	Transferrin saturation	< 16%
	Erythrocyte protoporphyrin	> 70 ug/dl RBC
	MCV	< 80 fl
	Serum Iron	< 60 ug/dl
	Anemia (Hemoglobin)	
	Adult male	< 13 g/dl
	Nonpregnant female	< 12 g/dl

Source: Cook and Skikne (1989).

When bone marrow stores are depleted serum ferritin levels fall below 12 ug/l. Iron stores can be estimated from the serum ferritin as 1ug/l of serum ferritin = 8 mg of storage iron (Haymes, 1987). Transferrin saturation is a measure of iron supply to the tissues. Increased saturation of iron binding capacity above the normal laboratory range indicates increased iron absorption (Herbert, 1993). Serum iron falls below 60 ug /dl after the iron stores are depleted. Transferrin increases when iron stores are depleted and higher concentrations are an indication of iron deficiency. Transferrin saturation, the ratio of serum iron to transferrin, falls below 16% in iron deficiency (Gibson, 1990). Beutler (1988) advocates the use of the absolute level of the unsaturated iron binding capacity (UIBC) as a guide, a level of over 54 umol/l (300 ug/dl) indicates sure iron deficiency.

IRON STATUS IN THE ELDERLY

Although anemia is the most common problem associated with aging, data on iron nutriture in the elderly are limited. Iron deficiency is second to anemia of chronic disease, as the most common cause of anemia in the elderly. In women older than age 59, iron deficiency occurs as frequently as in women of child bearing years (Ahmed, 1992; Guyatt et al., 1990; Lipschitz, 1991; Lynch et al., 1982). Inadequate intake, gastrointestinal blood losses and changes in iron absorption secondary to atrophic gastritis are the most common causes of iron deficiency in the elderly (Lipschitz, 1991; Lynch et al, 1982). The presence of anemia in the elderly may be a result of an age related reduction in hematopoietic cell reserves (Ahmed, 1992; Garry et al 1982). There is a progressive decline throughout the adult life in many physiological functions accompanied by changes in body composition and in the metabolism of nutrients. An age dependent decline in energy intake can thus lower iron intake (Finch and Schneider, 1985; Zheng et al., 1989). Changes in gastrointestinal function such as decreased taste and hypochlorhydria due to atrophic gastritis occur with aging. The decreased gastric acid secretion can impair digestion and absorption of dietary ferric iron (Russell, 1992). However, the bulk of evidence has indicated that healthy elderly can remain in iron balance on intakes below the RDA and suggests that iron absorption does not decline significantly with normal aging (Marx, 1979). Munro and Danford (1989) compared iron absorption in active elderly persons and young adults, all with normal iron status, and concluded that the aged may

have decreased red cell uptake of iron which may contribute to anemia in the presence of adequate iron stores.

The RDA for iron for men and women 51 years or older is 10 mg (NRC, 1989). Many elderly over 55 years of age consume less than the RDA of iron and Ahmed (1992) reports that surveys conducted in the U.S. showed that about 5% of men and women over 45 years of age had insufficient levels of stored iron and about 2% showed apparent iron deficiency anemia. In elderly men and women the sources of dietary iron may change from meat to the less available iron of cereals (Lynch et al., 1982). Koehler et al. (1992) studied nutrient intake and the meat, poultry and fish consumption in the healthy elderly by examining three day diet records. The mean dietary energy intake was 82% and 94% of the RDA for women and men respectively. However, iron intake was above 100% of the RDA. Thus, a moderate but not excessive use of meat, poultry and fish made a substantial contribution to nutrient intake. Morley (1993) states that the total energy expenditure and food intake are reduced only minimally in the older female compared to younger females. It has been suggested that a low dietary iron intake and anemia may not be a consequence of aging. Although Boykin et al. (1976) found that Hb levels and food habits reflected inadequate dietary iron intake in postmenopausal women, the low intake was similar to that seen in the adolescent female population.

Additionally, Garry et al. (1983), on examining the biochemical measures of iron status in healthy elderly persons also concluded that anemia or impaired iron status was no more prevalent in the elderly than in younger adults when identical criteria were used for

assessment. Butterworth et al. (1993) examined the relationship between moderate exercise training and changes in energy and nutrient intake in elderly women at risk for suboptimal intake. They reported that energy intake was 20% below the RDA in the sedentary elderly subjects while the highly active women averaged more than 100% of the RDA and demonstrated better nutrient intake.

In older men and women, bone marrow iron stores and serum ferritin concentrations increase with age. In older women following menopause, iron balance improves with an increase in body iron stores from an average of 300 mg to 800 mg. In older men, tissue iron stores average 1200 mg. There is a threefold increase in mean serum ferritin levels which reflect the difference in iron stores between adult men and women. Thus, the increased serum ferritin may represent a further increase in body iron stores, a higher prevalence of inflammatory disorders in the elderly or a gradual redistribution of iron from the functional to the storage compartment (Cook and Skikne, 1989; Lipschitz, 1991; Lynch et al., 1982; Schlenker, 1992). In the elderly, the body's need for iron is determined primarily by the rate of excretion since growth and menstruation losses no longer occur. It is difficult to measure total body iron excretion which involves losses from the bowel, skin, and urine. Iron losses in older men and women do not exceed those in the younger population. Iron losses of less than 1.0 mg/d occur in older men and postmenopausal women (Lynch et al., 1982; Russell, 1992; Schlenker, 1992).

Hemoglobin concentration in healthy elderly men and women remains unchanged

until the age of 70, after which there is a significant fall in men while in women it appears to remain stable into the mid eightys (Lynch et al., 1982; Yip et al, 1984). Lipschitz et al. (1981) found 21.4% of healthy elderly females had decreased Hb and Hct levels. The authors concluded that the overall reduction in hematopoietic reserves may be due to a direct effect of senescence.

An analysis of the NHANES II survey demonstrated a significant reduction in Hb levels with advanced age in men and a minimal although significant decrease in elderly women (Yip et al., 1984). Garry et al. (1983) found that none of the elderly women, in a study assessing the iron status of the elderly, had Hb levels below 12.0 g/dl. Documenting poor iron status in the elderly may need the use of criteria other than the Hb/Hct measures. Yip et al. (1984) suggest the development of an age specific criteria or a reference standard for use in the elderly. Joosten et al. (1993) found iron deficiency was a significant problem in anemic and nonanemic elderly inpatients and suggested serum ferritin levels be part of the routine biochemical investigation of elderly inpatients.

Serum ferritin levels below 45 ug/l and a transferrin saturation ratio of less than 11% have been suggested as better cut off limits to identify iron deficiency in the anemic geriatric population (Guyatt et al., 1990; Patterson et al., 1985). The TIBC levels also decrease with aging and are affected by chronic disease, more common in the elderly. The Iron/TIBC ratios are higher in males than in females and remain so in the elderly (Joosten et al., 1993; Yip et al., 1984).

Physical exercise and training for improved overall function in the elderly has been well promoted (Astrand, 1992). However research on the effects of dietary iron deficiency and exercise in the aged is minuscule. Ericsson (1970) studied the effect of an oral iron supplement (120 mg/d) on the physical work capacity of men and women, aged 58-71 years. The elderly subjects showed an increase in work capacity on iron supplementation without any significant changes in Hb or serum iron concentration. The author concluded that in healthy people, the increase in physical work capacity during moderate training is related to the availability of iron.

EXERCISE AND IRON METABOLISM

Dallman (1989) postulates that impaired work performance, as a manifestation of iron deficiency occurs as a result of decreased hemoglobin concentration or decreased muscle oxygen consuming capacity. Studies reviewed indicate that due to both the direct and indirect role of iron in sustaining the physiologic processes involved in physical activity and exercise performance, there is an iron cost and increased risk of iron deficiency associated with exercise (Clement and Sawchuk, 1984; Lyle, 1992; Prasad and Pratt, 1990; Sherman and Kramer, 1990; Szygula, 1990). Iron deficiency in athletes has been ascribed to inadequate dietary intake (Sherman and Kramer, 1990), impaired absorption (Ehn et al 1980), increased iron losses (Pate, 1983), hemolysis (Hunding et al., 1981; Clement and Asmundson, 1982; Hallberg, 1984), increased skeletal myoglobin (Strause et al., 1983), hemodilution (Weaver and Rajaram, 1992) and a shift in red cell catabolism from the reticuloendothelial system to the hepatocytes as proposed by Hallberg (1984).

Intensification of the erythropoietic processes and alterations in the hematological and iron parameters occurs with exercise and intense physical training (Szygula, 1990; Spodaryk, 1993). Effect of exercise on iron metabolism as indicated by compromised iron status, suboptimal Hb concentrations and presence of iron deficiency in athletes involved in physical exercise and intense training activity has been observed (Clement and Asmundson, 1982; Edgerton et al., 1972; Ehn et al 1989; Magazanik et al., 1991;

Magnusson et al 1984b; Weaver and Rajaram, 1992). Various animal and human studies suggest that iron deficiency with or without anemia can affect maximal physical performance or endurance capacity (Clement and Sawchuk, 1984; Hunding et al., 1981; Klingshirn et al., 1992; Pate, 1983; Perkkio et al., 1985).

IRON HEMATOLOGY

Hematological adaptations to exercise are expressed as a decreased Hb concentration or an increased red cell mass, especially in the elite athlete (Eichner, 1986). Reduced Hb levels in athletes have been attributed to iron deficiency, increased plasma volume and red cell hemolysis, or an increased concentration of 2,3 diphosphoglycerate (DPG) (Newhouse and Clement, 1988; Spodaryk, 1993). Decreased Hb values occur as a physiological adaptation, secondary to plasma volume expansion resulting from intense training (Eichner, 1992; Magnusson et al., 1984a; Pate et al., 1993, Sherman and Kramer, 1990; Spodaryk, 1993; Watts, 1989). Use of hematological parameters such as serum iron, TIBC, red cell protoporphyrin, transferrin saturation measures and iron stores, besides Hb and Hct, have been suggested as discerning parameters for iron deficiency screening in athletes (Dalongville et al, 1989; Grandjean and Ruud, 1990).

Yoshimura (1970) first suggested an occasional transient anemia with the onset of chronic endurance training. This sports anemia or pseudoanemia characterized by a

reduction in individual Hb, packed cell volume (PCV), red blood cell counts (Eichner, 1992; Hunding et al., 1981; Sherman and Kramer, 1990), has been defined as a Hb concentration below that which is optimal (Hb ≤ 15-16 g/100 ml) for oxygen delivery (Pate, 1983). To determine the presence of sports anemia, Hunding et al. (1981) found systemic iron deficiency in 56% of men and women joggers and competitive runners aged 18-78 years of age. Forty percent of the women and 13% of the men had latent anemia, characterized by Hb between 12-13 g/dl. On assessing the iron status of 8 crosscountry runners during and post season, Frederickson et al. (1983) found a 8.8% transient decrease in Hb concentration and an 8.3% decrease in PCV concentration compared with nonrunners.

Reports examining Olympic teams and athletes involved in endurance training have found decreased Hb and Hct values indicative of latent anemia, as well as the occurrence of anemia in a small percentage of athletes (Clement and Asmundson, 1982; Clement and Sawchuk, 1984; Pate, 1983). Ten percent of males had clinical anemia and a substantial number of men and women long distance runners showed evidence of real or latent iron deficiency (Clement and Asmundson, 1982). Training-induced changes in hematological parameters such as iron, Hct, and ferritin have been noted to affect performance in female athletes (Blum et al; 1986; Frederickson et al., 1983; Lyle, 1992; Manore et al., 1989; Snyder et al., 1989). Endurance trained athletes have a Hb level about 0.5 g/100 ml lower than untrained controls (Watts, 1989). Studies reviewed by Newhouse and Clement (1988) found a high incidence of decreased Hb values indicative of reduced iron status in

athletes. Other reports indicate that suboptimal Hb concentrations are more typical of the male endurance athlete, (Balaban et al., 1989; Newhouse and Clement, 1988) and upto 80% of the elite women endurance athletes are iron deficient (Clement and Asmundson, 1982; Eichner, 1992). Incidence of iron deficiency anemia in athletes may be more than twice what is normally expected (Haymes, 1987). However Balaban et al. (1989) compared the hematological parameters of male and female runners and concluded that iron depletion and anemia are not common events and that iron status in runners is similar to that of the general population. Iron deficiency anemia and other less deficient iron states occur occasionally in physically active persons (Clement and Asmundson, 1982; Ehn et al., 1980; Lampe et al., 1986; Parr et al., 1984; Risser et al., 1988). Subclinical iron deficiency may impair athletic performance and prolong injury healing times (Loosli, 1993; Rowland et al., 1988). Compromised performance due to an iron deficiency anemia has been noted in the endurance athlete (Haymes, 1987; Lyle, 1992; Weaver and Rajaram, 1992). However data on effects of the different stages of iron deficiency on exercise performance are controversial. Some studies have suggested nonanemic iron deficiency may impair running performance while others suggest that the nonanemic state does not represent a true iron deficiency (Jensen et al., 1991; Magnusson et al., 1984 b; Matter et al., 1987; Newhouse and Clement, 1989; Rowland et al., 1988; Tumbi and Dodd, 1990; Schoene et al, 1983).

Iron deficiency or reduced iron status has been associated with intense training and activities of longer duration (Ehn et al 1980; Frederickson et al 1983; Magazanik et al

1991; Robertson et al., 1992). On studying the impact of iron deficiency on performance, Risser et al. (1988) found an increased prevalence of iron deficiency in the non-endurance female athlete. Prevalence of iron deficiency without anemia has been noted much more than the presence of anemia in the female athlete (Clement and Asmundson, 1982; Clement and Sawchuk, 1984; Hunding et al., 1981; Parr et al., 1984). Studies have suggested that athletes with minimal decreases in hemoglobin or mild iron deficiency have mild impairments in maximal performance (Risser et al, 1988; Schoene et al., 1983). Distance runners in particular have shown a greater prevalence of iron deficiency and latent iron deficiency (Clement and Asmundson, 1982; Ehn et al., 1980; Hunding et al., 1981; Lampe et al., 1986; Manore et al., 1989; Pate et al., 1993). Studies examining the effects of a short term moderate exercise program on previously sedentary women have reported a compromised iron status (Blum et al., 1986; Jensen et al., 1991; Lyle et al., 1992). Lyle et al. (1992) found an initial decrease in hemoglobin levels that stabilized after 4-12 weeks of moderate exercise in college age women. However 62% of subjects reached stage III iron deficiency. Jensen et al. (1991) found hemoglobin concentrations declined significantly at week 4 and 8 between groups of sedentary young women in a 12 week exercise program.

Information on female athletes in other sports is scarce. Jacobsen et al. (1993) compared the hematological parameters of female basketball players and found no significant decrease in hemoglobin or hematocrit levels in the players. Similarly Lukaski et al. (1990) reported decreased hemoglobin levels but no adverse effects from physical

training on iron nutriture in male and female swimmers. Female swimmers had slightly decreased hematocrit and hemoglobin values compared with controls. Interval exercise resulted in significant modulations of iron status parameters in trained men (Gray et al., 1993).

Iron deficient erythropoiesis has been observed in men and women involved in physical exercise or training (Balaban et al., 1989; Clement and Asmundson, 1982; Frederickson et al., 1983; Gray et al., 1993; Hunding et al., 1981; Magazanik et al., 1991; Manore et al., 1989; Snyder et al., 1989; Syguyla, 1990). Other studies have shown no significant differences or very little changes in Hb, Hct, serum iron, % TS or red cell protoporphyrin (RCP) and a decreased TIBC during or after training in athletes (Ehn et al., 1980; Lampe et al, 1986; Pate et al, 1993). Endurance trained athletes have been found to have an increased MCV, decreased MCHC and increased reticulocytes (Brotherhood et al., 1975; Spodaryk, 1993). Seasonal deteriorations in iron levels such as a decreased Hb, PCV, serum iron, and %TS which declined during and increased on cessation of training have also been noted (Frederickson et al., 1983; Risser et al., 1988).

Although these deteriorations may not alter performance, it has been suggested that with the addition of physiological stress, they may lead to iron deficiency in the future (Sherman and Kramer, 1990). Intravascular hemolysis as suggested by reduced haptoglobin (Hp) levels has been reported in runners (Clement and Asmundson, 1982; Magnusson et al, 1984a). Spodaryk (1993) found that hemolysis does not accompany strength or endurance training as a chronic phenomenon. However in the trained athlete,

hemolysis, due to daily exercise with or without impact stress results in increased erythrocyte turnover and accelerated erythropoiesis and may precipitate an iron deficiency due to the increased iron requirement (Spodaryk, 1993; Weight et al., 1991).

TISSUE IRON STORES

Training appears to bring about adaptations in iron metabolism and distribution to allow for increased hemoglobin and myoglobin for improved oxygen delivery. Iron costs of these changes are met by mobilizing iron reserves (Sherman and Kramer, 1990). Prelatent iron deficiency is characterized by an impoverishment of tissue and ferritin iron reserves in the liver, spleen, and bone marrow while latent iron deficiency indicates further depletion of iron reserves in the bone marrow, although the Hb may be normal (Szygula, 1990). The detrimental effects of strenuous physical activity, as indicated by the presence of prelatent and latent iron deficiency, as well as decreased iron stores in athletes have been documented by several investigators (Clement and Asmundson, 1982; Ehn et al., 1980; Frederickson et al., 1983; Hunding et al., 1981; Magazanik et al., 1991; Magnusson et al., 1984a; Weaver and Rajaram, 1992).

Causes for the incidences of low iron stores in athletes are not well understood. Magnusson et al. (1984b) propose that increased hemolysis leads to the form of a Hp-Hb complex that is taken up by the hepatocytes leading to a reduction in plasma ferritin and

bone marrow hemosiderin. A 13 week exercise training program of previously sedentary women resulted in decreased serum ferritin levels that stabilized at six weeks. None of the women, however, displayed iron depletion or iron deficiency and the authors concluded that the compromised iron stores occurred due to a physiological adaptation (Blum et al., 1986). Fig. 4 outlines the proposed mechanism of the body's response to the iron cost associated with exercise. The amount and intensity of physical training may also be a risk factor in the iron store depletion as suggested by the inverse relationship found between the number of training sessions and serum ferritin concentration of athletes (Dallongeville et al., 1989; Robertson et al., 1992; Telford et al., 1993). Runners and females in particular are at a greater risk for depleted iron stores than their sedentary counterparts (Frederickson et al., 1983; Pate et al., 1993; Risser et al., 1988; Rowland et al., 1988). Pate (1993) found that female runners had significantly lower serum ferritin compared to the inactive females. Fifty percent of the runners were categorized as iron depleted.

Marginal iron stores in the absence of anemia may not diminish athletic performance (Eichner, 1986). Iron depletion in the absence of anemia has been associated with impaired endurance performance while other studies have shown no detrimental effects on endurance capacity and physical work (Klingshirn et al., 1992; Matter et al., 1987; Newhouse et al., 1989; Rowland et al., 1988).

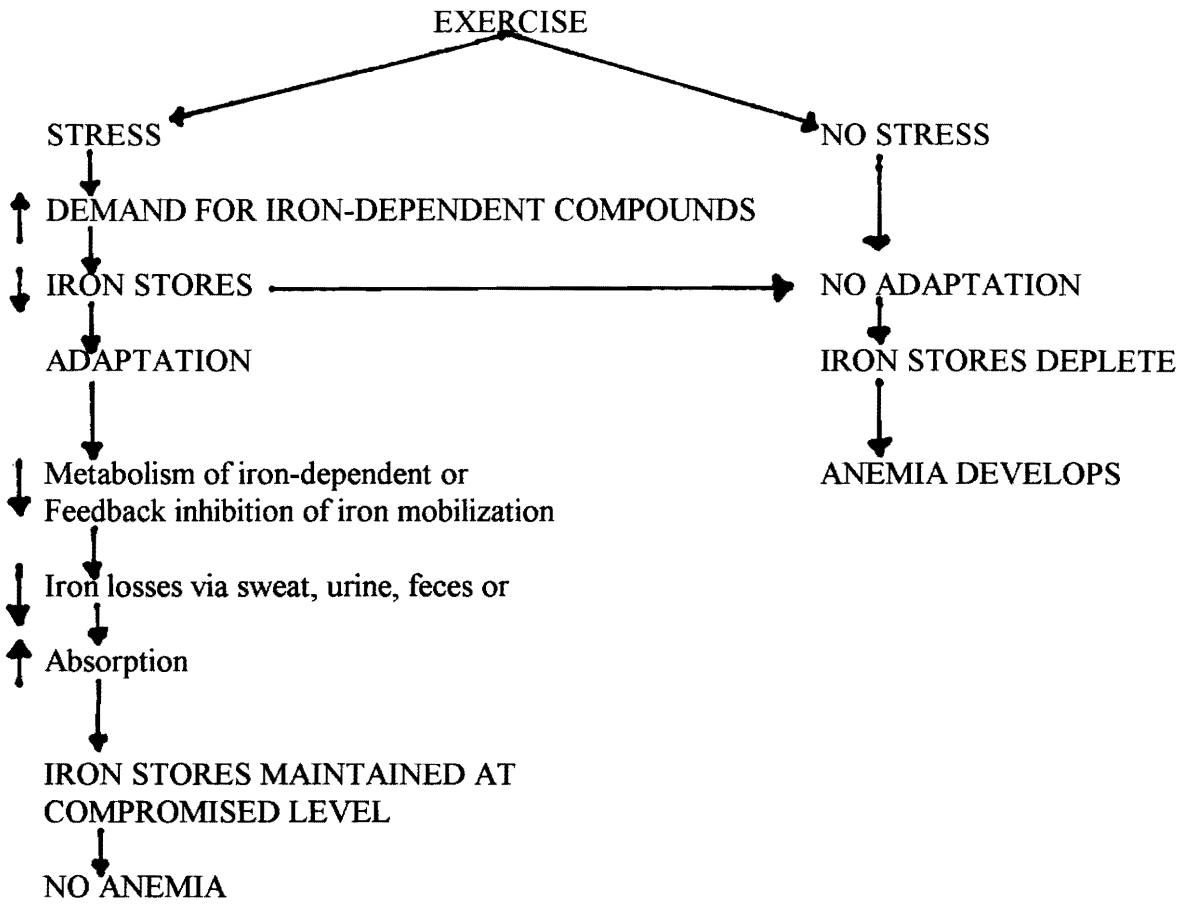


FIG.4: Proposed mechanism for exercise-induced iron cost.
Source: Blum et al., (1986).

Newhouse and Clement (1988) noted a high incidence of decreased serum ferritin values in the endurance athletes and nonendurance athletes.

Several studies have found decreased iron stores during exercise. Magazanik et al. (1991) found ferritin levels decreased 50% after four weeks of intense 8h/d physical training in women and men 20 years of age. Data on female long distance runners studied over a 9 week training period by Manore et al. (1989) indicated that 50-60% of the subjects had plasma ferritin levels indicative of Stage I iron deficiency. Clement and Asmundson (1982) using a plasma ferritin level of 25 ug/l as the critical value, found incidence of iron deficiency as high as 29% for male endurance runners and 82% in female distance runners. Comparing the 3 stages of iron deficiency in athletes and controls immediately after menstruation and at mid cycle, Parr et al. (1984) noted stage I and II iron deficiency in female athletes in the absence of anemia. Eighteen athletes of 29 and 4 controls of 8 had a serum ferritin value of less than 12 ug/l. Spodaryk (1993) studied male endurance and strength trained athletes and found a pronounced reduction in iron stores. Serum ferritin levels may be falsely elevated following a race. During an eleven week training period, 8 of 9 women averaged serum ferritin levels below 50 ug/l, indicative of a prelatent iron deficiency. However serum ferritin levels were significantly elevated three days post-race compared to pre-race levels and returned to pre-race levels at day 4 (Lampe et al., 1986).

Progressively declining serum ferritin levels through the season suggest an influence of running training on iron stores (Magazanik et al., 1991; Rowland et al.,

1988). Risser et al. (1988) found that 15.6% of the 45 female college athletes who had normal ferritin levels preseason ended the season with a serum ferritin concentration of less than 12 ug/l. However, iron depletion was comparable in non-endurance female athletes and nonathlete control subjects participating in various collegiate sports. Comparing the changes in iron status of female basketball players across a competitive season, Jacobsen et al. (1993) found 13% of the players had decreased serum ferritin levels preseason which decreased significantly postseason.

IRON INTAKE, ABSORPTION AND EXCRETION

Inadequate iron intake, increased losses and decreased absorption have been implicated as causes of iron deficiency or sports anemia in athletes (Pate, 1983; Williams and Anderson, 1993). Premenopausal women and adolescent athletes are of particular concern because of the blood loss associated with menses in the former and the extra demand with increased blood volume and lean muscle mass in the latter (Weaver and Rajaram, 1992). As shown in Fig. 5, Weaver and Rajaram (1992) propose that a true iron deficiency exists if exercise induces increased iron losses, decreases the dietary iron utilization and/or increases iron demands.

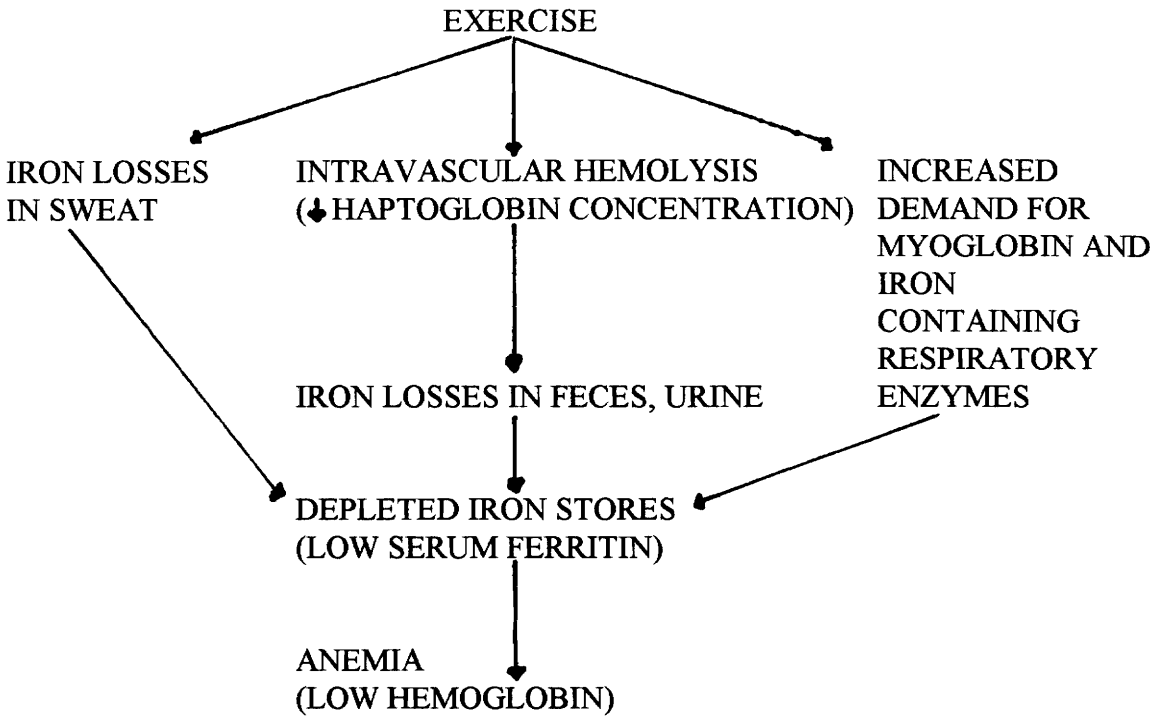


Fig.5. Possible mechanism for exercise-induced iron deficiency.
Source: Weaver and Rajaram (1992).

IRON INTAKE AND ABSORPTION

Inadequate iron intake, due to a restricted energy intake as well as improper dietary practices, makes the athlete more susceptible to iron deficiency anemia (ADA report, 1987; Dallongeville et al., 1989; Frederickson et al., 1983; Pate, 1983; Weight et al., 1992). This is especially true for women, since iron absorption is dependent on the bioavailability of iron. Increasing the amount and form of iron along with an adequate dietary intake will help promote a positive iron balance as well as improve athletic performance (Lloyd et al., 1992; Lyle et al., 1992; Snyder et al., 1989; Weaver and Rajaram, 1992; Weight et al., 1992). With dietary iron intake averaging 6 mg/1000 kcal, obtaining the RDA of 15 mg/d for most women on 2000 kcal/d seems difficult but has been emphasized especially during exercise training (Lyle et al., 1992; Monsen, 1988; Weaver and Rajaram, 1992).

Dietary surveys of female athletes as reviewed by Pate (1983) have generally shown adequate iron intake. However higher dietary iron needs for women endurance athletes compared to sedentary women have been suggested by various investigators (Clement and Asmundson, 1982; Haymes, 1987; Lampe et al., 1986; Lloyd et al., 1992; Loosli, 1993; Manore et al., 1989; Powell and Tucker, 1991). Lloyd et al. (1992) reported an average energy intake of 1850 kcal/d in 108 recreational swimmers and 37 controls. Of the overall study sample, 80% of runners did not meet the RDA for iron.

The average iron intake in female distance runners was 12.5 mg/d (Clement and Asmundson, 1982). Lampe et al. (1986) evaluated the iron status of 9 female marathon runners during 11 weeks of training and following a race. Results of the three day food diary recorded midway in the training indicated that iron intake averaged only 78% of the RDA. In a study of premenopausal women runners over a 9 week training period, Manore et al. (1989) reported mean iron intake of 11.9 mg/d. Inadequate dietary iron intake well below the RDA has been demonstrated more often in female athletes than in men (Clement and Asmundson, 1982; Risser et al., 1988; Snyder et al., 1989; Weight et al., 1992). Dietary iron intake in the male athletes has generally been found to meet the RDA (Dallongeville et al, 1989; Weight et al., 1992). However it has been suggested that total iron intake may not be an important determinant of iron status in athletes (Dallongeville et al, 1989; Telford et al., 1993). Telford et al. (1993) found a trend towards a negative correlation between plasma ferritin concentration and daily iron intake in non-anemic athletes of both sexes involved in various sports. Dallongeville et al. (1989) found no significant difference in total iron intake per day in athletes with either low, medium or elevated iron stores. Various studies have documented iron store depletion due to dietary inadequacy in athletes (Clement and Asmundson,1982; Haymes, 1987; Jensen et al, 1991; Lamanca and Haymes, 1992; Lampe et al., 1986; Manore et al., 1989).

Although the percentage of dietary iron absorption is increased with iron deficiency (Haymes, 1987), evidence suggests that this regulatory mechanism may be impaired in athletes (Clement and Asmundson, 1982; Ehn et al, 1980) placing the athlete

at a greater risk of developing iron deficiency anemia. However other studies of iron deficient and non deficient athletes have reported no significant differences in percentage of iron absorption (Dallongeville et al, 1989; Magnusson et al 1984a). Using radioactive iron to determine iron absorption in endurance runners, Ehn et al. (1980) reported iron absorption was only 16% compared to the 30% absorption seen in the iron deficient controls. Magnusson et al. (1984a) measured iron absorption by whole body counting two weeks after oral administration of 3 mg of elemental iron as ferrous ascorbate in both iron deficient and iron replete groups of long distance runners and found no significant differences in the percentage of iron absorption. Using an iron tolerance test, Dallongeville et al. (1989) found absorption was inversely correlated with iron stores and the iron deficient athletes had higher absorption suggesting a normal regulation mechanism.

Various dietary factors influence iron absorption. Serum ferritin was significantly correlated with vitamin C intake than iron intake or the hemoglobin concentration (Robertson et al., 1992). Inhibition of nonheme iron absorption due to polyphenols present in coffee or tea as well as a high intake of carbohydrates has also been ascribed to the decreased serum ferritin levels seen in athletes (Pate et al., 1993; Snyder et al., 1989; Telford et al., 1993). Since foods containing heme iron are the best sources of readily absorbable iron, studies have reported lower ferritin concentration and depleted iron stores in male and female athletes consuming decreased meat/heme iron (Dallongeville et al., 1989; Lamanca and Haymes, 1992; Telford et al., 1993; Weight et al., 1992). Three day

food diaries of women endurance athletes with normal or low plasma ferritin concentration in a study by Lamanca and Haymes (1992) revealed lower daily absorbable iron intake by the decreased ferritin group compared to normal ferritin group. Ferritin concentration was significantly related to absorbable iron and total iron intake. Form rather than amount of dietary iron appears to influence status. Worthington et al. (1988) evaluated the dietary iron intake and iron status of premenopausal women. Superior iron status was maintained by women consuming red meat in the diet as reflected in increased serum ferritin concentration. Increased ingestion of muscle foods in exercising women led to complete compensation on initial decreases whereas ingestion of iron supplements nearly offset initial decreases. Meat supplements were more effective in protecting Hb and ferritin status than iron supplements (Lyle et al., 1992). Iron intake in the form of meat was lower in iron deficient athletes as compared to those with normal or replete iron stores and findings suggest heme iron rich foods might have a protective effect against iron deficiency in some active men (Dallongeville et al., 1989). As more athletes tend towards vegetarian diets they may be at increased risk of iron depletion because of the poor absorption of nonheme iron (Eichner, 1986). Snyder et al. (1989) studied the dietary patterns and iron parameters in middle aged female runners. Using three day analyses to compare 9 women who consumed a modified vegetarian diet with women who ate red meat, they found a marked reduction in ferritin which they felt was due to the low iron bioavailability.

IRON EXCRETION AND LOSSES

Increased iron excretion through gastrointestinal blood losses, urine, sweat, feces and the menses in women athletes has also been implicated in the depletion of iron stores and iron deficiency anemia (Clement and Asmundson, 1982; Haymes, 1987; Pate, 1983; Weaver and Rajaram, 1992). Total urine and sweat iron losses are approximately 1.75 mg/d compared with the reference value of 1.4 mg/d in female endurance trained athletes due to additional iron losses with menses. Although exercise training does not increase losses through menstruation, all these factors combined increase the risk for iron deficiency and or anemia in the female athlete (Pate, 1983; Weaver and Rajaram, 1992). The average male and female lose 1.0 mg and 0.8 mg iron/d respectively through skin and the gastrointestinal tract and females lose an additional 0.6 mg/d through the menses (Hallberg, 1984). Haymes (1987) suggests that male distance runners could lose iron at twice the rate of normal men, 2 versus 1 mg/d, respectively.

The predominant source of iron losses in male endurance athletes may be through sweat and a daily loss of more than 1 mg is possible in runners. Ehn et al. (1980) reported increased elimination of radioactive iron corresponding to approximately 2 mg iron/d in runners studied for 10 months. Profound sweating was suggested as an explanation for the increased iron losses. Although male cross country runners had a greater sweat rate, the higher sweat iron concentration of females led to similar rates of iron loss, 0.21 and 0.28 mg iron/h by male and females respectively (Lamanca et al., 1988). The authors

suggest sweat iron losses coupled with low dietary iron intakes in female runners may result in a negative iron balance. Similar values of 0.22 mg/l for sweat iron concentration were reported by Brune et al. (1986) in males. Results suggest that the minimal losses of iron through sweat had only a marginal effect on total body iron losses. Hematuria, the most visible form of blood loss, has been documented in runners. Urinary iron losses as reflected by presence of hemoglobinuria, a well recognized complication of running resulting from intravascular hemolysis, has been reported (Watts, 1989; Weaver and Rajaram, 1992). Urinary iron losses averaging 0.18 mg/24 h have been reported (Magnusson et al., 1984a). No hemoglobinuria was detected in iron deficient young men after a training session since the intensity of usual training sessions was insufficient to produce any bladder trauma (Dallongeville et al., 1989).

The normal range of daily gastrointestinal blood loss has been established as 0.5-2.0 mg Hb/g/d by radiochromium labelling method, while Robertson et al. (1987) used the hemoquant assay and found a similar range of 0.10-2.53 mg/d. Examining fecal blood loss in marathon runners and male walkers, Robertson et al. (1987) found that though prolonged walking had no effect on gastrointestinal blood loss, intense endurance exercise induced a significant but clinically unimportant increase. Stewart et al. (1984) studied gastrointestinal blood losses and anemia in trained athletes after running. Both mean blood hemoglobin and hematocrit were significantly decreased in runners than in nonrunner controls. Pre-race mean fecal Hb levels were 1.08 mg/g in controls and 0.99 mg/g in runners, which peaked at 3.96 mg/g after a race. Fecal Hb levels in 83% of

runners thus indicated that competitive long distance running induces a GI blood loss and may contribute to iron deficiency. Ruckman and Sherman (1981) suggested that the elevated secretion of endogenous iron in the bile may account for the increased fecal iron loss observed in exercising rats. Gray et al. (1993), assessed the effects of interval exercise in trained men and found exercise induced increases in serum iron. The authors (Gray et al., 1993) speculate that the post exercise increase may be associated with increased iron elimination.

IRON SUPPLEMENTATION AND EXERCISE PERFORMANCE

Prophylactic supplementation with 325 mg ferrous sulphate three times per week has been recommended for women endurance athletes who repeatedly develop iron deficiency anemia despite a proper diet (Eichner, 1992). Using data from the NHANES II survey, Looker et al. (1988) compared the dietary intake and biochemical indices of iron status of adults in regular supplement users versus nonusers and found no association of iron status with supplement use. Suboptimal iron status related to exercise has caused the routine use of iron supplementation in athletes (Balaban et al., 1989). Although iron supplementation in iron-deficient and anemic athletes may be beneficial in individuals without iron deficiency anemia, it may not be an ergogenic aid (Bucci, 1990; Matter et al., 1987). The use of short term supplementation for improved iron status in athletes has

been advocated by some investigators (Clement and Asmundson, 1982; Haymes, 1987; Lyle, 1992; Pate, 1983) while others caution against the indiscriminate or routine use in nonanemic athletes (Grandjean and Ruud, 1990; Magnusson et al., 1984a; Parr et al., 1984; Snyder et al., 1989). According to Haymes (1987), iron supplementation following heavy exercise is beneficial in reducing blood lactate concentration and the amount of iron in the supplement may influence the amount of increases in Hb and ferritin levels. Both animal and human studies have indicated that a reduced exercise performance due to iron deficiency anemia can be reversed with supplementation, resulting in an increased Hb, a reduced muscle lactate production and an improved endurance and maximal oxygen consumption (Davies et al.,1982; Edgerton et al.,1972; Haymes, 1987; Mclane et al., 1981; Perkkio et al., 1985; Schoene et al., 1983). Additionally, iron supplementation was found to be beneficial in maintaining or improving hematological indexes and iron stores in previously sedentary young women involved in moderate exercise programs (Lyle et al., 1992; Jensen et al., 1991; Rowland et al., 1988). Normal or improved iron store status (Hunding et al., 1981; Klingshirn et al., 1992; Newhouse and Clement, 1989; Rowland et al., 1988; Risser et al., 1988) has been noted with treatment therapy in athletes.

Iron deficiency without anemia affects muscle metabolism during exercise and can reduce endurance capacity (Davies et al., 1982; 1984; Lukaski et al., 1991). Reduction of body iron stores without overt anemia can affect exercise metabolism by reducing total aerobic energy production and increasing glycolytic metabolism. Iron therapy produced significant increases in Hb content which coincided with improvement in maximal oxygen

consuming capacity (Davies et al., 1982). Lukaski et al. (1991) found iron repletion and supplementation increased Hb, ferritin and iron balance in 11 women who had been iron depleted and underwent a maximal work capacity test. Iron depletion was associated with elevated post exercise lactic acid concentration and reduced oxygen utilization and total oxygen output. Studies examining the relation of iron depletion or mild iron deficiency to performance in athletes have produced conflicting results (Balaban et al., 1989; Jensen et al., 1991; Kingshirn et al., 1992; Matter et al., 1987; Newhouse and Clement, 1989; Rowland et al., 1988; Risser et al., 1988; Tumbi and Dodd, 1990). Tumbi and Dodd (1990) studied the effect of oral iron supplementation on the iron status and physical work capacity in anemic women for 10 weeks. Iron repletion increased serum ferritin and transferrin saturation along with increased physical work capacity thus indicating that mild to moderate anemia may impair work performance. The beneficial effects of oral iron therapy on nonanemic iron deficient adolescent runners was demonstrated by Rowland et al. (1988) as indicated by improved endurance times. Serum ferritin levels rose from 8.7 to 26.6 ug/l in the iron supplemented group with improved endurance and thus performance. Newhouse et al. (1989) studied the effects of 8 weeks of iron supplementation in prelatent and latent iron deficient female endurance runners. Although there was a significant increase in serum ferritin and UIBC, no difference in maximal oxygen consuming capacity was seen and iron supplementation was not associated with a significant improvement in work capacity. However other studies indicate that although iron therapy in iron deficient nonanemic runners improved iron stores, treatment did not

always influence the maximal exercise performance or alter blood lactate and Hb levels during exercise (Matter et al., 1987; Risser et al., 1988). Klingshirn et al. (1992), examined the effect of oral iron supplementation in initially iron depleted, nonanemic female distance runners. Although iron status improved, the increase in iron stores did not enhance endurance capacity or alter the lactate concentration. Schoene et al. (1983) studied trained women athletes with Stage II iron deficiency versus those with normal iron status. Iron therapy improved the abnormal measures of iron status in iron deficient groups and resulted in lower lactate levels at end of exercise possibly due to the increased Hb levels which improved aerobic metabolism during high levels of work. However exercise performance remained unchanged in both groups.

Balaban et al. (1989) found conflicting results regarding the impact of iron repletion on running performance. On assessing the impact of iron supplementation in runners, no significant differences in iron storage parameters among runners on or off supplements and controls on or off iron were seen. Magazanik et al. (1991) investigated effects of a daily oral iron supplement administered during an intense 7 week physical training program on iron status and maximal aerobic capacity of women. Iron therapy to iron-reserve depleted women improved blood Hb, PCV, serum ferritin concentration and maximal aerobic capacity which increased significantly in the iron supplemented group on 21 days of training. Although iron supplementation increases work capacity in iron deficient anemic individuals its effect on work capacity or performance in common prelatent or latent deficiency states remains unclear (Newhouse and Clement, 1988).

Administration of a high dosage iron supplement to iron depleted, nonanemic female cross country runners with low iron stores but normal hematological parameters did not significantly change any of the blood iron indices or improve work performance. There were no significant changes in oxygen consumption, ventilation or lactate production with iron supplementation (Powell and Tucker, 1991).

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The study was designed to determine the effects of exercise and iron nutrition on biochemical indices of iron status in the 18-20 month old adult female rat. A 2 x 2 factorial design with fixed effects (two levels of dietary iron and two levels of exercise) was used. Fig. 6 presents an illustration of the experimental design. The animals were randomly assigned to one of five groups in order to obtain an average weight per group. There were twelve animals per group except for the baseline group which had only nine animals. Baseline animals were sacrificed a day after the randomization to obtain pre-treatment levels of blood and tissue samples. The four experimental groups received one of two purified diets, high iron (42 ppm) or a moderate iron (7 ppm) diet. The animals either received an exercise treatment which involved swimming five times per week for a period of six weeks or remained sedentary. The five experimental groups referred to in the study were noted as follows, baseline (BL), iron sufficient-exercise (HE), iron sufficient-sedentary (HS), moderate iron deficient-exercise (LE), moderate iron deficient-sedentary (LS).

BASELINE GROUP (BL) : n = 9		
ACTIVITY	DIET	
	HIGH IRON 42 PPM (H)	MODERATE IRON 7 PPM (L)
EXERCISE (E)	HE : 12	LE : 12
SEDENTARY (S)	HS : 12	LS : 12

FIG : 6 EXPERIMENTAL DESIGN

The four groups received the respective diets and deionized (DI) water ad libitum for a period of six weeks, at the end of which the animals were sacrificed. Food intakes and body weights were monitored throughout the experiment. Analysis of the following parameters was performed at the end of the six week experimental period.

1. Hematological analysis for hemoglobin, hematocrit, serum iron and total iron binding capacity (TIBC).
2. Tissue iron stores in liver, spleen, soleus and the gastrocnemius muscles.
3. Heart muscle citrate synthase enzyme activity
4. Total feed consumption, body weight and organ weights were also determined.

ANIMALS:

Fifty-seven 10 month old retired breeder rats of the Harlan Sprague Dawley strain were utilized in this study. They were housed in individual wire-mesh stainless steel cages at the University vivarium and fed a stock diet of laboratory chow ad libitum for 7 months until they were 18 months old. Room temperature was maintained at 21°C with a 12-hour light-dark cycle. At the end of this period, on the basis of an average mean weight for all the groups, the animals were randomly assigned to one of the five experimental groups. Animals in the BL group were fasted and then sacrificed the day the other animals were randomly assigned to the four experimental groups, to obtain pretreatment blood and

tissue samples for analysis. The animals received 45-50 g of the diet every two days taking into consideration the spills as well as the amount consumed. Average feed intake for each animal throughout the study was recorded. All protocols were approved by the Virginia Tech Animal Care Committee.

EXPERIMENTAL DIETS

The experimental diets used were an iron sufficient diet (42 ppm) and a moderate iron deficient diet (7 ppm). A dietary iron level of 35 ppm/kg diet is recommended by the National Academy of Science-National Research Council (1978) as adequate. The diets were formulated in the laboratory. The composition of the diets is presented in Table 4. The mineral mix in the iron deficient diet did not contain iron. Cellulose, as a source of fiber was omitted because of variable iron content and was replaced with cornstarch (Willis et al; 1990). To establish validity, iron contents of the diets were determined using the wet ashing procedure and analyzed by the Perkin-Elmer 2100 atomic absorption spectrophotometer. The average iron contents of the high and moderate iron deficient diets were 42 ppm and 7 ppm respectively. At the end of the seven month period all the rats in the experimental groups were switched to one of the two types of diet for a six week period.

TABLE: 4 COMPOSITION OF EXPERIMENTAL DIETS

PERCENT OF DIET COMPONENTS

DIETARY COMPONENTS	42 PPM IRON DIET	7 PPM IRON DIET
Cornstarch	35.0	35.0
Sucrose	30.0	30.0
Casein[1]	20.0	20.0
DL-Methionine[2]	0.30	0.30
Choline Bitartarate[2]	0.20	0.20
Corn Oil[3]	10.0	10.0
Vitamin Mix[4]	1.0	1.0
Mineral Mix[5]	3.5*	3.5**

1. ANRC Vitamin-Free Casein, Nutritional Biochemicals Corp., Cleveland, Ohio.
2. Nutritional Biochemicals Corp., Cleveland, Ohio.
3. Mazola, Best Foods, CPC International; Inc., Englewood Cliffs N.J.
4. AIN Vitamin Mixture 76. Nutritional Biochemicals Corp., Cleveland, Ohio.
5. AIN Mineral Mixture 76. Nutritional Biochemicals Corp., Cleveland, Ohio.

* Mineral mixture with iron.

** Mineral mixture without iron.

EXERCISE PROGRAM

The exercise protocol involved swimming five times per week for a period of six weeks. The method used by Gagne et al. (1994) was followed. Six rats from each group swam at the same time each day in plastic tanks (60 x 90 x 56 cm), filled to a water depth of 28 cm. The water temperature was maintained between 32-35°C.

Swimming protocol:

Week	I	II	III	IV	V	VI
Min/day	10-15	20-25	30-40	45-55	60	60

The animals swam progressively from 10 minutes per day at week I until they were swimming continuously for 1 hour/day by week V. After each swim the rats were placed in barrels, lined with towels and heated with overhead lamps, for drying. Exhaustion signs were noted for a few animals when swimming midweek at 30-40 min. The nonexercised groups remained in their cages throughout the study and received the same handling for feeding and weight measurements as the exercised group.

ANIMAL NECROPSY, BLOOD AND TISSUE HANDLING

At the end of the six week period, each animal in the experimental group was anesthetized with carbon dioxide gas and then sacrificed using sodium pentobarbital. The dosage level for sodium pentobarbital was based on body weight (40-60 mg/kg) and was administered 24 hours after the last exercise session. Blood was collected by cardiac puncture into 25 ml plastic polyethylene syringes (Serum monovette, Walter Sarstedt, Inc. Princeton, New Jersey), and gently transferred to polypropylene collecting tubes. Aliquots of blood samples were immediately transferred into two anticoagulant treated (Heparin) eppendorf capillary tubes and two hemocap tubes for Hb and Hct measurements, respectively. Hemoglobin and Hct determinations were made the same day. Each remaining sample of whole blood was allowed to stand at room temperature for 30 minutes to allow clot formation, followed by centrifugation at 3000 rpm for 30 minutes at 5°C for serum separation. For the analysis of serum iron and TIBC, the serum was aspirated using Pasteur pipettes into clean 5ml polypropylene tubes, capped and stored at -20°C until further use.

After blood collection, the liver, spleen and left and right gastrocnemius (mixed red and white) muscles and the left and right soleus (red) muscles were removed for histological examination, trimmed, blotted, weighed and frozen immediately in separately labeled plastic bags. For the analysis of the citrate synthase enzyme, the heart muscle was removed and treated in the same manner but was wrapped in aluminum foil and

submerged in liquid nitrogen for 8 seconds to prevent any enzyme degradation during the freezing process. The rat hearts were then stored frozen in plastic bags. More enzyme can be extracted from frozen tissue than from the fresh tissue (Srere and Kosicki; 1961).

HEMATOLOGICAL ANALYSIS

Duplicate analyses were performed for hemoglobin, hematocrit serum iron and total iron binding capacity (TIBC). Hematocrit analyses were performed in triplicate. Average values for each animal were then calculated.

HEMOGLOBIN

Hemoglobin was determined using the cyanmethemoglobin method which involves treatment of whole blood with a dilute solution of potassium ferricyanide and potassium cyanide to oxidize the iron in the hemoglobin molecule. In the reaction hemoglobin is converted to methemoglobin; the cyanide then reacts to form cyanmethemoglobin. This method measures all of the derivatives of hemoglobin. Hemoglobin concentration was determined and read on a spectrophotometer. The following procedure was used :

1. Make standards of the following concentrations:

15 mg/dl, 30 mg/dl, and 45 mg/dl (Sigma Diagnostics).

2. Transfer the solutions to cuvettes and measure the absorbance of each dilution against a blank at 540 nm. Zero the spectrophotometer with the blank (reagent).
3. Prepare a calibration curve by plotting the absorbance of the standards against their concentrations.
4. Mark 2 tubes for each animal, and pipette 5.0 ml of cyanmethemoglobin reagent into each tube.
5. Fill 2 hemocap tubes with blood and carefully drop one into each of the 2 tubes of reagent, and centrifuge for a few seconds, to get all the blood out. Let stand for at least 10 minutes at room temperature.
6. Transfer the contents of the tubes to cuvettes and measure the absorbance against the reagent at 540 nm. Transfer the reading to the standard curve and obtain the hemoglobin concentration in gm/dl.

HEMATOCRIT

Blood hematocrit levels were determined using the microcapillary hematocrit estimation. The following procedure was used :

1. Draw blood into two microhematocrit (capillary) tubes. Fill tubes to 2/3 to 3/4 full, and seal the filled end with "seal-ease".

2. Place tubes in the micro-centrifuge making sure that the sealed ends of all the tubes are touching the outside edge of the centrifuge.
3. Note the positions of the tubes, secure the lid and centrifuge for 15 minutes at 3000 rpm. Allow the centrifuge to stop without use of the brake.
4. Set the top of the meniscus of the plasma at 100, the bottom of the packed red cells at 0 and then read the top of the packed red cells.
5. Express the volume of red blood cells as percent of whole blood.

SERUM IRON AND TIBC

Quantitative colorimetric determination of iron and total iron-binding capacity in serum was done using a commercially prepared kit of serum iron/UIBC from Stanbio Laboratories Inc. (San Antonio, TX). The procedure for serum iron is based upon its release from combination with transferrin in acid medium, reduction to the ferrous form by hydroxylamine and its reaction with ferrozine to form a violet colored complex which is measured at 560 nm. Since only about one third of serum iron is normally bound to the globulin, transferrin, the unsaturated iron-binding capacity (UIBC) is determined by saturating the transferrin with a known excess of iron. Serum total iron-binding capacity (TIBC) is thus the sum of Serum Iron and UIBC.

TISSUE IRON ANALYSIS

The liver, spleen, left and the right soleus and gastrocnemius muscles were freeze-dried to a constant weight and the weight was noted. The tissues were then ground using a mortar and pestle before being analyzed. Samples of the tissues were them wet-ashed. Dilute samples of the wet ashed tissues and iron standards were then analyzed using the Perkin-Elmer 2100 atomic absorption spectrophotometer.

Procedure for Wet Ashing:

1. Weigh 0.05-0.15 gms of sample tissue in duplicates and add 3.0 mls of redistilled nitric acid, cover the tubes and leave overnight.
2. Turn on the perchloric hood and heat block. Add 1 ml of redistilled perchloric acid to the blanks and the samples and place test tubes on heating block.
3. Allow samples to reflux in acid. Swirl samples every 15 minutes until the brown smoke that appears has disappeared. Samples will remain yellow.
4. Continue to swirl samples. Prior to completion of the process, white smoke will appear in the test tubes. When samples are slightly below curve at bottom of the tube and turn clear upon swirling without formation of bubbles, the tubes can be removed and placed on rack under the hood for cooling.
5. Add 10% HCL to the samples to make up volume to 12.5 ml. Cap samples tubes with paraffin and allow it to set in 10% HCL for two hours. Transfer the solution

to labeled disposable tubes.

6. Samples can then be read on Perkin-Elmer 2100 Atomic Absorption spectrophotometer. Further dilutions of samples with 10% HCL may be obtained before reading on the spectrophotometer.

Procedure for calculating iron content on reading sample :

1. Multiply the atomic absorption reading (ug/g) by the total volume and the dilution factor (Total volume of HCL / sample amount) if used.
2. Divide this value by dry sample weight to obtain ug/g of dry sample.
3. Microgram/g of dry sample x dry tissue weight / wet tissue weight = ug/g wet sample.
4. Obtain an average value of ppm iron content for each sample.

CITRATE SYNTHASE ANALYSIS

Analysis of the citrate synthase activity, a marker enzyme of the Krebs cycle was conducted in the cardiac muscle to assess physiological development of aerobic fitness from the swimming-exercise treatment. Citrate synthase activity was determined by the method of Srere (1969). This reaction involves the measurement of citrate synthase activity by linking the release of COASH to the colorimetric agent DTNB (5,5'-dithiobis-

2-nitrobenzoate). Changes in absorbance are followed at 412 nm. Calculation of units of enzyme activity involves the use of the extinction coefficient 13.6. The limited pH range for this assay is 7.4-9.0.

Homogenate preparation for cardiac muscle tissue :

1. Remove heart muscle from freezer and place on ice. Clean the tissue of all connective, adipose tissue and trim the atria and valves. Finely mince the tissue with surgical scissors.
2. Weight 0.1-0.2 gms of the minced cardiac tissue into a test-tube. The muscle weight (gms) is then multiplied by 19 to yield the volume of KCL-EDTA necessary to prepare a 1:2 dilution of homogenate. Add 3/4 of this to the test tube and the tissue is then homogenized on the Polytron unit. Use the remaining KCL-EDTA to rinse the shaft of the polytron.
3. Transfer the homogenate to microcentrifuge tube, and centrifuge at 1200 rpm for 5 minutes. Collect the supernatants and hold on ice until assayed.

Procedure used:

1. Prepare spectrophotometer by entering blank reference into reference chamber. Blank will consist of :

   0.70 ml TRIS

   0.10 ml DTNB

0.15 ml Acetyl CoA

0.05 ml OAA (Oxaloacetic acid).

Span Spectrophotometer to 100% transmittance.

2. Add the following to cuvette :

0.74 ml TRIS

0.10 ml DTNB

0.10 ml Acetyl CoA

0.01 ml Sample

Follow absorption at 412 nm for 3 minutes to measure background acetyl-CoA activity.

3. Start citrate synthase reaction by adding 0.05 ml of OAA. Follow the reaction for 3 minutes for changes in absorbance (ΔA).

Calculations :

1. Figure the change in concentration (ΔC) which is amount of product formed: $\Delta C=\Delta A/13.6\ mM^{-1}$ $\Delta A=\Delta OD$/minutes (Subtract baseline values). To arrive at ΔOD (optical density) the recorder units must be converted from transmittance to absorbance, this will give absorbance units for $\Delta OD2$.
2. Multiply ΔC by the dilution factor to obtain the total gms of muscle in cuvette.
3. Figure citrate synthase activity per gm of muscle. CS activity (units/gm or umoles/ min/ g)=ΔC/gms of muscle. One unit of enzyme activity catalyzes the formation of 1 umole of product per minute.

STATISTICAL ANALYSIS

Mean values with standard error of means were calculated for body weight, intake, organ weights, hemoglobin, hematocrit, serum iron, TIBC, iron concentrations in liver, spleen, soleus and gastrocnemius muscle and citrate synthase enzyme activity in the heart muscle. The data were tested using the two way analysis of variance procedure (ANOVA) in order to assess the significance of diet and activity. The data were analyzed using a general linear model with diet and exercise as the independent variable and all the blood and tissue parameters as the dependent variables. When statistically significant F values were obtained ($P \leq 0.05$), the Least Square Means Multiple Comparison Procedure (Duncans Multiple Range Test) was used to determine the location of the differences between means within a group. All analyses were performed using the Statistical Analysis System (SAS Inst.Inc; Cary N.C.).

RESULTS AND DISCUSSION

FOOD INTAKE AND BODY WEIGHT

Total food intake and body weights are presented in Table 5. Analysis of variance procedures indicated that the total intake was significantly affected by activity as well as diet ($p \leq 0.05$). No significant interaction effect was observed. Average intake in the HE group was the lowest among the groups. Intakes were comparable in the LE and HS groups but were significantly higher in the LS group. The post-hoc comparison test (Table 6) revealed that intake was significantly higher in the sedentary animals receiving a moderate iron deficient diet in comparison with their exercising counterparts, as well as in comparison with both the sedentary and exercised animals receiving an iron sufficient diet. Intake was also 4% higher in the HS group compared to the HE group. Higher intakes in the sedentary than in the exercised animals suggest that physical activity affects total food intake. In contrast, Ruckman and Sherman (1981) observed a trend towards increased food intake in the female exercised rats with comparable intakes between the exercised and sedentary male rats.

TABLE 5. MEANS AND F VALUES FOR FOOD INTAKE AND BODY WEIGHTS OF EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	TOTAL INTAKE (g)	BODY WEIGHT (g)
BL	CHOW	296.49
HE	629.13 ± 17.19	306.33 ± 8.95
LE	659.72 ± 17.19	305.97 ± 8.95
HS	657.69 ± 17.19	319.76 ± 8.95
LS	710.03 ± 17.19	339.04 ± 8.95
ANOVA F VALUE		
ACTIVITY	5.26*	6.76*
DIET	5.81*	1.12
INTERACTION	0.40	1.20

* F values significant at $p \leq 0.05$

TABLE 6. LEAST SQUARE MEANS FOR TOTAL INTAKE IN EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p≥T Ho:LSM(i) = LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.2150	-		
HS	3	0.2464	0.9340	-	
LS	4	0.0018*	0.0445*	0.0369*	-

* significant at p≤0.05

Dietary iron consumption may also affect total intake as indicated by higher intakes in both the sedentary and exercising animals receiving the moderate iron deficient diet. Intake was 5% and 7% higher in the LE and LS groups respectively compared to the HE and HS group.

Final body weights in the experimental groups were higher than the BL group with significant differences observed among the groups. A significant effect of the activity level on body weight was observed as indicated by the ANOVA F values. No effect of iron intake on body weight was seen. No interaction effect was noted. The least square means multiple comparison test (Table 7) found a significant difference between the LS and HE group as well as the LS and LE group. Although initial body weights were comparable among the four experimental groups, the sedentary group had a significantly greater weight gain while the total weight gain in the exercising group was similar over the six week period. Higher intake in the LS group was reflected in its higher body weight in comparison with the other groups. Despite comparative intakes in the HS and LE group, as expected body weight was 4% greater in the HS group. Inspite of higher intake in the LE group, final body weights were comparable in both the exercised groups. Tobin and Beard (1989) also failed to observe a significant depression in body weight attributable to iron deficiency in sedentary and exercised male rats receiving two levels of dietary iron.

TABLE 7. LEAST SQUARE MEANS FOR BODY WEIGHT IN EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p>T Ho:LSM(i)= LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.9770	-		
HS	3	0.2938	0.2810	-	
LS	4	0.0131*	0.0122*	0.1349	-

* significant at $p \leq 0.05$

Prasad and Pratt (1990) and Gagne et al. (1994) found no significant differences in food intake and body weight among the sedentary and exercised female rats receiving the iron sufficient and iron deficient diets. Several other investigators have also found either similarities or no significant differences in body weight and intake of rats fed both the iron sufficient and iron deficient diets (Bowering and Norton 1981; Mclane et al., 1981; Ruckman and Sherman, 1981; Strause et al., 1983; Perkkio et al., 1985).

In contrast to our results, various studies have shown that dietary iron deficiency may decrease body weight (Davies et al, 1982; Perkkio et al., 1985). Willis et al. (1990) observed a 20% lower body weight in female weanling rats receiving an iron deficient diet (3 mg fe/kg diet) compared to rats receiving iron sufficient diets (53 mg fe/kg). Similarly body weights were approximately 18% lower in iron deficient male rats than those of controls. Following dietary repletion, the difference in weight though still significant had decreased to approximately 12% in the iron deficient rats (Davies et al, 1982). Weanling male rats assigned to diets of varying iron content for six weeks showed a significant difference in body weight (Borel et al., 1991). The highest body weight occurred in the group receiving the 40 mg fe/kg diet while the group receiving the 4 mg fe/kg diet had the lowest body weight. Our results do not show a detrimental effect of dietary iron deficiency on the body weight of female rats, although physical exercise decreased body weight in the exercised animals.

Willis et al. (1992) found that dietary iron deficiency resulted in a 14% lower body weight in sedentary rats while exercise-training amplified the difference by 22% in trained

animals compared to the iron sufficient rats. Rats made severely anemic by blood removal had significantly lower body weight than control rats however no significant difference was seen in the moderate anemic group (Koziol et al., 1978). The level of exercise had no effect on body weight and food intake of rats (Bowering and Norton,1981; Strause et al., 1983). Following 12 weeks of exercise training Tobin and Beard (1989) observed a 5.6% reduction in body weight in the iron sufficient exercised rats and a 9.5% attenuation in iron deficient exercised rats compared to their sedentary counterparts. Thus, similar to their results, this study also found that after 6 weeks of exercise training the final body weight of iron deficient exercised rats was 90% of that of iron deficient sedentary rats.

ORGAN WEIGHTS

Table 8 presents the dry weights of the liver, spleen, heart and the left and right gastrocnemius and soleus muscles respectively. In comparison with the baseline (BL) group no significant differences in the tissue and muscle weight were seen among the experimental groups except for the left soleus muscle weight. The left soleus muscle weight was significantly lower in both the exercised and sedentary animals receiving the moderate iron deficient diet (LE, LS) as well as the sedentary animals receiving an iron sufficient diet (HS). Dietary iron intake and level of activity had no significant effect on tissue and muscle weights. No significant interaction effect was observed. No significant differences in the liver and spleen organ weights occurred among the experimental groups.

Although comparable to the BL group, liver weight in the iron deficient exercised group was 8% higher then the iron sufficient exercised group. Liver weights were comparable between the iron sufficient groups while among the moderate iron deficient groups, the exercised group had a higher weight than the sedentary group. Spleen tissue weights were comparable among the groups. Koziol et al. (1978) observed both cardiomegaly and splenomegaly in the severe and moderately anemic rats. Edgerton et al. (1972) also found that spleen weights tended to be high with chronic iron deficiency. Other animal studies have found no effect of dietary iron intake or exercise on liver and spleen weights (Bowering and Norton, 1981; Gagne et al., 1994; Prasad and Pratt, 1990; Ruckman and Sherman, 1981; Strause et al., 1983).

TABLE 8. MEANS AND F VALUES FOR TISSUE AND MUSCLE WEIGHTS OF EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON.

GROUP	LIVER (g)	SPLEEN (g)	HEART (g)	GN (L) (g)	GN (R) (g)	SOL (L) (g)	SOL (R) (g)
BL	7.7 ± 0.35	0.59 ± 0.03	1.14 ± 0.04	2.00 ± 0.07	2.02 ± 0.07	0.13 ± 0.00	0.13 ± 0.00
HE	7.1± 0.27	0.60 ± 0.02	1.19 ± 0.03	1.95 ± 0.06	1.96 ± 0.05	0.12 ± 0.00	0.12 ± 0.00
HS	7.1 ± 0.27	0.61±0.02	1.10 ± 0.03	1.98 ± 0.06	2.04 ± 0.05	0.11 ± 0.00*	0.12 ± 0.00
LE	7.7 ± 0.27	0.60 ± 0.02	1.18 ± 0.03	1.91 ± 0.06	1.89 ± 0.05	0.11 ± 0.00*	0.11 ± 0.00
LS	7.2 ± 0.07	0.58 ± 0.02	1.19 ± 0.03	1.95 ± 0.06	1.97 ± 0.05	0.11 ±0.00*	0.13 ± 0.00
ANOVA F VALUES							
A	1.28	0.14	2.13	0.45	3.15	1.05	1.45
D	1.87	0.58	2.27	0.44	2.12	1.10	0.21
I	1.08	0.48	2.43	0.00	0.01	0.05	0.75

GN = Gastrocnemius muscle; SOL = soleus muscle; L = left; R = right
A = Activity; D = Diet; I = Interaction

* Means ± SEM are significantly different from BL at $p \leq 0.05$

Cardiomegaly as a functional consequence of iron deficiency has been observed in iron deficient rats. Significant differences in rat cardiac weight have been reported by some investigators (Borel et al., 1991; Bowering and Norton,1981; Gagne et al.,1994; Koziol et al.,1978; Ruckman and Sherman, 1981; Willis et al.,1988) while others have reported no changes (Prasad & Pratt, 1990; Strause et al., 1983). As shown in Table 9, the Least Square Means Multiple Comparison Test indicated there was a significant difference in heart muscle weight between the experimental groups. Among the animals receiving an iron sufficient diet the exercised animals (HE) had significantly higher heart weights than their sedentary counterparts (HS). Regardless of the activity level, cardiac weight was comparable between the animals receiving the moderate iron deficient diets (LE,LS) and was significantly higher than the iron sufficient sedentary group (HS).

Gagne et al. (1994) found cardiomegaly in exercised animals receiving both the adequate iron diet as well as the low iron diet, compared with the sedentary groups. Results of this study point towards cardiomegaly in the exercised and iron deficient animals. Severe iron deficient diets (7ppm) produced a 14% cardiac hypertrophy in male and female weanling SD rats (Bowering & Norton, 1981). A slight cardiomegaly was observed in rats subjected to an exercise program involving swimming for 9 weeks compared with the sedentary controls (Ruckman and Sherman, 1981).

TABLE 9. LEAST SQUARE MEANS FOR HEART WEIGHTS OF EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p>T Ho:LSM(i)= LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.9714	-		
HS	3	0.0385*	0.0418*	-	
LS	4	0.9730	0.9444	0.0357*	-

* significant at $p \leq 0.05$

Increased oxygen need for oxidative phosphorylation and ATP generation by the muscle may cause the development of an exercise induced cardiac hypertrophy. This allows greater blood delivery and hence oxygen to the working muscle due to an enhanced stroke capacity (Bowering and Norton, 1981; Ruckman and Sherman, 1981). Borel et al. (1991) found the mean heart (g) to body weight (kg) ratio was significantly higher in the iron deficient group compared to the other groups. Willis et al., (1988) found that iron deficiency resulted in a significantly greater cardiac ventricular mass and ventricle to body weight ratio in the weanling female rats but exercise training had no additional effect.

No significant differences were seen among the experimental groups in the left and right soleus muscle. Although lower than BL group, the left gastrocnemius muscle weights were comparable between the experimental groups. Prasad and Pratt (1990) and Strause et al. (1983) observed no significant differences among the experimental groups in the soleus and gastrocnemius muscle weights. However similar to observations by Prasad and Pratt (1990) gastrocnemius muscle weights were higher than the soleus muscle weights. Weights of the left and right gastrocnemius muscle were lower than BL and although not statistically significant, were greater in the iron sufficient group compared to the iron deficient group. Weights were also lower in the exercised groups compared to their sedentary counterparts. The least square means multiple comparison test (Table 10) revealed that the right gastrocnemius muscle weight was significantly higher in the sedentary animals receiving an iron sufficient diet versus the exercised animals receiving the moderate iron deficient diet.

TABLE 10. LEAST SQUARE MEANS FOR GASTROCNEMIUS (RIGHT) MUSCLE WEIGHT IN EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p≥T Ho:LSM(i)= LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.2818	-		
HS	3	0.2381	0.0272*	-	
LS	4	0.8221	0.1950	0.3375	-

* significant at p≤0.05

In contrast to these results, Gagne et al. (1994) found that although weights of the right gastrocnemius muscle in the iron deficient exercised and sedentary groups were comparable, the right gastrocnemius muscle was significantly lower in the iron sufficient sedentary animals. Variations in the types of muscle fibers, body size and composition and exercise performance may affect the variations in muscle weights as demonstrated by Keul et al. (1972). Although the gastrocnemius muscle weight was significantly lower in the iron deficient female Sprague Dawley rats, the gastrocnemius weight in relation to body weight was maintained in the untrained iron deficient rats and increased significantly with training (Willis et al., 1990). Based on these results, Willis et al. (1988) suggest that the exercise trained, iron deficient skeletal muscles are not diminished in mass through proteolysis.

EVALUATION OF AEROBIC CONDITIONING

The activity of citrate synthase (CS), a marker enzyme of the Krebs cycle, was determined in the heart muscle. Table 11 presents the cardiac citrate synthase enzyme activity in the four experimental groups. Citrate synthase (EC 4.1.3.7), a respiratory enzyme, shown previously (Baldwin et al., 1977) to undergo adaptative increases in skeletal muscle fibers from exercise was used as a training marker in the present study. No significant alteration in citrate synthase activity were noted among the four experimental groups. Physical activity and diet had no significant effect on enzyme activity. No significant interaction effect was observed. Significant differences may not have been seen due to the small cell size. Gagne et al. (1994) found significant increases of 27% and 38%, in skeletal muscle citrate synthase activity of the iron sufficient and iron deficient exercised groups respectively, compared to their sedentary counterparts at the end of the six weeks. Although not statistically significant, cardiac citrate synthase activity was increased by 2% and 7% at the end of the six week exercise program in the HE and LE groups respectively compared to their sedentary counterparts. Previous studies involving swimming programs of endurance training have also reported increases in CS activity plus other skeletal muscle and cardiac adaptations such as increases in myosin and actomyosin adenosine triphosphate activity and cardiac enlargements (Baldwin et al., 1977; Oscai, 1971).

TABLE 11. EFFECT OF 6 WEEK SWIMMING EXERCISE PROGRAM ON CARDIAC CITRATE SYNTHASE ENZYME ACTIVITY IN 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON.

GROUPS	CITRATE SYNTHASE ACTIVITY (umol/g/min)
HE	151.22 ± 5.34
HS	147.98 ± 5.33
LE	157.88 ± 5.97
LS	146.88 ± 5.97
ANOVA F VALUES	
ACTIVITY	1.41
DIET	0.24
INTERACTION	0.47

HE/HS: n =5; LE/LS: n=4

* Means±SEM and F values significant at $p \leq 0.05$

Baldwin et al. (1977) found that both steady state and high speed training conditions induced respiratory enzyme adaptations in the skeletal muscle fibers. However there was no marked increase in CS activity at various stages of training in the cardiac muscle. The authors surmise that the half-life of certain cardiac mitochondrial components (5-6d) is somewhat shorter than the half life of skeletal muscle mitochondrial components (8-10d) and hence the exercise stress was insufficient to appreciably alter the respiratory enzyme activity of cardiac muscle at any stage in the two training programs.

Citrate synthase, as an enzyme marker for oxidative capacity was analyzed in the human skeletal muscle to evaluate the effect of short term iron deficiency and the results indicated that with severely depleted body iron stores, endurance capacity remains intact and the maximal activities of various glycolytic and oxidative enzymes are not altered (Celsing et al., 1986). Similarly in a later study, Celsing et al. (1988) determined the effects of various degrees of chronic iron deficiency anemia on enzyme activity in the human skeletal muscle. Maximal activities of the iron dependent and non-iron dependent glycolytic and oxidative enzymes and Mb showed similar values in iron deficient group and controls.

Johnson et al. (1990) investigated the combined effects of endurance training and iron deficiency in weanling female rats. Although dietary iron deficiency had no effect on the TCA (tricarboxylic) cycle enzymes - CS and malate dehydrogenase, the activity in the soleus muscle was 50% higher in the iron deficient trained groups compared to the other groups. Thus the authors suggested that the decrease in mitochondrial iron-containing

electron transport enzymes and cytochromes is associated with an increase in the TCA cycle enzymes. In a similar study model Willis et al. (1988) found that iron deficiency in 21 day old female rats had no effect on the activity of the TCA cycle enzymes in skeletal muscle. Four weeks of mild training in iron-deficient rats resulted in improved TCA cycle enzymes of skeletal muscle (27-58%) and heart (29%) but did not affect these parameters in the iron sufficient animals. Endurance training uniformly increased the CS activity by 27% in the GN muscle of iron deficient rats and by 39% in the plantaris muscle. Physiological adaptations such as hypertrophied hearts and increases in TCA activity in skeletal muscle are associated with a markedly improved work capacity in iron-deficient animals. Other investigators have also found increases in the CS activity in the skeletal muscle of rats undergoing exercise training (Holloszy et al., 1970; Terjung et al., 1972).

IRON HEMATOLOGY

Hematological indices of iron status are presented in Table 12. Hemoglobin (Hb) concentrations in the experimental groups were not significantly different from the BL group. No significant effect of diet or activity on the Hb concentration was seen. No significant interaction effect was observed. Hematocrit (Hct) concentrations were significantly higher than BL in both the exercised groups. Analysis of variance procedure indicated that the Hct concentration was significantly affected by activity but not by diet ($p \leq 0.05$). No significant interaction effect was observed. Earlier studies by Bowering and Norton (1981) and Koziol et al. (1978) have noted Hb levels of 7.2-12.7 g/dl indicating moderate iron deficiency and below 7.2 g/dl indicating severe iron deficiency. Hemoglobin concentrations noted in this study were well below the normal average Hb values while the Hct levels for all groups were at the lower end of levels reported by Mitruka and Rawnsley (1981) for the Sprague-Dawley rats, thus indicating existence of moderate iron deficiency. Hemoglobin levels in animals receiving the moderate iron deficient diet were comparable to initial BL values and higher in animals receiving the iron sufficient diet. Slightly lower erythrocyte and Hb values have been reported in female than in male rats (Mitruka and Rawnsley, 1981). Severe iron deficiency constituted by a 74% and 61% decrease in Hb and Hct levels respectively was seen in rats fed an iron deficient diet compared to controls. Dietary iron repletion increased Hb and Hct levels by 13 and 14% respectively (Davies et al., 1982).

TABLE 12. MEANS AND F VALUES FOR HEMATOLOGICAL INDICES OF IRON STATUS IN EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUP	HEMOGLOBIN (g/dl)	HEMATOCRIT (%)	SERUM IRON (mcg/dl)	TIBC(mcg/dl)
BL	12.45 ± 0.41	40.97 ± 1.12	347.72 ± 33.97	528.56 ± 18.21
HE	12.83 ± 0.31	44.81 ± 0.85*	387.92 ± 25.94	505.01 ± 13.91
HS	12.71± 0.31	43.58 ± 0.85	401.13 ± 27.09	484.26 ± 14.53
LE	12.59± 0.31	44.51± 0.85*	311.65 ± 25.94	473.61 ± 13.91*
LS	12.21± 0 .31	42.74 ± 0.85	381.68 ± 25.94	501.24 ± 13.91
ANOVA F VALUES				
ACTIVITY	0.61	3.73**	2.49	0.08
DIET	1.34	0.54	3.49	0.32
INTERACTION	0.16	0.13	1.18	3.07

* Means ± SEM are significantly different from BL at $p \leq 0.05$

** F values significant at $p \leq 0.05$

Similar results were seen when iron deficient male rats fed a low iron diet for 8 weeks and treated with iron over an 8 day period showed a 53% increase in Hb (Mclane et al., 1981). In the present study although not statistically significant, higher Hb/Hct levels were observed in the iron sufficient diet groups than the moderate iron deficient groups. This may suggest that the higher iron content of the diet was being absorbed and utilized by the rats. The low Hb/Hct levels observed in the animals consuming the moderate iron deficient diet indicate existence of a moderate iron deficiency.

Gagne et al. (1994) also observed higher Hb/Hct in animals receiving iron sufficient diets. Significantly decreased Hb and Hct values have been reported in rats receiving iron deficient diets compared to animals receiving an iron sufficient diet (Borel et al., 1991; Koziol et al., 1978; Mclane et al., 1981; Perkkio et al., 1985; Willis et al., 1990; Willis et al.,1988). Willis et al. (1990) evaluated Hb concentration and exercise endurance within the first 18 hours following iron treatment in animals receiving iron deficient and control diets. Endurance increased three fold in the iron deficient treated animals without a significant increase in Hb concentration, although the values were significantly lower compared to iron sufficient animals. In one of the experiments to determine the relation of Hb and exercise endurance during the early progression of iron deficiency, Willis et al. (1990) found 36 day old rats fed low iron diets for 15 days were severely anemic compared to control animals. By 46 days of age concentrations had declined slightly but significantly ($p \leq 0.05$). Borel et al. (1991) studied the impact of varying degrees of iron nutriture on functional consequences of iron deficiency. Their finding demonstrated that

certain physiological manifestations of iron deficiency occurred even at a moderate to mild degree of anemia. A linear increase in the mean Hb concentration was evident with an increase in the iron content. Dietary iron intake of 11 mg fe/kg diet or less resulted in anemia, while as iron intake increased above 11 mg fe/kg, the Hb concentration stabilized at normal levels.

No significant differences in Hb/Hct levels have been observed in previous studies between exercised and sedentary female weanling rats (Bowering and Norton, 1981; Ruckman and Sherman, 1981; Strause et al., 1983). However in this study higher levels were observed in exercised animals compared to their sedentary counterparts. Prasad and Pratt (1990) found Hb concentrations were significantly affected by activity but not diet. Ruckman and Sherman (1981) observed higher Hb and Hct levels in exercised and sedentary male rats than in the female rats. In female rats fed iron deficient diets and exercised for 21 days, Strause et al. (1983) found that although the exercised group had higher Hb concentrations, levels did not differ significantly from sedentary female rats over the experimental period. Although an iron deficient diet significantly reduced Hb levels by 61%, exercise training did not significantly affect Hb concentration of iron deficient rats (Willis et al., 1988). Iron deficiency and exercise training may interact to adversely alter iron physiology, as observed by Tobin and Beard (1989) on studying the effect of dietary iron deficiency and 12 weeks of exercise training on Hb concentration in male rats. Exercise training did not improve the anemia of iron-deficiency anemia, nor did it affect the hematological values of control animals. The study failed to demonstrate that

submaximal exercise training lessens the impact of iron deficiency on circulating Hb or red cell mass in iron deficient animals as has been suggested by an earlier study. Johnson et al. (1990) found no significant difference in Hb levels of exercised and sedentary female rats fed iron sufficient (50 mg fe/kg) diets. Animals receiving iron deficient diets (6 mg fe/kg) diets had Hb levels that were significantly lower (46%) than those of the iron sufficient groups, there was however no difference in Hb levels between the sedentary and exercised groups. Effect of exercise on Hb, Hct and blood volume have produced controversial results with increases, decreases or no effect on hematological concentrations. Ruckman and Sherman (1981) concluded that increased Hb/Hct levels in the exercised male rats were not a result of a decreased blood volume but may be due to accelerated iron mobilization and transport to provide iron for erythrocyte synthesis in the bone marrow.

Serum iron levels in the experimental groups were not significantly different from the BL group. No significant effects of dietary intake, activity or an interaction effect were observed among the groups. The least square means multiple comparison test (Table 13) revealed significant differences among the groups. The exercised animals receiving the moderate iron deficient diet, had a significantly lower serum iron level compared to animals receiving the iron sufficient diet. Sedentary animals receiving an iron sufficient diet had a significantly higher serum iron level than the moderate iron deficient-exercised animals. Among the exercised group values were 24% higher in the HE group indicating that an iron sufficient diet compensated for the demands imposed by exercise.

TABLE 13. LEAST SQUARE MEANS FOR SERUM IRON IN EXERCISED AND NON-EXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p>T Ho:LSM(i)= LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.0428*	-		
HS	3	0.7254	0.0211*	-	
LS	4	0.8651	0.0620	0.6053	-

* significant at $p \leq 0.05$

Prasad and Pratt (1990) found a significant effect of dietary iron intake on serum iron levels. Serum iron levels were significantly decreased in the iron deficient group than in the iron sufficient group. The exercised animals had lower serum iron levels compared to their sedentary counterparts. Although not statistically significant, Prasad and Pratt (1990) also found higher serum iron levels in the nonexercised groups compared to the exercised groups. Low serum iron levels with intense training may suggest the gradual depletion of iron stores (Frederickson et al., 1983). Gagne et al. (1994) also found a 32.7% and 7.8% reduction in serum iron of the iron deficient exercised and iron sufficient exercised animals respectively. Mclane et al. (1981) reported an 80% reduction in serum iron of iron deficient male rats compared to rats on control diet. In rats on iron deficient diet and supplemented with iron or with 8 days of iron therapy, serum iron levels were about 13% and 29% lower than serum iron levels in control rats. Ruckman and Sherman (1981) found that although exercised or sedentary female rats had a significantly higher serum iron level then the exercised and sedentary male rats, there was no significant difference in serum iron levels between exercised and sedentary animals. Strause et al. (1983) found that serum iron levels were significantly higher in exercised rats, however levels returned to initial values upon 2 weeks of rest.

TIBC levels in the exercised animals receiving iron deficient diets were significantly lower than BL levels. No significant effect of diet or activity was seen. No interaction effect was observed. TIBC levels were 6% higher in the LS groups than the LE groups. In the iron sufficient groups a 4% increase in the HE group compared to the

sedentary group was seen. Prasad and Pratt (1990) found TIBC levels were 9% higher in the iron deficient non exercised group compared with the exercised group, as well as a 3% reduction in TIBC levels in exercised animals compared to sedentary group in iron sufficient animals. Both lower and unchanged levels of serum iron and TIBC have been noted with exercise since serum iron and TIBC are thought to be more accurate indicators of available iron than Hb or Hct (Ruckman and Sherman, 1981).

Gagne et al. (1994) found higher TIBC levels in exercised groups. Mclane et al. (1981) found a 40% increase in TIBC levels in rats made iron deficient by feeding them a low iron diet compared with controls. Ruckman and Sherman (1981) and Strause et al., (1983) found no significant differences in TIBC levels in both the exercised and sedentary male and female rats. In male rats receiving varied levels of dietary iron for 6 weeks, impaired erythropoiesis as indicated by significantly lower mean plasma iron concentration and transferrin saturation and a significantly higher TIBC was noted in rats receiving the low iron diets (4 and 6 mg fe/kg) compared to the other groups (Borel et al., 1991).

TISSUE IRON STORES

Mean iron concentration in the liver, spleen, left and right gastrocnemius and soleus muscles respectively are presented in Table 14. Similar to results of Prasad and Pratt (1990) liver iron concentration were comparable in BL and HS groups and lower in the other groups. Liver iron concentration was significantly lower in the LE group compared to the BL group. Concentration of iron in liver showed a significant effect of diet ($p \leq 0.05$) but not activity. No interaction effect was observed. Among the exercised animals liver iron concentration, as indicated by the Least Square Means Multiple Comparison Test (Table 15), was significantly higher in the iron sufficient animals. Among the iron sufficient exercised and sedentary groups, the liver iron concentrations were higher by 23% and 13% respectively, compared to the iron deficient exercised and sedentary groups. Additionally, the liver iron concentrations were greater by 13% and 12% in the iron sufficient and iron deficient sedentary groups respectively, compared to the exercised iron sufficient and iron deficient groups. A significant difference was also found in the HS group versus the LE group which was lower. Liver iron concentrations were lower in the exercised groups versus their sedentary counterparts and also lower in the iron deficient animals versus the iron sufficient group. Prasad and Pratt (1990) and Gagne et al. (1994) also found that the liver iron stores were significantly lower in the iron deficient groups, as well as in the exercised animals.

TABLE 14 MEANS AND F VAULES FOR TISSUE IRON CONCENTRATION IN TISSUES (WET WEIGHT) OF EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUP	LIVER (mcg/g)	SPLEEN (mg/g)	GASTROC L (mcg/g)	GASTROC R (mcg/g)	SOLEUS (mcg/g)
BL	548.8 ± 32.8	4.5 ± 0.32	25.00 ± 3.1	22.6 ± 1.0	41.8 ± 4.4
HE	531.3 ± 25.1	3.3 ± 0.25*	23.8 ± 2.4	22.9 ± 0.80	38.6 ± 3.2
HS	547.9 ± 25.1	3.1 ± 0.25*	26.8 ± 2.4	20.5 ± 0.80	40.0 ± 3.1
LE	432.0 ± 25.1*	3.1 ± 0.25*	24.0 ± 2.4	23.3 ± 0.80	40.9 ± 3.1
LS	485.0 ± 25.1	2.7 ± 0.25*	23.7 ± 2.4	21.3 ± 0.80	34.6 ± 3.2
ANOVA F VALUES					
A	1.76	1.30	0.29	10.85*	0.78
D	9.55*	1.90	0.34	0.69	0.35
I	0.48	0.17	0.42	0.07	2.08

A = Activity; D = Diet; I = Interaction
Gastroc = Gastrocnemius muscle; L=left; R= right

* Means ± SEM are significantly different from BL at $p \leq 0.05$
** F values significant at $p \leq 0.05$

TABLE 15. LEAST SQUARE MEANS FOR LIVER IRON CONCENTRATION IN EXERCISED AND NON-EXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p>T Ho:LSM(i)= LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.0104*	-		
HS	3	0.6569	0.0032*	-	
LS	4	0.2187	0.1603	0.0971	-

* significant at $p \leq 0.05$

Although statistical significance was not reached, Ruckman and Sherman (1981) noted a trend towards decreased liver iron concentrations in the male exercised compared to the sedentary rats. Strause et al. (1983) also found that the total iron in the liver of exercised rats decreased by 61% over the 21 day exercise regimen and remained depressed after two weeks of rest. Authors suggested that the iron mobilized from the liver and spleen was not a response to iron deficiency since only the exercised animals showed this redistribution. Bowering and Norton (1981) reported that the moderate deficient and severe iron deficient diets significantly affected the liver nonheme iron in both the male and female rats. Although no exercise effect was noted the liver nonheme iron levels in the severely iron deficient rats of both sexes were very low, possibly reflecting a basal level of non-mobilizable iron (Bowering and Norton, 1981). A decline in tissue storage iron was also seen in weanling male rats fed three levels of dietary iron - 4, 6, and 11 mg fe/kg diet respectively (Borel, 1991). With increase in dietary iron content above 11 mg fe/kg diet, a linear increase in the liver iron concentration was observed.

There is a continuous deposition of liver iron as the animal grows older until they attain a weight of 450 gm and are approximately 6 months old (Kaufman et al., 1962). Iron content of liver, spleen and bone marrow is reduced below normal in dietary iron deficiency and in hemorrhagic anemia (Morris, 1986). Female rats have a higher total body iron. In contrast to humans, on examining the rates of ferritin synthesis and degradation in male and female rats, Linder et al. (1973) found female rats were better able to accumulate and retain iron fed them than the corresponding male rats, although

both had been depleted to the same degree. Bowering and Norton (1981) also reported female rats having greater iron stores than males.

Iron concentrations in the spleen for all experimental groups were significantly lower than BL. No significant effect of diet or activity on spleen iron concentration was seen. No interaction effect was observed. Although not statistically significant spleen iron concentrations were higher in the exercised and sedentary animals receiving the iron sufficient diet compared to their counterparts receiving the iron deficient diet. However the exercised group in comparison with their sedentary counterparts showed no depletion of iron stores. Gagne et al. (1994) observed that the spleen iron concentration was significantly higher in the exercised, adequate iron group. These results are in contrast to Strause et al., (1983) and Ruckman and Sherman (1981) who reported reduced iron content of the spleen in exercised rats despite an iron sufficient diet. Prasad and Pratt (1990) observed that intake of an iron deficient diet as well as exercise resulted in a reduced spleen iron concentration. Strause et al. (1983) observed that the spleen iron concentration decreased 36% over a 35 day exercise regimen, while in the sedentary rats it gradually increased over a 21 day period. According to Ruckman and Sherman (1981) the overall trend of decreased organ iron levels, noted in the exercised animals, could be related to the increased Hb levels also noted in the study. The authors speculate that there may be a shift of iron from storage sites to Hb. The low levels of iron might also be related to the higher levels of fecal iron excretion noted in the exercised male rats. As suggested by Gagne et al. (1994) the combination of consuming a low iron diet and

exercise may have altered the functional capacity of the spleen to clear red cells from circulation, since the lowest concentration of spleen iron was found in the iron deficient group.

Due to the small sample amounts the left and right soleus muscle were pooled together for analysis of the muscle iron concentration. Iron concentrations in the soleus muscle had decreased compared to BL levels, however there were no significant differences among the BL and experimental groups. Iron concentrations were 4% lower in the iron sufficient exercised group compared with the sedentary group. However iron concentration in the LE group was 16% higher than the LS group. Two way analysis of variance procedure revealed no significant effect of diet, activity or interaction ($p \leq 0.05$). Prasad and Pratt (1990) observed iron concentrations in the soleus muscle were significantly higher in BL compared to experimental groups but there were no significant differences in the exercised and sedentary groups. No significant differences were observed in the iron concentration of the left and right gastrocnemius muscle among the experimental groups compared to the BL group. Two way analysis of variance procedure indicated a significant effect of activity on the right gastrocnemius muscle. No significant effect of diet or an interaction effect was noted on the left and right gastrocnemius muscle iron concentration. Iron concentration in the right gastrocnemius muscle was significantly lower in the sedentary animals (HS, LS) compared to their exercised counterparts (HE, LE) in both the iron sufficient and iron deficient groups by 11% and 19% respectively. Iron concentrations were significantly higher in the LE group compared to the HS group (Table 16).

TABLE 16. LEAST SQUARE MEANS FOR GASTROCNEMIUS (RIGHT) MUSCLE IRON CONCENTRATION IN EXERCISED AND NONEXERCISED 19-MONTH-OLD FEMALE RATS FED TWO LEVELS OF DIETARY IRON

GROUPS	i/j	p≥T Ho:LSM(i)= LSM(j)			
		1	2	3	4
HE	1	-			
LE	2	0.6881	-		
HS	3	0.0157*	0.0056*	-	
LS	4	0.0886	0.0375*	0.4448	-

* significant at p≤0.05

In contrast to the results of Prasad and Pratt (1990) dietary iron intake had no effect on gastrocnemius muscle iron concentration nor did exercise reduce the iron concentration of the muscle. Thus regardless of iron intake exercise seemed to have caused an increase in iron concentration in the right gastrocnemius muscle whereas the opposite effect was seen in the left gastrocnemius muscle in the iron sufficient group. Although Gagne et al. (1994) found no significant difference in the gastrocnemius iron concentration among the groups there was a trend towards higher muscle iron values in the exercised animals. The considerable variation among individual rats may explain this status outcome. Elevation of muscle iron content is indicative of higher muscle Mb content, seen as an effect of training (Ruckman and Sherman, 1981; Hunding et al., 1981). Muscle myoglobin concentration increases after exercise training in iron sufficient rats (Hickson,1981; Strause et al., 1983) and remains constant (Bowering and Norton, 1981; Koziol et al., 1978) or becomes reduced (Hagler et al., 1980; Hickson, 1981) in exercised animals during iron deficiency. Ruckman and Sherman (1981) found that the muscle iron concentration was significantly higher in the male exercised rats compared to the sedentary while no effect was seen in the female exercised rats. It has been reported that muscle Mb is elevated in those muscles being directly stressed by an exercise regimen (Hagler et al., 1980). The increase in muscle iron was most likely caused by the increase in muscle Mb content.

SUMMARY AND CONCLUSIONS

The purpose of this study was to examine the effects of exercise and dietary iron intake on the iron status in 19-month-old adult female rats. Female rats received diets containing one of two levels of dietary iron, an iron sufficient diet (H=42 ppm) or a moderate iron deficient diet (L=7 ppm). The animals were either exercised (E) or remained sedentary (S). Fifty-seven female Sprague-Dawley rats with similar weight distribution were randomly assigned to one of the five experimental groups: Baseline (BL), an iron sufficient-exercise group (HE), iron sufficient-sedentary group (HS), moderate iron deficient-exercise group (LE) and the moderate iron deficient-sedentary group (LE). Swimming exercise sessions were conducted five days per week for a period of six weeks, after which the animals were sacrificed. The liver, spleen, heart, soleus and gastrocnemius muscles were analyzed for iron content and blood was also analyzed for Hb, Hct, TIBC and serum iron parameters. Citrate synthase, a respiratory enzyme used as a training marker was measured in the heart for verification of aerobic capacity development from exercise training.

Body weights of the female rats showed a significant effect of the activity level. Dietary iron deficiency did not lower the body weight of female rats and no interaction effect of diet and activity was noted. Six weeks of exercise training had a detrimental effect on body weight. Final body weights of the female rats were depressed in both the iron sufficient and moderate iron deficient exercised groups in comparison with their

sedentary counterparts. Body weight in the LS group was significantly higher then the LE and HE groups. Total intake was significantly affected by activity as well as diet. No interaction effect was observed. Intake was significantly higher in the LS group compared to the LE group, as well as the HE and HS groups. Dietary iron also affected total intake as indicated by significantly higher intakes in the LS group than the HS and HE groups. Thus the results indicate that exercise training affects food intake and body weight gain of female rats as evidenced by higher total intake and body weight in the sedentary rats. Dietary iron deficiency had a significant effect on total intake as indicated by higher intake in the iron deficient group.

Results showed no significant differences in liver and spleen weights among the experimental groups. Cardiac muscle weights were significantly different among the experimental groups. The HE group had a significantly higher heart weight then the HS group. Cardiac weights were comparable among the LE and LS groups and were significantly higher then the HS group. Since cardiomegaly maybe characteristic of iron deficiency and occurs as a adaptational change in response to exercise and iron deficiency these results indicate that moderate iron deficiency as well as exercise training resulted in cardiac hypertrophy in the female rats. The left soleus muscle weight was significantly lower in HS, LE and LS groups as compared to the BL. While the right gastrocnemius muscle weight was significantly higher in the HS then the LE group. These differences may be a result of individual variation among the animals.

Cardiac CS enzyme activity did not differ significantly among the experimental

groups. No significant effect of diet, activity or an interaction effect was noted. Although the trend towards improved levels in the exercised group may suggest a physiological adaptation, results indicate that the exercise stress was insufficient to significantly alter CS enzyme activity in the cardiac muscle.

Hemoglobin concentration in the experimental groups was not significantly different from BL. No significant effect of diet, activity or an interaction effect was noted. Hematocrit concentrations showed a significantly affected of activity and were significantly higher in the exercised group as compared with the BL levels. No significant effect of diet or an interaction effect of diet and activity was observed. Hemoglobin and Hct levels were higher in the iron sufficient animals and also tended to be higher in the exercised animals. These higher levels in the exercised rats may be attributed to an increased iron mobilization and transport to provide iron for erythrocyte synthesis. Levels of both Hb/Hct were at the lower end of the average levels for all groups indicating a moderate iron deficiency. Serum iron levels did not differ significantly from BL. No significant effect of diet, activity or an interaction effect was noted. Among the exercising animals the moderate iron deficient animals had a significantly lower serum iron level compared to animals receiving an iron sufficient diet. The HS group had a significantly higher serum iron then the LE group. Results imply that an iron sufficient diet compensates for the demands imposed by exercise. When storage iron levels are depleted, serum iron levels fall if the iron cost exceeds the iron storage levels. The exercised animals had a lower serum iron than their sedentary counterparts, suggesting the gradual

depletion of iron stores. TIBC levels in the LE group were significantly lower than BL levels. TIBC levels were not affected by the diet, activity or an interaction of diet and activity.

Liver iron concentrations were significantly lower in the LE group compared to BL and demonstrated a significant effect of diet. No significant effect of activity or an interaction effect of diet and activity was observed. Liver iron concentrations were significantly higher in the HE group than the LE group. Concentration of liver iron were also significantly higher in the HS group compared to the LE group. A trend towards lower liver iron concentrations in the exercise group compared to the sedentary group was observed. These results suggest that exercise results in a reduction of liver iron stores and a low level of dietary iron may also deplete liver iron stores. This overall trend of decreased iron in organs of exercised animals may be related to the increased Hb levels also observed in this group suggesting that there may be a shift of iron from storage sites to Hb. Spleen iron concentrations were significantly lower than BL in all the experimental groups. No significant effects of diet, activity or an interaction effect was observed. Spleen iron concentrations tended to be higher in the animals receiving an iron sufficient diet compared to the moderate iron deficient groups. Soleus and the left gastrocnemius muscle iron concentration were not significantly different among the groups. No significant effects of diet, activity or an interaction effect were observed. The right gastrocnemius muscle iron concentration showed a significant effect of activity. No significant effect of diet or an interaction effect was observed. Iron concentrations in the

right gastrocnemius were significantly lower in the sedentary animals compared to their exercised counterparts in both the iron sufficient and iron deficient groups. Iron concentration was significantly higher in the LE group compared to the HS group. These results indicate that exercise training causes an elevation in muscle iron concentration in the gastrocnemius muscle. The increased muscle iron values in the exercised animals are indicative of a higher muscle Mb content which occurs as a effect of training.

These results suggest a trend towards redistribution of iron stores as a result exercise as evidenced by alterations in iron concentrations in some tissues and a trend towards elevated Hb/Hct levels with exercise. Thus dietary iron deficiency and exercise training adversely affect various components of iron metabolism in the aged female rat, enhancing the characteristics of a moderate iron deficiency. Although iron stores may be adequate in the elderly, and no overt anemia may be displayed, exercise as well as a dietary iron deficiency can affect overall iron metabolism and induce a moderate iron deficiency. The body undergoes physiological adaptations in response to exercise stress and prevents development of anemia by maintaining iron stores at compromised levels.

Implications for future research may thus involve (1) Investigating the effects of an increase in the amount and intensity of exercise on iron store depletion. (2) Examining an even older age group to look at the effects of exercise and dietary iron status in the older old population since the 24-36 month old rats may be representative of humans 80 years or older. (3) Investigating the role of exercise in improving cardiovascular fitness and the effects of excessive and iron deficient intake in the elderly humans.

Further research is needed to determine the exact needs of the active elderly versus the inactive elderly and identify the iron cost associated with exercise over a prolonged period of time, quantify the cost and determine appropriate nutrition intervention strategies.

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Vita

Charusheela M Chitale was born in Pune, India, on September 29, 1965. She received a B.Sc. degree in Foods and Nutrition from Maharaja Sayajirao University, Baroda, India in 1986. She graduated with a M.Sc.degree in Dietetics and Institution Management in 1988. She worked as a Research Associate at M.S University until she came to the U.S in 1989 and enrolled in the M.S. program in Human Nutrition and Foods at Virginia Polytechnic Institute and State University, Blacksburg, Virginia. She became a Registered Dietitian in 1992 after completing a Dietetic Internship with the Maryland Department of Health and Mental Hygiene in 1991. She has since worked as a Clinical Dietitian at Crownsville Hospital Center, Maryland.