THE EFFECTS OF EXERCISE ON IRON METABOLISM IN ADULT FEMALE RATS

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(ABSTRACT)

The effects of exercise training and iron intake on iron metabolism in adult female rats were investigated. Adult female Sprague-Dawley rats were assigned to either an exercise (E) or sedentary (S) group and fed either a diet containing 9 ppm (9) of dietary iron (low iron level), or 40 ppm (40) of iron (a level slightly above the National Research Council recommendations). The exercise animals were subjected to a program of swimming, 5 days/week, over a 6-week period.

Total food intake and final body weight were similar between the E and S groups. In both 40-E and 9-E animals, concentration of serum iron was significantly ($P<0.05$) lower while total iron binding capacity was significantly elevated, when compared to sedentary counterparts. Saturation of transferrin was significantly reduced in the 9-E group. Liver and spleen weights did not differ but significant increases in cardiac weights were noted in both E groups. Gastrocnemius muscle weights were similar in both E groups and 9-S, but significantly lower in the 40-S group. In organ tissues, liver iron concentration was significantly reduced in the 9-E animals, while spleen iron level was highest in the 40-E

group. Cardiac iron concentration was significantly reduced in both E and low iron diet groups while levels of iron in gastrocnemius muscle did not differ among experimental groups. In both groups of exercised rats, bone marrow iron was significantly lower when compared to sedentary animals. In response to exercise training, an increase in skeletal muscle citrate synthase activity was observed in both E groups.

This study suggests that exercise affects various parameters of iron metabolism. Regardless of iron intake, physical training appeared to alter distribution of iron stores, that may be associated with alterations of hematological iron transport and iron-containing proteins. The Combination of a low iron intake and intense exercise training appeared to enhance early characteristics of a latent iron deficiency.

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INTRODUCTION

Both nutrition and physical fitness during adulthood and in later life have become areas of clinical and research interest in the past decade. As the U.S. population increases in age, and as the number of elderly individuals increase within the population, additional research will be required to understand the nutritional needs that pertain to the adult who chooses a physically active lifestyle.

The relationship between iron status, exercise training and physical performance has both theoretical and practical significance. Iron, found both in porphyrins and non-heme sulfur centers, performs an essential role in exercise performance, since it is involved with practically every aspect of oxygen utilization. In iron deficiency anemia, the degree of tissue oxygenation is generally reduced, due to reduced hemoglobin levels, causing a reduction or impairment in physical and exercise performance (Ohira and Gill, 1983; Gardner et al., 1977; Finch et al., 1976; Ericsson, 1970). Strenuous, habitual physical training for endurance-type activities such as triathlons and marathon races, have been associated with decreases in red blood cell counts, hemoglobin concentrations and packed-cell volumes (Fredrickson et al., 1983; Clement and Asmundson, 1982; Dressendorfer et al., 1981; Ehn et al., 1980). This phenomenon has been called

"sports anemia", because of its association with physical training. However, unlike a clinical anemia, in sports anemia, red blood cells usually remain normocytic and normochromic.

Several studies have reported various incidences of suboptimal levels of hemoglobin, hematocrit, serum iron, transferrin saturation percentages and total iron-binding capacity, in various endurance athletes (Stewart et al., 1984; Fredrickson et al., 1983; Dufaux et al., 1981). In addition to reduced levels of iron hematological status, a number of endurance-skilled athletes maintain low body iron stores, another predisposing factor for anemia, despite adequate iron intakes (Dufaux et al., 1981; Hunding et al., 1981). Some researchers have suggested that reduced estimates of iron hematology are due to expansion of plasma volume, a phenomenon that commonly accompanies endurance training, causing a hemodilution effect. However, changes in plasma volume cannot explain reduced levels of iron storage, nor changes in erythrocyte size and fragility that have been observed in various athletes with sports anemia.

There is evidence that endurance training affects iron levels in various storage sites, possibly due to alterations in iron utilization, distribution, and/or absorption and excretion. In exercised, iron-sufficient animals, a reduction in iron stores from liver, spleen and possibly cardiac tissue are observed, in comparison to sedentary control animals

(Strause et al., 1983; Ruckman and Sherman, 1981). It has been suggested that iron from these organs is being mobilized and utilized for myoglobin synthesis in the working skeletal muscle. Current evidence indicates that exercise alters the absorption and excretion of iron. The low rates of iron absorption, and marked elevation in fecal iron ex--cretion observed in exercised humans and animals, have been suggested to stimulate the rate of iron mobilization from various storage sites.

Most studies to date, have investigated the effects of exercise on various iron status indices in iron-sufficient, young growing animals or young adults. Little is known about iron status, physical training and the mature adult, since it is generally assumed that adult humans have sufficient iron stores to accommodate iron needs. It is not known whether a reduction in dietary iron alone, or with exercise training, affects various components of iron metabolism in the mature animal model. The purpose of this investigation was to examine the effects of exercise training and iron intake on iron metabolism in adult female rats. Animals were assigned to either an exercise or sedentary treatment, and consumed either a low iron diet or a sufficient level of iron. Exer cise treatment involved swimming sessions that trained for endurance performance. Assessment of endurance training was considered. Food intake and body weight were monitored throughout the experiment. The following parameters were

analyzed at the end of the 6 week trainig period:

a. Final body weight and feed consumption

b. Organ weights- heart, liver, spleen and gastrocnemius muscle

c. Iron hematology- hemoglobin and hematocrit levels, serum iron, percent transferrin saturation and total iron-binding capacity

d. Tissue iron- heart, liver, spleen and gastrocnemius muscle

e. Bone marrow iron stores

REVIEW OF LITERATURE

Overview of Iron Metabolism

Body Iron Content. Total body iron concentration of various animal species vary between 25-75 mg/Kg body weight, estimated on the basis of the sum of individual iron fractions (Bothwell and Finch, 1962). The adult human male contains approximately 49 mg/Kg while the adult female has approximately 38 mg/Kg. For an 80 Kg male and 65 Kg female, these estimates are equivalent to a total body iron content of about 4.0 g and 2.5 g, respectively. A total body iron concentration of 3 g has been reported for the adult rat (Underwood, 1971). Variations in iron content within individuals of one species and among other vertebrate species are explained by differences in sex, body weight, circulating hemoglobin concentration and overall iron stores.

In the human adult, approximately 70% of body iron is classified as functional or essential iron, and the remaining 30% as storage or nonessential iron. Functional body iron is relatively proportional to lean body mass (National Research Council, 1979). The storage forms of iron are generally created in humans between the ages of 15 and 30. Iron stores of some 500-1000 mg are found in males whereas females maintain low iron stores of about 300 mg until menopause. After menopause, the level of iron stores increases to levels found in males.

Physiological Distribution and Function of Body Iron. Classification and quantitative distributions of various iron-containing compounds are listed in Table 1. Most of the body iron exists as protein chelates in which heme protein predominate such as hemoglobin and myoglobin, or as nonheme protein-bound complexes such as ferritin and transferrin.

Red cell hemoglobin constitutes about 80% and 70% of the total body iron content in the adult female and male, respectively. The primary function of hemoglobin is to carry oxygen in the erythrocyte, to the tissues where it is released to take part in oxidatives processes. Myoglobin, found only in muscle tissue, also is an oxygen carrier that serves as a reservior of oxygen to muscle cells and in the removal of carbon dioxide. Iron transported in the serum is bound to the B-globulin protein, transferrin, which therefore serves as an iron transporter. The concentration of transferrin in the serum is influenced by the availability of body iron storage and rate of erythropoiesis (Pike and Brown, 1975). Its quantitative ability to bind serum iron is referred as the iron binding capacity of the serum. No ionic iron exists in the animal body.

Though in smaller amounts, the remaining quantities of functional iron exist in various tissue enzymes, particularly the catalases, peroxidases, and cytochromes. Iron serves as the active component of the enzyme and becomes alternatively oxidized and reduced during cellular respiration.

Table 1. Physiological distribution of iron compounds in an adult male and female

Iron-containing compound	Total body content (mg)	
	80 Kg male	65 Kg female
Functional iron forms:		
Hemoglobin	2400	1800
Myoglobin	340	245
Heme enzymes	85	60
Nonheme enzymes	105	71
Transferrin iron	4	4
Storage iron forms:		
Ferritin	746	236
Hemosiderin	320	84
Totals	4000	2500

[a] Adapted from National Research Council, 1979.

Ferritin and hemosiderin represent the storage forms of iron, which together form the second largest fraction of iron following hemoglobin (National Research Council, 1979). These iron storage compounds are found in largest concentrations in the parenchymal cells of liver and reticuloendothelial cells of bone marrow, spleen and liver. Ferritin is also found in the serum, normally, and is related to iron storage status. Serum ferritin may also function in the transport of iron, as with transferrin.

The distribution between and amount of iron, stored as ferritin and hemosiderin differ according to the total amount of iron stored within the cell. At low concentrations of tissue iron, ferritin predominates, at higher concentrations most of the iron found is in the form of hemosiderin.

Metabolism of Iron. Hallberg (1982) summerized a schematic representation of the metabolism of iron (Figure 1). The metabolic pathways of iron can be described as two loops an internal loop and an external loop. The internal loop describes a continuous reutilization of iron from cells catabolized in the body, primarily the red blood cells. Mature erythrocytes live about 120 days and are then trapped by the spleen and phagocytosed by reticuloendothelial cells. The iron is released from hemoglobin, taken up by transferrin in the plasma and transported to the bone marrow for incorporation into hemoglobin and synthesis of new erythrocytes. A smaller portion of recycled iron is utilized for formation

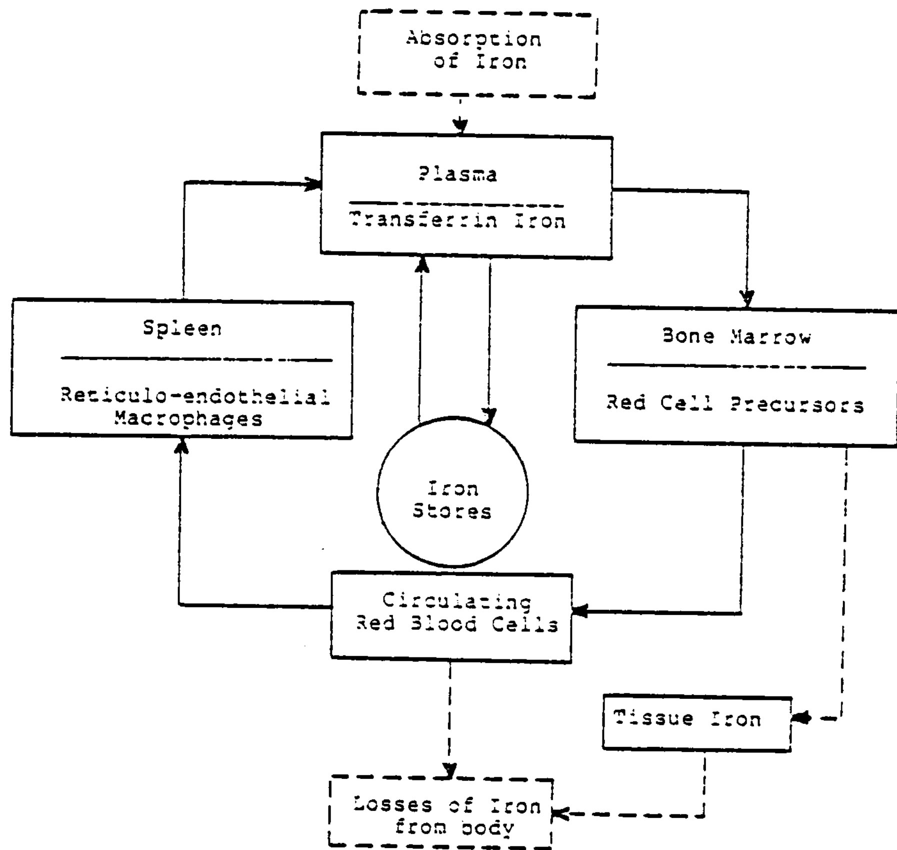

Figure 1. Schematic representation of iron metabolism. The main internal loop (solid line) describes the continuous reutilization of iron and the external loop (broken line) represents dietary iron absorption and iron losses from the body. Adapted from Hallberg, 1982.

of cellular enzymes and incorporation into ferritin and hemosiderin.

The external loop represents losses of iron from the body and the absorption from the diet. Basal physiological losses of iron from the body are predominately an obligatory phenomenon, due to the exfoliation of cells from its surfaces. Losses of iron are primarily through the skin, gastrointestinal tract, and in urine. Green et al. (1968) calculated basal physiological losses of iron as 14 ug/Kg body weight/day which amounts to about 1 mg in a 70 Kg adult male and 0.8 mg in a 65 Kg female. In the gastrointestinal tract alone, the summation of iron content from exfoliated intestinal cells, bile and erythrocytes can account up for to 70% of the total body iron losses. Daily urinary iron losses of 0.1 mg or less are considered insignificant to the total body iron losses (Man and Wadsworth, 1969; Green et al., 1968). Iron losses through the skin generally range from 0.2 to 1.2 mg/day except in cases of excessive sweating where studies have reported several mg of iron loss daily (Vellar, 1968; Prasad et al., 1963). However, evidence indicates that high values of iron in sweat result from technical difficulties in the chemical analyzes used to measure steady-state iron losses (Hallberg, 1982).

Additional Iron Losses in Females. Iron losses during menstruation and pregnancy must be added to the basal losses. Menstrual blood iron losses have been shown to average

approximately 0.6 mg/day when distributed over a 28 day cycle (Cole et al., 1972; Hallberg et al., 1966). Therefore, the mean total iron loss in menstruating females could increase to 20 ug/Kg/day or 1.4 mg in a 65 Kg female. Menstrual iron losses are consistent in a particular woman but vary markedly between women because of variation in menstrual blood flow. In the study of Hallberg et al. (1966), out of a randomly selected group of 476 women, 25% of these women exceeded a menstrual iron loss of 0.9 mg/day; and 10% 1.4 mg/day. Exception to these figures are women with menstrual irregularity, amenorrheic conditions and/or using oral contraceptives.

During pregnancy, iron requirements are enhanced due to the basal losses of the mother (240 mg), expansion of her red-cell mass (500 mg) and requirements for the placenta and fetus (300 mg) (Beaton, 1974). At delivery, about 200 mg of iron is lost through blood. Therefore, a total iron requirement of 1000 mg or 3.5 mg/day over 9 months is estimated in pregnancy. During lactation, daily iron loss is reduced if cessation of menstruation continues. The sumation of basal iron losses (0.8 mg) and iron lost into breast milk (~ 0.25 mg/day), which adds up to an approximate 1 mg/day, is estimated for the daily iron requirement during this period.

Iron Intake and Absorption. The absorption of iron the diet is a function of several factors. These include body iron status, health status, the bioavailability of the iron ingested, the presence and amount of other dietary components

such as fiber and phytic acid, and such luminal factors as gastric acidity and gastrointestinal motility (Hallberg, 1982). In healthy people with adequate iron stores and intake, iron is absorbed at a rate to maintain body iron content at a relatively constant level. Iron absorption generally decreases as iron stores increase while depletion of iron store causes elevation in iron absorption. In iron deficient people, rate of iron absorption may increase two to three times above that in healthy individuals (National Research Council, 1979). Conditions that stimulate erythropoiesis such as severe blood loss, hemolysis, or exposure to decreased ambient oxygen tension also have been shown to increase iron absorption (Marsh and Koenig, 1982; Hathorn, 1971; Jacobs and Finch, 1971).

Luminal factors such as gastric acidity, the presence of ascorbic acid, sugar and amino acids enhance iron absorption by enhancing the reduction of ferric iron to the ferrous form, to create low-molecular iron chelates (Cook et al., 1964; Jacobs et al., 1964). Iron absorption is affected by the motility of the gastrointestinal tract. Operative procedures on the stomach that increase stomach emptying time and/or reduce transit time, also reduce the rate of iron absorption (Bezwoda et al., 1976; Magnusson, 1976).

The varied bioavailability of food iron corresponds to the form of iron: heme versus non-heme iron, and composition of the meal ingested. Heme iron, derived from hemoglobin and

myoglobin in meat, fish, and poultry, is readily absorbed regardless of meal composition (Bothwell et al., 1979). Non-heme or inorganic iron is found in vegetable foods such as legumes, vegetables, whole grains, dried fruit and nuts, in varying concentrations. The absorption of non-heme iron is less efficient than heme iron and is affected by the presence of several dietary compounds. Inhibitors of non-heme iron absorption include dietary fiber, phytate and inorganic phosphates, found also in vegetal food sources, while ascorbic acid and animal tissues have been shown to enhance non-heme iron absorption in a given test meal (National Research Council, 1979).

Iron Deficiency. In iron deficiency, total body iron content is decreased. A deficiency may range in severity from diminished body iron stores without any restriction on erythropoiesis to a severe anemia causing multiple deficiencies in iron-containing tissue enzymes (Bothwell et al., 1979).

Three stages of iron deficiency have been identified. The earliest stage is iron depletion or referred to as prelatent iron deficiency. Storage iron becomes depleted but essential body iron is not affected. During iron storage depletion, a parallel fall in serum ferritin is observed at the time plasma transferrin concentration, along with the total iron-binding capacity, increases. However, values of transferrin saturation remain normal during this period since

concentration of serum iron increases proportional to storage iron release (Marsh and Koenig, 1982). Also in response to storage depletion, gastrointestinal iron absorption increases although most of the iron that is needed is derived from the storage compartments.

The second stage, iron deficient erythropoiesis, or latent iron deficiency, begins when the supply of iron is insufficient to the erythroid marrow. Hemoglobin level, hematocrit or red blood cell indices may decrease but remain within normal ranges although an increase in microcytic cells is noted (Wintrobe et al., 1981). An increase in red cell concentration of free protoporphyrin is observed at the same time a reduction in the marrow sideroblast count occurs.

The third stage, iron deficiency anemia, begins arbitrarily when the hemoglobin concentration or hematocrit fall below normal ranges. Most of the circulating cells are normocytic and normochromic but are gradually replaced by microcytic hypochromic cells as the anemia progresses. A number of functional iron-containing compounds such as myoglobin, the cytochromes and various iron tissue enzymes also become depleted during the anemic stage (Beard et al., 1981).

Iron Status in the Elderly

Hemoglobin concentration and serum iron levels tend to be lower in apparently healthy elderly men (55 years and older) than younger males, 20 to 25 years of age (Gershoff et

al., 1977; Morgan, 1962; Hawkins et al., 1954; Shapleigh et al., 1952). For women, McDonough et al. (1965) found that hemoglobin and serum iron increased after menopause and then remained relatively constant. More recent surveys carried out on elderly people also report slightly lower levels of hemoglobin and various hematological indices and that sex differences diminish with age (Dallman et al., 1984; Htoo et al., 1979; Kelly and Munan, 1977; Milne and Williamson, 1972).

In the Ten-State-Nutrition Survey (DHEW, 1972) indication of deficient hemoglobin levels were reported in 20% of the elderly studied. Serum iron and percent transferrin saturation levels were also reported in the low ranges. Low values of hemoglobin concentration had been reported in the first Health and Nutrition Examination Survey, HANES 1971-1972, (DHEW,1975) and a study conducted on the elderly in Boston (Gershoff et al., 1977) though transferrin saturation levels were not at levels to suggest anemia. Examinations conducted on the second National Health and Nutrition Examination Survey (NHANE II 1976-1980) report that relative prevalence of hemoglobin concentration depression due to anemia or inflammatory disease was largest in infants, teenage girls, young women and elderly men (Dallman et al., 1984).

Inflammatory disorders and disease conditions in the elderly have been documented to be major contributors to iron

deficiency (Dallman et al., 1984; Nordstrom, 1982; Douglas and Adamson, 1975). Decreased iron absorption may occur due to chronic loss of blood from bleeding in the gastrointestinal tract, chronic diarrhea, steatorrhea, gastric or duodenal ulcers, achlorhydria, and total or partial gastrectomy (Walsh et al., 1981).

It is hypothesized that reduced hemoglobin and hematocrit levels could represent a normal physiological process of aging. The progressive changes in body composition, when an increase in body fat and a decrease in lean body mass are observed with aging (Gregerman and Bierman, 1974; Novak, 1972; Forbes and Reina, 1970), suggest a decreased requirement for oxygen on the cellular level. This in turn may initiate an adaptation response causing a reduction in circulating hemoglobin (Smith and Bidlack, 1982).

Effects of Exercise on Iron Metabolism

Iron Hematology. Various hematological assessments conducted on total circulating hemoglobin, hematocrit, blood volume, blood flow and distribution have been studied in young active humans and rats. Both hemoglobin and hematocrit levels in young athletes have been documented to increase (Karvonen and Saarela, 1976; Costill and Fink, 1974), decrease (Frederickson et al., 1983; Hunding et al., 1981; Puhl et al., 1981; Lindemann et al., 1978; Brotherhood et al., 1975; Brown et al., 1974; Ashida, 1972; DeWijn et al., 1971;

Yoshimura, 1970) and remain unaltered (Ehn et al., 1980; Wirth et al., 1978; Boning et al., 1976) after prolong physical training.

Data on total blood volume has been shown to increase (Brotherhood et al., 1975; Dill et al., 1974; Oscai et al., 1968; Holmgrem et al., 1960) or remain constant (Glass et al., 1969; Dill et al., 1966) after exercise training. An increase in plasma volume is presumed to temporarily lower values of hemoglobin and hematocrit by hemodilution. However, previous studies that have reported reduced estimates of iron hematology cannot be explained by a hemodilution phenomenon because of other concomitant changes as mean erythrocyte size and a decrease in erythrocyte fragility (Puhl et al., 1981; Puhl and Runyan, 1980). Reduction of plasma volume, due to thermal dehydration from intense exercise and elevated environmental temperatures, has shown to elevate hemoglobin and hematocrit levels immediately after the exercise (Karvonen and Saarela, 1976; Costill and Fink, 1974).

There are indications that blood flow and distribution of blood become altered as a result of cellular adaptation to exercise training (Rowell, 1971; Saltin, 1969). With training, muscle cells extract and utilize oxygen from the circulating blood at a greater capacity, as indicated by increases in arteriovenous oxygen differences (Saltin, 1969). It is hypothesized that with this enhanced cellular capacity to extract oxygen, less blood would be needed to meet the

muscle's oxygen needs and would then be distributed and therefore oxygenate other organ tissues. This in turn would promote fatigue resistence during prolong submaximal physical activity (McArdle et al., 1981).

Exercise has been shown to reduce serum iron and transferrin saturation percentages in some studies (Frederickson et al., 1983; Haymes, 1973; Bottiger et al., 1971; DeWijin et al., 1971; Kilbom, 1971) and also in a study when hemoglobin and hematocrit values remain within normal ranges (Heinrich, 1968). On the other hand, other investigators (Ehn et al., 1980; Wirth et al., 1978) have reported that physical training does not affect iron status via alteration of hemoglobin, hematocrit, or serum iron indices. The disparity of these studies could be due to differences in initial body iron stores among subjects and differences in training intensity. Reductions of iron hematological parameters become more pronounce only when iron stores become diminished (Jacobs and Worwood, 1971). Slight reductions (Frederickson et al., 1983; Haymes, 1973; DeWijn et al., 1971; Kilbom, 1971) and no changes (Lindemann et al., 1978) in total iron binding capacity have been reported in humans and rats (Strause et al., 1983; Ruckman and Sherman, 1981) from various exercise regimes.

Tissue Iron Content and Internal Redistribution. Of the limited amount of research conducted on the effect of exercise on iron stores, Ruckman and Sherman (1981) found an

overall trend toward iron depletion in the liver, spleen, and hearts of exercised male rats. Strause et al. (1983) reported a 61% reduction in liver iron and a 36% reduction in spleen iron over a 35 day exercise regime in exercised female rats compared to their sedentary-control counterparts. Strause et al. (1983) and other investigators (Hickson, 1981; Hickson and Rosenkoetter, 1981; Ruckman and Sherman, 1981; Hagler et al., 1980; Pattengale and Holloszy, 1967) found that myoglobin levels in the heart and soleus muscle increased in response to exercise. These studies suggest that in iron-sufficient animals, liver and spleen iron are mobilized in response to exercise and utilized for myoglobin production in cardiac and skeletal muscles to thereby increase the oxygen-carrying capacity of these working muscles.

In humans, several descriptive studies indicate certain athlete groups have reduced body iron stores. Low iron stores have been reported primarily in male runners (Stewart et al., 1984; Dufaux et al., 1981; Hunding et al., 1981; Ehn et al., 1980) and various groups of female athletes (Hunding et al., 1981; Haymes, 1973; Kilbom, 1971). While the incidence of low iron storages in certain athletes is well documented, the cause of this phenomenon is not well understood. Variations in iron storage estimates could be explained by different laboratory procedures in measuring body iron stores. Ehn et al. (1980) estimated status of iron stores from measures of bone marrow iron; Dufaux et al.

(1981) by serum ferritin; while Hunding et al. (1981), Kilbom (1971), and Haymes (1973) utilized the method for serum iron/total iron-binding capacity.

Ericsson (1970) found that initial values for non-heme iron in the bone marrow decreased about 10% in elderly humans who underwent repeated physical work tests over a short period of time. In the same study, another elderly group which had been given iron supplements, reported an increase in non-heme iron content of about 7%.

Absorption, Excretion, and Elimination. Recent studies provide evidence that exercise alters the absorption and excretion of iron. In the study of Ruckman and Sherman (1981), fecal iron excretion was significantly higher thereby causing apparent iron absorption to decrease by 50% in exercised male rats compared to sedentary male rats. No differences in iron absorption or excretion were found between exercised and sedentary female rats. In contrast, Strause et al. (1983) found that exercise enhanced retention of iron in female rats, confirmed by both radioiron tracer and whole body radioiron measurements. It was suggested by Ruckman and Sherman (1981) that an increased excretion of endogenous iron through the bile could have caused the elevation in fecal iron excretion. A simultanous analysis on copper balance, reported by Ruckman and Sherman (1981), showed no alteration in absorptive capacity or intestinal transit time. Furthermore, since iron stores tended to be lower in the exercised

rats, it is unlikely that iron need was reduced.

Investigations of iron absorption in human athletes are limited. Ehn et al. (1980) found low rates of both heme iron and a greater extent non-heme iron absorption in 8 long distance runners studied over a period of 2 years. In the same study, a high rate of iron elimination was identified, which corresponded to 2 mg iron/day. The large rate of iron elimination could not be explained by intravascular bleeding, hemolysis or hemoglobinuria. These investigators suggest that loss of iron via sweat could explain the accelerated iron loss and therefore caused the exhaustion of bone marrow iron stores. In an earlier study, Velar (1968) reported in cases of extreme sweating, iron losses via sweat could amount up to 40 ug/100 ml sweat. Athletes or active persons engaged in heavy physical workouts have been shown to sweat between 1 to 3 liters/day (Cade et al., 1972). Thus, using these figures, iron elimination through sweat could amount to 0.4-1.0 mg/day.

Effects of Iron Supplementation on Physical Work Capacity. Several investigators have shown that humans (Pate et al., 1979; Cooter and Mowbray, 1978; Gardner er al., 1977, 1975; Weswig and Winkler, 1974; Davies et al., 1973; Anderson and Stavem, 1972; Anderson and Barkue, 1970; Ericsson, 1970) and rats (Finch et al., 1976; Wranne and Woodson, 1973; Edgerton et al., 1972; Jacobs and Glover, 1972; Woodson et al., 1972a) with iron deficiency anemia or low levels of

hemoglobin cannot physically perform at standard levels of fitness. Upon iron supplementation either in oral form or intravenous infusion, the anemic rats showed work capacity improvements within three days of treatment. The majority of the physical impairment can be attributed to suboptimal tissue oxygenation, due to inadequate concentrations of circulating hemoglobin (Woodson et al., 1972a; Linman, 1968) but also a deficit in iron-dependent tissue enzymes that affect muscle performance in the absence of overt anemia (Finch et al., 1976).

Investigators indicate that hemoglobin production cannot be responsible at first, for the improvement observed in work performance upon iron supplementation since work capacity improvements are noted prior to enhanced hemoglobin synthesis. Ericsson (1970) reported a 4-12% increase in physical work capacity in iron deficient but not anemic subjects after oral iron supplementation when there was no change in hemoglobin or serum iron levels. Intravenous infusion of iron dextran to human subjects with iron deficiency anemia has also shown to improve work performance and lower submaximal heart rate within 4 days of treatment prior to changes in hemoglobin concentration (Ohira et al., 1979). Similar studies with rats have shown that the effect of iron deficiency anemia on physical work capacity is not related to hemoglobin level or myoglobin or total cytochrome concentration in either the myocardium or skeletal muscle

(Edgerton et al., 1972).

Initiation of metabolic pathways in other areas such as red cell 2,3-diphosphoglycerate (DPG), skeletal muscle a-glycerophosphate, and serum ceruloplasmin activity, have been examined during iron deficiency and iron administration when vigorous physical activity is tested. The increase of DPG concentration in red blood cells occurs in response to hypoxic conditions, shifting the oxygen dissociation curve to the right and thereby facilitates the release of oxygen to the tissues (Harper et al., 1979). Hypoxic conditions, such as anemia and intense physical exertion combined, influence red cell DPG activity in compensation of oxygen delivery adaptation.

Previous workers have shown that an inverse relationship exists between hemoglobin levels and DPG content (Gardner et al., 1977; Woodson et al., 1972b; Shappell et al., 1971). In the study of Gardner et al. (1977) subjects who had the lowest hemoglobin levels (6-13 g/100 ml) showed significantly greater oxygen delivery adaptation through larger levels of DPG when compared to subjects with highest levels (13 g/100 ml). Gardner et al. (1977) estimated for every 1 g of hemoglobin/100 ml below normal, DPG could expect to increase by 0.8 umol/g of hemoglobin.

Alterations in the concentration of skeletal muscle a-glycerophosphate has been associated with changes in muscle function. At a hemoglobin concentration compatible with

normal work performance, iron-deficient rats showed marked impairment of running ability as compared with control rats (Finch et al., 1976). Iron administration corrected this disability within four days with a parallel increase in a-glycerophosphate activity in skeletal muscle rather than in the cytochrome pigments or myoglobin.

An increase in serum ceruloplasmin in response to physical training has been reported in both humans (Haralambie and Keul, 1970) and rats (Ruckman and Sherman, 1981; Dowdy and Dohm, 1972; Evans et al., 1969). In one study, Dowdy and Burt (1980) reported that serum ceruloplasmin decreased by 32% during the second month of training and remained at low levels throughout the remainder of the study.

MATERIALS AND METHODS

Experimental Design. The effects of exercise on several iron metabolic parameters during a low iron intake in adult female rats was investigated. The rats were fed one of two purified diets, 40 ppm (40) or 9 ppm (9) iron and either received an exercise (E) treatment or remained sedentary (S) (Figure 1). Reference to the four experimental groups will be notated as follows: 40-E, 40-S, 9-E, and 9-S.

Food consumption and body weight were measured during the study while blood and tissue samples were examined at the end of the experimental period.

Animals. Forty-eight, 6-8 months of age, female rats of the Sprague-Dawley strain were utilized in this study. The rats were housed in individual mesh stainless steel cages and fed Purina Laboratory Chow ad libitum for 4 days. At the end of this period 12 rats were randomly assigned to each of the four experimental groups based upon their weight to make the average rat weight per experimental group 345.4 ± 3.8 g. Feed intake and body weight were measured every other day. The environment was maintained at 22°C, with a 12 hr

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[1] Charles River Breeding Laboratories, Wilmington, Massachusetts.

[2] Purina Laboratory Chow, Ralston Purina Company, St. Louis, Missouri.

Iron Level	Experimental Groups (# of Rats)	
	Exercise(E)	Sedentary(S)
40 ppm (40)	12	12
9 ppm (9)	12	12

Figure 2. Experimental Design

light-dark cycle. Each rat was provided with the experimental ration and distilled water ad libitum throughout the study.

Experimental Diets. The composition of the experimental diets is presented in Table 1. The two iron concentrations were commercially formulated in two separate mineral mixes, in the form ferric citrate. The 40 ppm iron diet was considered an adequate level of iron for the rat's requirement while the 9 ppm iron diet was moderately deficient. A dietary iron level of 35 ppm/Kg diet is presently recommended by the National Academy of Science-National Research Council (1978).

Exercise Program. The exercising groups (40-E, 9-E) were subjected to a program of swimming over a period of 6 weeks. The rats swam 5 days a week at the same time each day. They swam in groups of 6, in plastic tanks (62.2 x 92.7 x 71.1 cm) filled to a water depth of 69 cm. The water level was such that the animals could not rest by supporting themselves on the tank's edge. The water temperature was maintained between 32 - 35°C. During the first week, the animals swam for 10 minutes. From week 2 to 6, the duration of the swimming sessions was progressively increased by 10 minutes/week until the animals were swimming continuously for 1 hr/day. After each swim, the rats were placed in dry barrels that were heated with overhead lamps. Once they were placed in the barrel, each rat laid in a supine position for at

Table 2. Composition of Experimental Diets

Dietary Component	Percent of Diet Components	
	40 ppm iron diet	9 ppm iron diet
Cornstarch	30.0	30.0
Sucrose	30.0	30.0
Casein[1]	20.0	20.0
DL-Methionine[2]	0.3	0.3
Choline Bitratate[2]	0.2	0.2
Cellulose[2]	5.0	5.0
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, New Jersey.

[4] AIN Vitamin Mixture 76. Nutritional Biochemicals Corp., Cleveland, Ohio.

[5] AIN Mineral Mixture 76. Nutritional Biochemicals Corp., Cleveland, Ohio.

least 5 minutes, indicating that they were exhausted due to the exercise.

The sedentary groups of rats (40-S, 9-S) remained in their cages throughout the experiment and received the same amount of handling, for feed and weight measurements, as the exercising groups.

Animal Sacrifice, Blood and Tissue Preparation. Each rat was anesthetized with chloroform 24 hr after the last exercise session. Blood was collected by cardiac puncture in a 25 ml plasic polyethylene syringe (Serum Monovette, Walter-Sarstedt, Inc., Princeton, NJ). The 21 guage 1.5 inch needle was immediately removed with a luer cone (Walter-Sarstedt, Inc., Princeton, NJ) and the blood was gently transferred to a polypropylene collecting tube.

Before the blood was allowed to clot, alloquots of blood were transferred into 2 anticoagulant-treated capillary tubes and 2 hemocap tubes for hematocrit and hemoglobin concentration determination, respectively. Both determinations of hematocrit and hemoglobin concentration were conducted on the day of blood collection.

Each remaining sample of whole blood stood at room temperature for 30 minutes to complete clot formation. The blood specimens were then centrifuged at 2000 g (3000 rpm) for 30 minutes, at 5 ^{o}C. Serum was aspirated using Pasteur pipettes into 5 ml polypropylene tubes, and were recentrifuged under the same conditions to assure removal of all

cells from the specimen. Serum was transferred into clean 5 ml polypropylene tubes, capped and stored frozen until analysis.

After blood collection, the heart, liver, spleen, and right gastrocnemius muscle were removed, rinsed with physiological saline, blotted, weighed and frozen immediately in separate polycarbonate specimen cups with a snap-on lid. For the analysis of citrate synthase activity, the left gastrocnemius muscle was removed and treated in the same manner but was wrapped in several layers of aluminum foil and submerged in liquid nitrogen for 8 seconds to prevent any enzymatic degradation during the freezing process. These muscles were also stored frozen at -18 °C until use.

After the gastrocnemius muscles were removed, the proximal portion of the femurs were broken to collect samples of bone marrow. Marrow specimens were manually removed with a stainless steel spatula and fixed in 10% buffered formalin.

Hematological Analyses. Hematocrit levels were determined by microcapillary hematocrit estimation. Hemoglobin concentration was estimated using the cyanmethemoglobin method and read on a Bausch and Lomb Spectronic 21 spectrophotometer. Analysis of serum iron and total iron-binding capacity (TIBC) was conducted with commercially prepared Serum Iron/UIBC kits (American Monitor Corp., Indianapolis, IN.) which supplied all neccessary reagents and standards.

Values for hematocrit, hemoglobin concentration, serum

iron, and unsaturated iron-binding capacity were determined in duplicates and the main value was calculated for each animal. In the analysis of serum iron and TIBC, measurements were repeated if any serum iron value was less than 50 ug/dl or greater than 250 ug/dl and if any unsaturated iron-binding capacity value was below 120 ug/dl or above 360 ug/dl. Known values of serum iron and unsaturated iron-binding capacity in control serum samples (American Monitor Corporation, Indianapolis, IN.) were analyzed along with each assay to assure accuracy of method performance.

Tissue Iron Analysis. Hearts, livers, spleens and right gastrocnemius muscles were freeze-dried to a constant weight and then ground with a mortar and pistol, before being analyzed. Samples of ground-dried tissues were wet-ashed with 2 ml of redistilled nitric acid and 1.5 ml of 70% perchloric acid. Diluted samples of the wet-ashed tissues and prepared iron standards were analyzed for iron using the Perkin-Elmer model 503 atomic absorption spectrophotometer.

Bone Marrow Analysis. The bone marrow specimens were sent to the Virginia-Maryland Regional College of Veterinary Medicine for preparation and staining of bone marrow iron content. Each specimen was processed for paraffin and bedding and sectioned at 5 microns in thickness, for consistent light microscope examination. Several block sections were prepared on a given slide. The slides were then stained using the Perl's test for ferric iron (hemosiderin granules)

(Perls, 1967).

The slides were studied by one examiner in a microscope (American Optical Microstar Series 10) with the oil immersion (100X) objective of the microscope. The amount of hemosiderin granules found in a minimum of 5 sections were summed and averaged for each sample slide. The results were expressed as the mean number of iron-positive bone marrow cells per microscopic oil field.

Citrate Synthase Analysis. Analysis of skeletal muscle citrate synthase activity, a marker respiratory enzyme, was conducted to assess physiological development of aerobic fitness from the swimming-exercise treatment. Due to error, the left gastrocnemius muscle was weighed in the frozen state since initial wet weight was not obtained. Muscle samples from a baseline group of rats did not show any weight differences before or after the same storage period. Therefore the muscle weight was determined in the frozen state and considered an estimate of wet weight, even though moisture loss may have occurred during storage.

After weight determination, the frozen muscle samples were diluted (1:10) with 100 mM KPO_4, pH 7.4 and were homogenized on ice until the muscle fibers were blended in a uniform suspension. Each muscle received the same grinding treatment, removing tendons, hair, etc., during the procedure. The homogenates were centrifuged at 2000g (3000 rpm) for 20 minutes at 5°C. Supernatants were collected and held

on ice until assayed. Citrate synthase activity was determined spectrophotometrically by the method of Srere (1969).

Statistical Analysis. Mean values with standard error of the means were determined for all parameters in each experimental group. Analysis of variance procedures were performed to assess the significance of exercise or diet treatments. Where an F value was significant at $P < 0.05$, Duncan's Multiple Range Test was employed to determine differences between any four means within a group.

RESULTS AND DISCUSSION

Evaluation of Aerobic Conditioning. Citrate synthase, a respiratory enzyme, shown previously (Baldwin et al., 1977, 1972) to undergo adaptive increases in skeletal muscle fibers from exercise, was used as a training marker in the present study. As shown in Table 3, citrate synthase activity was increased after 6 weeks by 27% in the 40-E group and 38% in the 9-E, compared to their sedentary counterparts. Previous studies involving swimming programs of endurance training also report increases in citrate synthase activity plus other skeletal muscle and cardiac adaptations such as increases in myosin and actomyosin adenosine triphosphate activity and cardiac enlargement (Baldwin et al., 1977, 1972; Bhan and Scheuer, 1975, 1973; Oscai et al., 1971).

As shown in Table 4, heart weights of both E groups were significantly larger than both S groups, suggesting a physiological adaptation of the myocardium, in response to the increased work load. The development of cardiac hypertrophy, induced by exercise, allows for greater blood delivery (and therefore oxygen) to the working muscle, due to an enhenced stroke capacity (McArdle et al., 1981). Since only total heart weight was measured in the present study, it is not known whether the increase in heart weight was due to an actual increase in myocardium tissue, and/or residual red blood cells remaining in cardiac tissue, at the time of

Table 3. Effect of 6 week swimming program on skeletal muscle citrate synthase activity in adult female rats fed two levels of dietary iron

Experimental Group	Citrate Synthase Activity (umol/g/min)
40-E	49.7 ± 3.0[a]
9-E	53.2 ± 6.1[a]
40-S	36.2 ± 3.4[b]
9-S	32.9 ± 4.5[b]

[1] Means ± S.E.M. with same letter are not significantly different ($p<0.05$).

Table 4. Heart, liver, spleen and right gastrocnemius muscle weights of adult female rats fed two levels of dietary iron with and without exercise training

Organ (dry wt, g)	Experimental Groups[1]			
	40-E	9-E	40-S	9-S
Heart	0.300 ± 0.032^a	0.293 ± 0.009^a	0.257 ± 0.007^b	0.259 ± 0.007^b
Liver	2.445 ± 0.095^a	2.853 ± 0.280^a	2.621 ± 0.105^a	2.541 ± 0.274^a
Spleen	0.127 ± 0.005^a	0.131 ± 0.006^a	0.136 ± 0.005^a	0.134 ± 0.006^a
Gastrocnemius[2] Muscle	0.554 ± 0.014^a	0.565 ± 0.016^a	0.507 ± 0.019^b	0.567 ± 0.013^a

[1]Means $\pm$ S.E.M. with same letter are not significantly different ($p < 0.05$).

[2]Removed from right lower hind-leg.

autospy. On the other hand, cardiomegaly has been observed in iron-deficient rats (Bowering and Norton, 1981; Cusack and Brown, 1965) though is probably unlikely in this study since larger hearts were also found in exercising animals receiving adequate iron diets.

Organ Weights. Absolute dry weights of heart, liver, spleen, and right gastrocnemius muscle are presented in Table 4. As discussed earlier, heart weights of both E groups were significantly larger than either S groups. Cardiac hypertrophy has been produced in several studies using various swimming protocols (Bowering and Norton, 1981; Ruckman and Sherman, 1981; Oscai et al., 1971; Dawson and Horvath, 1970). In agreement with previous reports (Strause et al., 1983; Ruckman and Sherman, 1981), liver and spleen weights of rats were not affected by either diet or exercise.

Similar weights of the right gastrocnemius muscle were observed in both E groups and the 9-S group, while significantly lower weight values were identified in the 40-S group (Table 4). Variations in muscle weights could be due to differences in initial types of muscle fibers, body size and composition, as well as variations in exercise performance (Keul et al., 1972).

Food Consumption, Iron Intake and Body Weights. In agreement with previous work (Bowering and Norton, 1981; Ruckman and Sherman, 1981), food intake and body weight of female rats fed either adequate or moderately iron deficient

diets, did not differ significantly with exercise (Tables 5 & 6). Daily and total food intake did not differ between E and S groups, regardless of iron intake (Table 5). Based on food intake figures, estimates of daily iron intake ranged between 0.1512 - 0.1566 mg iron/day in the 9-S and 9-E group while rats receiving the 40 ppm iron diet ingested between 0.6520 - 0.6720 mg iron on a daily basis. Although the 9-E and 9-S animals were consuming approximately 77% less iron than the 40 (E and S) animals, no physical manifestations of iron deficiency anemia (i.e., matted-discolored fur, skin lesions, brown-tinged incisor teeth) were observed.

Final body weights among experimental groups did not differ significantly at the end of the 6 week period (Table 6). Final body weight was greatest in the 40-S group (21.5 g higher) while the 9-S and 9-E animals only increased their body weight by 2.1 and 4.1 g, respectively. In comparison to male exercised rats, other investigators (Ruckman and Sherman, 1981) have reported that animals tend to lose weight despite larger feed intakes. Ruckman and Sherman (1981) suggest that differences in the effect of exercise-swimming on weight gain between sexes are likely due to a lower degree of physical activity in female rats, because of greater buoyancy, due to larger percentages of body fat. However, the degree of physical activity in the present study was sufficient enough to augment a training effect, as indicated by skeletal muscle citrate synthase activity (Table 3).

Table 5. Daily and total food consumption in adult female rats fed two levels of dietary iron with and without exercise training

	Food Intake[1]	
Experimental Group	g/day/rat	Total Kg/Group
40-E	16.3 ± 2.3[a]	8.2[a]
9-E	17.4 ± 2.3[a]	8.8[a]
40-S	16.8 ± 2.4[a]	8.5[a]
9-S	16.8 ± 2.8[a]	8.5[a]

[1] Means ± S.E.M. with same letter are not significantly different ($p<0.05$).

Table 6. Initial and final body weights of adult female rats fed two levels of dietary iron with and without exercise training

Experimental Group	Body Weight (g)[1]	
	Day 0	Day 42
40-E	345.0 ± 10.0[a]	341.7 ± 9.9[a]
9-E	349.2 ± 8.2[a]	353.3 ± 7.3[a]
40-S	340.4 ± 7.8[a]	362.1 ± 11.7[a]
9-S	347.1 ± 6.4[a]	349.2 ± 7.9[a]

[1] Means ± S.E.M. with same letter are not significantly different ($p<0.05$).

Iron Hematology. Hemoglobin and hematocrit levels were similarly higher in the 40-E and 40-S group than the 9-E and 9-S (Table 7). In iron-sufficient, exercised female rats, 21 day old (Ruckman and Sherman, 1981) and 2 months of age (Strause et al., 1983), hemoglobin concentration and hematocrit levels have remained unaltered after exercise training. However, in rats consuming the low iron diet (9 ppm), lower hemoglobin and hematocrit levels indicate the existence of a mild iron deficiency. Hemoglobin concentrations generally were between 7.2 - 12.7 g/dl in moderate iron deficiency and below 7.2 g/dl in severe iron deficiency anemia (Bowering and Norton, 1981; Koziol et al., 1978) while hematocrit values decrease 9% and 25% in moderate and severe iron deficiency anemia, respectively, from normal levels (Edgerton et al., 1972). In comparison to average hematological values of Sprague-Dawley rats from other reports (Mitruka and Rawnsley, 1981; Koziol et al., 1978; Schalm et al., 1975), lower values of both hemoglobin and hematocrit in the present study were below the normal ranges.

In humans, it has been substantiated that total blood volume is either increased (Brotherhood et al., 1975; Dill et al., 1974) or remained constant (Glass et al., 1969; Dill et al., 1966) after exercise training. Although examination of blood volume in exercised rats has not been assessed, it cannot explain lower hemoglobin and hematocrit levels observed in the present study since both low iron diet groups,

Table 7. Hematological indices of iron status in adult female rats fed two levels of dietary iron with and without exercise training

	Experimental Groups[1]			
	40-E	9-E	40-S	9-S
Hemoglobin (g/dl)	15.7 ± 0.7^{a}	13.5 ± 0.4^{b}	$15.5 \pm 1.0^{a,b}$	$14.2 \pm 0.6^{a,b}$
Hematocrit (%)	39.1 ± 0.6^{a}	35.9 ± 0.8^{b}	$38.4 \pm 1.3^{a,b}$	$36.3 \pm 1.0^{a,b}$
Serum Iron (ug/dl)	170.6 ± 9.2^{a}	130.4 ± 6.3^{b}	185.1 ± 10.2^{a}	193.9 ± 7.1^{a}
TIBC[2] (ug/dl)	376.3 ± 23.9^{a}	389.4 ± 12.8^{a}	321.3 ± 14.4^{b}	$344.4 \pm 10.6^{a,b}$
Transferrin Saturation (%)[3]	48.6 ± 5.1^{a}	33.7 ± 1.7^{b}	58.8 ± 4.8^{a}	56.8 ± 2.8^{a}

[1]Means ± S.E.M. with same letter are not significantly different ($p < 0.05$).

[2]TIBC = total iron-binding capacity.

[3]Transferrin Saturation = serum iron/TIBC x 100.

exercise and sedentary (9-E and 9-S) demonstrated low values.

In the 9-E group, concentration of serum iron was lower by 32.7% while a 7.8% reduction occurred in the 40-E, compared to both sedentary counterparts. Increases in TIBC were observed in both 40-E and 9-E groups (17.1 and 13.1% respectively) while saturation of transferrin was lowest in the 9-E group. Frederickson et al. (1983) and others (Haymes, 1973; Bottiger et al., 1971; Kilbom, 1971) reported lower serum iron concentrations and percent transferrin saturations in women during intense physical training, suggesting that iron stores were gradually becoming depleted, despite adequate daily iron intakes. In addition, Frederickson et al. (1983) found that after the women stopped exercising, serum iron and percent transferrin saturation returned to pre-training levels within 1 week and that the largest values of TIBC also occurred during this period. These iron hematological alterations suggest that metabolic adjustments were being made to restore the iron reserves that appeared to diminish during training.

The combination of a low dietary iron intake and exercise regime (9-E group) appeared to enhance the early characteristics of a mild iron deficiency without anemia, based on hematological parameters alone. Evidence indicates that a reduction of iron proteins in blood and tissues could occur, during latent iron deficiency, well before the exhaustion of

mobilizable iron stores (Dallman et al., 1982).

Tissue Iron Content. Mean iron concentrations in heart, liver, spleen and right gastrocnemius muscle are shown in Table 8. Cardiac iron concentrations were similar in both E and low iron diet groups while largest in the 40-S group. Although statistically insignificant, both rat groups consuming 9 ppm of dietary iron had lower heart iron concentrations, the largest reduction in the 9-E group. Liver iron concentration was significantly lower in the group 9-E when compared to all other experimental groups. In agreement with previous reports (Strause et al., 1983; Ruckman and Sherman, 1981), both cardiac and liver iron content were reduced in the exercised rats indicating a trend toward iron depletion in these tissues.

Strause et al. (1983) and Ruckman and Sherman (1981) reported reduced contents of spleen iron in exercised rats, despite sufficient iron intake. However, in the present study, concentration of iron in spleen was significantly higher in the adequate iron-exercise rat group (40-E). Yoshimura et al. (1980) has postulated that intense exercise causes the release of a hemolyzing factor, secreted by the spleen, that increases red blood cell destruction. Increased rates of erythrocyte destruction during heavy physical training has been reported in exercised humans (Hunding et al., 1981; Radomski et al., 1980; Yoshimura et al., 1980, 1970; Siegal et al., 1979;) and animals (Gollnick et al., 1965;

Table 8. Heart, liver, spleen and right gastrocnemius muscle iron concentration in adult female rats fed two levels of dietary iron with and without exercise training

	Experimental Groups[1]			
	40-E	9-E	40-S	9-S
Heart (ug/g dry wt)	378.4 ± 4.6^a	367.0 ± 5.5^a	398.8 ± 6.0^b	373.8 ± 5.4^a
Liver (ug/g dry wt)	670.1 ± 44.1^a	555.8 ± 26.4^b	649.3 ± 28.4^a	676.5 ± 22.5^a
Spleen (mg/g dry wt)	6.23 ± 0.29^a	5.11 ± 0.24^b	5.27 ± 0.15^b	5.50 ± 0.19^b
Gastrocnemius[2] Muscle (ug/g dry wt)	87.8 ± 2.3^a	91.1 ± 2.9^a	82.4 ± 3.4^a	80.9 ± 5.4^a

[1] Means $\pm$ S.E.M. with same letter are not significantly different ($p < 0.05$).

[2] Removed from right lower hind-leg.

Hiramatsu, 1960), manifested by the incidence of hemoglobin-urea. Observation of erythrocyte size and fragility alteration during intense physical training (Puhl et al., 1981; Puhl and Runyan, 1980) may play a role in increasing the rate of erythrocyte clearance from circulation, via a mechanism in which the hemolyzing factor, suggested by Yoshimura et al. (1980) may be initiating. In the present study, whether or not a net accumulation of iron in the spleen occurred in the 40-E rats, due to an enhanced lysis reaction of red blood cells, is not known. Measurement of free hemoglobin in the urine was not measured to research this hemolytic theory.

Variations in spleen iron values could have also been attributed to low iron intakes. The combination of consuming a low iron diet and exercise regime could have altered the functional capacity of the spleen to clear red blood cells from circulation for lowest concentrations of spleen iron were found in the 9-E group. However, mean spleen iron content was similar in rats fed low iron and adequate iron who remained sedentary (9-S, 40-S).

No significant differences were observed in gastrocnemius muscle iron concentration between the 4 experimental groups. Although there was a trend toward higher muscle iron values in the exercised animals, the considerable variation among individual rats could explain the statistical outcome. Elevation of muscle iron content is indicative of increased muscle myoglobin content (Ruckman and Sherman, 1981).

Myoglobin concentration commonly increases after exercise training in iron-sufficient animals (Strause et al., 1983; Hickson and Rosenkoetter, 1981) and remains constant (Bowering and Norton, 1981; Koziol et al., 1978) or becomes reduced (Hagler et al., 1981, 1980; Hickson, 1981) in exercised animals during iron deficiency.

Bone Marrow Iron. Table 9 shows the results of the bone marrow examination for iron content. In both groups of exercised rats, stainable bone marrow iron was significantly lower compared to their sedentary counterparts. The incidence of low iron stores in trained individuals has been observed in a number of studies (Stewart et al., 1984; Dufaux et al., 1981; Hunding et al., 1981; Ehn et al., 1980; Haymes, 1973; Kilbom, 1971). In the study of Ehn et al. (1980), bone marrow iron of 8 male long distance runners was either totally depleted or had only traces of iron detected, while hemoglobin and serum iron values fell within normal ranges.

In physically-trained individuals, it has been suggested that low iron storage could be due to 1) inadequate iron intake 2) low iron absorption 3) increased iron loss and/or 4) an increased iron demand (Pate, 1983; Ehn et al., 1980). Although diet patterns and practices vary greatly among recreational and competitive athletes, some studies have reported adequate dietary iron intakes, according to current Recommended Dietary Allowances (R.D.A.s) (Frederickson et al., 1983; Ehn et al., 1980), while other studies have

Table 9. Examination of bone marrow iron content in adult female rats fed two levels of dietary iron with and without exercise training

Experimental Group	Mean No. Iron-Positive Bone Marrow Cells / Microscopic Oil Field[1,2]
40-E	1.77 ± 0.33[a]
9-E	2.76 ± 0.40[a]
40-S	6.53 ± 2.12[b]
9-S	6.42 ± 1.21[b]

[1] Means ± S.E.M. with same letter are not significantly different ($p<0.05$).

[2] Stain: Prussian blue; Magnification: 100X

reported low iron intakes due to weight control or reduction (Clement and Asmundson, 1982; Wirth et al., 1978), in certain athletes.

Low rates of iron absorption, in response to exercise, have been observed in both humans (Ehn et al., 1980) and animals (Ruckman and Sherman, 1981), as well as high rates of iron elimination through fecal excretion (McMahom et al., 1984; Stewart et al., 1984; Ruckman and Sherman, 1981) and urine (Hunding et al., Radomski et al., 1980; Siegel et al., 1979), primarily in the form of hemoglobin, and loss of elemental iron through sweat (Vellar, 1968). Because a number of iron metabolic parameters become altered, in response to physical training, researchers speculate there is an iron cost of physical training and therefore an increased iron requirement in individuals who habitually train. In response to this iron cost of physical training, iron stores appear to be drawn upon, despite adequate iron intakes, intakes that meet two-thirds or more of the R.D.A. (Frederickson er al., 1983; Pate, 1983; Ehn et al., 1980).

SUMMARY AND CONCLUSIONS

The present study was conducted to investigate the effects of exercise on iron metabolism during a low iron intake in adult female rats. Forty-eight adult female Sprague-Dawley rats, age 6-8 months, were assigned on a weight basis to either one of 2 exercise (E) or one of 2 sedentary (S) groups. One group from each E and S was fed an adequate level of dietary iron, 40 ppm (40) and the other was fed a low level of dietary iron, 9 ppm (9). The exercising rats swam 5 days a week for 6 weeks. The duration of the exercise sessions were progressively increased over a 6-week period until the animals were swimming continuously for 1 hour/day. Food intake and body weights were monitored throughout the 6 weeks. The rats were anesthetized on Day 43 and blood was collected by cardiac puncture, then heart, spleen, liver, gastrocnemius muscle, and bone marrow were removed. Samples of whole blood were used for the determination of hemoglobin concentration and microhematocrit while iron concentration, total iron-binding capacity (TIBC) and percent transferrin saturation were determined in serum. Organ weight and iron concentration in heart, liver, spleen and right gastrocnemius muscle were determined while specimens of bone marrow were stained for non-heme iron stores. Verification of aerobic capacity development from the exercise treatment was conducted by measurement of skeletal muscle citrate synthase

activity, a training-marker, respiratory enzyme, in all rats.

After 6 weeks of exercise-swimming, skeletal muscle citrate synthase activity increased 27% and 38% in the 40-E and 9-E group respectively, compared to their sedentary counterparts. Significant increases in cardiac dry weight were also noted in both E groups, compared to both S groups. These findings suggest that the exercise of swimming for aerobic conditioning can be used for adult rats to induce respiratory enyzme adaptation, conducive to a physical trained effect.

Exercise and reduced dietary iron intake did not alter food consumption or body weight in the adult female rats. Total food intake and final body weights were similar between the E and S groups, regardless of iron intake. Animals that consumed the 9 ppm iron diet showed no physical characteristics of iron deficiency anemia. Because these rats were 6-8 months of age, adequate body iron stores probably prevented the development of an overt anemic state during the 6 week period.

Absolute dry weights of liver and spleen did not differ between experimental groups. Cardiac weights were significantly larger in both E groups than either S groups. Weights of the right gastrocnemius muscles were similar in both E groups and the 9-S group, but were significantly lower in the 40-S group. While a reduced iron intake had no effect on organ weights, exercise in the form of swimming developed

cardiac enlargement but had minimal effect on gastrocnemius muscle hypertropy.

Hemoglobin concentration and hematocrit level were similarly higher in both E and S rat groups consuming the 40 ppm iron diet, than rat groups on the 9 ppm iron diet. Concentration of serum iron was 32.7% and 7.8% lower in the 9-E and 40-E group respectively, compared to their sedentary counterparts. Increases of TIBC were found in both 40-E and 9-E animals, 17.1 and 13.1% respectively, while saturation of transferrin was lowest only in the 9-E group. These results indicate that the combination of a low dietary iron intake and exercise regime appear to induce early characteristics of a mild iron deficiency without anemia.

Cardiac iron concentration was significantly reduced in both exercise and low iron diet groups. Liver iron concentration was significantly reduced in the 9-E group. Concentration of iron in spleen was highest in the 40-E group. No significant differences were observed in gastrocnemius muscle iron concentration among experimental groups. Reduced levels of both cardiac and liver iron in exercised animals suggest that tissue iron may become redistributed or altered, in response to chronic physical stress. Elevation of spleen iron in iron-sufficient, exercised rats could have been caused by an increased rate of erythrocyte destruction. Exercise and iron intake had no effect on iron content in gastrocnemius muscle.

Regardless of iron intake, bone marrow iron was significantly lower in the exercised animals, compared to their sedentary counterparts. The incidence of low iron reserves in subjects who train chronically has been associated with inadequate iron intake, reduced iron absorption, increased iron loss, and/or increased iron requirement. Relative rate and extent of iron store depletion would likely depend on levels of iron stored, prior to physical training.

In response to exercise alone, alteration of iron storage, iron transport and iron-containing proteins suggest there is an iron cost involved with physical training. If this is the case, further research is indicated to (1) determine how iron is utilized in response to chronic physical exercise, (2) define a quantity of iron "cost" (reutilization, absorption, excretion) and (3) relate these components to nutritional intervention for effective application.

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