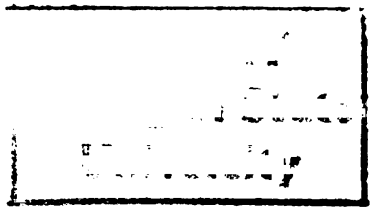

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RELATIONSHIPS AMONG DIET, EXERCISE AND HEMATOLOGICAL PARAMETERS OF YOUNG COMPETITIVE RUNNERS VERSUS NONRUNNERS

presented by

Elaine Ryder

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RELATIONSHIPS AMONG DIET, EXERCISE AND HEMATOLOGICAL PARAMETERS OF YOUNG COMPETITIVE RUNNERS VERSUS NONRUNNERS

By

Elaina Ryder

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ABSTRACT

RELATIONSHIPS AMONG DIET, EXERCISE AND HEMATOLOGICAL PARAMETERS OF YOUNG COMPETITIVE RUNNERS VERSUS NONRUNNERS

By

Elaina Ryder

Hematological status and nutrient intake were evaluated for elite runners versus nonrunners, 8-16 years of age, in relation to developmental stage and physical capacity. Subjects were participants in a study on fitness and performance conducted by the Youth Sports Institute. Fasted venous blood samples were drawn for determination of hematological values, by automated procedures. Three day dietary records were evaluated for nutrient content using the Michigan State Nutrient Data Bank. Respiratory variables were obtained by treadmill testing.

Runners were not at risk of iron deficiency anemia, as indicated by hematological parameters. Runners consumed greater energy, protein and iron intakes than controls. Hematological parameters were similar between iron-supplemented and non-supplemented subjects. No significant relationships were observed of physical capacity with hematological values. Percent of iron absorbed from a meal, calculated according to its bioavailability, was similar to subjects' %RDA for iron, if subjects were assumed to have 250 mg. iron storage levels.

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INTRODUCTION

Iron deficiency anemia is the most common nutritional problem among adolescents (1). There is an increased requirement for iron for this age group because of rapid growth and an increase in hemoglobin production for expanding blood volume, occuring during sexual maturation (2). Combined with this increased need, a low intake of dietary iron by adolescents has been frequently reported in nutritional surveys (3-6).

Several investigators (7-11) have reported that mature, elite runners have a greater tendency to develop anemia than less active individuals. Possible causes for this, as suggested in these investigations, include; sports anemia, disturbance in iron absorption, greater iron loss through excess sweating, and hemoglobinuria and hematuria. Thus far, there have been no studies conducted comparing the hematological status of adolescent runners and non-runners.

The question of iron supplementation of the diet has aroused considerable controversy during the recent years. Conflicting results have been reported in studies conducted to determine the effectiveness of prophylactic iron supplementation in athletic and sedentary individuals (12-15). More research must be done to determine if iron supplementation may or may not be necessary for athletes.

Iron absorption from foods depends not only upon the amount of

iron supplied, but by the nature of that iron and the composition of the meal in which it is consumed (16). Total iron intake, therefore, provides only a rough approximation of the amount available for absorption (17). Monsen (18) developed a model to calculate the amount of absorbable iron in the diet based on the iron status of the individual and the amount of enhancing factors, meat and ascorbic acid, present in the meal. The main objective of this method was to classify the quality of iron in a meal as having high, medium, or low availability.

The purpose of this study was to determine if a sub-population group of elite runners, 8-16 years of age, had a higher incidence of marginal iron status than normal teenage controls of similar age and gender.

The objectives of this study were:

-- To assess the hematological status and nutrient intake in relation to developmental stage and physical capacity over a two year period.

-- To compare the hematological status with quantitative changes in growth, physical capacity, and anaerobic metabolism.

-- To determine the effect of prophylactic iron supplementation on hematological status and performance capability.

-- To evaluate the quality of subjects' diets in terms of the bioavailability of iron.

REVIEW OF LITERATURE

GENERAL METABOLISM OF IRON

IRON UTILIZATION AND STORAGE

The nutritional requirement for iron in humans is derived from the central role that this metal plays in the energy metabolism of cells. Most of the body iron exists in complex forms bound to protein, either as porphyrin or heme compounds including; hemoglobin, cytochromes a, b, and c and P-450, and myoglobin. Iron also exists as nonheme, protein-bound compounds including; ferritin, transferrin, hemosiderin, and flavoprotein enzymes (19). The total body iron in an adult (70 kg. male) is approximately 4 to 5 grams of which 65% is bound as hemoglobin, 15-20% as ferritin, 3-5% as myoglobin, and the remainder as hemosiderin and iron-containing enzymes (20). Iron occurs in blood bound to hemoglobin in the erythrocytes and to transferrin in the plasma in a ratio of nearly 1000:1 (19). Hemoglobin consist of four ferroprotoporphyrin or "heme" moieties linked to four polypeptide chains. Each heme can reversibly bind to one molecule of oxygen functioning as a carrier to supply oxygen to all cells in the body. Myoglobin contains one heme moity and has a higher affinity for oxygen than hemoglobin. This heme compound is found in muscle cells. It functions as an oxygen store, releasing oxygen to cytochrome oxidase when the supply of oxygen is insufficient for the needs of the tissues

(21). Mitochondria contain an electron transport system which transfers electrons from substrates to molecular oxygen - with the simultaneous generation of adenosine triphosphate (ATP). The cytochromes are components of this system. The iron atoms of cytochromes and iron-sulfer enzymes are alternately oxidized and reduced in the process of electron transport. The iron sulfur proteins or flavoprotein enzymes consist of non-heme iron in the active center, which also participate in the electron transport chain (21). Transferrin is a glycoprotein with two almost identical iron-binding sites - each capable of binding one atom of ferric iron. Transferrin serves as the principle carrier of iron in the blood, and therefore, plays a central role in iron metabolism. In normal individuals only 30-40% of the transferrin carries iron, the remainder being known as the latent iron-binding capacity (19).

The reserve or storage iron of the body occurs predominantly as ferritin and hemosiderin. These occur widely in the tissues, with the highest concentrations normally present in the liver, spleen, and bone marrow. The two compounds are chemically dissimilar although intimately related in function. These compounds are involved in the maintenance of iron homeostasis. When the supply of dietary iron becomes inadequate, iron is mobilized from ferritin and hemosiderin and serves to maintain the production of hemoglobin and other iron compounds with known metabolic functions. Not until these products become restricted is there likely to be any impairment of body function (1). The main factor affecting the relative distribution of iron between ferritin and hemosiderin in mammals is the total storage iron concentration. When total storage iron in the liver and spleen

is below 500 mg./g. of tissue, more iron is stored as ferritin than as hemosiderin. When the storage level is above 1000 ug./g., more is stored as hemosiderin (1). Ferritin is the soluble iron storage protein found in all cells of the body. Ferritin is a ferroxidase, catalyzing the oxidation of Fe^{+2} to Fe^{+3} during its incorporation into the iron core. Small quantities of ferritin are present in the erythrocytes, serum, and leukocytes. The levels of ferritin in serum vary with the iron status of the individual and with certain disease states. This form represents only 0.2-0.4% of the serum iron normally present in the adult (21).

Hemosiderin is a term applied to iron which, after staining with potassium ferrocyanide, can be seen as blue granules in sections of liver or bone marrow. Histochemical examination of aspirated samples of bone marrow provides a useful index of body iron stores (19).

IRON ABSORPTION

The three main phases in the absorption of iron from the gut include: the intraluminal phase, where food is digested by the gastric and pancreatic enzymes and iron is released in a soluable form; the mucosal phase, in which iron is taken up by the mucosal cell and transported across to the serosal side or retained as ferritin; and the corporeal phase, in which iron is taken up by transferrin in plasma on the serosal side of the mucosal cell and carried to liver and hemapoietic tissues (22). The absorption of iron is affected by: age, iron status, and state of health of the individual; conditions within the gastrointestinal tract; the amount and chemical form of the iron ingested; and the amounts and proportions of various other components of the diet, both organic and inorganic (19). The most

important known stimuli to iron absorption include the rate of erythropoiesis and the levels of tissue iron stores. These two factors regulate absorption particularly at the level of serosal transfer. The way these stimuli inform the duodenum to transfer appropriate amounts of iron into the plasma is unknown (20). It appears likely that homeostatic mechanisms within the body affect the populations of brush border receptors - according to iron status - at the time of mucosal cell formation. Changes in transport across the serosal surface, occurring with a somewhat shorter time lag, respond more quickly to changes in iron status. Iron can also enter the mucosal cells from the plasma and pass back into the lumen by active extrusion, particularly in the lower part of the small intestine (23).

The maximal absorption of iron takes place in the duodenum. The efficiency decreases from the proximal to the distal part of the small intestine. The amount of iron transferred from the gut lumen to the mucosa depends upon the abundance of receptors on the brush border. The receptor population increases in iron deficiency, the increase being more in the distal than in the proximal part of the intestine (22). Once taken up by the mucosa, some of the iron passes rapidly into the circulating plasma (21). The excess iron in the mucosal cell, not transferred to plasma transferrin on the serosal side, is taken up by apoferritin and stored as ferritin (22). Cellular iron may enter the body to meet current body requirements or may remain within the cell to limit mucosal uptake of iron. This iron may be excreted when the cells are sloughed from the villus. In iron-deficient subjects little iron is incorporated into these cells from body stores so that absorption is

enhanced and excretion is diminished (20).

IRON METABOLIC PATHWAYS

The major movement of iron in the body is unidirectional; absorbed iron is attached to transferrin, which delivers it to the erythroid precursors in the bone marrow. Iron is utilized to form hemoglobin, which in turn, is then incorporated into the red blood cell. The hemoglobin remains within the red cell for its 120-day life-span. The red blood cell is then phagocytized in the reticuloendothelial system and the iron is released. Approximately 85% of the iron derived from the catabolism of red blood cells is promptly returned to the plasma. The remaining iron is stored in the reticuloendothelial cell (20). The subsidiary metabolic pathways involve the plasma iron attaching to transferrin and then being delivered to cells throughout the body for the synthesis of ferritin, hemosiderin, myoglobin, and the iron-containing enzymes (19).

IRON EXCRETION

Previously it was believed that the quantity of iron in the body was controlled solely by regulation of absorption and that excretion played a passive role. However, most cells contain iron somewhat in proportion to the quantity of iron in body stores. Thus the daily obligatory loss of cells from skin and gut secretions - such as bile and sweat - provide a limited but selective loss of body iron (20). The major rate of loss of iron was found to be through the gastrointestinal tract with mean losses of 0.38 mg. per day from blood loss, 0.25 mg. per day from the bile, and 0.1 mg. per day from exfoliated epithelial cells. Losses in the urine were approximately 0.1 mg. per day (21). The total amount of iron lost daily in the

sweat depends on the individual, the ambient temperature, and the dermal cell content in sweat loss. The average loss of iron through dermal cell loss of a healthy adult has been assessed as about 0.5 mg. per day. The total quantity of iron lost in the urine, feces, and sweat (excluding iron from the dermal cell in sweat) amounts to 0.6 - 1.0 mg. per day in most individuals. A loss of this magnitude is appreciable when it is realized that the average amount of iron absorbed from ordinary mixed diets is only 1.0 - 1.5 mg. per day (19).

IRON DEFICIENCY

PREVALENCE

Iron deficiency anemia is the most common nutritional disease among adolescents. Several studies have indicated a high prevalency among teenage males - more so than females before the onset of menses. According to the 1968-1970 U.S. Public Health Service, Ten State Nutritional Survey (3), 5 - 10% of teenagers had below normal hemoglobin and hematocrit levels. In that survey, the normal ranges for hemoglobin were 14.0 $\pm$ 2g/dl for females and 16.0 $\pm$ 2 g/dl for males. The normal ranges for hematocrit were 42 $\pm$ 5% for females and 47 $\pm$ 5% for males. In the 1970-1972 U.S. Department of Health, Education, and Welfare, Health and Nutritional Examination survey (NHANESI) (24), it was reported that 10% of the boys and 5% of the girls were iron deficient based on hemoglobin, hematocrit, serum iron, and serum transferrin levels. In the 1976-1980 NHANESII survey (25), 2-3% of the male and 4-6% of the female adolescents had hemoglobin and hemacrit values indicating iron deficiency anemia. In other studies (26), the incidence of iron deficiency was reported to be in the range of 10 - 27% for female and 13 - 50% for male adolescents. Iron

deficiency anemia, as a term, has been almost interchangeable with nutritional anemia and has represented the most prevalent deficiency among children in the U.S. Its frequency has even prompted the suggestion that it has been the most frequent disorder seen in clinical medicine (27).

DESCRIPTION OF IRON DEFICIENCY AND METHODS OF DETECTION

Iron deficiency anemia is a progressive condition of negative iron balance that is created when the physiological demand for iron is in excess of the iron ingested. Exogenous iron loss, iron absorbed from food, and iron stores available in the body are factors that affect iron balance. Iron deficiency can be due to one or more conditions such as acute or chronic blood loss or destruction, decreased iron intake, impaired absorption and/or increased requirements due to rapid growth (1). Iron deficiency is considered to be a state in which the iron supply is inadequate to permit normal synthesis of essential iron compounds (28). Although usually considered benign, iron deficiency anemia may have serious debilitating effects. These include: decreased resistance to infection, impaired immune response, symptoms of irritability and fatigue, alterations in temperature regulations, a diminished capacity for work and activity, and lowered intellectual motivation and performance (27,29,30). Functional gastrointestinal abnormalities are also associated with iron deficiency including; a reduction of acid secretion by the stomach, an impairment in iron, fat, and xylose absorption, an occult gastrointestinal bleeding in infants, and varying degrees of histologic changes in the duodenal mucosa (29,31). In the past, physicians and investigators have been too concerned with the

circulatory hemoglobin level and not enough with the other effects associated with tissue iron deficiency. More recent investigations have indicated abnormalities associated with muscle function and resistance to infection in iron-deficient animals and man. It seems likely that an element such as iron, which is involved in so many essential tissue reactions, would be found to be vital to body functions other than oxygen transport. Such tissue effects of iron deficiency require further examination (28).

Iron depletion exists in varying degrees which extend from the mild depletion of iron stores to the development of a severe anemia (32). If the body goes into negative iron balance, iron is removed from the body stores (ferritin and hemosiderin) for metabolic needs. Simultaneously, iron absorption is enhanced and there may be some increase in the iron binding capacity of the plasma (33). During this phase, plasma iron concentration is not appreciably altered. When iron stores become exhausted, however, plasma iron levels fall and erythropoiesis is curtailed. Normocytic and normochromic anemia is often seen with iron deficiency when the anemia is mild or developing rapidly (32). No single iron parameter monitors the entire spectrum of iron status (33). These parameters of iron status reflect changes in different body iron compartments and are affected at different levels of iron depletion.

It is convenient to define iron deficiency as progressing through three stages (34). Distinctions between the three levels of iron deficiency are entirely arbitrary, because iron stores in a population form a continuum, ranging from severely iron deficient to iron overload (33). The least severe stage of iron deficiency, "iron

depletion", occurs when iron stores (in the liver, spleen, and bone marrow) fall to less than 100 mg. as indicated by a marked reduction in ferritin and hemosiderin (30,33,35). Quantitative estimates of iron status can be made from tissue by making chemical assessments of samples removed from bone marrow or by making determinations of the serum ferritin concentration of a blood sample (33,36). The serum ferritin concentration is directly proportional to the level of available storage iron in the liver and bone marrow. Levels of serum ferritin are inversely related to iron absorption; absorption increases when iron stores are depleted (34). Measurement of serum ferritin allow the estimation of iron stores by noninvasive radio or enzymatic immunoassay procedures (35). A low concentration of serum ferritin is characteristic only of iron deficiency. However, when inflammatory disease and iron deficiency coexist, serum ferritin values may be within the normal range (1). Once the iron stores have been depleted, serum ferritin levels will not reflect advanced stages of iron deficiency (35).

With continued iron loss, iron stores become exhausted and the second stage of iron deficiency erythropoiesis ensues. During this stage, the level of the heme precursor (protoporphyrin) rises in the red cells. This rise indicates an insufficient supply of serum iron for hemoglobin synthesis and red blood cell production (34). Indicators of this stage include an increased level of free erythrocyte protoporphyrin (FEP) and total iron binding capacity (TIBC), and a decreased level of serum iron and percent transferrin saturation (33,36). The FEP can be measured rapidly by a simple fluorescence assay using a fluorometer. The serum iron and TIBC are

most commonly measured by atomic absorption or spectrophotometric techniques. The percent of transferrin saturation is derived simply by dividing the serum iron concentration by the TIBC value and multiplying by 100 (1).

During the final stage of iron deficiency, "iron deficiency anemia", the diminishing iron supply will further impair red cell production. This phase is identified by a significant fall in the circulating hemoglobin and alterations in the hematological indices (33,36). Laboratory diagnosis can be made by analyzing the results from a complete blood count, including a differential smear, and by measuring the concentrations of the hematological parameters. If the analysis of the blood smear shows small cells (microcytic) and undercolored cells (hypochromic), the diagnosis usually is iron deficiency anemia. The hematological parameters measured include hemoglobin, hematocrit (or packed cell volume - PCV), red blood cell count (RBC), and mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), and mean cell hemoglobin (MCH) (33). The concentration of hemoglobin is measured by diluting a blood sample with a solution that converts the hemoglobin to cyanmethemoglobin, which is then quantified spectrophotometrically (37). The hematocrit is measured by centrifugation of a minute amount of blood that has been collected in a heparinized capillary tube. The hematocrit is then calculated by comparing the height of the column of packed red cells to that of the plasma (38). Electronic coulter counters are commonly used to accurately measure red blood cell count, MCV, MCH, and MCHC directly (1). Table 1 illustrates the development of iron deficiency and the various parameters indicating each level of iron status.

Table 1. STAGES OF IRON DEFICIENCY

STAGE	METHOD OF DETECTION	PHYSIOLOGICAL CONSEQUENCES
1. Iron Depletion	Serum Ferritin	Depletion of iron stores in liver, spleen, and bone marrow.
2. Iron Deficiency Erythro-poiesis	Plasma Iron TIBC Transferrin Saturation	Iron stores have been depleted; levels of iron carried in the plasma decrease and transferrin formation in the liver increase. Total iron-binding capacity increases to levels of 400-500 ug/dl. Percent saturation of transferrin with iron falls from a mean of 30% to about 15% to 18%.
3. Iron Deficiency Anemia	Hemoglobin RBC Count Hematocrit MCV MCHC	Hemoglobin concentration falls below 12 g./dl. The degree of iron deficiency anemia can be evaluated with additional blood data.

In a recent survey of 1,564 subjects, living in Northwestern U.S. (33), it was observed that if only one of the three parameters - serum ferritin, transferrin saturation, or hemoglobin (each being from a different stage of iron deficiency) - was abnormal, the prevalence of anemia was only slightly higher (10.9%) than in the population as a whole (8.8%). However, when any two of these three parameters were abnormal, the prevalence of anemia increased to 28% and when all three parameters were abnormal, to 63%. Thus, the cause of anemia can be reasonably attributed to iron deficiency only when at least two iron parameters fall within the iron deficient range. The magnitude of analytic errors and the within subject biological variations are less than 4% for hemoglobin, hematocrit, and red cell indices (39). Higher coefficients of variation are characteristic of serum iron, TIBC, serum ferritin, and erythrocyte protoporphyrin (41). Remarkably consistent results with less than 2% experimental error) can by obtained by experienced laboratories for hemoglobin, hematocrit, and red blood indices (39). This facilitates the detection of mild anemia. Furthermore, the relatively small biological variations in these laboratory measurements make it easier to distinguish even a relatively small response to therapy from a random fluctuation. In of serum ferritin, TIBC and eythrocyte protoporphyrin, analytic variation can be drastically decreased by the use of automated equipment, which decreases environmental contamination. Variations due to biological factors are much greater than analytic variations with an automated method. The variations due to diurnal factors can be minimized by sampling in the morning or early afternoon; values can normally fall to very low levels at night. There is an impression that the

biological variation in measurements of iron diminishes in iron deficiency, resulting in less fluctuation of low values. With improvements in methodology, a major remaining problem will relate to the overlap of subjects, making it difficult to identify individuals with mild iron deficiency, but not a major obstacle when characterizing groups of subjects (39). Perhaps the most reliable criterion of iron deficiency anemia is the hemoglobin response to an adequate therapeutic dose of iron. A therapeutic trial allows the recognition of an individual whose hemoglobin value, although within the reference range, is low for him/her (1). When screening a large segment of a population, hematocrit and hemoglobin are the common procedures to determine iron status (34).

Therapy of the anemia itself involves reversal of the sequence of iron depletion, first repletion of functional body iron compounds and then of iron stores. Repletion of the stores will occur slowly when iron is given orally. This form of iron therapy must, therefore, be continued for many months after the hemoglobin level has returned to normal to fully replete the stores. In most serious cases of iron deficiency, the subject will usually recieve injections of an iron dextran to allow a more rapid replacement of iron stores (32).

ADOLESCENCE

The demands of the body for iron are greatest during three periods - the first two years of life, the period of rapid growth and hemoglobin increase of adolescence, and throughout the child-bearing period in women (19). The acceleration of growth rate and weight gain, particularly during the years of sexual maturation, impose increased requirements for iron, primarily for the production of

hemoglobin (1). Iron deficiency is a common finding among adolescents of both genders due to the increased need for iron and the low iron content of the foods most commonly eaten by this age group. This nutritional disease is more prevalent in boys than in girls because of their greater expansion of blood volume and lean body mass associated with growth (40). For example, during the peak year of their adolescent growth spurt, boys gain an average of ten kilograms. This can be calculated to require a net increase of approximately 300 mg. of iron merely to maintain a constant concentration of hemoglobin in an expanding blood volume (1). In a group of 14,000 subjects who had a constant iron intake of 6 - 9.5 mg. per day after infancy, the rate of anemia increased from 2% in the 11 year old male to 30% in the 15 year old male largely due to growth in lean body mass. In the girls, the rate of anemia was not as great, but approximately two times higher in the 15 year old than in the 11 year old females (40).

In the adolescent girls, iron needs are also large, but their growth rate does not peak as sharply as in boys. The maximum yearly weight gain is somewhat less than in boys and the concentration of hemoglobin in girls increases only slightly during this period. The greatest average weight gain of 9 kg. per year in girls requires approximately 280 mg. iron for the maintenance of a constant concentration of hemoglobin (1). The onset of menses usually follows the peak of adolescent growth. The median menstrual blood loss of approximately 30 ml. per menstrual period in 15 year old girls involves a net loss of about 175 mg. of iron per year (1). Adolescent females are considered to be nutritionally vulnerable because of the rapid growth rate combined with marginal nutrient intake and menstrual

loss. The three major factors influencing the status of iron nutrition throughout the adolescent period for both males and females include; the amount and bioavailability of iron consumed, the rate of body growth, and the amount of iron loss. Each factor affects the amount of iron available for both metabolism and storage (34).

Until relatively recently, young adults were the usual basis for reference ranges of laboratory tests. Increasingly, the use of age-specific criteria for children has become accepted, particularly in relation to hemoglobin, hematocrit, RBC indices, serum iron, TIBC, and transferrin saturation (41). One basis for this conclusion is related to the fact that the ranges of laboratory values in children, with the exception of erythrocyte protoporphyrin, tend to be narrower than those in adults (25). Laboratory results from the second National Health and Nutrition examination survey (NHANES II) have been used to define age-related changes in values used in the diagnosis of anemia and iron deficiency. Analyses included hemoglobin, hematocrit, RBC, red cell indices, serum iron, TIBC, transferrin saturation, and erythrocyte protoporphyrin which had been uniformly performed on a representative sample of 15,093 subjects, 1 to 74 years of age. The median value and the 95% range of each parameter measured for age categories, 9 to 11, 12 to 14, and 15 to 17 year old males and females are listed in Appendix A (25).

IRON STATUS AMONG RUNNERS

SPORTS ANEMIA

The reduction of red blood cells during strenuous exercise has been recognized in human beings, dogs, and rats. The anemia occurs at an early period of physical training but eventually disappears after

one to three weeks from when the exercise was initiated. The blood properties return to the initial level shortly after the exercise is discontinued. In a normal individual, a practically constant balance is maintained between blood destruction and blood formation. This is not the case when subjects have been kept under sedentary conditions for a long time and then suddenly subjected to a strenous exercise for several days. In this situation, the blood cells are destroyed more rapidly than the hematopoietic tissue can replace them resulting in a marked fall in volume of red blood cells and circulating hemoglobin This exercise-induced reduction of red blood cell has been termed "sports anemia" (42).

Increased destruction of red blood cells during strenous muscular exercise has been known since the beginning of this century. Studies to determine the mechanism were first done on dogs by G.O. Brown in 1922 (7). Brown postulated that the reduction in resistance of the red cell membranes was caused by the wear and tear of increased circulation through the capillaries. J.E. Davis concluded (43) that this decreased resistance in the membranes was due to the high body temperature brought on by heavy muscular work.

Sports anemia is characterized by a transient decrease in hemoglobin concentration, RBC and PCV, but rarely results in clinical anemia. The red blood cells remain normocytic and normochromic (8). Associated with the red cell reduction is a concomitant decrease in serum protein (42). These reductions are not merely due to the hemodilution which results from an expanding plasma volume, which commonly occurs in athletes while training for a sport. There is an absolute reduction of hemoglobin as indicated by a significant

decrease in the total circulating hemoglobin detected after one week of training. An observed increase in osmotic fragility of the erythrocyte membrane has been associated with this reduction in hemoglobin and hematocrit This has been reported to occur as early as the first day of training, and remaining elevated throughout an entire seven-day, exercise period. The decrease in the integrity of the red blood cell membrane has been attributed to the increased circulation rate, temperature and acidity of the blood and the greater compression on the cell which results from training (44).

Although the lysis of erythrocytes occurring with severe work has been generally widely accepted at the present time, it has not been possible to clearly explain the exact physiological reasons for this increase in intravascular hemolysis. A number of theories have been proposed as reasons for this temporary disorder (44). Shirahi demonstrated (45) that this increase in fragility and reduction of red cells during exercise could be prevented by removing the spleen in dogs. In this report, it was also shown that the effete red cells - with cell membranes having higher osmotic fragility resulting from the exercise - appearing in exercising dogs could be normalized by incubating these in the plasma of a resting or splenectomized dog. Shirahi concluded that the sports anemia was caused by the liberation of some hemolysing factor (termed lysolecithin) from the spleen. When a subject has undergone strenous muscular exercise, an increase in epinephrine secretion would be promoted by the stress. This would cause an acceleration in the contraction of the spleen allowing the hemolysing factor to flow out into the circulating blood. This has been the proposed mechanism which initiates sports anemia (45).

Several investigators believe that the increase in lysis of red cells may be an adaptive process to strenous muscular exercise. The heme component of the destroyed red cells and the serum protein may be utilized by the muscles to meet the increased demand for protein metabolism during exercise (42,44). Yoshimura (7) demonstrated that the myoglobin in limb skeletal muscle of rats exercising for two weeks increased as compared with no change in amount in resting controls. Thus, he concluded that the hemoglobin in red cells has been utilized to produce muscle protein and myoglobin as well as new red cells. Hiramatsu (7) found that after one week of hard physical training, the spherical index of the subjects' red blood cell had increased; thus decreasing the fragility of the cell. This may be regarded as an adaptive reaction to promote growth or hypertrophy of muscles and regeneration of new and strong red cells capable of withstanding strenous physical training. In another study (45), Hiramatsu found a faster decay rate in the specific activity of hemin ^{59}Fe in red blood cells in exercising versus sedentary rats, indicating an increase in destruction of labeled red blood cells. In the training group, the rate of incorporation of hemin ^{59}Fe was sharply accelerated in all tissues (especially in the skeletal and heart muscle, spleen, and bone marrow where hemoglobin or myoglobin are contained) as compared to that in the sedentary control. The rate of incorporation of ^{59}Fe was much greater from red blood cell hemin than from serum injected into the muscles, spleen and bone marrow. These results demonstrated how ^{59}Fe could be incorported more easily into these organs when obtained from hemoglobin than from serum. Therefore, the hemoglobin iron may facilitate the expansion of muscles and synthesis of new red blood

cells associated with training. Thus, Hiramatsu had presumed that the hemin molecule, labeled with ^{59}Fe hemoglobin, was directly incorporated into the myoglobin in the muscle. There seems to be a temporary alteration of priorities for iron needs during exercise. Increased levels of exercise stimulate the increased production of myoglobin in the hypertropic muscle. A greater amount of iron would be required to synthesize the myoglobin, taking precedence over erythropoiesis, so oxygen delivery to exercising muscle would not be compromised. If iron was relatively unavailable, the new generation of red blood cells might be produced without a full complement of hemoglobin (46).

A low-grade runner's hemolysis could create and sustain a negative iron balance, especially in subjects with low iron stores and low dietary iron absorption. In a recent study of 16 marathon runners (47), a poor overall correlation had been observed between hematocrit and performance, but the faster runners had significantly higher pre-race hematocrits. (mean 49%) than the slower group (mean 45%). These results indicate that runner's hemolysis may prevent the attainment of optimal red cell mass for maximal race performance.

PREVALENCE OF LOW IRON STATUS AMONG ATHLETES

Runners have a greater tendency to develop anemia than less active individuals due to several factors associated with the exercise. In one investigation (8), the effect of running on indices of iron status in young female cross-country runners was studied during their training and competitive season. The runners experienced sports anemia as indicated by a decrease in hemoglobin and PCV during the first week of training. All indices of iron status (hemoglobin, PCV,

FEP, transferrin saturation, serum iron) returned to initial values between the first and eighth week of the season except for TIBC, which was significantly greater than preseason values. Results suggested that the young women's recovery from sports anemia could impose a demand on their body iron reserves. Although serum iron and percent transferrin saturation returned to their initial values within one week after training, the fact that TIBC peaked at that time suggested that the runners' bodies were actively attempting to restore iron reserves that had been diminished during training.

Several investigations on long distance runners were conducted to analyze the levels of iron status (7,9,10). Clement et. al. (9) reported that 29% of the men and 82% of the women long distance runners had plasma ferritin concentrations at risk for iron deficiency. The male runners had an adequate dietary intake of iron (mean intake was 18.5 mg per day). In contrast, the female runners had an inadequate intake (mean intake was 12.5 mg per day) which may have contributed to the high percentage of those subjects having low plasma ferritins. Kilbar (11) investigated the effects of seven weeks of training on the serum iron levels in three age groups (including ages 9-31, 37-48, and 51-64 years) of relatively inactive women. Serum iron levels significantly decreased in each group indicating that there was a significant iron cost associated with physical training. However, the lack of a control group and failure to account for dietary and menstrual factors - each of which may affect serum iron levels - must be taken into account when interpreting these results. If there had been an iron cost of physical training, serum iron levels would decrease only if this cost exceeded storage iron

levels.

Due to the high incidence of latent iron deficiency among runners, many researchers have studied possible causes of this. Ehn et. al. (10) found indications of latent iron deficiency, as measured by bone marrow iron, in all eight of the male long distance runners studied, even though each had an adequate dietary intake. Ehn suggested that the iron deficiency may be caused by a disturbance in iron absorption among these individuals due to the running. Iron absorption was measured with the aid of ^{59}Fe labeled ferrous sulphate, ^{59}Fe labeled hemoglobin, and a whole body counter in this group of elite runners and compared to eight nonrunning control subjects having similar iron status. The mean absorption of the ferrous sulfate was 16.4% in the runners, which was much lower than that of the control group which was 30.0%. The difference in absorption of hemoglobin iron was less pronounced; the runners absorbed 4.3% less than the controls. Plasma iron clearance was 20% greater in the runners than controls, indicating a greater iron loss. This was measured by the rate of disappearance of an intravenous injection of trace amounts of radioiron $^{59}FeCl_3$ in the plasma. Labeled iron incorporation into red blood cells was slightly greater in control subjects, indicating a higher rate of erythropoiesis.

Another contributing factor for a lower iron balance could be the result of excess sweating in conjunction with running. In one study (48), Paulev measured an additional iron sweat loss of 0.4 - 1.0 mg. per day increasing normal daily losses of 1 mg. Veller et. al. (49) found no relationship between the iron concentration of cell-rich or cell-free sweat and hematological indices or serum iron levels, with

the exception of a positive correlation between the iron concentration of cell-free sweat and the serum iron values after sweat collection. These findings indicate that the iron lost through sweating could not be controlled by an individual with iron deficiency. In another study (50), similar results were reported to occur. No significant differences were noted in the iron content of cell-free sweat between the normal and the iron-deficient groups. Normal subjects had a mean iron content of 1.2 mg. per liter of sweat. Hot climate and/or exercise could increase sweat loss by 2 to 11 liters, therefore, increasing the range of total amounts of iron loss to 2.5 - 13 mg. per day.

In the event of a hemolytic state, as indicated by sports anemia, some of the hemolysis would occur intravascularly resulting in an elevation of free hemoglobin. This hemoglobin would combine with haptoglobin to form a complex molecule, and then be removed from the circulation by the reticuloendothelial system and eventually be used again in the synthesis of new iron-containing compounds, thus conserving iron. Circulating free-haptoglobin levels, therfore, would also decrease. If the hemolysis continued and/or exceeded the hemoglobin-haptoglobin binding capacity, excess hemoglobin would appear in the urine, after the reabsorbing capacity of the kidney tubules has been exceeded, producing hemoglobinuria. However, there could be varying degrees of exercise-induced hemolysis before the hemoglobin-haptoglobin binding capacity was exceeded (51). Hemoglobinuria has been the focus of many studies on exercise-induced hemolysis. Nine out of 50 males completing a marathon showed gross or microscopic hematuria; all abnormalities cleared up within 48 hours

(52). Gross hematuria has been previously reported to be an infrequent occurrence after running. In contrast, recent investigations have described up to 21 such cases among the participants of a long distance running event in which no intrinsic urinary tract cause was found (53). One investigator (10) suggested that this hemoglobinuria and hematuria could account for the iron loss producing the latent iron deficiency found in runners.

Low serum haptoglobin values - indicating increased intravascular hemolysis - has been commonly reported among athletes (8,10,47,53,54,55). In a more recent hypothesis (56), the investigator suggested that this increased level of hemolysis, producing an increase in the hemoglobin-haptoglobin complex, caused a shift in red cell catabolism from the reticuloendothelial system to the hepatocytes. This would be a reasonable explanation of a reduced content of hemosiderin in the bone marrow cells and low serum ferritin levels because these values reflect the content of iron in the reticuloendothelial system. Thus, it was concluded that runners "anemia" was not caused by an increased iron loss as detected by low iron stores. No obvious explanation could be given for the single divergent laboratory values which indicated low iron stores. The low serum ferritin and low bone marrow hemosiderin values often found in athletes indicate the need for further studies on iron kinetics in this group to clearly explain these differences (55).

IRON DEFICIENCY - EFFECTS ON PERFORMANCE

Three fundamentally different questions are relevant to the potential biological effects of iron deficiency anemia. First, to an athlete the important question is related to maximum performance

capacity. Second, to the worker whose survival depends on his/her job performance, the daily work productivity is often a crucial factor, particularly in developing countries. A third question is related to a person's general sense of well-being or vitality, though it remains to be determined to what extent this is affected by iron deficiency anemia (57). The impact of iron deficiency on endurance athletes is illustrated by the fact that a person with a hemoglobin concentration of 12 g/dl can carry only 75% of the oxygen that a person with 16 g/dl can carry with equal red blood cell volumes (36). The total body oxygen needs are the summation of all the individual requirements of tissues and organs and vary as a consequence of functional changes occurring in everyday life. Under normal conditions, physical activity is the most important factor in determining total oxygen requirements, at least in quantitative terms. This is so because it induces significant increments in the metabolic rate of skeletal muscle and to a lesser degree in that of myocardium (58). The lower levels of hemoglobin in anemia impair oxygen delivery to the tissues to a degree which depends on the severity of the anemia and on the energy demands. Therefore, a sedentary person or someone engaged in light and intermittent work may experience no symptoms of anemia with a moderate hemoglobin deficit and may function in essentially the same manner as a normal individual; whereas someone with more severe anemia and/or who engages in physically demanding activities must resort to various physiological compensatory mechanisms. This type of individual may be forced to reduce his/her work output or modify his/her work pattern if the physiological compensation proves to be insufficient (57). In several studies (57,59,60,61,62,63,64),

investigators have shown that physical working capacity in different populations, measured as oxygen consumption, significantly decreases in iron deficiency anemia and improves when hemoglobin levels reach normal values. Edgerton et. al. (64) measured selected parameters related to work tolerance in 31 adult subjects with hemoglobin from 2.5 to 14 g per dl. Work tolerance was closely related to hemoglobin concentration regardless of the adequacy of storage iron level. The data strongly suggested that the decrement in work performance capacity in iron-deficient and anemic subjects was in a large part, a reflection of the level of anemia rather than other nonhemoglobin-related biochemical changes that could accompany prolonged iron deficiency anemia.

Iron deficiency of short duration and of moderate degree can be associated with hemoglobin concentration in the normal range. Therefore, there may be an overlap of iron-deficient and non-deficient individuals with normal and minimally decreased hemoglobin levels. Several studies (31,47,61,65,66,67) have demonstrated that iron deficient, but not anemic subjects, could benefit from iron treatment, producing an increase in physical work capacity without significant change in hemoglobin level. Administering an iron supplement to a deficient subject reduces the stress of physical activity more than can be expected by the improvement in oxygen carrying capacity of blood alone (68). Iron may be incorporated into tissue in a manner functionally beneficial to the extent that work tolerance can be improved (67).

In the past, the investigation of iron deficiency anemia has been focused on hemoglobin. However, it has been becoming quite clear that

the symptomatology of iron deficiency anemia reflects a complex systemic condition involving almost all cells in the body (69). Symptomatic improvement in response to iron treatment before a significant increase in hemoglobin concentration would indicate a possibility of an early repair of tissue heme protein deficiency (70). Several experiments in rats (29,61,71,72,73) demonstrated that iron deficiency was associated with a decreased concentration of cytochrome c oxidase in various tissues, a decrease in concentration of muscle myoglobin, and in certain instances a diminshed activity of iron-containing enzymes such as succinic dehydrogenase and aconitase. Severe iron deficiency caused a general decrease in the concentration or activities of iron-containing components of the electron transport chain in mitochondria isolated from rat skeletal muscles. It also caused a loss of mitochondrial protein from that tissue (72). Because of these reductions, the iron deficiency produced a decrease in skeletal muscle capacity for aerobic metabolism, and, by this mechanism, an increase in susceptibility to fatigue (73). It has been suggested (66) that the dramatic improvement in symptoms that often occurs prior to a significant rise in hemoglobin concentration during iron therapy was due to early repair of tissue iron deficiency. In all muscle tissue examined (of iron-deficient Sprague-Dawley rats) the ^{59}Fe preferentially entered the mitochondria (74). The enhanced mitochondrial uptake of iron, prior to any detectable changes in the hemoglobin level, in experimental animals could be indicative of nonhemoglobin related biochemical changes and/or decrements in work capacity (74).

In one experiment (75), rats were raised on a severely iron-

deficient diet and then exchange-transfused to a normal hemoglobin level. These animals demonstrated an impaired work capacity (as measured by length of treadmill running time) accompanied by an increased lactate production, as compared to control animals on a chow diet. Given parenteral iron, the deficient animals regained a "normal" running ability within 3 to 4 days. Concentrations of the cytochrome pigments and myoglobins and rates of oxidative phosphorylation with pyruvate-malate, succinate, and alpha-glycerophosphate as substrates, were all reduced in mitochondrial preparations from skeletal muscles of iron-deficient rats. Only the rate of phosphorylation with alpha-glycerophosphate as substrate increased significantly and in parallel with the recovery of work performance of the deficient rats treated with iron. The iron deficiency apparently caused a depletion in the iron-containing mitochondrial enzyme, alpha-glycerophospate oxidase, which in turn impaired glycolysis. This resulted in an excess lactate formation, which at high levels leads to cessation of physical performance observed in iron deficiency (29).

Iron deficiency alone could cause an elevation of resting blood lactate levels in human beings and rats. Findings have suggested that iron deficiency with or without anemia has caused an increase in tissue anaerobic glycolysis, resulting in an accumulation of lactate (77). Blood lactate concentrations following a period of exercise provide an estimate of the amount of energy obtained from anaerobic sources. It may be used to assess the relative levels of skeletal muscle hypoxia incurred during these activities (78). Post exercise lactate concentrations also appear to be directly related to the

degree of anemia. Lower lactate concentrations have been observed after exercise in the subjects with the highest hemoglobin concentration, in spite of the fact that they worked longer and at higher work loads than those subjects with hemoglobin concentrations below 13 g. per dl. (79). Iron-deficient rats with adequate hemoglobin levels displayed muscle activity associated with a higher blood lactate concentration than that observed in iron-repleted animals. The accumulation of lactate appears to be the result of excessive production, as lactate clearance from the blood has been shown to be unaffected (76). Two weeks of iron therapy in minimally iron-deficient women athletes resulted in lower lactate levels at the end of an exhaustive exercise (47). Aerobic metabolic capacity can be restored by iron treatment. The lower post-exercise lactate and higher partial pressure of carbon dioxide found after iron treatment may be caused by such improvements of mitochondrial oxidative phosphorylation, changing the relative metabolism from anaerobic to aerobic (77).

In a brief hard exercise, maximal oxygen consumption (VO_2 Max) would decrease in a subject with iron deficiency. Additionally, the ability to perform prolonged submaximal endurance exercise would also be diminished. Perkkio et. al. (80) had shown that the depressed VO_2 Max in rats with severe iron deficiency anemia was virtually corrected by red cell transfusion whereas endurance remained impaired. The results suggested that the concentration of hemoglobin was a major determinant of VO_2 Max, whereas mitochondrial oxidative capacity was more likely to be the limiting factor in endurance capacity. Severely impaired duration of performance in a submaximal exercise (endurance)

was related primarily to a decreased muscle capacity for oxygen utilization (due to a decrease in iron-containing compounds) rather than dimished oxygen delivery (due to a lower hemoglobin concentration) in the blood. This study indicated that endurance exercise had become substantially impaired even under conditions of moderate iron deficiency where VO_2 Max was virtually uneffected.

IRON NUTRITURE

DIETARY SELECTION

The average U.S. dietary intake, reported forty years ago, indicates that dietary intake supplies 14 to 20 mg. of iron per day in man. There is some evidence that iron intakes are falling as modern food handling processes reduce the opportunities for contamination (19). The typical western diets provide approximately 6 mg. total iron per 1000 kilocalories (32). A decrease in total caloric intake and a reduction in use of iron pots may also contribute to lower iron intake today. The more important change, however, may be related to the availability of iron to be absorbed from food (28).

The adolescent's nutritional status has been of increasing concern in the past two decades. The data on nutriture of the adolescent population has shown that intakes of iron were lower than intakes of any other nutrient (19). Earlier studies (4) have established that adolescent girls have the highest prevalence of unsatisfactory dietary intake of iron in all age groups surveyed. The average intake among girls has been reported in many studies as 10 to 12 mg. per day. In the Ten State Nutritional survey (3), 80% of females of all age groups had iron intakes below the recommended daily allowance (RDA) of 18 mg. Approximately three-fourths of the boys, 12 to 16 years of age,

consumed less than the RDA of 18 mg. of iron per day, while more than one-third of the boys, 10 to 11 years of age, consumed less than the RDA of 10 mg. of iron. According to the U.S. Department of Health, Education, and Welfare dietary intake findings from the Health and Nutritional Examination survey of 1971 - 1974 (5), mean intakes of dietary iron were 73.2% and 57.7% of the RDA for male and female adolescents, 10 - 14 years, respectively. According to the U.S. Department of Agriculture survey of 1977 - 1978 (6), 36% of adolescent males and 64% of females had less than 70% the RDA of 18 mg. dietary intake of iron per day. The Recommended Dietary Allowances have been set to afford a sufficient margin above the physiological requirement to cover variation among essentially all individuals in the general population (81). That this objective with respect to iron has been rarely met by intake of ordinary foods, at any age during adolescence, has been a well accepted fact (4).

Adolescents tend to consume a large portion of their daily energy intake as snacks. In the latest household food consumption survey (U.S.D.A.,1980) 9 to 11 year old males and females consumed up to 12.7% and 12.0% of the total daily energy, respectively, from snacks (82). According to the Ten State Nutrition Survey, both male and female teens obtained a substancial proportion of their recommended kilocaloric intake - approximately 23% - from between-meal snacks. The mean nutrient intake per 100 kilocalories from between-meal snacks did not meet the RDA for iron, contributing only 12% of their total iron intake (3). In their quest for independence, adolescents spend more time away from the home and consume more of their meals and snacks outside of the home. Fast-food restaurants and vending

machines are popular choices because they provide inexpensive foods in a short period of time. The iron density of many of these foods (except for the red meat hamburgers) in relation to the kilocaloric intake, is low in comparison with the iron requirements of teenagers. Certain meal combinations, from vending machines and fast food restaurants, are excessive in energy when compared with the amount of nutrients provided. These foods have been acceptable nutritionally when consumed judiciously and as part of a well-balanced diet. When these snack foods have become the mainstay of the diet, however, there has been cause for concern and some form of iron supplementation or enrichment would then be suggested (40).

Elite athletes' diets may have important effects on their responses to their endurance training programs (9). Limited information on the dietary patterns, in relation to nutrient intake, in runners is available. Blair et. al. (83), compared the nutrient intake of middle-aged male and female (35 - 59 yrs.) runners and controls, of the same age and gender. The runners were leaner and had higher energy intake; 40 to 60% more kilocalories per kilogram body weight than the heavier sedentary controls. The increased energy intake of the runners was associated with higher intakes of fats and carbohydrates than by the controls. The runners consumed relatively less protein per 1000 kcal, although the absolute amount of protein in their daily diet was similar to that of the controls. Clement and Asmundson studied (9) the nutritional intake in 52 middle-distance and distance runners, and reported adequate levels of mean energy intake for both males and females. The women had a mean iron intake of only 69% of the recommended daily intake of 18 mg per day. The men had a

mean intake which was adequate at 18.5 mg. per day; the recommended daily intake is 10 mg. per day. These data support the hypothesis that runners eat typical American diets, with the only difference being a higher energy intake than nonrunners to cover the increased energy expenditure associated with the participation in the sport (83).

IRON SUPPLEMENTATION

Several investigators (12,13,14,15) have reported the effectiveness of prophylactic supplementation of iron in athletic and sedentary children, adult men and women, both anemic and normal. The dose of iron given in the form of ferrous sulphate or ferrous fumarate has varied from 5 mg. to 400 mg. a day and the duration of supplementation from one month to three years. The results have been variable depending upon the dose of iron used, duration of supplementation, and iron status of the population studied (22). In two studies (56,71), where hematological investigations and iron status measurements were done on middle and long-distance runners, no differences were found in these measurements between the athletes taking iron supplements and those that did not. Cooter and Mowbroy (12) investigated the effects of iron supplementation on serum iron, TIBC, hemoglobin, and MCHC in female college basketball players divided into two groups; one received a multivitamin with iron and the other received a multivitamin without iron. No significant changes were observed in any of the experimental variables, including hematological parameters, blood iron indices, and performance capability, over the duration of the four month study. In another study (13), College athletes randomly divided into an iron

supplemented group and a control group were followed during their training and competitive seasons. No significant changes were observed in any of the hematological indices, serum iron, or percent saturation of transferrin measured in each group throughout the nine week season. Contrary to the previously mentioned investigations, results from one study (14) showed significant difference between the supplemented and control groups. In this study, higher dosed supplements of 250 - 300 mg. of iron per day, along with vitamin C (to increase the availability of the iron from the supplement) were given to female athletes. The group receiving iron supplementation showed significantly greater hemoglobin levels, with a mean increase of 1.0 g/dl, than the placebo-supplemented group.

Two investigators (13,15) compared the performance capability of an iron supplemented group with that of a nonsupplemented group in association with their state of iron nutriture. No significant differences were found in the hematological and iron status between the groups, but measures of physical work capacity and performance capability were greater in the iron-supplemented group. That no significant difference was found between groups has been consistant with the statement that athletic training differs primarily in the extra energy requirement and the needs for hypertrophy of the muscle. Improvement in performance capability may be the only indication that prophylactic iron supplementation during training would be needed by top athletes (13).

Suboptimal hemoglobin (which designates a hemoglobin concentration that is lower than could be considered optimal for oxygen transport purposes) is not a major concern to the non-athletic population.

Therefore, relatively little is known about how to treat it. Researchers are not even sure that hemoglobin concentration in the normal range can be increased with standard noninvasive techniques. It seems apparent that a larger scale clinical trial of iron therapy in athletes with suboptimal hemoglobin is needed to answer some of these questions (85). It would be premature to conclude from studies conducted thus far that iron supplementation should be given to athletes as a way of improving performance capabilities. More definitive research must be done in this area before such a recommendation can be made. At present, the prudent recommendation regarding prevention of sports anemia and suboptimal hemoglobin seems to be: to ensure that athletes ingest a diet that provides at least the RDA of iron, protein, vitamin C, vitamin B_{12}, and folic acid; to screen athletes routinely for iron deficiency and hemoglobin concentration; and to prescribe iron and/or other dietary supplements for athletes who manifest low hemoglobin levels or iron deficiency (85).

IRON BIOAVAILABILITY

IRON ABSORPTION

The average American diet contains over five times the total amount of iron needed to maintain iron balance, but only a small percent of this is absorbed (86). The nutritive iron value of special foods or categories of food should be based on at least 3 considerations: concentration of iron, form of the iron in terms of its availability, and influence on iron availability by foods ingested simultaneously (87). Bioavailability is a measurement of the potential use of a mineral, nutrient or drug by an organism. Since

the conversion of the nutrient to an active species is essential, bioavailability thus encompasses more than just intestinal absorption. Factors which influence the measurement of bioavailability of iron from different meal combinations include solubility, pH, chemical form of iron, oxidoreductive activity of iron, concentration of iron in the meal, capacity of iron to form complexes (with inhibitors and promoters), food digestibility, food processing conditions, and nutrient interactions. Although this list is far from complete, it can readily be seen that these factors are multifactorial and interdependent. Therefore, the control of all but a single variable is difficult in food systems (86).

Absorption of dietary iron is regulated in the normal individual by physiological need (iron status) and will vary from meal to meal as modified by the composition of each meal (88). This regulation of absorption is closely tied to the level of iron stores (absorption increases with reduced stores and decreases as stores enlarge) and the rate of erythropoiesis (30). With respect to its availability, food iron can be divided into two main parts: heme and nonheme iron, each of them forming a different pool of iron (86). The availability of heme iron, which is present as hemoglobin and myoglobin in animal tissue, is high in comparison to nonheme iron, averaging 15% for iron-replete men to 35% absorbed in those lacking iron stores (89). Although heme iron constitutes only 5 to 10% of dietary iron ingested in a Western diet, it accounts for nearly 30 to 40% of the iron absorbed from the diet because of the high assimilation of heme iron by the mucosal cells (90). This increased availability is due to both the physiochemical nature of heme iron and its mode of absorption

(89). Heme iron is more soluble in the neutral conditions of the small intestine than in the acid environment of the stomach. It is thought to be absorbed directly into the intestinal mucosa as the intact iron porphyrin complex. The enzyme, mucosal heme oxygenase, splits the iron from the porphyrin ring in the intestinal mucosal cell (23). The iron then enters the same storage or transit pathways as the nonheme iron fraction. However, in another hypothesis (91), a different pathway for the hemoglobin iron absorption is suggested. During meat digestion, heme is split from the globin in the stomach. After further digestion in the intestine, the heme ring is cleaved with the released iron, becoming loosely bound to one of the many fragments derived from the digestion of the meat proteins. This iron may then enter the intestinal mucosa as inorganic iron or may even be absorbed as an amino acid or small peptide chelate.

Wheby et. al. (92) studied the hemoglobin iron absorption kinetics in iron-deficient dogs. He found very little free iron acculmulation in the mucosal cell during the absorption of ^{59}Fe labeled hemoglobin, suggesting the absence of a feed back inhibition on the heme oxygenase. The mucosal ^{59}Fe heme had accumulated over time while the free ^{59}Fe did not, indicating rapid transport of the ^{59}Fe split from heme. These results suggested that the rate limiting step in absortion of hemoglobin iron in iron-deficient dogs was the splitting of iron from heme in the mucosa. This control mechanism could explain the fact that iron-deficient humans absorb more iron from a dose of elemental iron than from the same amount of iron given as hemoglobin.

Except for the facilitating effect of meat on the absorption of heme iron, the type of meal has little influence on its absorption

because the iron remains within the porphyrin complex until it is absorbed by the mucosal cell (93). From studies involving different dose levels of heme iron, it appears that the bioavailability of heme iron in meals containing meat has a maximum absorption of about 25% while the bioavailability of heme iron given without meat or liver has a maximum absorption of only 10% (16). Using in-vitro studies with simulated intestinal loops, Hazell et. al. (94) found that when meat or hemoglobin was ingested with a large amount of protein, the end products were low molecular weight, nonheme compounds which were easily absorbed. Hazell also found that hemoglobin remained as the end product to be absorbed only when it was digested alone. Thus, hemoglobin was absorbed intact when digested alone, but absorbed as a nonheme iron complex when digested with meat in simulated digestion studies. More in-vitro studies need to be conducted to explain the differences in digestion and absorption of iron from hemoglobin or meat. Thus, the chemical nature of the heme-iron absorption phenomenon is still not clearly understood (89).

Iron from foods such as vegetables, fruits, cereals, eggs, dairy products, as well as nonheme iron of meats, poultry, and fish and from soluble iron supplements all form a common nonheme iron pool - representing the largest fraction of iron in the meal (23). Assimilation depends on the extent to which iron remains soluable within the lumen of the upper small intestinal tract - largely affected by the general composition of the meal.

DIETARY FACTORS INFLUENCING BIOAVAILABILITY

During the passage of food from stomach to duodenum, the pH increases from 1.5 to 7.0 due to duodenal secretions. As a result,

most ferric iron is precipatated as ferric hydroxide or phosphate unless this is prevented by the presence of chelating agents. Ferrous iron, however, is not so readily precipated in the duodenum, and a significant proportion of it is still soluble even at a pH of 7.0 and hence is better absorbed than the ferric iron. It is the iron which is present in the duodenum and upper parts of the jejunum in a soluble form that is available for absorption (22). While increasing amounts of inorganic iron are absorbed from larger test doses, the percent absorbed progressively decreases. This indicates that intraluminal iron molecules compete with each other for either: binding to intraluminal substances which facilitate absorption; passage through absorptive pores in intestinal mucosal cells; or a combination of these possibilities (95). The acceptor sites on the brush-border surface compete for iron with the ligands present in the gut lumen. Some ligands, mainly derived from foods, promote iron absorption by keeping it in solution and others inhibit absorption by precipatating iron. The results depend upon the balance of these opposing factors (22). Therefore, it is not the biological form of iron in the food, but rather the composite effect of the inhibitors or enhancers in a complete meal that determines nonheme iron availability (96).

Certain compounds such as organic acids, sugars, amino acids, dicarboxylic acids and hydroxy acids, normally present in food, can form chelates with ferric iron to keep it in solution and prevent its precipitation at the neutral or alkaline pH in the duodenum. These compounds are considered to be enhancing factors for iron absorption (22). Contrary to these agents, compounds such as calcium-phosphates, phytates, and tannins can precipitate iron at the neutral pH, and

therefore, are considered inhibiting factors (88). Iron can be absorbed without chelating agents, but only at low pH. Besides maintaining iron in available form, the chelating agents also provide a specific transport mechanism for iron chelates similar to heme (97). Ascorbate acts as a ligand for the common nonheme iron pool. Enhancing effects of ascorbate are directly proportional to the quantity added to the meal. This vitamin can also reverse the inhibitory effects of tannins, calcium, and phosphates on nonheme iron absorption (96). The effect of ascorbate depends upon its presence in the meal and is unrelated to the ascorbate status of the individual (98). Cook et. al. (90) reported that vitamin C had negligible effects on iron stores when large quantities of ascorbate were included in subjects' diets for up to two years. There was a fivefold enhancement of iron absorption from foods when 1000 mg. ascorbate supplements were taken with the meal. If this enhancement had been maintained for 2 years in a normal adult male, with a basal absorption of 1 mg. iron daily, the body iron stores should have increased to greater than 3 grams. These results were not observed among the subjects. Apparently, the regulatory mechanism controlling body iron reserves overrides any pronounced alterations in food iron availability.

In several studies (93,97,99), the presence of animal flesh in the diet has been shown to result in an increase in the absorption of nonheme iron, possibly by stimulating the digestion of food or counteracting the inhibiting factors on iron uptake. The absorption-promoting effect of meat seems to be of the same magnitude for both kinds of iron. This fact suggests that there is a common mechanism of

action of meat on the absorption of both heme and non-heme iron (16). The animal tissues; beef, pork, lamb, liver, chicken, and fish each increase iron absorption to a different degree. Because dairy and animal products such as milk, cheese and eggs inhibit absorption, the enhancing effect of meat on nonheme iron absorption may be due to some component of animal tissue unrelated to the protein content. Alternatively, it may relate to the amino acid composition of the administered protein (100). The mechanism of animal protein enhancement on dietary iron still remains unanswered (87). The enhancing effects of ascorbate and meat are directly proportional to the quantity present in a meal - but the combination of both in facilitating iron absorption is not additive (101). This suggests that animal tissue and ascorbate enhance iron absorption by the same mechanism (98).

Sub-optimal iron nutriture may become an increasing concern if the consumption of plant foods continues to rise (33). Iron absorption from grains and vegetables is markedly inhibited by the concommitant ingestion of dietary components such as dietary fiber and phytic and tannic acids present in plants (7,88,102). Fiber binds minerals in the digestive tract, possibly rendering them unavailable for absorption in the body. Absorption of minerals might also be decreased because of the diluting effects from the extra water taken up with the fiber or because of the faster transit (due to the fiber) through the intestines (103). Iron binds to tannates and phytates to form complexes which precipate, making them unable to be absorbed (16). Calcium and phosphorous individually, cause slight inhibition on nonheme iron absorption, but not as effectively as calcium-

phosphate complexes. The phosphate precipitates more efficiently by iron with calcium present especially at the higher pH of the small intestine (104). Other inhibitors of iron absorption include; the chelating agent, EDTA, used to prevent metal oxidation in many foods, tannins found in tea, polyphenolics found in coffee, phosphoproteins found in eggs and cows' milk, and lectins found in soy proteins (88). Even though the percent absorbed of nonheme iron is considerably less than heme iron - due to inhibiting actions of many dietary components - the quantity of nonheme iron in the diet is many fold above that of heme iron, therefore, the major contribution of available iron is made by nonheme (17).

MEASUREMENTS OF DIETARY BIOAVAILABILITY OF IRON

Various methods have been used to study the dietary bioavailability of iron in man. The chemical balance technique is the only method that directly measures iron absorption from the whole diet. This method, however, is insensitive, imprecise, and time consuming, and it gives no information on iron absorption from different meals (16). Other in-vivo techniques measure: the increase in serum iron concentration following administration of an oral dose of iron from different iron compounds; changes in body iron stores as an indicator of iron utilization vs. iron availability from foods; and the rate of hemoglobin repletion in iron-deficient animals after eating various dietary sources of iron (105).

The introduction of radioisotopes has made it possible to label single food items biosynthetically with radioiron (16). In recent studies (101,106), investigators found that there is an almost complete isotopic exchange between the non-heme iron compunds in the

foods and the added inorganic radioiron tracer. The absorption of such a tracer mixed into the diet can thus be expected to give a true measure of the total absorption of non-heme iron from a diet. The absorption of radioiron labeled hemoglobin mixed into the diet may give a fairly true measure of the total absorption of heme iron from the diet. The absorption of food iron might thus be considered to take place from two pools - one heme iron and one non-heme iron pool (101). After administration of a radioiron-labeled food or meal, blood samples are taken over a period of time to measure the amount of radioiron in either whole blood or hemoglobin. Instead of taking blood samples, one can use whole-body counting to calculate the percent of retention vs. time after the radiolabeled food or meal is eaten (105). This method has greatly facilitated iron nutrition studies of various kinds. Further studies are necessary - especially on the exchangeability of nonheme iron in a wider variety of foods involving different preparation techniques - to permit application of the method in studies involving different main dietary iron sources (101,105).

ESTIMATION OF AVAILABLE DIETARY IRON

Because iron absorption varies so markedly according to the diet, recommended dietary allowances merely provide rough guidelines. It is possible, however, to develop iron deficiency while ingesting large amounts of iron-rich foods in which the iron is poorly absorbed, or conversely, to escape deficiency despite ingesting far less than the recommended amount but in forms that are particularly well absorbed (1). The current Recommended Dietary Allowance for food iron intake has been established assuming an absorption of 10% of the total

amount present in the daily intake (82). However, this amount can rarely be achieved with the ordinary foods available (17). The 1980 Food and Nutrition Board (81) has recognized the importance of iron bioavailability; the critical issue relating diet and iron status is not the total amount of iron ingested but, rather the amount of iron available for absorption. Research on iron absorption has extended further than that on the absorption of other trace minerals, and thus iron is the first trace mineral for which a model to estimate bioavailability has been developed (18).

Accumulating evidence demonstrates that the amount of iron potentially available from foods depends not only upon the amount supplied but the nature of that iron and the composition of the meal. The total iron content of the diet is thus a relatively poor indicator of the adequacy of the diet with regards to this mineral. Although numerous questions remain to be answered about iron bioavailability and iron needs, sufficient information is now at hand so that better estimates of iron need in relation to diet can be made. Such information should be used in the development of diets and in making dietary recommendations (17).

Monsen (17) developed a model which categorizes the meal as high, medium, or low availability of the nonheme iron pool with a certain percentage associated with each, indicating the amount of iron able to be absorbed. These categories are based on the amount of ascorbate and meat present and the level of iron stores in the individual. In order to derive the percent of absorbable iron present in a meal one must: calculate the iron content of individual meals and snacks; calculate the amount of heme iron in these meal units and the amount

of bioavailable heme iron; calculate the amount of non-heme iron in these meal units; assume absorption of the non-heme iron to be at a low level unless there is concomitant ingestion of enhancing factors; sum up the enhancing factors and calculate the amount of bioavailable nonheme iron one would anticipate; and finally sum up the bioavailable heme and non-heme iron to arrive at the total amount of iron absorbed (18). Appendix B illustrates this model.

Several assumptions have been made with this model which restricts its application. All types of heme iron are assumed to be equivalent in percentage of the iron found in the heme complex of the animal tissue. Certain forms of nonheme iron are not entirely exchangeable with the nonheme pool - particularly fortification iron in compounds of low availability - which should not be included into the calculations. Inhibitory factors are not taken into account. Iron status of the individual is split into three separate categories with one specific percentage associated with each group. All of these assumptions decrease the credibility of the model in calculating a specific amount of absorbable iron in the meal (17). In that the goal is to increase the quality of the diet by increasing the quantity of bioavailable iron, it makes no difference in comparative analysis of diets which level of body iron stores one utilizes for calculations. It is suggested, however, that the individual with 500 mg. iron stores be selected as a basis for comparison. For the individual with zero iron stores, one would anticipate approximately twice as much iron to be bioavailable as would be calculated for the reference individual with 500 mg. iron stores. One can, therefore, use this model to gage the quality of the diet in facilitating iron absorption. In the

effort to improve the iron nutriture of an individual one can; increase the content of factors which will enhance the absorption of nonheme iron, decrease the ingestion of inhibiting agents, and improve food choice to increase ingestion of dietary iron and improve its bioavailability (18).

METHODS

SUBJECTS:

Subjects for this investigation were part of an extensive study on fitness and performance conducted by the Youth Sports Institute, through Michigan State University. The sample included; subjects evaluated in Phase II of the study, August, 1983, consisting of 23 male and 15 female runners and 9 male and 7 female nonrunners, and in Phase III of the study, September, 1984, consisting of 21 male and 13 female runners and 11 male and 10 female nonrunners. Nineteen male runners, 10 female runners, 10 male controls, and 6 female controls participated in both Phase II and Phase III. One male runner and 1 female runner in Phase II had stopped running and became control subjects in Phase III. All except 2 subjects were caucasian (the other 2 subjects were hispanic), ranging in age from 8.5 to 16.9 years. The runners were selected if they were one of the top three finishers in their age group in road races in the state of Michigan during the year of testing or during the previous year, represented by Phase I of the study. The controls were selected to match the ages and heights of the runners for the first year, Phase I, of the study - Fall, 1982. The control subjects were normally active, but did not undergo heavy training for running road races. The control subjects were selected from among the participants of an ongoing motor

performance study conducted at Michigan State University. Due to differences in growth rate and discontinuation of some subjects, pair-matched controls could not be maintained throughout the course of the study. Hematological parameters were not measured in blood samples obtained from subjects in Phase I of the study. Hence, data from the other tests (treadmill and dietary records) conducted in Phase I were not included in this study. Written informed consent was obtained from all subjects and their parents prior to testing. The study was approved by the Committee on the Use of Human Subjects, Michigan State University.

BLOOD ANALYSIS:

The subjects participated in a full day of testing at the Center for the Study of Human Performance, beginning with blood collection at 7:30 a.m. Blood was drawn with stasis from the anticubital vein of each subject after a 12-hour fast. Five milliliters of blood were drawn into vacutainers containing disodium edetic acid anticoagulant for determinations of hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and red blood cell count (RBC). These measurements were determined by an eletronic coulter counter. This and the other determinations were done at the Lansing Clinical Laboratory, Lansing, MI. Seven milliliters of blood were collected in heparinized vacutainers for mineral analysis. The tubes were inverted to prevent clotting and cooled to $4^{o}C$ immediately, by being placed in a refrigerator ($4^{o}C$) at the testing site. Three to seven hours later, the heparinized tubes of blood were spun down - at 2400 RPM (1300 g) for 15 minutes

at 5° in an IEC model PR-6000 centrifuge[1] - to harvest the plasma. Plasma samples were frozen in polystyrene storage tubes for later analysis of iron(107).

Plasma iron was determined by atomic absorption spectrophotometry using an Instrumentation Laboratory (model 951) spectrophotometer[2] following the method of Olson and Hamlin (108). Duplicate aliquots of 0.5 milliliters plasma from each sample were deproteinized with trichloracetic acid (Mallinkrodt[3]) and incubated in a water bath at 90°C for 15 minutes. Immediately afterwards, the samples were refrigerated at 4°C to cool down, then centrifuged at 2000 RPM (1080 g.) for 15 minutes. Supernatants were harvested, and used undiluted for iron determinations which were done the same day, and directly after preparation of the standard curve for iron. Samples were read against aqueous standards prepared from iron stock solution (JT Baker Chemical Co.[4]). Any duplicate samples which were less than 90% of each other were discarded and determination of iron was made on freshly prepared samples. Due to the limited amount of plasma, repeat analyses to obtain lower percentage of error was not possible.

ANTHROPOMETRIC MEASUREMENTS:

Heights and weights were measured on each child during the day of testing. Heights were measured to the nearest

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[1]Damon/IEC Division. Needham Hts., MA.
[2]Instrumentation Laboratory, Inc. Wilmington, MA.
[3]Mallinkrodt, Inc. Paris, KY.
[4]J.T. Baker Chemical Co. Phillipsburg, NY.

millimeter with the subjects, without shoes, eyes straight ahead, with heels and shoulders touching the wall. Measurements were made with a free-standing anthropometer. Weights were measured to the nearest gram with a medical beam balance, with the subjects in bare feet and bathing suits. All subjects were weighed on the same balance. A Tanner rating (109) on each child was evaluated by a physician in Phase II, 1983. Hand and wrist x-rays were taken on some of the subjects during the spring of 1984; after Phase II and before Phase III of testing. These x-rays were used to determine the skeletal age of each subject. This was done by matching his/her x-ray to a standardized atlas of roentgenograms compiled by Greulich and Pyle (110). Recorded weights and heights in Phase III were plotted per age of each subject on the NCHS growth charts (111) for boys and girls. Relative weights and heights were calculated by dividing the actual measurements by the measurements for the 50th percentile of their age/sex group times 100. The height measurements of each subject, for each phase of the study in which they participated, (four years for those subjects who participated since the first year and two or three years for those who joined the study the following years) were plotted individually on the NCHS growth chart (111) to determine the stage of growth of each individual. The three stages of growth were: the "pre" category which included those subjects who had not reached their growth spurt, gradually increasing in height at a constant rate along a growth curve percentile on the NCHS growth chart (111); the "peak" category which included those subjects who were undergoing a

rapid increase in height, accompanied by all the changes associated with puberty; and the "post" category which included those subjects who had slowed down their rate of growth, indicating near completion of puberty. To illustrate how subjects were categorized, a line representative of growth for each of the three stages is plotted in Figure 1.

Phase IV data were available but not included in this study. However, the heights of each subject in Phase IV (Fall, 1985) were plotted to give a better indication of the stage of the growth spurt for each subject in Phase II and Phase III.

The Tanner ratings and skeletal bone ages (of those subjects who had hand and wrist x-rays taken) were referred to, to check the accuracy of the prediction of each subject's growth category.

Figure 1. Growth curve lines for the 3 growth categories; pre-, peak-, and post-growth spurt, plotted on a NCHS growth chart.

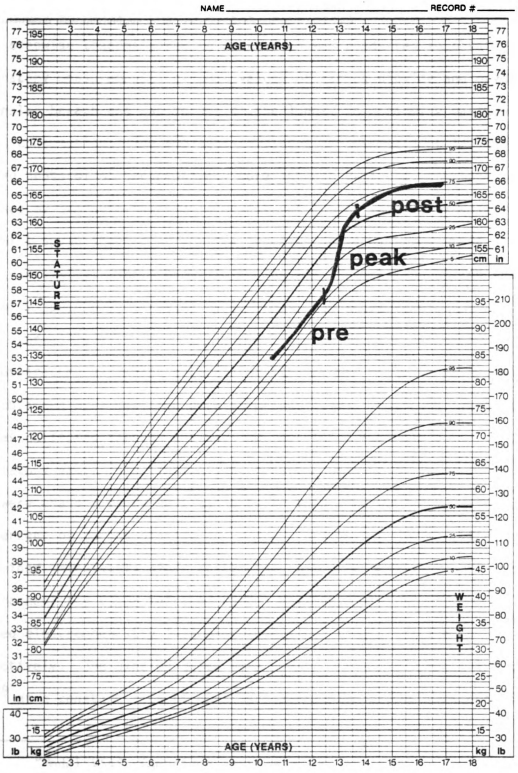
NAME
RECORD #
AGE (YEARS)
STATURE
WEIGHT
post
peak
pre
in
cm
lb
kg
AGE (YEARS)

Figure 1.

PERFORMANCE CAPABILITY AND ANAEROBIC METABOLISM:

A stepwise treadmill run to exhaustion was conducted to obtain the respiratory measurements; percent values of carbon dioxide and oxygen in exhaled breath and gas volume of the lungs, to determine maximum oxygen consumption. The subjects completed an intermittent treadmill running protocol of progressively more intense 3-minute work intervals followed by 3-minute resting intervals until they reached exhaustion. Treadmill speed started at 6 miles per hour (MPH) with the grade set at 0%, initially. The treadmill incline was increased by 1% and the treadmill speed was increased by 1 MPH at each subsequent work-interval level.

Respiratory variables determining the maximum oxygen consumption were obtained by a modified Douglas method (112). Subjects' expired air was collected through a 2-way low-resistance (Daniels[5]) respiratory valve into lightweight, neoprene weather balloons (113). Bags were changed every 30 seconds by an automated switching valve[6]. The percent of carbon dioxide and oxygen contents of the air were determined immediately after collection, using an applied electrochemistry CD-38 infrared carbon dioxide analyzers and an applied electrochemistry S-3A electrochemical oxygen analyzer[7], respectively. The exhaled gases were pumped through a DTM-115 dry gas meter[8] at a constant rate of 50 liters per minute for measurement of

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[5]Model VS4S, Cambridge Instrument Co.
[6]Van Huss Wells Automated Switching Valve.
[7]Applied Electrochemistry. Sunnyvale, CA.
[8]American Meter Co. (Singer).

gas volume. Pulmonary ventilation, oxygen uptake, and maximum oxygen uptake were calculated by the equations of Consolazio, Johnson, and Perera (112). The heart rate was determined from a 3-lead electrocardiogram and recorded continuously on a Sargent recorder to determine the maximum rate. Before work, immediately following each test level, immediately following exhaustion, and at 5, 10, and 15 minutes of recovery, fingertip, arterialized (arterialized by keeping the hand warm at 45^{o}C) blood samples were obtained for determination of whole blood lactate. Lactate levels were determined by the Roch Lactate Analyzer 640[9] (114). Immediately after the blood sample was taken, 50 microliters of this anticoagulated whole blood was added to 450 microliters of a diluting solution, (Oxford Laboratory Solution pre-prepared containing 1 gram per liter of sodium azide[9]) into the haemolysis tube, mixed, and then read. Subjects' lactate levels were corrected for the training effect by dividing their maximum lactate levels by their maximum oxygen consumption (50).

NUTRIENT ANALYSIS:

Nutrient intake was estimated from a 3-day dietary record kept by each subject prior to the day of testing. The record included 2 week-days and 1 week-end day. At the test site, subjects were interviewed (with a parent when possible) on each food record to clarify portion sizes, condiments, recipe ingredients, brands of food selected, and if any vitamin or mineral supplement was taken. Dietary records were coded and evaluated for several nutrients by the Michigan State

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[9]Bioelectronics. Switzerland.

University Nutrient Data Bank (115). Nutrient intakes for each group were compared with each other as well as with the Recommended Dietary Allowances (81) for the appropriate gender and age. Subjects' nutritional intakes were evaluated to detect any significant differences in amounts of protein, animal and plant protein, and energy intake, and percent RDA for energy, protein, iron, vitamins A, C, B_{12}, and folic acid. Other nutrients were also examined to note any extreme levels of intake.

Subjects ingesting iron supplements were compared to those who did not in each group in relation to hematological and nutriture iron status. The amount of iron in supplements was added to food nutrients to arrive at total daily consumption, which was compared to the nutrient consumption levels excluding the supplements. Those individuals who took an iron suplement in one phase but not in the other were also evaluated.

Iron bioavailability for each meal was calculated - according to Monsen's model (18) - for each subject at each level of iron status (0 mg., 200 mg. and 500 mg. of iron stores). Appendix B describes this method to calculate the amount of absorbable iron. This analysis was done to evaluate and compare this method with the general method of assuming an overall 10% absorption of the total amount of iron present in foods as done with estimating the percent RDA. The percent of the required 1.8 mg., as suggested by the Recommended Daily Allowance (81), absorbed was calculated using Monsen's model and compared to the percent RDA for iron. The percent of total dietary iron intake which was absorbed at 3 specified iron storage level (according to Monsen's

model; 0 mg, 250 mg., and 500 mg.) was compared with the percent RDA in each group. The quality of each subject's diet was assessed through use of this model by recording the amounts of absorption enhancing factors (dietary heme and ascorbic acid) present in each meal. The inhibiting factors; calcium, phosphate, tea, coffee, and dietary fiber were also recorded, though Monsen's model did not include these factors in the calculations for the amount of absorbable iron.

STATISTICAL ANALYSIS:

The effects of running versus developmental stage (breaking the sample into three groups; pre, peak, and post growth categories) on the dependent variables were asessed by an F ratio test, analysis of variance for a 3 by 4 multiple classification table (116). Based on the procedure by Grubbs (117), outliers were excluded in some statistical analyses. The Bonferroni t-test for unequal sample size (118) was used in the comparison of variables where appropriate. Physical characteristics, hematological measurements and dietary intake of kcal, protein, iron and vitamin C were evaluated by the Bonferroni t-test for unequal sample size (118), and compared in each of the four groups (male runners, male controls, female runners, and female controls) for both phases of the study. Hematological and plasma iron values of each group were compared to the normal median values reported for adolescents in the NHANES II survey (25). The median and range values are presented in Appendix A. Subjects with low hemoglobin and hematocrit values were individually evaluated to determine whether the low level persisted in more than one phase and

whether or not a specific cause of the low level could be identified. Two of these subjects had been reclassified according to their activity patterns, from being a runner in Phase II to a control subject in Phase III.

The following correlation coefficients were determined: (1) gain in height vs. change in hemoglobin and change in hematocrit from Phase II to III; (2) In Phase II, VO_2 Max vs. hemoglobin and hematocrit; (3) In Phase III, VO_2 Max vs. hemoglobin, hematocrit, and plasma iron; (4) In Phase III, VO_2 Max vs. maximum lactate level and total lactate production (maximum lactate production - resting lactate level); (5) In Phase III, maximum lactate level vs. hemoglobin, hematocrit and plasma iron; (6) In Phase III, total lactate production vs. hemoglobin, hematocrit, and plasma iron. All correlations were done by the Pearson product-moment correlation coefficient (117).

Hematological measurements, VO_2 Max, and dietary iron intake were compared and evaluated by the Bonferroni t-test for unequal sample size (119), between supplemental iron users and nonusers within each group. Those individuals who took supplements in one phase but not in the other were analyzed separately. A paired-data t-test (120) was used to evaluate the significance of the change in the hemoglobin, hematocrit, VO_2 Max, age, height, and dietary iron intake values of when they took the supplement from when they stopped using the supplement.

The amount of iron absorbed, as calculated by Monsen's Model (18), was compared between each specified iron storage level (0mg., 250 mg., and 500 mg.) versus the percent of the RDA (81) for iron in each group. Percent values for bioavailable iron were evaluated by the

Bonferroni t-test for unequal sample size (118) for each group to assess differences in the quality of their diets. Differences in vitamin C intake, total protein, animal protein, plant protein, and percent of dietary iron intake in the form of heme were determined for each group to compare the amount of enhancing factors in their diets. Differences in calcium, phosphate, and dietary fiber were determined for each group to compare the amount of inhibiting factors in their diets. These differences were evaluated by the Bonferoni t-test for unequal sample size (118).

RESULTS

CLASSIFICATION OF SUBJECTS:

In Phase II (Table 2), runners and controls of each gender were similar in age and height. Male runners (MR) were significantly lighter ($p<0.01$) than male controls (MC), and there was a trend ($p<0.10$) for the female runners (FR) to be lighter than the female controls (FC). The males and females of each group of runners and controls were similar in age, height and weight. However, there appear to be meaningful differences, although not detectable statistically.

Although male and female runners were slightly younger than their respective controls, there were no significant differences in their ages in Phase III (Table 3). However, MR tended to be shorter than MC, and FR were significantly shorter than FC ($p<0.05$). Controls of each gender weighed significantly more ($p<0.01$) than runners of the same gender.

When subjects were assesed for their stage of growth based upon their peak growth spurt (Table 4) the highest percentage of male runners and controls fell in their peak growth spurt. The fact that there were no MC in the pre growth spurt may explain why the height of MC was greater than MR (Table 3). The highest percentage of FR also fell in their peak growth spurt, but the highest percentage of FC fell

in their post growth category (Table 4). This could explain why the height of the controls was greater than FR (Table 3). In Table 4, although female subjects in the pre and peak groups had similar mean increases in height, the group in the pre growth category was not placed in the category of those who were in their peak growth because their pattern of growth had been steady, at a constant rate, and they have not yet undergone a sharp acceleration in growth, as indicated from their plotted heights (from the past 3 phases and the phase following Phase III) on the NCHS growth charts (111). The spurt of growth indicates that the adolescent has reached puberty. The Tanner ratings were checked for these two subjects, and may also indicate that the 2 subjects were pre-puberty (1 subject had a Tanner rating of I and the other subject had a rating of II), reenforcing the classification of these subjects in the pre growth spurt category. There was one FC in the pre growth spurt category with an increase in height of 8.3 cm. from Phase II to Phase III (dcm/dt). This individual had been growing at a steady, but rapid rate; her growth spurt did not occur until the following year (as indicated by her height measurement taken in Phase IV, Fall, 1985). Her Tanner rating (Tanner rating was I) also indicated that she had not yet reached puberty.

When subjects were classified according to growth categories, based on the shape of their growth curve, it appeared that runners and controls reached their growth spurt at the same age for both genders (Table 5). In any case, runners did not have a delayed growth spurt, because there was a trend for them to be younger when they reached their growth spurt than their counterparts of the same gender. Both

male and female controls tended to be taller than runners during the peak growth period but height equalized in the post growth category. Controls were significantly heavier than runners of the same gender in the peak growth period. In the post growth period there was a similar trend but it was not significant.

In Phase III, the mean relative weight for age of MC was significantly greater ($p<0.01$) than that of the MR (Table 6). MR had a mean relative weight 8% below the 50th percentile of weight for age and MC had a mean value 12% above the 50th percentile of weight for age. Heights for both groups were at the 50th percentile height for age. FR had a mean relative weight 15% less ($p<0.10$) than the FC. FR were 9% lighter than the 50th percentile weight, while the FC were 6% heavier than the 50th percentile weight; heights for both groups were at the 50th percentile. When subjects were divided into the three growth categories, the same trend was true (Table 7). That is, runners of both genders even though they were close to the 50th percentile for height were below the 50th percentile for weight, while controls for both sexes were usually above the 50th percentile for weight even though they ranged close to the 50th percentile for height.

HEMATOLOGICAL PROFILE

Mean hemoglobin values for all groups, in Phase II as well as in Phase III, were normal (Table 8). Thirty-eight percent of the subjects in Phase II and 26% of the subjects in Phase III had hemoglobin values below the median values for males and females, 12-14 years of age, reported in the NHANES II survey (25), illustrated in Figure 2. Hemoglobin values were similar for runners and controls

(Table 8). There was a trend for females to have lower hemoglobin values than males. One male runner, in Phase II, had a low hemoglobin value of 10.6 g/dl. In Phase III, a different male subject and one female had low hemoglobin values (11.4 and 11.7 g/dl, respectively). These subjects will be discussed in more detail later in the case study section of this chapter. In both Phases II and III, 33% of the group (Figure 3) had hematocrit values below the median values of males and females, 12-14 years of age, reported in the NHANES II survey (25). Mean hematocrit values for all groups in Phases II and III were normal (Table 8). In Phase II, the MR who had a low hemoglobin value also had a low hematocrit value (28.5%). In Phase III, 2 MR had low hematocrit values, less than 37%. The MR with the lowest hematocrit value (34.3 %) was the same individual with the low hemoglobin value mentioned previously. The mean hematocrit values of each group were similar for Phase II and III (Table 8). The mean hematocrit values were similar between the runners and controls of each gender in both phases. The other hematological indices, listed in Table 8, include red blood cell count (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). These hematological indices were similar between Phase II and Phase III for each group. The mean values of all of these measurements were normal and were similar between the runners and the controls of each gender in both Phases II and III. Thirty-five percent of the group (Figure 4) had plasma iron values (measured only in Phase III) below the median values of males and females, 12-14 years, reported in the NHANES II survey (25). Mean plasma iron values for all groups were normal (Table 8). Mean plasma iron values were

similar between the runners and controls of each gender.

In Table 9, mean hematological values were given for each category of growth (pre, peak, and post) of MR, MC, FR, and FC. The mean values of each indice for each group (MR, MC, FR, and FC) were similar among growth categories (pre, peak, and post) except for the hematocrit values in the FC. The mean hematocrit value of the peak growth spurt category was significantly lower ($p<0.05$) than that of the pre and post growth categories of FC. Mean values of the hematological values were similar between runners and controls of each gender. MC in their peak growth spurt had mean hemoglobin and hematocrit values which were significantly greater ($p<0.01$) than that of FC in their peak growth spurt. There was a trend ($p<0.10$) for FR in their peak growth spurt to have a lower mean hematocrit value than that of MR in their peak growth spurt.

In Table 10, the r values for the correlations of the values for gain in height with the change in hemoblobin and with the change in hematocrit, from Phase II to Phase III were presented for all subjects and for just the subjects in their peak growth spurt of each group. There was a significant inverse relationship between gain in height and change in hemoglobin and hematocrit for FR. In other words, hemoglobin and hematocrit values became lower as the individuals gained in height. There were no significant correlations of the values for the control groups. There was a significant positive correlation ($p<0.05$) of the gain in height with the change in hemoglobin and hematocrit for MR in their peak growth spurt and a significant inverse correlation ($p<0.05$) of the gain in height with the change in hemoglobin for FR in their peak growth spurt. There

were no significant correlations of values for controls.

MEASUREMENTS OF PHYSICAL CAPACITY

In Table 11, the mean values of aerobic capacity measurements for each group in both Phases II and III were given. In Phase II, MR had a 11% greater ($p<0.05$) level of oxygen consumption than MC and FR had a 22% greater ($p<0.01$) level of oxygen consumption than the FC. Mean VO_2 Max values of male runner and control groups were each significantly greater ($p<0.01$) than respective female runner and control groups. VO_2 Max values were similar between Phases II and III for each group. In Phase III, male and female runners had a 15% and 20% greater ($p<0.01$) level of oxygen consumption than male and female controls, respectively. VO_2 Max values were usually greater (though not significant) for males than females in each group of runners and controls. Mean measurements for resting and maximum heart rates were similar between runners and controls of each gender in Phase III (Table 11).

When subjects were divided into growth categories, runners in each category (pre, peak, and post) had significantly greater levels of oxygen consumption than their control group counterpart of each gender (Table 12). Male subjects in the post growth category tended to have higher VO_2 Max values than subjects in pre or peak growth category, whereas female subjects in the post growth category had lower VO_2 Max values than those in pre and peak growth categories. Resting and maximum heart rates were similar between growth categories for female runners and controls (Table 12). There was a trend for older female subjects (post growth category) to have lower resting heart rates than younger subjects (pre growth category).

In Table 13, the r values of the correlations of VO_2 Max with hemoglobin and hematocrit, were listed for Phase II, and of VO_2 Max with hemoglobin, hematocrit, and plasma iron for Phase III. In Phase II, there were no significant correlations observed between these parameters in any group. In Phase III, no significant relationships were found in male runner or control groups. There was a significant positive correlation ($p<0.05$) of VO_2 Max with hemoglobin in FR and a significant inverse correlation ($p<0.05$) of VO_2 Max with hemoglobin in FC.

Mean values for various parameters related to anaerobic capacities of each group in Phase III were listed in Table 14. Mean values for resting and maximum blood lactate levels (before and after the treadmill run) and the amount of lactate produced (difference between maximum and resting lactate levels) were similar between runners and controls for each gender. When maximum lactate levels were corrected for differences in VO_2 Max values MR and MC still had similar values. The post growth spurt category of FR (Table 15) tended to have a higher mean maximum lactate level (25%), lactate production level (32%), and maximum lactate/VO_2 Max meaurement (17%), than the post category of FC. There was a lactate value for only one MR in his post growth spurt. This subject had the highest maximum lactate and maximum lactate/VO_2 Max levels of all subjects.

In Table 16, the r values for the correlations of values VO_2 Max with maximum lactate and total lactate production were given for each group. There was a trend ($p<0.10$) for a positive correlation of VO_2 Max with maximum lactate level in MR. The maximum lactate levels and total lactate production levels were both significantly inversely

correlated ($p<0.05$) with plasma iron concentrations in MR. In FC, there was a significant inverse correlation ($p<0.05$) of maximum lactate levels and total lactate production levels with hematocrit concentrations.

NUTRIENT INTAKE

In general, the percentage of dietary energy which came from protein, fat and carbohydrate was similar for all groups of subjects in both phases of the study (Table 17). Several exeptions might be noted. In Phase II, FC tended to consume a higher mean percentage of energy as fat ($p<0.10$) than MC. FR tended to consume a higher mean percentage of energy ($p<0.10$) as carbohydrate than FC. When subjects were divided into growth categories similar consumption of carbohydrate, protein, and fat was observed (Table 18).

In Phases II and III, mean energy consumption of runners was above 100% of the RDA, while energy intake of controls was below 100% of the RDA for each gender (Figure 5). FC had a low mean energy intake (63% of the RDA) in Phase II; their mean energy intake (90% of the RDA) was adequate in Phase III. In Phase II, MC consumed 32% more ($p<0.10$) energy than FC; male and female controls had similar mean energy intakes in Phase III. Male and female runners consumed similar mean energy intakes in both phases. When subjects were divided into growth categories (Figure 6), there were some exceptions for the trends in energy consumption, observed in Phase III. Post growth spurt FR consumed a mean energy intake below 100% of the RDA, while post growth spurt MC and pre growth spurt FC consumed mean intakes above 100% of the RDA. Peak growth spurt MR tended to consume more energy ($p<0.10$) then peak growth spurt MC. Post growth spurt MR tended to consume

more energy ($p<0.10$) than post growth spurt FR.

In Phases II and III, FR consumed nearly twice as much energy/kg. body weight as female controls (Figure 7). In Phase II, mean kcal. intakes/kg. body weight were similar for MR and MC. However, in Phase III, MR consumed approximately 50% more energy (28 kcal./kg. body weight; $p<0.05$) than MC. Although males usually consumed more energy per kg. body weight than their female counterparts, there was a lot of variability within the groups and there were no significant differences in any of the four groups. Intakes between Phases II and III were not statistically compared.

When subjects were categorized into pre, peak and post growth categories, it was obvious that subjects consumed fewer kcal./kg. body weight in the post growth category than they did in the other two categories (Figure 8). In addition, differences in energy intake per kg. body weight between runners and controls which were observed in Phase III (Figure 7) no longer existed in the post growth spurt period. However, they appeared to exist in the pre and peak growth spurt periods, but the sample sizes were too small to show significance.

Mean daily consumptions of protein for all groups were above 100% of the RDA in Phases II and III (Figure 9). In Phase II, FC had the lowest mean protein intake, 40% less than MC ($p<0.01$) and 36% less than FR ($p<0.10$). In Phase III, MR consumed more protein ($p<0.01$) than MC and FR. When subjects were divided into growth categories; all groups consumed over 100% of the RDA for protein (Figure 10). Peak growth spurt MR consumed more protein than peak growth spurt MC ($p<0.01$) and peak growth spurt FR ($p<0.01$). Post growth spurt males

consumed more protein than post growth spurt females for runners ($p<0.01$) and controls ($p<0.05$).

In Phases II and III, mean consumption of dietary iron for all groups were less than 100% of the RDA (Figure 11). Mean dietary iron intake for FC was less than 70% of the RDA for iron in Phases II and III. In Phases II and III, FR tended ($p<0.10$) to consume greater mean dietary iron intakes than FC. In Phase III, MR tended ($p<0.10$) to consume more dietary iron than MC. When subjects were divided into growth categories (Figure 12), post growth spurt males consumed mean iron intakes greater than 100% of the RDA, while post growth spurt females consumed mean intakes less than 70% of the RDA for iron. Peak growth spurt FC also consumed a mean intake less than 70% of the RDA for iron. Although runners usually consumed more dietary iron than their corresponding control group, no significant differences were observed, probably due to small sample sizes.

In Figures 13 and 14, the percentage of subjects in each group consuming mean daily intakes for each level of dietary iron, in Phases II and III, respectively, have been illustrated. In Phase II, 5% of the MR, 10% of the MC, 15% of the FR and 56% of the FC consumed less than 50% (9 mg.) of the RDA of 18 mg. Twenty-four percent of MR, 20% of MC, 16% of FR, and 11% of FC consumed dietary iron intakes greater than 18 mg. In Phase III, 5% of MR, none of MC, 27% of FR, and 20% of FC consumed less than 50% of the RDA. Twenty-five percent of MR, 36% of MC, 9% of FR, and none of FC had dietary iron intakes greater than 18 mg.

SUPPLEMENTAL IRON USE

Subjects who took a multi-vitamin/mineral supplement containing iron and vitamin C, 3 to 7 times a week, were compared to their counterparts who did not take supplements for Phase II. Twenty-four percent of MR, 10% of MC (Table 19), 15% of FR, and 33% of FC (Table 20) took some form of iron supplement, which also contained vitamin C. Mean dietary iron intake and mean hemoglobin and hematocrit concentrations were similar between supplement users and non-users in each group. VO_2 Max measurements were also similar between users and nonusers of iron supplements. When runners and controls for each gender were grouped together (to increase the sample sizes of supplement and non-supplement groups since hematological values were similar between runners and controls for each gender) mean values for dietary iron intake and hemglobin and hematocrit concentrations were similar for supplement and non-supplement users within each gender (Table 21).

In Phase III, 30% of MR, 18% of MC (Table 22) 36% of FR, and 30% of FC (Table 23) took some form of an iron supplement which also contained vitamin C. Mean dietary iron intakes (expressed in %RDA) were similar for supplement and non-supplement users in all groups. Taking the iron supplement increased the intake of iron to over 100% of the RDA. Mean hemoglobin, hematocrit and plasma iron concentrations of supplement users were similar to those of non-users in each group. In general, supplement users usually had higher VO_2 Max values (though not significantly higher) than nonusers in each group. The small sample size of each group increased the difficulty in determining statistical significance. When runners and controls of

each gender were grouped together (Table 24), mean dietary iron intakes (expressed in %RDA) were similar between supplement users and non-users for each gender. When the amount of iron in the supplement was included, males and females had 47% and 42% greater mean iron intakes than that of male and female non-users, respectively. Mean hemoglobin, hematocrit and plasma iron concentrations were similar for supplement users and non-users of each gender.

Changes in hematological measurements and VO_2 Max values for those subjects who took an iron supplement in one phase but not in the other phase (change in value = value when using supplement - value when not using supplement) were presented in Table 25. Three subjects took an iron supplement in Phase II but not in Phase III, and 5 subjects took an iron supplement in Phase III but not in Phase II. The differences in age and in height (between the phase when they took the supplement and the phase they did not) of the group were not significant. The differences in dietary iron intake and VO_2 Max values of the group were similar when they took the supplement compared to when they did not. There was a significant increase ($p<0.05$) in hemoglobin and hematocrit values for subjects when they took the supplement compared to when they did not. One subject, a FC, had a relatively low hemoglobin value (12.8 g/dl) before taking an iron supplement; the rest of the subjects had hemoglobin values greater than 13 g/dl. This subject, along with two other subjects, had the highest increase in her hemoglobin value (+1 g/dl) after taking the supplement.

CASE STUDY REPORTS

In Phase II, there was one male runner who had low hemoglobin and hematocrit values, indicating iron deficiency anemia (Table 26). In

Phase II, he ran 6-8 miles/day, but in Phase III, he discontinued running and became a control subject; his VO_2 Max value decreased 1.5 ml./kg./min. He was completing his growth spurt in Phase II and had completed it in Phase III. Most of his hematological indices were low and increased from Phase II to Phase III by 5.4 g./dl., 17.2%, 1.8 ($x10^6/mm^3$), and 3.5 u^3 for hemoglobin, hematocrit, RBC, and MCV indices, respectively. His MCH and MCHC decreased 0.3 ug. and 1.9% , respectively. His plasma iron concentration was normal in Phase III. His mean dietary iron and energy intakes increased from Phase II to Phase III. He did not take any iron supplement during either phase.

In Phase III, there was one male subject who had low hemoglobin and hematocrit values indicating iron deficiency anemia (Table 27) (a different subject than the subject with low hemoglobin and hematocrit values in Phase II). This subject was a male runner, 11.0 yrs. of age in Phase III. He had gained 2.9 kg. in weight and 6.1 cm. in height from Phase II to Phase III. He had not reached his growth spurt or puberty yet, growing steadily close to the 50th percentile growth curve of height for age/gender on the NCHS growth chart (111). His hemoglobin dropped 2.5 g./dl. and his hematocrit dropped 4.9% from Phase II to Phase III. His RBC, MCH, and MCHC decreased 0.8 ($x10^6/mm^3$), 0.8 ug., and 2.1%, respectively, but his MCV increased 2 u^3. His plasma iron concentration was normal. His mean dietary iron and energy intakes increased from Phase II to Phase III. He did not take any form of supplementation during either of the phases. He had been running road races throughout the past two phases and maintained a VO_2 Max of 64 ml./kg./min. in both phases of the study.

There was one female subject in Phase III (Table 28) who had a low

hemoglobin value. She was a runner in the Phase II but had stopped running and became a control subject in Phase III. She remained active though by playing other sports such as basketball. This could explain why her VO_2 Max increased by 6.1 ml./kg./min. from Phase II to Phase III. She gained 8.8 kg. in weight, and 4 cm. in height from Phase II to Phase III. She began her growth spurt in Phase II which continued through Phase III. Her hemoglobin dropped 1.6 g/dl, although her hematocrit remained the same from Phase II to Phase III. Her RBC, MCH, and MCHC decreased by 14%, 5%, and 11%, respectively and her MCV increased by 7% from Phase II to Phase III. Her plasma iron concentration was normal. Her mean dietary iron and energy intakes increased from Phase II to Phase III. She took a multi-vitamin/mineral supplement (containing 18 mg. of iron) 1-2 times/week in Phase III, but not in Phase II.

IRON BIOAVAILABILITY

In Figure 15, mean total, animal, and plant protein daily intakes were plotted for each group (MR, MC, FR, and FC). The mean total protein consumption of MR was significantly greater than that of FR ($p<0.01$) and MC tended ($p<0.10$) to have a greater mean consumption than FC. Runners and controls within each gender had similar mean intakes of total protein, animal protein, and plant protein. Males tended ($p<0.10$) to have greater mean intakes of animal protein than females for both runners and controls. MR usually consumed a higher percent of their dietary iron intake in the form of dietary heme than the other groups. Each group had over 100% of the RDA for vitamin C (Figure 17).

In Table 29, the mean value for dietary iron absorbed, as

calculated by Monsen's Model (105), for the iron storage levels of 0 mg., 250 mg., and 500mg., are presented for each group. The greatest amount of dietary iron absorbed was at the 0 mg. iron storage level, while the lowest amount absorbed was at the 500 mg. storge level for all subjects. MR had significantly greater mean absorption values than FR at the 0 mg. ($p<0.05$) and 250 mg. ($p<0.10$) storage levels. MC tended to have greater mean absorption values at each storage level than FC. The percent values of the recommended 1.8 mg. of iron absorbed (suggested by the RDA to cover the body's needs and replenish daily losses of iron) as calculated by Monsen's model, were compared to the %RDA for iron (which assumes that 10% of the total dietary iron intake would be absorbed). Mean values for the percent of absorption of 1.8 mg. of dietary iron were calculated for each storage level and then compared to the %RDA for iron for each group (Table 30). Of the 3 storage levels, the mean percent at the 250 mg. storage level of iron absorption was most similar to the mean %RDA absorption value. The percent of subjects in each group who had absorption measurements less than 67% of the recommended 1.8 mg. (1.2 mg.) of iron were presented for each storage level in Table 31. The percentages of subjects in each group at the 250 mg. storage level were most similar to the percentages of subjects less than 67% of the RDA, as compared to the percentages of subjects at the other storage levels. In Figure 18, the percent of total dietary iron intake absorbed, as calculated by Monsen's Model, for each iron storage level was plotted for each group. The ranges of absorption were 14-18%, 10-12%, and 7-8% for the 0 mg., 250 mg., and 500 mg. iron storage levels, respectively. MR had a significantly greater mean percent absorption of dietary iron than

the FR at the 0 mg. ($p<0.01$) and 250 mg. ($p<0.05$) storage levels. In Table 32, the r values for the correlations of hemoglobin values with %RDA for iron and percent of 1.8 mg. iron absorbed (as calculated by Monsen's method for the 250 mg. iron storage level) were given for each group. There were no significant correlations observed between these parameters.

In Table 32, the mean dietary intake of nutrients inhibiting iron absorption, including dietary fiber, calcium and phosphorus, were presented for each group. Runners and controls, of each gender, consumed similar intakes for dietary fiber, calcium, and phosphorus.MC tended ($p<0.10$) to consume more dietary fiber than FC. MR tended ($p<0.10$) to consume more phosphorus (expressed in %RDA) than FR.

TABLE 2. AGES, HEIGHTS, AND WEIGHTS OF SUBJECTS IN PHASE II

MALES			FEMALES		
RUNNERS		CONTROLS	RUNNERS		CONTROLS
		NUMBER			
21		10	13		9
		AGE (decimal yrs)			
12.7±1.9#		13.1±1.1	13.0±1.8		14.0±1.9
		HEIGHT (cm)			
151.7±12.5		160.5± 6.5	151.9±11.4		157.9± 9.1
		WEIGHT (kg)			
40.0± 9.5	*	51.0± 6.4	41.9± 7.8	***	50.4±10.7

MEAN ± SD
* significant difference ($p<0.01$) between runners and controls of the same gender.
*** trend ($p<0.10$) for a difference between runners and controls of the same gender.

TABLE 3. AGES, HEIGHTS, AND WEIGHTS OF SUBJECTS IN PHASE III

MALES			FEMALES		
RUNNERS		CONTROLS	RUNNERS		CONTROLS
		NUMBER			
20		11	11		10
		AGE (decimal yrs)			
13.6±1.8#		14.3±1.1	13.4±1.8		14.6±1.8
		HEIGHT (cm)			
156.9±12.9	***	166.5± 7.8	154.2± 7.4	**	161.1± 9.1
		WEIGHT (kg)			
43.7±10.2	*	57.2± 3.4	42.0± 8.4	*	52.8± 3.1

MEAN ± SD
* significant difference ($p<0.01$) between runners and controls of the same gender.
** significant difference ($p<0.05$) between runners and controls of the same gender.
*** trend ($p<0.10$) for a difference between runners and controls of the same gender.

TABLE 4. GAIN IN HEIGHT OF SUBJECTS FROM PHASE II TO III CLASSIFIED ACCORDING TO STAGE OF GROWTH

	RUNNERS			CONTROLS		
	PRE	PEAK	POST	PRE	PEAK	POST
			MALES			
NUMBER	5	13	2	0	9	2
% GRP.	25	65	10	0	82	18
dcm/dt[a]	4.5±0.6[#]	7.4±2.2	3.4±0.8	---	6.5±2.3	2.1±0.4
RANGE[b]	3-6	5-12	2-4	---	4-10	1-2
			FEMALES			
NUMBER	2	6	3	1	4	5
% GRP.	18	55	27	10	40	50
dcm/dt[a]	6.4±1.1	6.3±1.9	2.2±2.2	8.3	4.3±0.9	0.7±1.0
RANGE[b]	5-7	4-10	0-4	8	4-5	0-2

MEAN ± SD
a increase in height (dcm) in 1 year (dt) from Phases II to III.
b range of subjects' increase in height (dcm/dt).

TABLE 5. AGES, HEIGHTS, AND WEIGHTS OF SUBJECTS IN EACH GROWTH CATEGORY IN PHASE III

	MALES		FEMALES	
	RUNNERS	CONTROLS	RUNNERS	CONTROLS
		NUMBER		
PRE	5	0	2	1
PEAK	13	9	6	4
POST	2	2	3	5
		AGE (decimal yrs)		
PRE	11.8±0.9#	---	11.0±0.5	11.2
PEAK	13.8±1.5	14.1±1.1	13.3±1.3	13.9±1.3
POST	16.5±0.6	15.4±0.7	15.1±1.0	15.8±1.1
		HEIGHT (cm)		
PRE	141.8± 6.3	---	151.3± 3.7	148.6
PEAK	160.1± 9.8[a]	165.4± 8.1[b]	152.2± 8.2[a] ***	165.9±10.3[b]
POST	173.9± 0.9[a]	171.4± 4.4[b]	159.9± 5.7[a]	159.9±10.3[b]
		WEIGHT (kg)		
PRE	30.3± 1.0	---	36.8± 8.2	38.7
PEAK	46.7± 7.0 **	55.2± 9.2	46.7± 7.0 **	54.1± 8.8
POST	57.2± 3.4	66.6± 2.3	51.8± 5.6	54.5±10.1

MEAN ± SD

* significant difference ($p<0.01$) between runners and controls of the same gender.

** significant difference ($p<0.05$) between runners and controls of the same gender.

*** trend ($p<0.10$) for difference between runners and controls of the same gender.

a significant difference ($p<0.05$) between male and female runners.

b significant difference ($p<0.05$) between male and female controls.

TABLE 6. RELATIVE WEIGHTS AND HEIGHTS TO AGE OF SUBJECTS IN PHASE III

MALES				FEMALES		
RUNNERS		CONTROLS		RUNNERS		CONTROLS
		RELATIVE WEIGHT[a] (%)				
92±14[#]	*	112±16		91±12	***	106±16
		RELATIVE HEIGHT[b] (%)				
100± 5	***	102± 6		100± 4		102± 5

MEAN ± SD
* significant difference ($p<0.01$) between runners and controls of the same gender.
*** trend ($p<0.10$) for difference between runners and controls of the same gender.
a Relative Weight = (subjet's wt/50th percentile wt for subject's age)x100
b Relative Height = (subjet's ht/50th percentile ht for subject's age)x100

TABLE 7. RELATIVE WEIGHTS AND HEIGHTS TO AGE OF EACH GROWTH CATEGORY IN PHASE III

	MALES			FEMALES		
	RUNNERS	CONTROLS		RUNNERS		CONTROLS
		RELATIVE WEIGHT[b] (%)				
PRE	81±11[#a]	---		102±18[a]		105
PEAK	97±14	111±17		86±11	**	113±17
POST	90± 9	116±15		95± 6		101±17
		RELATIVE HEIGHT[c] (%)				
PRE	97± 6	---		106± 0		102
PEAK	101± 5	103± 6		98± 3		105± 4
POST	100± 2	101± 6		99± 4		99± 4

MEAN ± SD
** significant difference ($p<0.05$) between runners and controls of the same gender.
a significant difference ($p<0.01$) between male and female runners.
b Relative Weight = (subjet's wt/50th percentile wt for subject's age)x100
c Relative Height = (subjet's ht/50th percentile ht for subject's age)x100

TABLE 8. HEMATOLOGICAL VALUE OF SUBJECTS IN PHASES II AND III

	MALES		FEMALES	
	RUNNERS	CONTROLS	RUNNERS	CONTROLS
		HEMOGLOBIN (g/dl)		
PHASE II	14.2±1.2[#]	14.4±1.0	14.0±0.6	13.6±0.6
PHASE III	14.0±1.0	14.5±1.0	13.3±0.5	13.0±0.9
		HEMATOCRIT (%)		
PHASE II	39.3±3.4	40.5±3.2	39.3±2.0	38.2±1.4
PHASE III	41.9±3.2	43.3±2.5	39.9±1.6	38.8±2.8
		RED BLOOD CELL COUNT (10^6/mm^3)		
PHASE II	4.83±0.43	4.94±0.29	4.78±0.19	4.59±0.14
PHASE III	4.86±0.38	5.01±0.34	4.67±0.20	4.44±0.20
		MEAN CELL VOLUME (u^3)		
PHASE II	82.1±2.6	83.1±4.0	83.6±2.7	84.8±3.0
PHASE III	86.1±2.6	86.3±3.5	86.0±2.1	88.1±2.8
		MEAN CELL HEMOGLOBIN (uug)		
PHASE II	29.5±1.2	29.1±1.4	29.5±0.9	29.7±0.8
PHASE III	28.7±0.9	28.9±1.5	28.6±1.1	29.2±1.3
		MEAN CELL HEMOGLOBIN CONCENTRATION (%)		
PHASE II	36.1±0.9	35.1±1.2	35.4±0.8	35.3±0.7
PHASE III	33.4±0.7	33.6±0.7	33.3±1.0	33.1±0.9
		PLASMA IRON (mcg/ml)		
PHASE III	1.13±0.39	0.97±0.19	1.08±0.32	1.10±0.32

#MEAN ± SD

TABLE 9. HEMATOLOGICAL VALUES OF EACH GROWTH CATEGORY IN PHASE III

	MALES		FEMALES	
	RUNNERS	CONTROLS	RUNNERS	CONTROLS
		HEMOGLOBIN (g/dl)		
PRE	13.3±1.4[#]	---	13.3±0.1	13.1
PEAK	14.3±0.8	14.3±1.0[a]	13.4±0.6	12.4±0.8[a]
POST	14.3±1.1	15.5±0.8	13.1±0.5	13.5±0.9
		HEMATOCRIT (%)		
PRE	42.4±2.6[b]	---	39.9±1.3[b]	40.2*
PEAK	42.4±2.6[b]	42.8±2.5[a]	39.7±1.5[b]	36.5±2.7[a]*
POST	44.1±4.0	45.3±0.6	40.5±2.2	40.4±1.9*
		RED BLOOD CELL COUNT (10^6/mm^3)		
PRE	4.69±0.56	---	4.76±0.23	4.82
PEAK	4.93±0.35	4.93±0.32	4.66±0.17	4.22±0.21
POST	4.91±0.50	5.34±0.16	4.61±0.31	4.53±0.10
		MEAN CELL VOLUME (u^3)		
PRE	84.6±1.6	---	83.3±2.1	83.4
PEAK	85.8±3.0	86.6±3.6	86.0±1.2	88.1±2.8
POST	89.8±0.9	84.8±3.9	87.7±2.0	89.1±2.2
		MEAN CELL HEMOGLOBIN (uug)		
PRE	28.1±1.0	---	28.0±1.1	27.3
PEAK	28.8±1.0	28.9±1.4	29.0±1.1	28.9±0.7
POST	29.2±0.8	28.9±2.6	28.3±1.3	29.7±1.6
		MEAN CELL HEMOGLOBIN CONCENTRATION (%)		
PRE	33.3±0.8	---	33.6±0.4	32.7
PEAK	33.6±0.5	33.4±0.5	33.7±1.0	32.8±0.3
POST	32.5±0.5	34.2±1.3	32.3±0.8	33.3±1.2
		PLASMA IRON (mcg/ml)		
PRE	1.16±0.26	---	---	1.09
PEAK	1.17±0.47	0.96±0.21	1.19±0.34	0.95±0.14
POST	0.88±0.24	1.01±0.14	0.85±0.10	1.22±0.42

MEAN ± SD

* significant difference ($p<0.05$) between categories of growth in female controls.

a significant difference ($p<0.01$) between male and female controls.

b trend for difference ($p<0.10$) between male and female runners.

TABLE 10. CORRELATION BETWEEN GAIN IN HEIGHT WITH CHANGE IN HEMOGLOBIN AND HEMATOCRIT FROM PHASE II TO PHASE III.

	Correlation Coefficients			
	MR	MC	FR	FC
	ALL SUBJECTS			
SUBJECTS (N)	15	10	7	5
GAIN IN HEIGHT WITH:				
CHANGE IN HB.	+.49	-.36	-.76*	-.16
CHANGE IN HCT.	+.40	-.31	-.76*	-.55
	PEAK GROWTH SUBJECTS			
SUBJECTS (N)	9	8	5	2
GAIN IN HEIGHT WITH:				
CHANGE IN HB.	+.57*	-.19	-.92*	--
CHANGE IN HCT.	+.60*	-.01	-.19	--

* significant correlation ($p<0.05$)

TABLE 11. AEROBIC CAPACITY MEASUREMENTS OF SUBJECTS IN PHASE II AND PHASE III

	MALES			FEMALES		
	RUNNERS		CONTROLS	RUNNERS		CONTROLS
			VO_2 Max (ml/kg/min)			
PHASE II	64.4±6.2[#a]	**	57.5±7.8[b]	56.7±5.3[a]	*	44.1±6.1[b]
PHASE III	63.7±6.2	*	54.1±7.5	60.5±5.1	*	48.6±5.3
			RESTING HEART RATE (beats/min)			
PHASE III	75±12		82±17	72±12		72± 7
			MAXIMUM HEART RATE (beats/min)			
PHASE III	206±11		208±20	201± 9		196±23

MEAN ± SD
* significant difference ($p<0.01$) between runners and controls of the same gender.
** significant difference ($p<0.05$) between runners and controls of the same gender.
a significant difference ($p<0.01$) between male and female runners.
b significant difference ($p<0.01$) between male and female controls.

TABLE 12. AEROBIC CAPACITY MEASUREMENTS OF SUBJECTS IN EACH GROWTH CATEGORY IN PHASE III

	MALES			FEMALES		
	RUNNERS		CONTROLS	RUNNERS		CONTROLS
			VO_2 Max (ml/kg/min)			
PRE	66.9±4.0[#]		---	61.6±5.4	*	52.8
PEAK	61.7±6.7	**	53.6±8.3	62.3±3.9	*	48.6±6.5
POST	72.2	*	55.9±4.4	56.2±6.1	**	46.7±4.1
			RESTING HEART RATE (beats/min)			
PRE	69±12		---	84±11		83
PEAK	77±12		86±18	69±12		70± 7
POST	76		70± 2	69±11		72±0
			MAXIMUM HEART RATE (beats/min)			
PRE	205± 6		---	198±11		210
PEAK	207±13		207±22	203± 8		203± 4
POST	204		212± 4	199±11		174±40

MEAN ± SD
* significant difference ($p<0.01$) between runners and controls of the same gender.
** significant difference ($p<0.05$) between runners and controls of the same gender.

TABLE 13. CORRELATIONS BETWEEN VALUES OF VO_2 MAX WITH HB., HCT., AND PLASMA IRON IN PHASE II[2]AND PHASE III

	Correlation Coefficient (r)			
	MR	MC	FR	FC
VO_2 MAX WITH:				
	PHASE II:			
HB.	+.32	+.23	-.26	+.35
HCT.	+.40	+.33	-.24	+.13
	PHASE III:			
HB.	+.06	+.18	+.61*	-.73*
HCT.	+.16	+.26	+.18	-.02
PLASMA FE	-.43	+.03	+.47	-.03

* significant correlation ($p<0.05$).

TABLE 14. ANAEROBIC CAPACITY MEASUREMENTS OF SUBJECTS IN PHASE III

MALES		FEMALES	
RUNNERS	CONTROLS	RUNNERS	CONTROLS
RESTING LACTATE LEVEL (mmol/l)			
2.4±0.6[#]	2.3±0.5	2.5±0.4	2.5±0.5
MAXIMUM LACTATE LEVEL (mmol/l)			
11.7±3.7	11.2±1.4	12.5±2.6	10.9±2.7
LACTATE PRODUCTION LEVEL[a] (mmol/l)			
9.3±3.6	8.9±1.5	10.4±2.4	8.9±2.7
MAXIMUM LACTATE/ VO_2 Max[b] (mmol/l/min)			
4.4±1.2	3.7±0.9	5.1±1.5	4.5±1.3

MEAN ± SD
a (Maximum lactate level) - (Resting lactate level)
b (Maximum lactate level)/(VO_2 Max)

TABLE 15. ANAEROBIC CAPACITY MEASUREMENTS OF EACH GROWTH CATEGORY IN PHASE III

	MALES		FEMALES	
	RUNNERS	CONTROLS	RUNNERS	CONTROLS
		RESTING LACTATE LEVEL (mmol/l)		
PRE	2.4±0.2[#]	---	1.9±0.3	1.9
PEAK	2.4±0.7	2.4±0.5	2.3±0.3	2.0±0.5
POST	3.0	2.0±0.5	1.9±0.5	2.0±0.1
		MAXIMUM LACTATE LEVEL (mmol/l)		
PRE	11.0±2.2	---	10.7±3.1	12.6
PEAK	11.4±3.7	10.8±1.2	13.6±2.6	11.9±3.1
POST	19.1	12.5±1.4	11.9±2.4	8.9±1.7
		LACTATE PRODUCTION LEVEL[a] (mmol/l)		
PRE	8.7±2.2	---	8.8±2.8	10.8
PEAK	9.0±3.8	8.4±1.4	11.3±2.5	10.0±2.9
POST	16.1	10.5±0.9	10.1±2.1	6.9±1.7
		MAXIMUM LACTATE/ VO_2 Max[b] (mmol/l/min)		
PRE	5.4±1.3	---	4.9±2.0	6.2
PEAK	3.9±1.0	3.7±1.0	5.8±1.4	4.6±1.2
POST	4.8	3.6±0.8	4.1±0.9	3.4±0.4

MEAN ± SD

a (Maximum lactate level) - (Resting lactate level)

b (Maximum lactate level)/(VO_2 Max)

TABLE 16. CORRELATIONS OF (1) VO_2 MAX VALUE WITH MAXIMUM LACTATE LEVELS & TOTAL LACTATE PRODUCED; (2) MAXIMUM LACTATE LEVEL WITH HB., HCT., & PLASMA IRON VALUES; & (3) TOTAL LACTATE PRODUCTION WITH HB., HCT., & PLASMA IRON VALUES IN PHASE III

	Correlation Coefficients (r)			
	MR	MC	FR	FC
VO_2 MAX WITH:				
MAX LACTATE	+.46**	+.26	+.08	+.37
LACTATE PRODUCED	+.26	+.14	+.05	+.29
MAXIMUM LACTATE LEVEL WITH:				
HB.	+.04	-.20	+.33	-.46
HCT.	+.17	-.19	+.39	-.66*
PLASMA FE	-.54*	-.50	+.56	-.24
TOTAL LACTATE PRODUCED WITH:				
HB.	+.04	-.31	+.35	-.41
HCT.	+.16	-.33	+.39	-.70*
PLASMA FE	-.51*	-.52	+.57	-.24

* significant correlation ($p<0.05$)
** trend for correlation ($p<0.10$)

TABLE 17. PERCENTAGE OF ENERGY CONSUMED FROM PROTEIN, CARBOHYDRATE, AND FAT FOR SUBJECTS IN PHASES II AND III

	MALES			FEMALES		
	RUNNERS	CONTROLS		RUNNERS	CONTROLS	
			PROTEIN (%)			
PHASE II	13.9±1.9#	15.6±2.5		13.7±2.7	16.4±6.2	
PHASE III	13.9±0.7	14.0±0.7		13.0±0.8	13.3±1.0	
			CARBOHYDRATE (%)			
PHASE II	51.3±5.7	51.5±6.6		53.3±8.5	*** 43.9±11.5	
PHASE III	51.4±2.2	55.2±1.5		52.7±2.4	50.9±2.8	
			FAT (%)			
PHASE II	36.9±4.9	35.1±4.8		35.7±7.3	40.1±7.4	a
PHASE III	35.4±1.6	32.2±1.1	a	36.5±2.3	37.1±2.9	a

MEAN ± SD

*** trend ($p<0.10$) for difference between runners and controls of the same gender.

a trend ($p<0.10$) for a difference between male and female controls.

TABLE 18. PERCENT OF KILOCALORIES CONSUMED FROM PROTEIN, CARBOHYDRATE, AND FAT OF EACH GROWTH CATEGORY IN PHASE III

	MALES		FEMALES	
	RUNNERS	CONTROLS	RUNNERS	CONTROLS
		PROTEIN (%)		
PRE	12.2±3.9#	---	13.7±5.1	12.1
PEAK	14.4±2.7	13.9±2.7	12.7±2.8	14.1±1.9
POST	14.1±2.1	14.4±1.1	13.1±1.8	12.9±4.4
		CARBOHYDRATE (%)		
PRE	52.3±13.3	---	47.4±3.2	57.0
PEAK	51.5±9.5	56.0±5.0	54.2±10.0	48.9±11.0
POST	48.6±7.9	51.8±4.2	53.1±5.3	51.2± 8.7
		FAT (%)		
PRE	32.6±8.1	---	40.8±8.4	31.9
PEAK	35.9±6.9	31.6±3.4	35.2±8.4	38.6± 8.6
POST	39.4±5.3	35.1±5.5	36.2±7.2	37.0±11.0

MEAN ± SD

TABLE 19. IRON INTAKE AND VO_2 MAX VALUES FOR USERS AND NON-USERS OF IRON SUPPLEMENT FOR MR AND MC (PHASE II)

	RUNNERS		CONTROLS	
	+SUPP	-SUPP	+SUPP	-SUPP
SUBJECT (N)	5	16	1	9
SUBJECTS (%)	24	76	10	90
DIETARY FE (% RDA)	93±52#	106±38	73	84±36
IRON INTAKES WITH SUPP. (%RDA)	176±62	--	173	--
SUPP. Fe (mg.)	13±7	--	18	--
HB. (g/dl)	14.6±1.2	14.1±1.3	12.7	14.6±0.9
HCT. (%)	40.3±2.6	38.9±3.7	36.1	41.1±2.9
VO_2 MAX (ml/kg/min)	69.4±6.1	63.2±5.8	50.7	58.2±7.9

MEAN ± SD

TABLE 20. IRON INTAKE AND VO_2 MAX VALUES FOR USERS AND NON-USERS OF IRON SUPPLEMENT FOR FR AND FC (PHASE II)

	RUNNERS		CONTROLS	
	+SUPP	-SUPP	+SUPP	-SUPP
SUBJECT (N)	2	11	3	6
SUBJECTS (%)	15	85	33	67
DIETARY FE (% RDA)	84±10#	81±28	62±37	44±29
IRON INTAKES WITH SUPP. (%RDA)	184±10	--	179±65	--
SUPP. Fe (mg.)	18±0	--	21±5	--
HB. (g/dl)	13.5±0.2	14.1±0.6	14.0±0.2	13.3±0.7
HCT. (%)	37.5±0.0	39.7±2.0	38.0±0.4	38.4±1.9
VO_2 MAX (ml/kg/min)	56.6±9.1	56.7±5.0	46.8±11.8	43.0±4.1

MEAN ± SD

TABLE 21. IRON INTAKE AND VO_2 MAX VALUES FOR USERS AND NON-USERS OF IRON SUPPLEMENT FOR MALES AND FEMALES (PHASE II)

	MALES		FEMALES	
	+SUPP.	-SUPP.	+SUPP.	-SUPP.
SUBJECT (N)	6	25	5	17
SUBJECTS (%)	19	81	23	77
DIETARY FE (% RDA)	90±47#	93±32	71±29	69±33
IRON INTAKES WITH SUPP. (%RDA)	168±59	---	181±47	---
HB. (g/dl)	14.2±1.3	14.3±1.1	13.7±0.3	13.9±0.7
HCT. (%)	39.5±3.0	39.7±3.5	37.8±0.4	39.4±2.0

MEAN ± SD

TABLE 22. IRON INTAKE AND VO_2 MAX VALUES FOR USERS AND NON-USERS OF IRON SUPPLEMENT FOR MR AND MC (PHASE III)

	RUNNERS		CONTROLS	
	+SUPP	-SUPP	+SUPP	-SUPP
SUBJECT (N)	6	14	2	9
SUBJECTS (%)	30	70	18	82
DIETARY FE (% RDA)	86±33#	97±37	112±80	81±22
IRON INTAKES WITH SUPP. (%RDA)	199±48	--	225±60	--
SUPPL. Fe (mg.)	19±5	--	7±4	--
HB. (g/dl)	14.5±0.5	13.9±1.1	14.2±1.4	14.6±1.0
HCT. (%)	43.4±1.7	41.4±3.5	44.0±2.5	43.3±2.4
PLASMA FE (mcg/ml)	1.15±.40	1.13±.40	0.86±.01	1.00±.21
VO_2 MAX (ml/kg/min)	66.7±5.2	62.3±6.3	57.5±0.9	53.2±8.3

MEAN ± SD

TABLE 23. IRON INTAKE AND VO_2 MAX VALUES FOR USERS AND NON-USERS OF IRON SUPPLEMENT FOR FR AND FC (PHASE III)

	RUNNERS		CONTROLS	
	+SUPP	-SUPP	+SUPP	-SUPP
SUBJECT (N)	4	7	3	7
SUBJECTS (%)	36	64	30	70
DIETARY FE (% RDA)	73±24#	79±36	70±24	57±17
IRON INTAKES WITH SUPP. (%RDA)	191±44	--	134±35	--
SUPPL. Fe (mg.)	19±6	--	12±5	--
HB. (g/dl)	13.5±0.8	13.2±0.4	13.2±1.6	12.9±0.7
HCT. (%)	40.6±2.0	39.5±1.2	39.8±2.4	39.1±1.8
PLASMA FE (mcg/ml)	1.01±.23	1.11±.38	1.06±.11	1.11±.39
VO_2 MAX (ml/kg/min)	61.7±8.0	59.8±3.1	54.3±2.1 *	46.4±4.3

MEAN ± SD

* significant difference ($p<0.05$) between female runners and controls.

TABLE 24. IRON INTAKE AND VO_2 MAX VALUES FOR USERS AND NON-USERS OF IRON SUPPLEMENT FOR MALES AND FEMALES (PHASE III)

	MALES		FEMALES	
	+SUPP.	-SUPP.	+SUPP.	-SUPP.
SUBJECT (N)	8	23	7	14
SUBJECTS (%)	26	74	33	67
DIETARY FE (% RDA)	100±41[#]	88±33	72±22	68±30
IRON INTAKES WITH SUPP. (%RDA)	187±42	---	161±42	---
HB. (g/dl)	14.4±0.7	14.1±1.1	13.3±1.1	13.1±0.5
HCT. (%)	43.3±2.1	42.1±3.1	40.4±1.9	39.3±1.5
PLASMA FE (mcg/ml)	1.05±.34	1.08±.34	1.03±.16	1.11±.37

MEAN ± SD

TABLE 25. CHANGES IN HEMATOLOGICAL MEASUREMENTS AND VO_2 MAX IN SUBJECTS WHILE TAKING SUPPLEMENTAL IRON AS COMPARED TO WHEN THEY DID NOT.

PHASE WHEN Fe SUPP WAS USED +SUPP/-SUPP	AGE* (yrs)	HT* (cm)	IRON SUPPL (mg)	DIETARY* IRON (%RDA)	HB* (g/dl)	HCT* (%)	VO_2 MAX* (ml/kg/min)
			MALE RUNNERS, N = 4				
II / III	-1.1#	-6.6	18	-62	-0.2	-3.0	+1.1
III / II	+1.2	+6.5	27	+26	-0.2	+2.4	-1.8
II / III	-1.1	-4.8	18	-25	+0.7	+0.4	--
III / II	+1.1	-12.8	18	+ 6	+0.7	+5.8	+1.8
			MALE CONTROLS, N = 1				
III / II	+1.1	+6.5	4	+10	+1.0	+5.5	-2.2
			FEMALE RUNNERS, N = 2				
III / II	+1.1	+3.7	18	+62	+1.0	+3.6	+4.6
III / II	+0.8	+2.4	18	+ 7	+1.0	+3.8	+3.9
			FEMALE CONTROLS, N = 1				
II /III	-0.9	-0.1	18	+44	+1.0	-0.8	--
			SIGNIFICANCE				
	[a]NS	NS	--	NS	[b]0.05	0.05	NS

* Change in measurement; use of supp. - not using supp.
\# (Value when using supp.) - (Value when not taking supp.)
a No significance between difference in values before and after using the iron supp.
b Significant difference ($p<0.05$) between values when subjects took iron compared to when the same subjects did not take iron.

TABLE 26. CASE STUDY REPORT ON SUBJECT WITH LOW HEMATOLOGICAL MEASUREMENTS IN PHASE II

MALE RUNNER - PHASE II/ CONTROL - PHASE III

	PHASE II	PHASE III	CHANGE IN VALUE
AGE (yrs)	15.0	16.2	
Wt (kg)	56.1	65.0	+8.9
Relative Wt (%)	98	105	
Ht (cm)	166.0	168.4	+2.4
Relative Ht (%)	98	97	
Growth CATEGORY	PEAK	POST	
HB (g/dl)	10.6	16.0	+5.4
HCT (%)	28.5	45.7	+17.2
RBC ($10^6/mm^3$)	3.42	5.22	
MCV (u^3)	84.0	87.5	
MCH (ug)	31.0	30.7	
MCHC (%)	37.0	35.1	
Plasma Fe (ug/ml)	--	1.11	
Dietary Fe Intake (% RDA)	87.4	117.6	
Kcal/Kg Body Wt	54.2	60.7	
Kcal (%RDA)	129.1	144.5	
Supplement (mg)	None	None	
Activity Level	6-8 miles/day	Not Running	
VO_2 MAX (ml/kg/min)	60.5	59.0	

TABLE 27. CASE STUDY REPORT ON SUBJECT WITH LOW HEMATOLOGICAL MEASUREMENTS IN PHASE III

MALE RUNNER

	PHASE II	PHASE III	CHANGE IN VALUE
AGE (yrs)	9.8	11.0	
Wt (kg)	30.3	33.2	+2.9
Relative Wt (%)	95	95	
Ht (cm)	145.3	151.4	+6.1
Relative Ht (%)	105	106	
Growth Category	PRE	PRE	
HB (g/dl)	13.9	11.4	-2.5
HCT (%)	39.2	34.3	-4.9
RBC ($10^6/mm^3$)	4.85	4.05	
MCV (u^3)	82.0	84.0	
MCH (ug)	28.8	28.0	
MCHC (%)	35.3	33.2	
Plasma Fe (ug/ml)	--	0.90	
Dietary Fe Intake (% RDA)	129.3	151.8	
Kcal/Kg Body Wt	66.6	97.6	
Kcal (%RDA)	78.4	114.8	
Supplement (mg)	None	None	
Activity Level	Runner	Runner	
VO_2 MAX (ml^1/kg/min)	64.9	64.2	

TABLE 28. CASE STUDY REPORT ON SUBJECT WITH LOW HEMATOLOGICAL MEASUREMENTS IN PHASE III

FEMALE RUNNER - PHASE II/ CONTROL - PHASE III

	PHASE II	PHASE III	CHANGE IN VALUE
AGE (yrs)	14.3	15.5	
Wt (kg)	52.2	61.0	+8.8
Relative Wt (%)	104	111	
Ht (cm)	175.1	179.1	+4.0
Relative Ht (%)	109	111	
Growth Category	PEAK	PEAK	
HB (g/dl)	13.3	11.7	-1.6
HCT (%)	37.2	37.2	0
RBC ($10^6/mm^3$)	4.65	4.01	
MCV (u^3)	82.0	88.1	
MCH (ug)	30.5	29.1	
MCHC (%)	37.1	33.0	
Plasma Fe (ug/ml)	--	0.94	
Dietary Fe Intake (% RDA)	79.5	96.7	
Kcal/Kg Body Wt	33.6	47.6	
Kcal (%RDA)	87.9	125.3	
Supplement (mg)	None	Multivitamin, 1-2/wk	
Activity Level	Runner	Playing Other Sports	
VO_2 MAX (ml/kg/min)	49.7	55.8	

TABLE 29. AMOUNT OF DIETARY IRON ABSORBED AS CALCULATED BY MONSEN'S MODEL FOR DIFFERENT IRON STORAGE LEVELS, 0 MG., 250 MG., AND 500 MG.

MALES		FEMALES	
RUNNERS	CONTROLS	RUNNERS	CONTROLS
	0 MG. OF IRON		
2.95±1.09#a	2.68±1.12	1.93±0.85[a]	1.76±0.94
	250 MG. OF IRON		
1.92±0.68[b]	1.76±0.75	1.32±0.60[b]	1.20±0.69
	500 MG. OF IRON		
1.34±0.46	1.25±0.54	0.95±0.44	0.82±0.51

MEAN AMOUNT OF IRON ABSORBED (MG.)± SD
a significant difference ($p<0.05$) between male and female runners.
b trend ($p<0.10$) for a difference between male and female runners.

TABLE 30. CALCULATED ABSORPTION OF IRON USING MONSEN'S MODEL AT 3 LEVELS OF IRON STORAGE AND 10% OF THE ACTUAL INTAKE (RDA ASSUMPTION)

MALES		FEMALES	
RUNNERS	CONTROLS	RUNNERS	CONTROLS
IRON STORAGE LEVEL:			
	0 MG. OF IRON		
164±62#a	149±62	107±50[a]	98±52
	250 MG. OF IRON		
106±39	98±42	73±35	67±38
	500 MG. OF IRON		
74±26	70±30	53±26	46±28
	% RDA FOR IRON[b]		
94±35	87±32	77±29	61±18

MEAN Percent ± SD of calculated iron absorption in mg. ÷ 1.8 mg. iron (10% of the RDA) x 100.
a significance difference ($p<0.05$) between male and female runners.
b The RDA assumes 10% of total dietary iron intake is absorbed.

TABLE 31. PERCENT OF SUBJECTS ABSORBING LESS THAN 1.2 MG. IRON PER DAY

MALES		FEMALES	
RUNNERS	CONTROLS	RUNNERS	CONTROLS
MONSEN'S MODEL:			
	0 MG. OF IRON		
5#	9	27	20
	250 MG. OF IRON		
10	36	46	80
	500 MG. OF IRON		
35	46	73	90
	RDA FOR IRON[a]		
15	36	36	70

Percent of subjects
a The RDA assumes 10% of dietary iron intake is absorbed.

TABLE 32. CORRELATION OF HEMOGLOBIN VALUES WITH %RDA FOR DIETARY IRON AND % OF 1.8 MG. IRON ABSORBED, CALCULATED BY MONSEN'S MODEL (FOR THE 250 MG. IRON STORAGE LEVEL)

	Correlation Coefficients (r)			
	MR	MC	FR	FC
SUBJECTS (N):	19	11	11	10
HEMOGLOBIN (g/dl) WITH:				
% RDA	+.11	+.09	+.20	-.57
% ABSORBED[a]	-.06	+.32	+.05	-.56

a (Calculated iron absorption in mg./1.8 mg. iron) x 100.

TABLE 33. DIETARY INTAKE OF NUTRIENTS INHIBITING IRON ABSORPTION

MALES		FEMALES	
RUNNERS	CONTROLS	RUNNERS	CONTROLS
	DIETARY FIBER (g)		
14± 6[#]	14± 6[a]	12± 6	8± 5[a]
	CALCIUM (% RDA)		
125±62	113±77	101±43	74±28
	PHOSPHORUS (% RDA)		
168±58[b]	142±39	124±48[b]	99±35

MEAN ± SD
a trend ($p<0.10$) for a difference between male and female controls
b trend ($p<0.10$) for a difference between male and female runners.

Figure 2. Hemoglobin concentrations for individual subjects in each group of MR, MC, FR, and FC, for Phases II and III. Median values of each group compared to the NHANES II (25) median values; 14 g/dl for males and 13.4 g/dl for females, 12 - 14 years old.

Phase II:	MR, N=17	MC, N=9	FR, N=11	FC, N=5
Phase III:	MR, N=20	MC, N=11	FR, N=11	FC, N=10

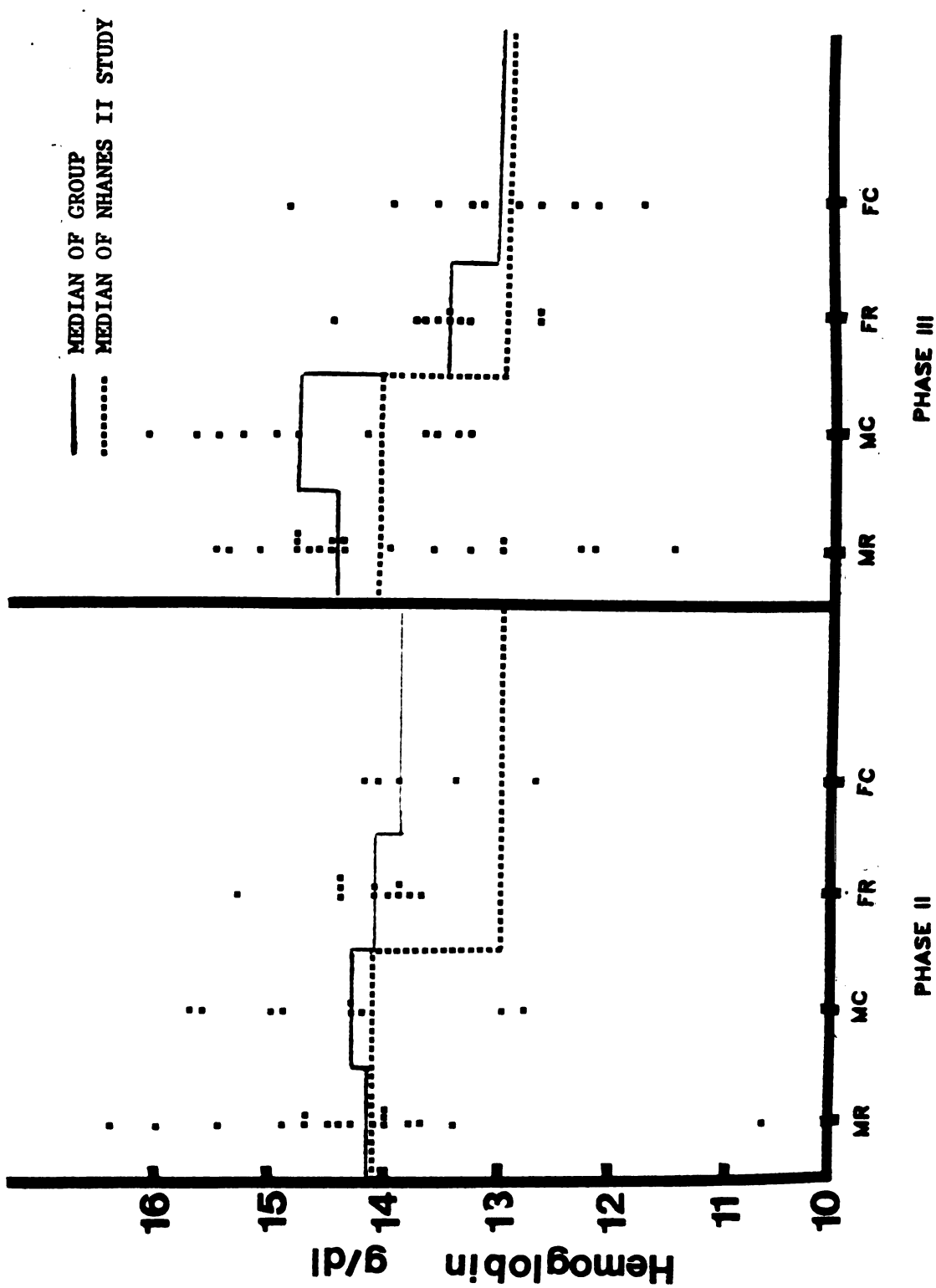

MEDIAN OF GROUP
MEDIAN OF NHANES II STUDY
Hemoglobin g/dl
16
15
14
13
12
11
10
MR
MC
FR
FC
PHASE II
PHASE III

Figure 2.

Figure 3. Hematocrit concentrations for individual subjects in each group of MR, MC, FR, and FC, for Phase II and III. Median values of each group compared to the NHANES II (25) median values; 40.5% for males and 39% for females, 12 - 14 years old.

Phase II:	MR, N=17	MC, N=9	FR, N=11	FC, N=5
Phase III:	MR, N=20	MC, N=11	FR, N=11	FC, N=10

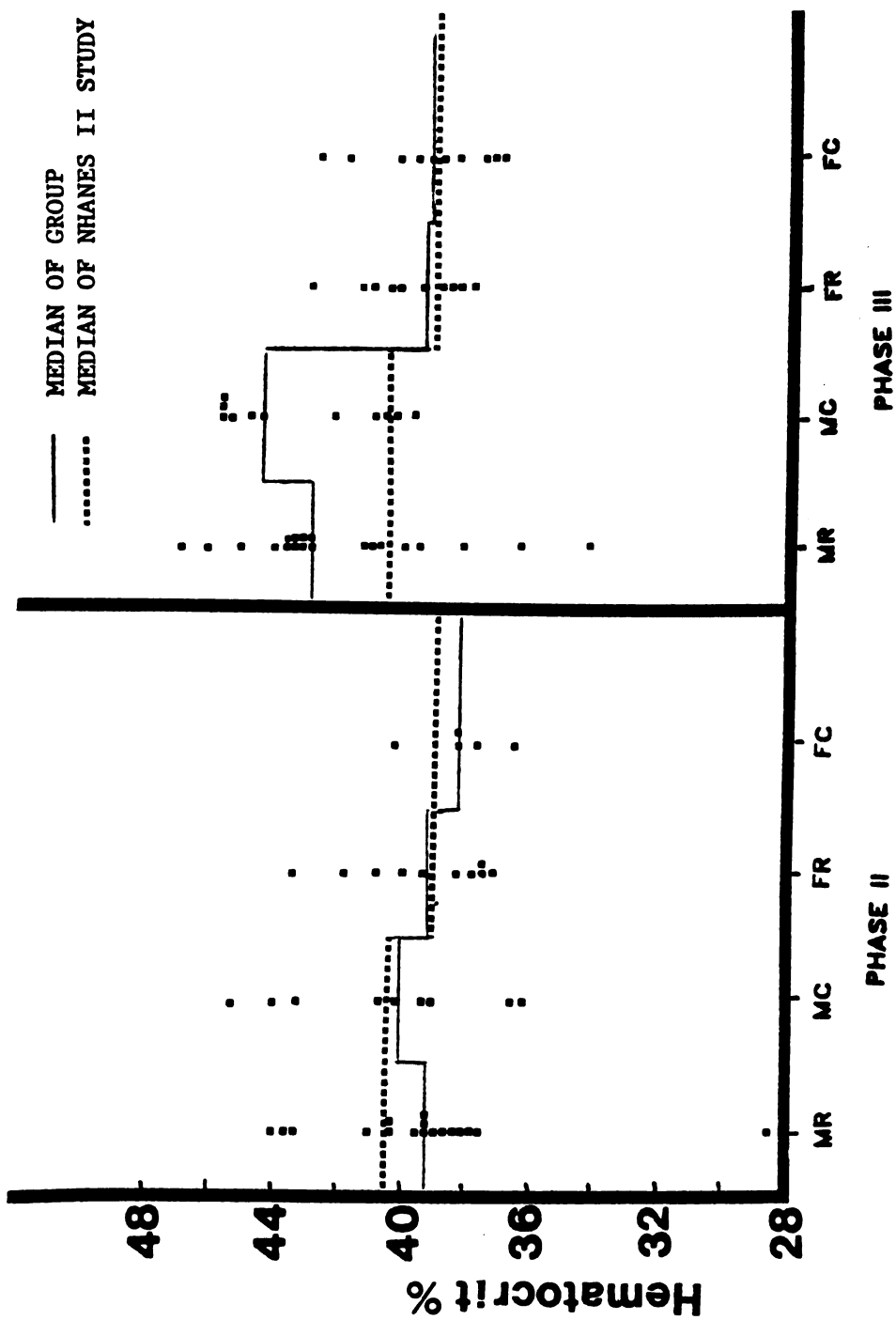

MEDIAN OF GROUP
MEDIAN OF NHANES II STUDY
Hematocrit %
48
44
40
36
32
28
MR
MC
FR
FC
PHASE II
PHASE III

Figure 3.

Figure 4. Plasma iron concentrations for individual subjects in each group of MR, MC, FR, and FC, for Phase III. Median values of each group compared to the NHANES II (25) median values; 95 mcg/dl for males and 96 mcg/dl for females, 12 - 14 years old.

PHASE III: MR, N=18 MC, N=11 FR, N=10 FC, N=10

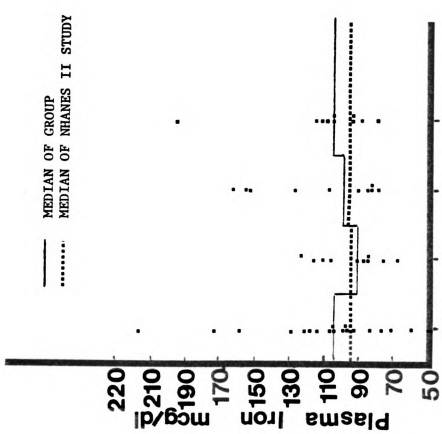

MEDIAN OF GROUP
MEDIAN OF NHANES II STUDY
Plasma Iron mcg/dl
220
210
190
170
150
130
110
90
70
50
MR
MC
FR
FC
PHASE III

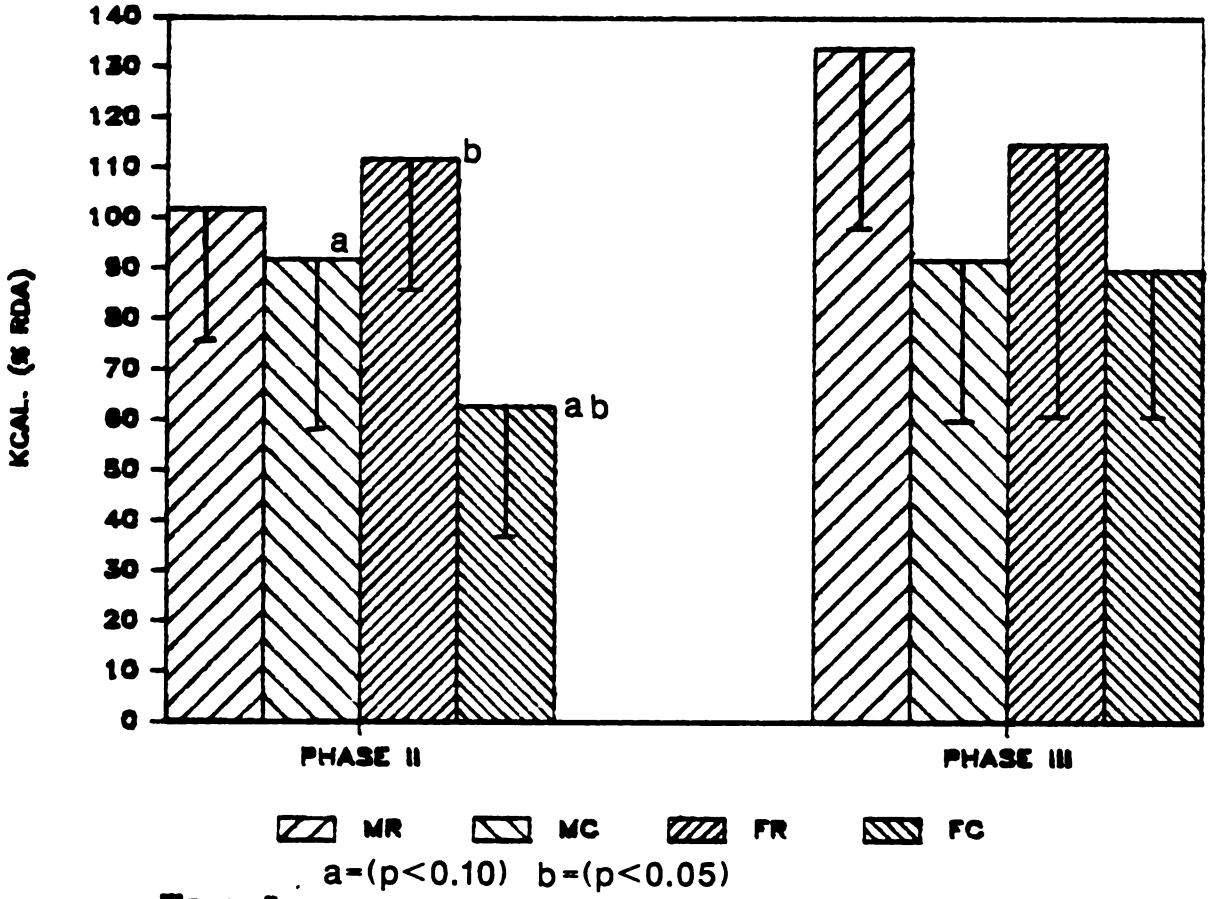
Figure 4.

Figure 5. Mean daily energy consumption, for MR, MC, FR, and FC for Phases II and III.

Phase II:	MR, N=21	MC, N=10	FR, N=13	FC, N=9
Phase III:	MR, N=20	MC, N=11	FR, N=11	FC, N=10

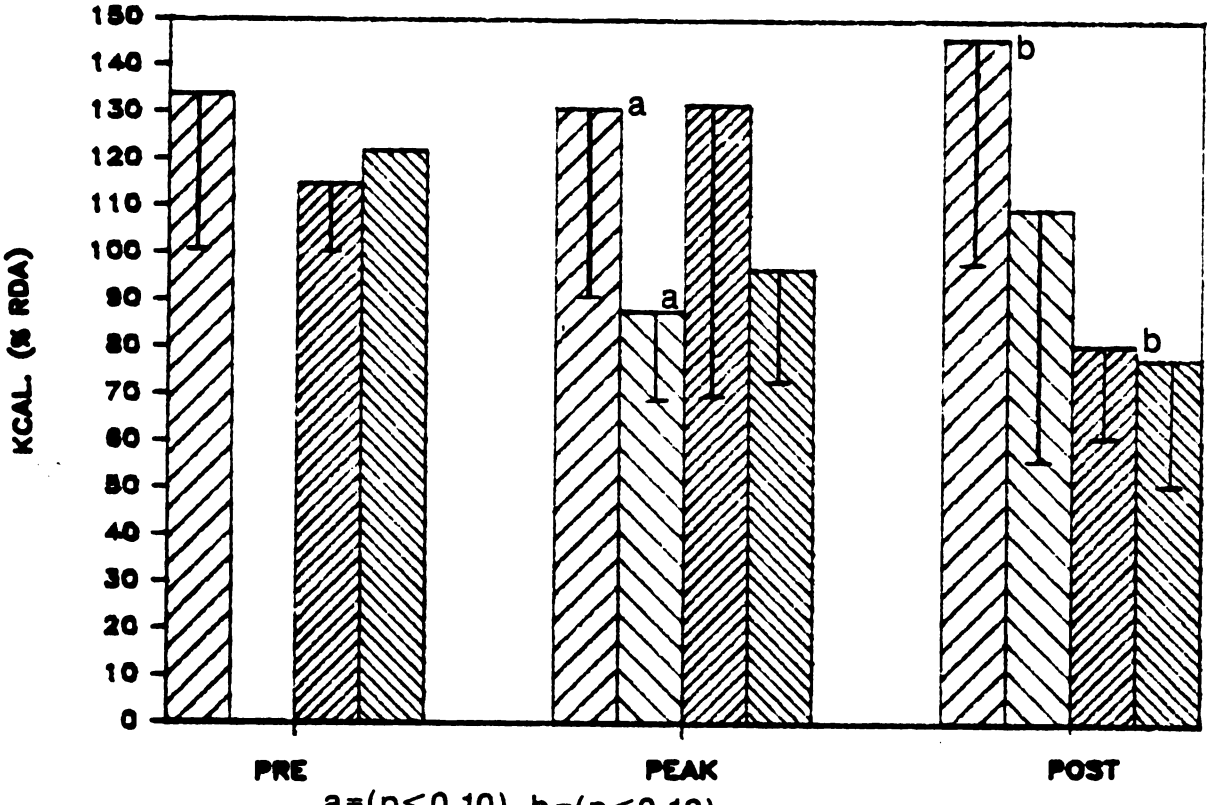
Figure 6. Mean daily energy consumption, for subjects categorized into pre- peak- and post growth spurt for MR, MC, FR, and FC for Phase III.

MR	Pre, N=5	MC	Pre, N=0	FR	Pre, N=2	FC	Pre, N=1
	Peak, N=13		Peak, N=9		Peak, N=6		Peak, N=4
	Post, N=2		Post, N=2		Post, N=3		Post, N=5



a=(p<0.10) b=(p<0.05)

Figure 5.



a=(p<0.10) b=(p<0.10)

Figure 6.

Figure 7. Mean kcal. intake /kg. body weight for MR, MC, FR, and FC for Phases II and III.

Phase II:	MR, N=21	MC, N=10	FR, N=13	FC, N=9
Phase III:	MR, N=20	MC, N=11	FR, N=11	FC, N=10

Figure 8. Mean kcal. intake/kg. body weight for subjects categorized into pre-, peak-, and post- growth spurts for MR, MC, FR, and FC for Phase III.

MR	Pre, N=5	MC	Pre, N=0	FR	Pre, N=2	FC	Pre, N=1
	Peak, N=13		Peak, N=9		Peak, N=6		Peak, N=4
	Post, N=2		Post, N=2		Post, N=3		Post, N=5

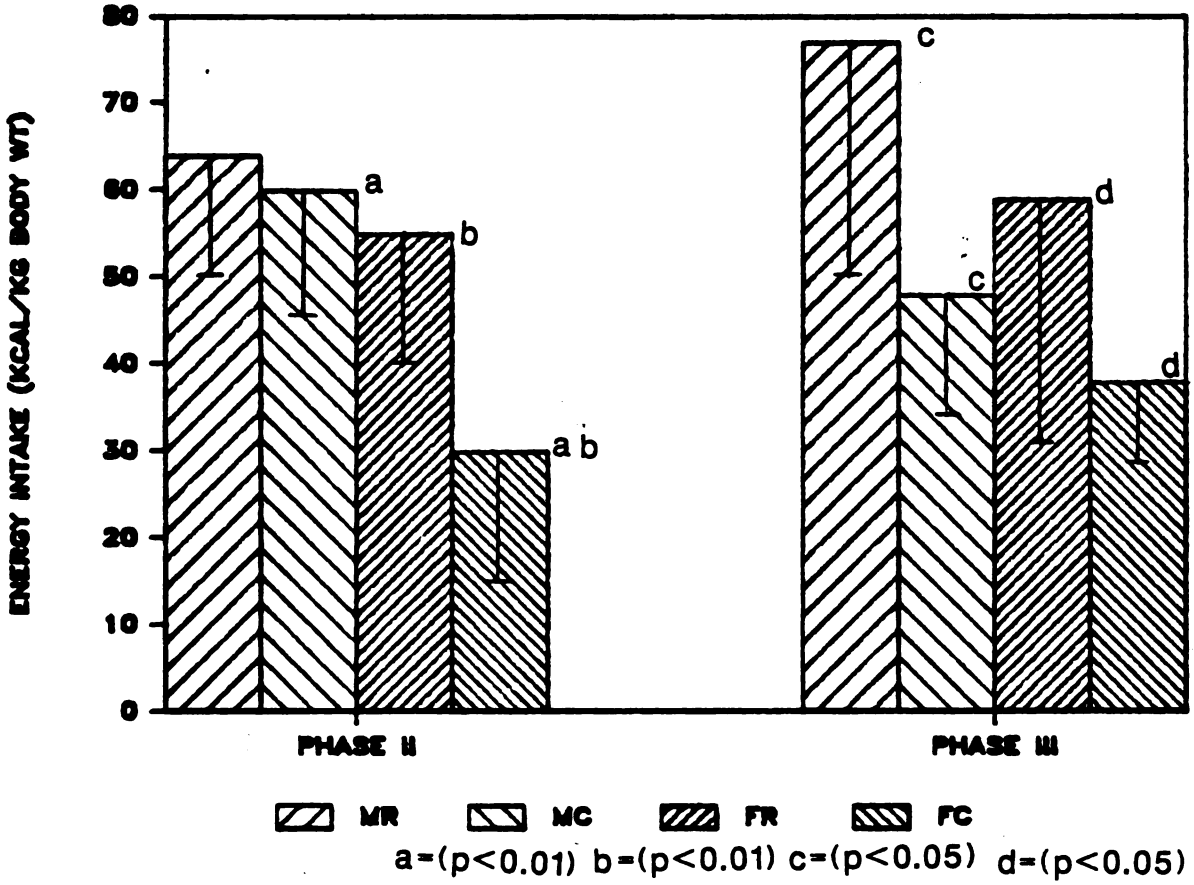


Figure 7.

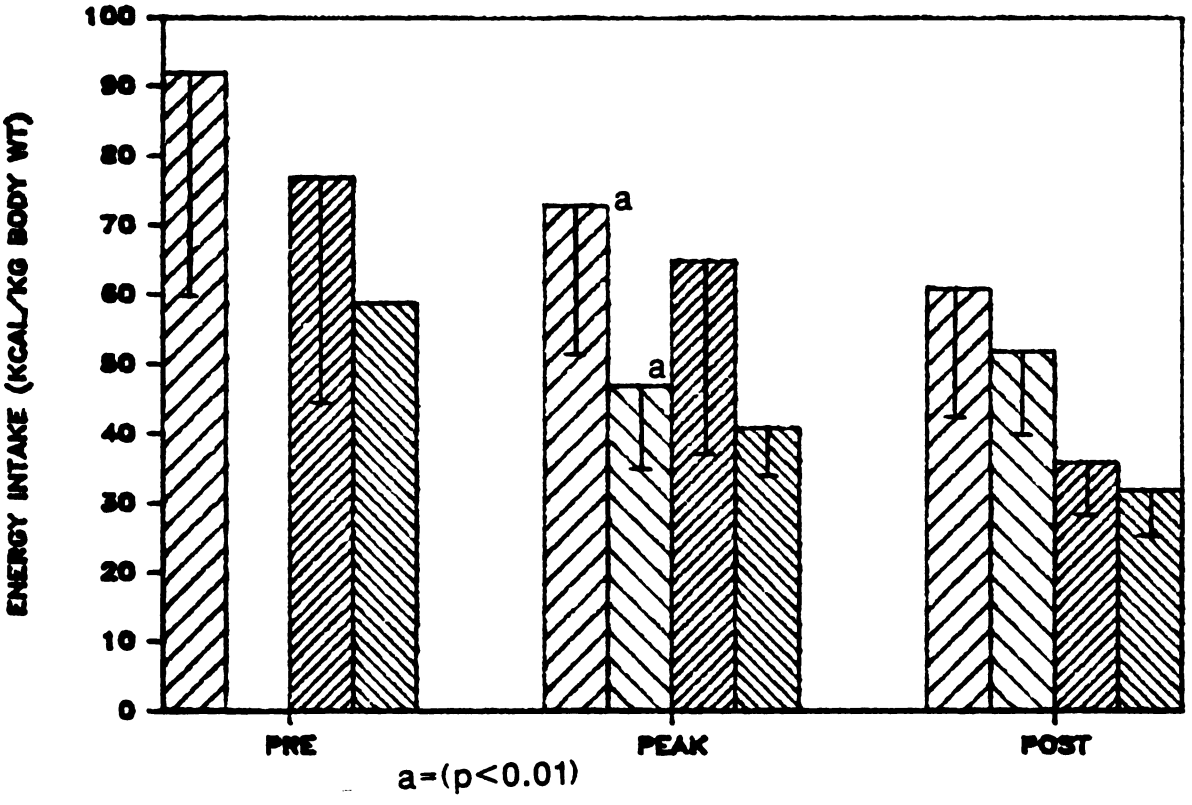


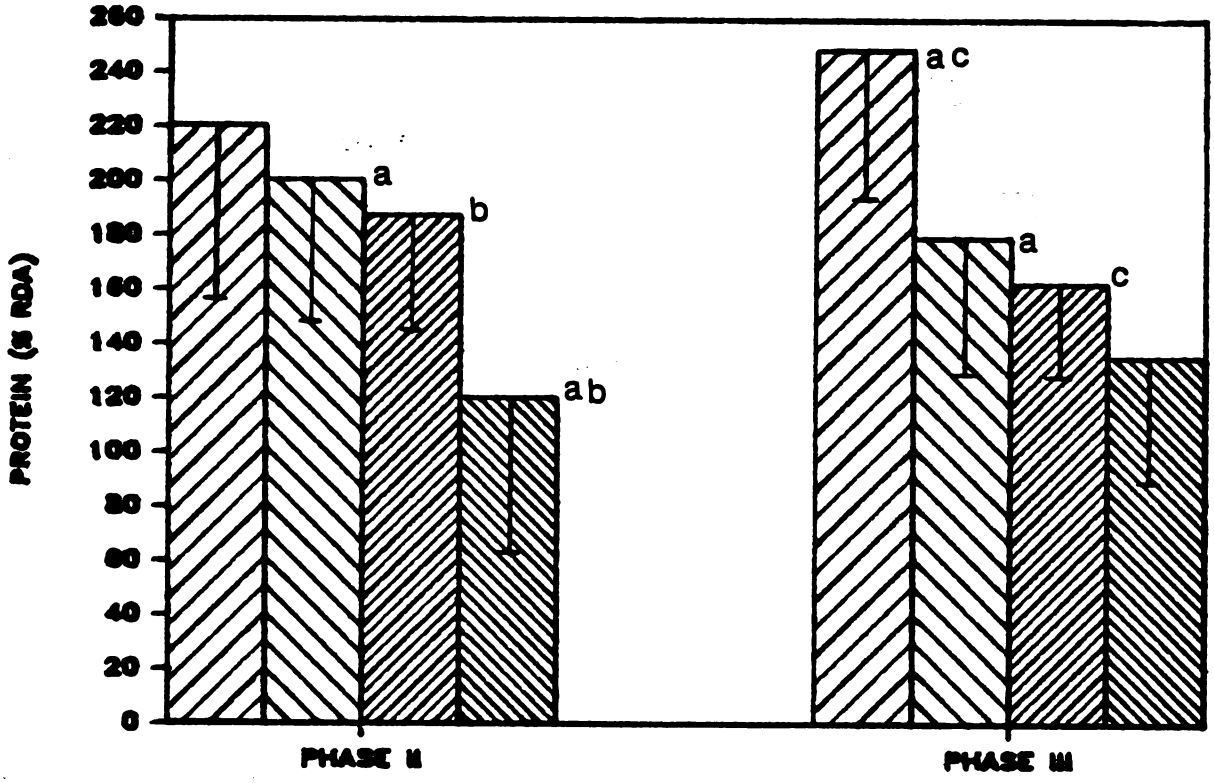
Figure 8.

Figure 9. Mean daily protein intake, for MR, MC, FR, and FC for Phases II and III.

Phase II:	MR, N=21	MC, N=10	FR, N=13	FC, N=9
Phase III:	MR, N=20	MC, N=11	FR, N=11	FC, N=10

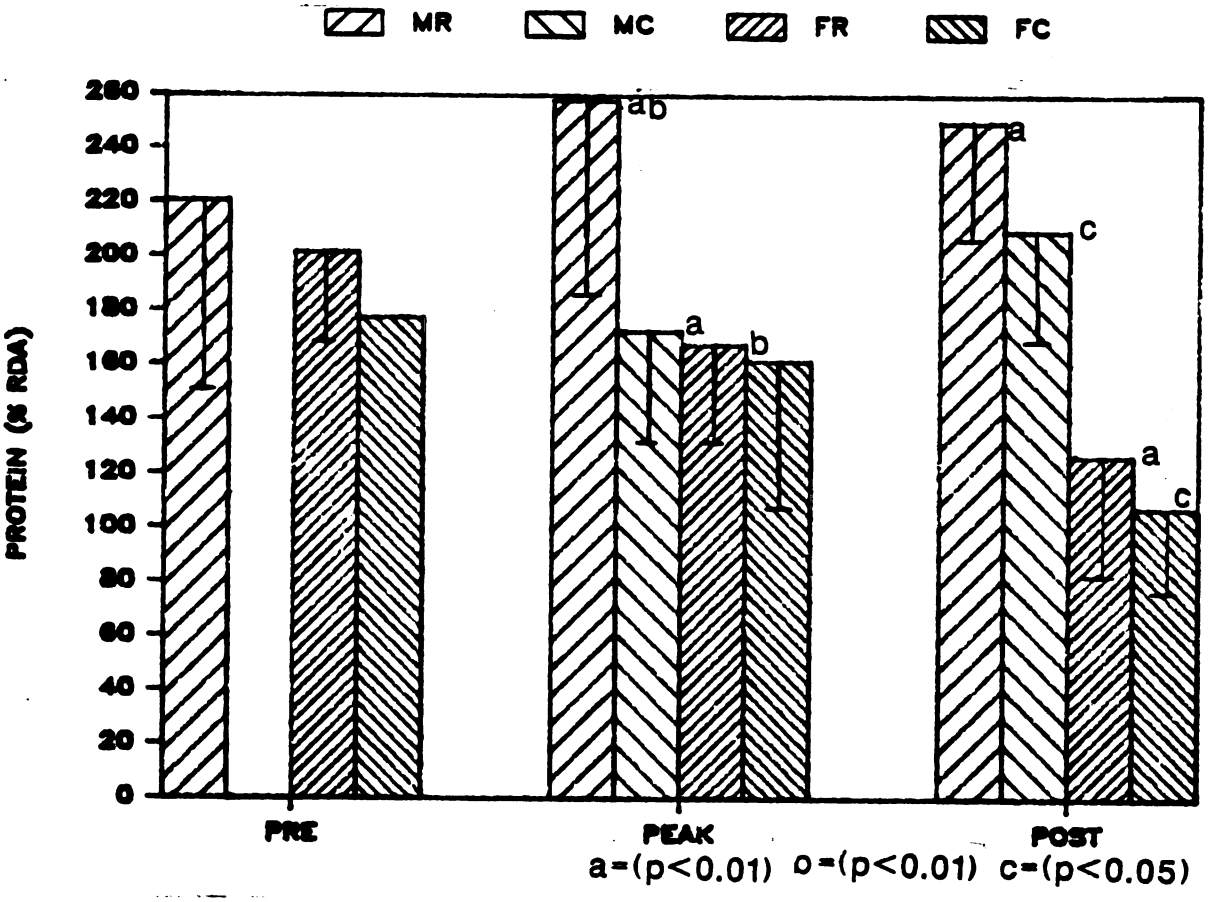
Figure 10. Mean daily protein intake, for subjects categorized into pre-, peak-, and post- growth spurts for MR, MC, FR, and FC for Phase III.

MR	Pre, N=5	MC	Pre, N=0	FR	Pre, N=2	FC	Pre, N=1
	Peak, N=13		Peak, N=9		Peak, N=6		Peak, N=4
	Post, N=2		Post, N=2		Post, N=3		Post, N=5



a=(p<0.01) b=(p<0.10) c=(p<0.01)

Figure 9.



a=(p<0.01) o=(p<0.01) c=(p<0.05)

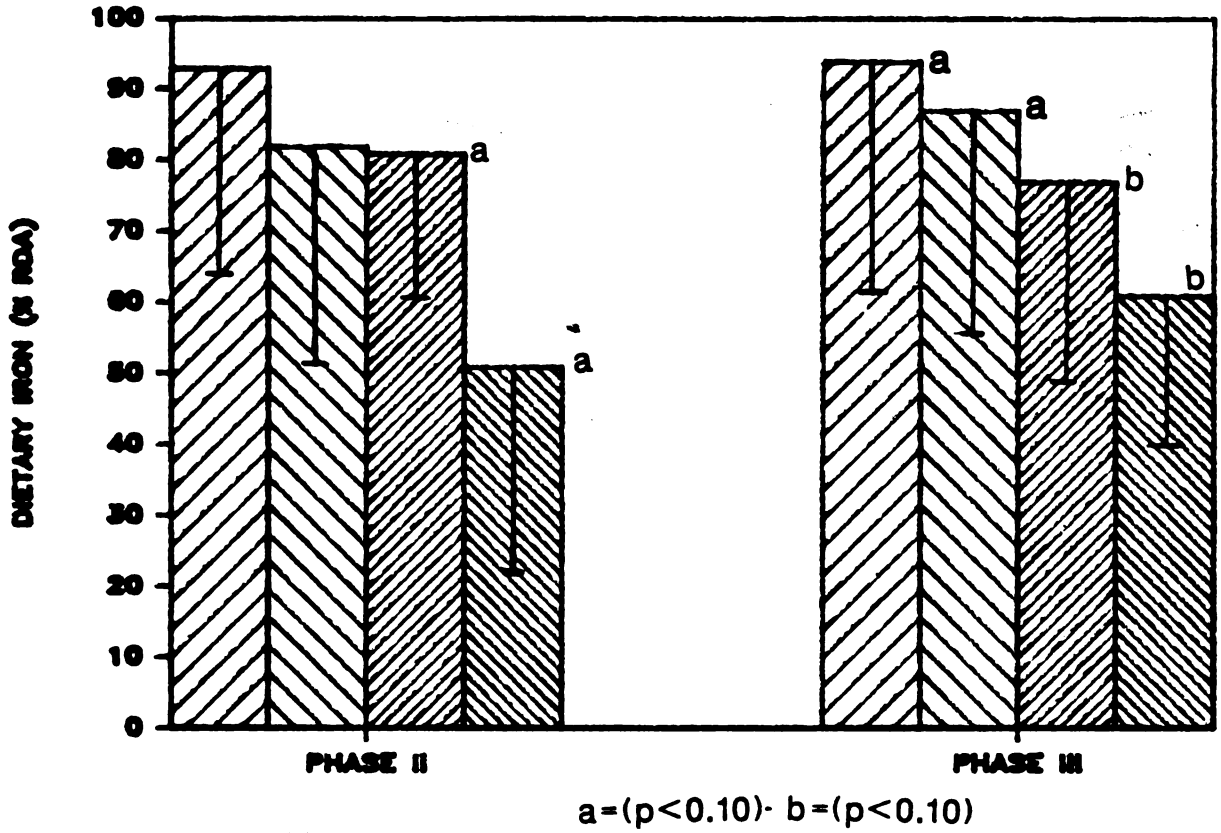
Figure 10.

Figure 11. Mean daily dietary iron intake, for MR, MC, FR, and FC for Phases II and III.

Phase II:	MR, N=21	MC, N=10	FR, N=13	FC, N=9
Phase III:	MR, N=20	MC, N=11	FR, N=11	FC, N=10

Figure 12. Mean daily dietary iron intake, for subjects categorized into pre-, peak-, and post- growth spurt for MR, MC, FR, and FC for Phase III.

MR	Pre, N=5	MC	Pre, N=0	FR	Pre, N=2	FC	Pre, N=1
	Peak, N=13		Peak, N=9		Peak, N=6		Peak, N=4
	Post, N=2		Post, N=2		Post, N=3		Post, N=5



a=(p<0.10)· b=(p<0.10)

Figure 11.

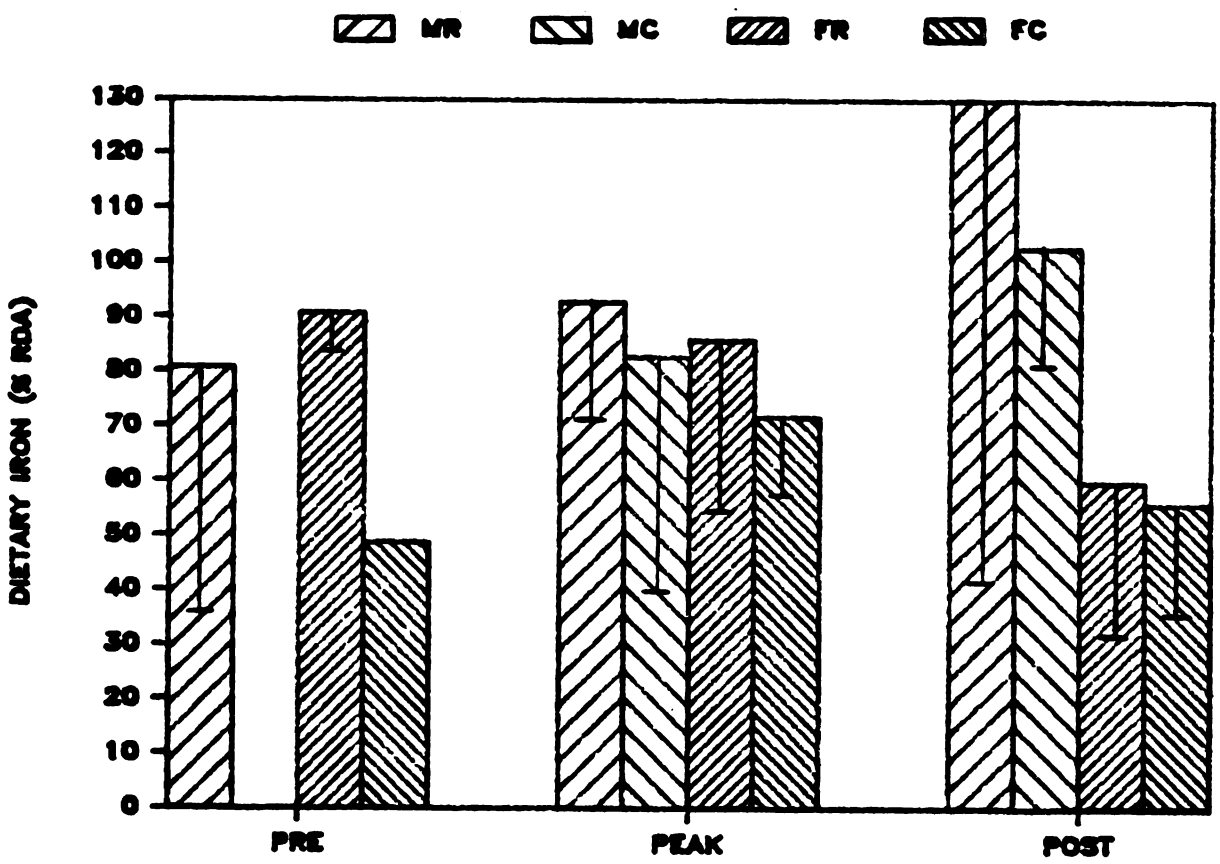


Figure 12.

Figure 13. Subjects were divided into 8 categories based upon their mean daily iron intakes in mg. This figure illustrates the percentage of subjects who fell within each category in Phase II.

Phase II: MR, N=21 MC, N=10 FR, N=13 FC, N=9

Figure 14. Subjects were divided into 8 categories based upon their mean daily iron intakes in mg. This figure illustrates the the percentage of subjects who fell within each category in Phase III.

Phase III: MR, N=20 MC, N=11 FR, N=11 FC, N=10

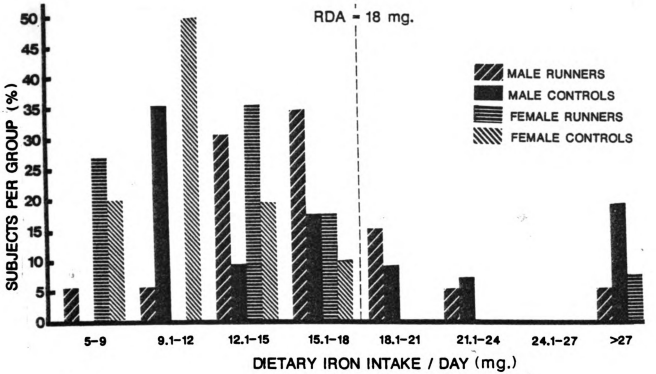


Figure 13.

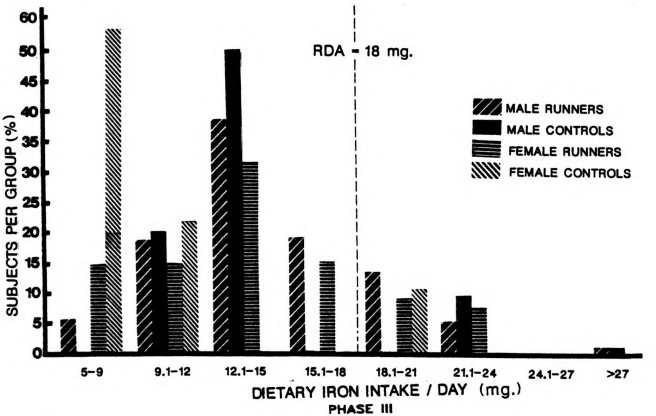


Figure 14.

Figure 15. Mean daily total protein, animal protein, and plant protein intakes for MR, MC, FR, and FC, for Phase III.

Phase III: MR, N=20 MC, N=11 FR, N=11 FC, N=10

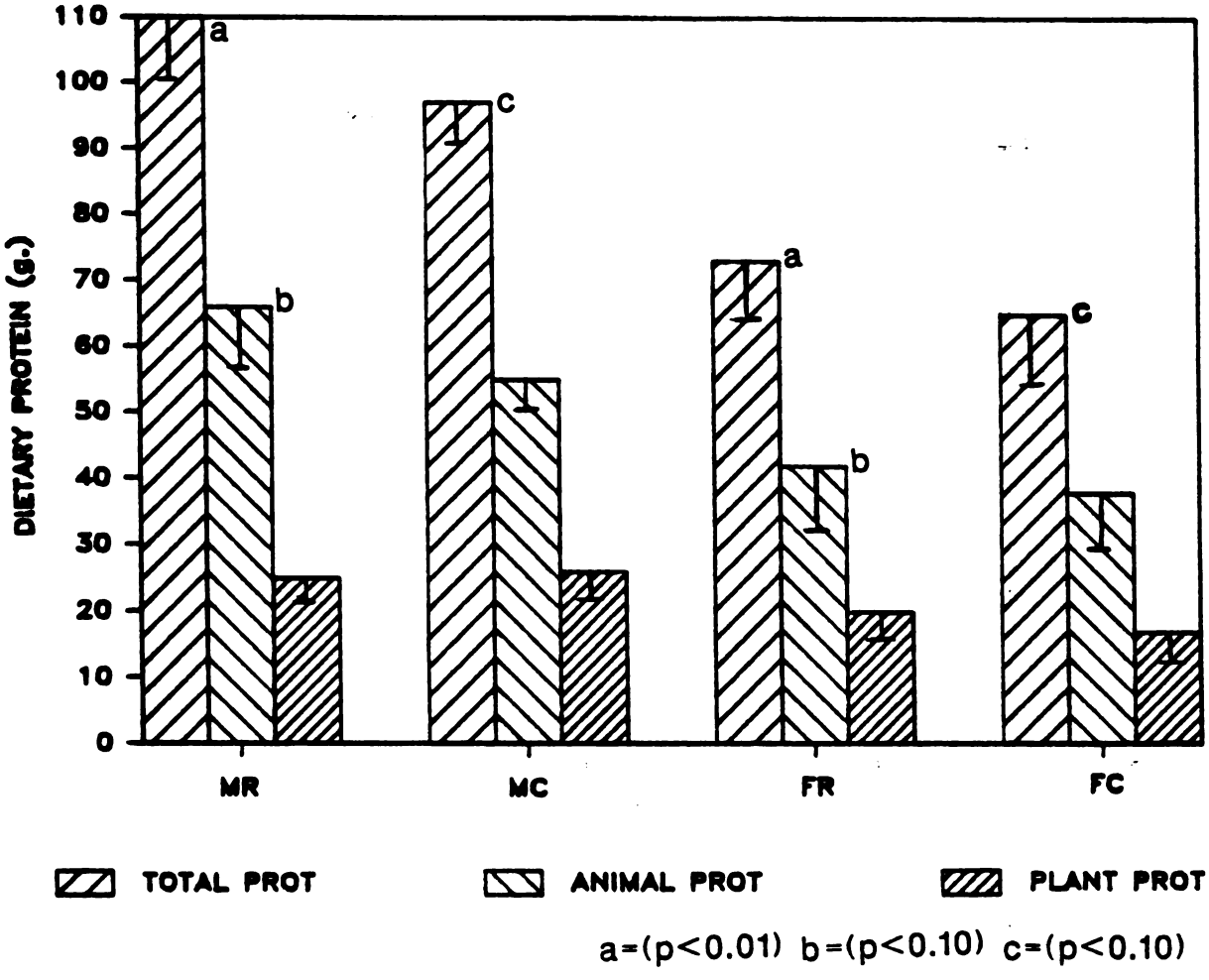

DIETARY PROTEIN (g.)
110
100
90
80
70
60
50
40
30
20
10
0
a
b
c
a
b
c
MR
MC
FR
FC
TOTAL PROT
ANIMAL PROT
PLANT PROT
a=(p<0.01) b=(p<0.10) c=(p<0.10)

Figure 15.

Figure 16. Percent of the mean daily dietary iron intake associated with dietary heme, for MR, MC, FR, and FC, for Phase III.

Phase III: MR, N=20 MC, N=11 FR, N=11 FC, N=10

Figure 17. Mean dietary Vitamin C intake, for MR, MC, FR, and FC, for Phase III.

Phase III: MR, N=20 MC, N=11 FR, N=11 FC, N=10

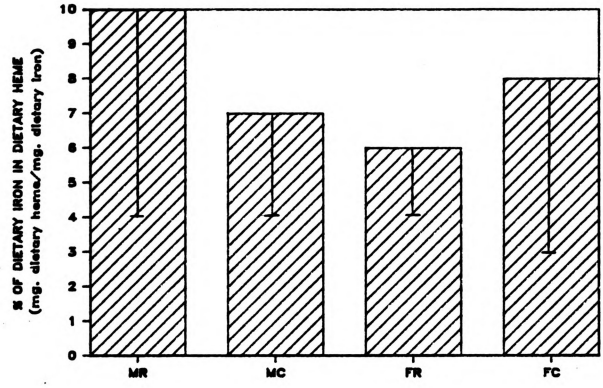


Figure 16.

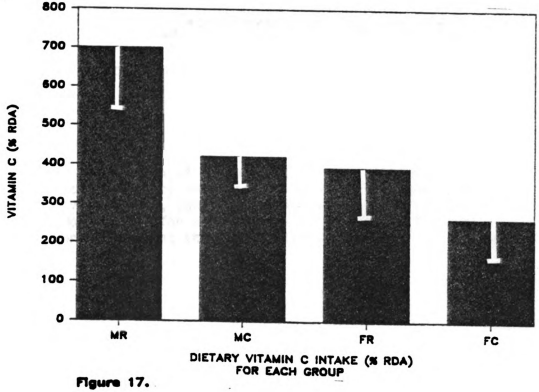
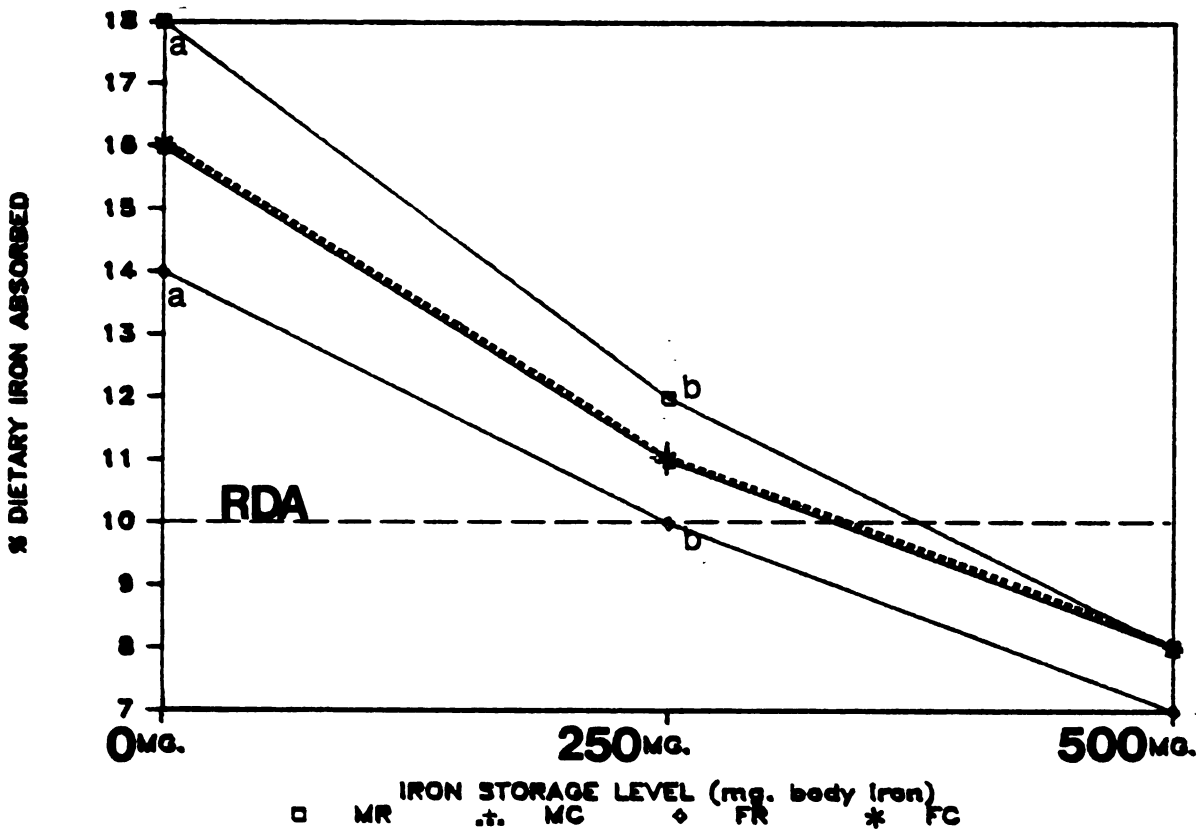


Figure 17.



a=($p<0.01$) b=($p<0.05$)

Figure 18. Comparison of the percent of total dietary iron intake calculated to be absorbed at the specified body iron storage levels (0 mg., 250 mg., and 500 mg.), according to Monsen's Model (18), for each group with the 10% absorption assumed for determining the RDA for iron.

Phase III: MR, N=20 MC, N=11 FR, N=11 FC, N=10

DISCUSSION

CLASSIFICATION OF SUBJECTS

Iron deficiency anemia, as indicated in several studies, is the most common nutritional problem among adolescents (1,2,26,121,123). Rapid growth is characteristic of adolescents and is associated with an increased need for iron (2). There is difficulty in evaluating this age group because of the large variation in stages of physical development, growth rate, body size, and physical activity. Because the time of onset of adolescence, the velocity of change, and the age at which somatic maturity occurs varies with each individual, chronological age provides little information about an individual's stage of biological growth and development. Therefore, the subjects in this report were grouped according to the stage of growth (as measured by increase in height over time) they were in during Phase III of the study.

A majority of the subjects were in the peak of their growth spurt. This is the stage when the body undergoes rapid changes in height and weight, as well as skeletal and somatic maturation. The average increases in height during the peak of the growth spurt, according to the 50th percentile values reported by the NCHS (2), were 9.5 and 6.7 cm./yrs. for boys and girls, respectively. These values were slightly greater than the mean measurements for peak growth spurt categories of

male and female runners and controls in this study. The average ages for the peak height velocity, according to the ages for the 50th percentile reported by the NCHS (2), were 13.5 years for boys and 11.5 years for girls. In this study, male subjects did not appear to be delayed in biological growth and development because the males in their growth spurt were similar in chronoloical age to the 50th percentile age reported by the NCHS. Female subjects appeared to have a slightly delayed growth spurt, since the females in their growth spurt were chronologically older than the 50th percentile age reported by the NCHS. The onset of puberty and age at menarche have been reported to be delayed in female athletes (120). Female controls were not competitively running, although they were fairly active in other sports, which could explain why the peak category of FC also appeared to have a delayed growth spurt.

Subjects were of average height, as indicated by the relative height values for each group, which were within 0-6% of the 50th percentile height for age, reported by the NCHS (111). Male and female runners were slightly below the average weight while controls were usually above the 50th percentile weight for age reported by the NCHS (111). In both phases, the runners were lighter than the controls. Because the runners had greater energy intakes than controls, the reason they were lighter was, presumably due to the greater energy expenditure associated with training and competing for their sport. The heights and ages of runners and controls were more similar when grouped according to growth category rather than on chronological age.

HEMATOLOGICAL AND PLASMA IRON PROFILE

The major laboratory tests used in the diagnosis of iron deficiency anemia include the analyses for hemoglobin, hematocrit, red blood cell count (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), serum iron, total iron binding capacity, transferrin saturation, erythrocyte protoporphyrin, and serum ferritin (25). Normal values for these laboratory tests are known to vary to some degree due to differences in age, gender, velocity of growth, and stage of adolescent maturation (2,25,41,121,123). The range of laboratory values in children tend to be narrower than those in adults (25). The differences in the hematological values for the pre, peak, and post growth categories were small, and therefore, more difficult to detect with a small sample size. Iron represents a special case in that the biological variations tend to override the analytic ones (39). Because of the biological variations within each group, mean values would obscure the fact that some subjects had hematological measurements indicating iron deficiency. When each subject's hemoglobin, hematocrit, and plasma iron values were plotted individually, 5% of the subjects had low hemoglobin values and 7% had low hematocrit values in Phase II, and 4% of the subjects had low hemoglobin and hematocrit values in Phase III. These percentages were comparable to the percent of subjects with low hematological values reported in the NHANES I and NHANES II and the Ten State Nutritional survey studies (3,24,25). The incidence of iron deficiency anemia was not more prevalent in the group of subjects in this study than in NHANES I or NHANES II. The median values of hemoglobin, hematocrit, and plasma iron for each group were similar or

slightly above the values reported for 12-14 yrs. old male and female adolescents, in the NHANES II study (25). These results provide further evidence that the subjects in this study were not at greater risk of iron deficiency than the adolescent population in the NHANES II survey.

Associated with the increase in height in boys, there is an increase in the muscle size, and in blood volume and therefore, an increase in the number of red blood cells, accompanied by a rise in the hemoglobin and hematocrit values (52). The change in muscle size during adolescence is proportionately less in girls than in boys; asociated with this are lower hematocrit and hemoglobin values in girls (121). In several studies (24,25,33,121), male adolescents were reported to have higher hemoglobin and hematocrit values than femle adolescents, but no differences in these values were reported for pre-pubertal boys and girls. In this study, male subjects in their peak and post growth categories had greater mean values for hemoglobin, hematocrit, and RBC, than their female counterparts in those growth categories. Pre growth spurt subjects of each group had similar values for each gender. Daniels (121) reported a significant positive correlation of hematocrit values with maturity ratings for adolescent boys, but not for the adolescent girls. In this study, there were significant positive correlations of the gain in height with the change in hemoglobin and with the change in hematocrit in MR in their peak growth spurt but not in MC in their peak growth spurt. Other factors could influence this relationship so that the expansion of blood volume and increase in height would not occur simultaneously. The velocity of growth, or the rapidity of puberty change, appears to

be the most important factor associated with increased nutrient demands of pubertal change. In a report by Gaines et. al. (4), the adolescent boys who passed through their growth spurt in a relatively short period consumed a greater quantity of kcal. than their slower-growing counterparts of the same chronological age. In this study, MC in their peak growth spurt consumed less kcal and had a smaller mean increase in height from Phase II to Phase III, than MR in their peak growth spurt. Because of their slower growth rate, other factors (ie. hormonal changes and nutrient intake) could play larger roles so that the increase in height would not be directly proportional with the change in hemoglobin and change in hematocrit. For FR and FC in their growth spurt, there were no positive correlations of these parameters, which corresponded with Daniel's (121) results. Female controls in their growth spurt had the lowest mean hematocrit value as compared to the rest of the growth categories in the study. During their growth spurt, their plasma volume may have expanded more rapidly than the rate of erythropoiesis (124). Their dietary iron intake was approximately 70% of the RDA, which was less than that of FR in their growth spurt. Due to an inadequate dietary iron intake, the peak group of female controls may have been at a greater risk for developing iron deficiency during their period of increased need (1,20,34).

Several investigators (7,8,9,10,11) have found that runners have a greater tendency to develop anemia than less active individuals, because of several factors associated with the exercise. Possible causes for this, as reported in these investigations, include; sports anemia, disturbance in iron absorption, greater iron loss through

excess sweating, and hemoglobinuria and hematuria. In this study, the hematological and plasma iron parameters were similar between male and female runners and controls of each gender. However, in subjects' post growth spurt, there was a trend for the male and female runners to have lower mean hemoglobin and plasma iron values than that of post growth spurt MC and FC. This trend was comparable to the results reported in the investigations concluding that iron deficiency anemia was more prevalent among runners than non-runners (7,8, 9,10,11). In these studies, the subjects investigated were mature, elite runners. There are no studies available to the auther which compared the hematological status of adolescent runners and controls. The high incidence of latent iron deficiency may be common only among those runners who have completed maturation. The many developmental changes associated with puberty, including the increased need for iron due to expanding blood volume and muscle mass, may have a stronger impact on the regulation of the hematological indices in the body than would running. The same factors would not apply to adults.

Endurance athletes have been reported to have a relatively large total blood volume, and endurance training has been found to be accompanied by an increase in plasma volume (125,126). In the results from these studies, the athletes had a greater total body content of hemoglobin than non-active individuals, and training was found to be associated with an increase in total hemoglobin. Nonetheless, a decrease in hemoglobin concentration could result from an increase in plasma volume that has not been matched by a proportional elevation of the red blood cell mass or total hemoglobin (85). Brotherhood et. al. (84) measured the blood volume and hematological profile of 40 male

middle- and long-distance runners and 12 non-athletes. The distribution of hemoglobin and hematocrit concentrations, red blood cell count, and total iron binding capacity were essentially the same in athletes and non-athletes. Blood volume and total body hemoglobin were, on average, 20% higher in the athletes than in the non-athletes. The hematological and iron indices of both groups were within the normal range. He concluded that there was no firm evidence for the belief - that a mild degree of anemia was more common among runners than non-runners - which may have arisen from the wide scatter of results obtained by unstandardized methods. The hematological values of the runners in this study were found to be similar to those of the controls, in both Phases II and III, supporting the findings of Brotherhood's investigation. An exception was possibly the trend for lower hematological values in post growth spurt runners than post growth spurt controls.

In a recent study reported by Magnusson et. al. (55), 43 male elite runners had lower hematocrit, serum iron, transferrin saturation, and serum ferritin values than 119 non-athletic controls. However, the runners had a normal sideroblast count and hemosiderin grade in their bone marrow, confirming an adequate supply of iron to the normoblasts. They also had normal MCV and red cell protoporphyrin values, indicating the lack of iron had not impaired erthropoeisis. The runners had normal Desferal tests, indicating normal iron reserves, adequate dietary iron intakes, and no excess in urinary iron excretion. Because of all of these observations, the investigators concluded that, despite low hematocrit, serum ferritin, and bone marrow hemosiderin values, long distance runners were not truly iron

deficient. A low serum haptoglobin has been commonly found among runners in several investigations (8,10,47,52,54,55), including those subjects in Magnusson's et. al. study. A low haptoglobin value is an indication of an increase in intravascular hemolysis. Magnusson (56) suggested that this decrease in haptoglobin was due to an increased formation of the hemoglobin-haptoglobin complex to prevent excretion of the free hemoglobin in the urine. This complex would subsequently be taken up exclusively by hepatocytes, interfering with the regular return of catabolized red cells to the reticuloendothelial system. The shift in return of iron, from red cell catabolism, from the reticuloendothelial system to the hepatocytes would produce a reduced content of hemosiderin in the bone marrow reticuloendothelial system cells of long distance runners. The level of ferritin in serum reflects the content of iron in the reticuloendothelial system, and hence it would also be reduced. Therefore, the differences in iron status between runners and non-athletes may be due to an altered metabolic pathway for iron as an adaptive response to the sport. A serum ferritin or bone marrow hemosiderin analysis would have to be conducted on the subjects in this study to detect if differences existed in iron status at the storage site between the adolescent runners and controls of this study.

AEROBIC AND ANAEROBIC CAPACITY

Maximal oxygen consumption is that point at which the exercise becomes increasingly difficult, but the individual is unable to deliver more oxygen to his or her muscles. Maximal oxygen consumption is significantly greater in trained than in untrained athletes, allowing them to sustain high-intensity exercise for longer periods

(127). The runners were more physically fit than the controls of each gender, as indicated by the mean VO_2 Max measurements which were significantly greater for the runners as compared to the values for the controls, in both phases of the study. VO_2 Max has been widely accepted as an important determinant of cardiovascular-respiratory fitness and as a predictor of success in endurance sports such as middle- and long-distance running (127). The mean VO_2 Max value of the male runners was comparable to the values reported by Daniels et. al. (127) and Kramer et. al. (128). These values were 61.5 ml./kg./min. for 20 male, middle distance runners, ages 10 to 18 years, in Daniel's et. al. study and 62.1 ml./kg./min. for 12 male competitive swimmers and bicyclists, mean age of 15.3 yrs., in Kramer's et. al. study. Male controls had a higher mean VO_2 Max value than the 26 boys (46.7 ml./kg./min), mean age of 15.2 yrs., serving as a control group in Kramer's et. al. study. FC had a mean VO_2 Max measurement similar to the mean value (49.5 ml./kg./min.), reported in Kramer's et. al. study, for six female "top-notch" swimmers, mean age of 14.7 yrs. FR, in both phases of this study, had a much higher mean VO_2 Max value. The five female controls, mean age of 13.9 yrs., in Kramer's et. al. study, had a lower mean VO_2 Max of 27.5 ml./kg./min. than the FC in this study. Even though the control subjects in this study were not running competitively, they were normal, active teenagers who participated in other noncompetitive sports. This may be why they had higher mean VO_2 Max measurements than the control subjects in Kramer's et. al. study.

There was a trend for male subjects, who had completed their

growth spurt, to have greater VO_2 Max values than the other subjects in each group. This type of trend has been reported in other studies (127,128,129). As the boys grow and mature, their lung volume capacity and lean body mass increase allowing them to reach higher levels of aerobic fitness. The post categories of female runners and controls had lower VO_2 Max values. As the girls reach maturity, their percent of body fat increases, increasing body weight at a faster rate than absolute oxygen uptake, therefore, reducing their VO_2 max value (130).

Total oxygen requirements, under normal conditions, are primarily determined by the amount of physical activity one does, because it induces significant increments in the metabolic rate of skeletal muscle and to a lesser degree in that of myocardium (58). Anemia represents a real threat to tissue oxidation and can alter an individual's productivity at work. In addition, the results of several clinical studies (29,15,67) have demonstrated the role of iron deficiency before the onset of anemia in the reduction of work performance; physical activity of non-anemic, iron-deficient patients who received iron therapy was clearly increased, without any significant increases in hemoglobin levels. On the contrary, Edgerton et. al. (64) reported that work performance capacity was closely related to hemoglobin concentration, regardless of the adequacy of storage iron level in 7 subjects, 27-55 yrs. old.

When VO_2 Max measurements were correlated with hemoglobin, hematocrit, and plasma iron concentrations, only FR (having a positive correlation between VO_2 Max and hemoglobin) and FC (having an inverse

correlation between VO_2 Max and hemoglobin) had significant relationships in Phase III. No significant correlations of the VO_2 Max value with hemoglobin and with hematocrit were found in Phase II. There are many other factors which affect the VO_2 Max value, interfering in the relationships with hemoglobin, hematocrit, and plasma iron. Daniels et. al. (127) reported that VO_2 Max was strongely related to training, muscle mass, weight and growth rate of the young individual. One of these factors could have changed in proportion to the change in hemoglobin in the females of this study, thus enforcing the correlation of hemoglobin with VO_2 Max values. Since no significant correlations of hemoglobin with VO_2 Max values were observed in the female groups in Phase II, the significant correlations in Phase III may have been incidental and not indicative of a true relationship.

The results of the studies (73,76,80), in which the hemoglobin concentration in rats were manipulated by exchange transfusion, have shown that VO_2 Max and maximal work load in a brief hard run were largely determined by the degree of anemia. In contrast, endurance capacity (duration until fatigue with a submaximal work load) seemed to be determined primarily by the oxidative capacity of the muscle itself. Perkkio et. al. (80) reported that the endurance capability became substantially impaired even under conditions of moderate iron deficiency in rats. They concluded that the endurance capacity was related primarily to the capacity of oxidative metabolism in the skeletal muscle. In several reports (62,66,69,70,76), a decrease of iron containing proteins in the skeletal muscle and of respiratory enzymes in the muscle mitochondria were reported for mildly iron

deficient rats, during their growth period, as compared to iron repleted animals. Muscle activity was associated with a higher blood lactate concentration than was observed in iron-replete animals.

Iron deficiency of short duration and of moderate degreee can be associated with hemoglobin concentrations in the normal range. Subsequently, there is an overlap of iron-deficient and nondeficient individuals with normal or minimally decreased hemoglobin levels (61). Even though they have normal hemoglobin levels, those individuals with iron deficiency could have decreased levels of heme proteins and iron containing enzymes in their muscle because of an inadequate iron supply. In this study, the treadmill run protocal was set up to measure the maximal oxygen consumption for endurance capability. To detect if there were any differences in the anaerobic capacity, resting and maximal lactate (before and after the treadmill run) concentrations were measured and compared among groups. There was a trend for FR to have greater levels of lactate production than for FC. One MR in his post growth spurt period had the highest lactate production of all subjects. The other MR had similar lactate production levels as MC. The runners in this study ran for longer lengths of time and had higher VO_2 Max measurements than the controls, which may have caused the higher levels of lactate production. When maximum lactate levels were corrected for differences in VO_2 Max, there still was a similar trend for FR and the only MR in the post category to have greater lactate levels than controls. This trend suggests that the runners had lower muscle oxidative capacity than the controls, possibly due to less heme proteins and iron enzymes (because of a lower levels of iron stores) in their muscle.

Schoene et. al. (61) studied the effect of 2 weeks of iron therapy on exercise performance and exercise-induced lactate production in trained women athletes: 6 control subjects with normal parameters of iron status and 9 with mild iron-deficiency anemia. Iron therapy normalized the low iron status in the second group. VO_2 Max (as measured by a bicycle ergometer, exercise protocol to exhaustion) was unchanged after iron therapy in both groups; however, blood lactate levels at maximum exercise in the iron-deficient group decreased significantly after therapy. There was no change in blood lactate levels in the control group.

The results from Schoene's study suggest that even iron depletion without severe anemia may affect the oxidative metabolism so that the muscles resort to anaerobic metabolism to meet energy needs, producing greater lactate levels at an earlier stage of exercise. Once an individual reaches his or her maximal oxygen consumption, exercise beyond that point must be supplied by energy from anaerobic reactions - those that operate in the absence of oxygen, producing lactic acid during ATP generation (124).

The results from Hurley's et. al. study (131) showed that intense endurance exercise training induced adaptations that resulted in a lower blood lactate level during exercise. The individual would have to exercise at a higher percentage of VO_2 Max in the trained than in the untrained state to attain the same blood lactate concentration. When the VO_2 Max measurements were correlated with maximum lactate levels and total lactate produced in each group, only the male runners had a significant positive correlation between VO_2 Max with maximum lactate levels. These results indicate that those individuals (other

than the male runner group) who had higher VO_2 Max values, (and therefore, more physically fit) did not necessarily have the highest maximum lactate and lactate production levels. Other factors such as the lactate removal and the oxidative capacity of the muscle can affect this relationship. One possible reason why the MR had a positive relationship of VO_2 Max with maximum lactate could have been because they ran for a longer length of time (due to a greater endurance capacity and stamina since they were more physically fit) on the treadmill test than the other subjects, and therefore, had higher levels of anaerobic metabolism. These subjects could also have been less efficient in removing and metabolizing the lactate that was produced resulting in greater levels accumulated in the blood. In one study (132), differences in lactate removal were reported for athletes and non-athletes relating to the body's anaerobic metabolic efficieny.

When maximum lactate and total lactate production levels were correlated with hemoglobin, hematocrit, and plasma iron values, significant relationships were observed in the MR and in the FC groups. A significant inverse correlation of plasma iron with maximum lactate and total lactate production levels were found in MR. In FC, there was a significant inverse correlation of the hematocrit values with maximum lactate and total lactate production levels. If those individuals with the lower hematocrit and plasma iron levels also had lower iron storage levels, then they would have decreased concentrations of iron containing proteins and enzymes in their skeletal muscle (29). Since muscle biopsies could not be done on the subjects, it was not possible to identify if specific biochemical reactions were compromised by a limited iron supply. Without

measurements of the iron storage levels (as measured by serum ferritin or bone marrow hemosiderin) the differences in iron status in relation to the hematological and plasma iron values could not be proven. Because no significant inverse correlations were observed in MC and FR, and significant inverse correlations were observed with only one of the hematological values in MR and in FC, these significant inverse correlations observed in MR and FC may be incidental and may not represent true relationships. The anaerobic metabolism during a full exertion sprint test (rather than an endurance run test used in this study) would be more closely related to the hematological values of the subjects (80). Other facters, such as the removal of the lactate produced, the level of training, or body composition could be more important in determining the level of anaerobic metabolism.

DIETARY EVALUATION

Adolescents are particularly susceptible to nutritional deficiencies because of the maturational changes and rapid growth rate, thereby increasing their bodies' needs for nutrients (4). Data from the Ten-State Nutrition Survey (3) indicate that nutritional deficiencies are more prevalent among adolescents than in other age groups. Most subjects in nutritional surveys are categorized by chronological ages. However, because of the large amount of variation in growth and development among adolescents, an increased range of values would be found among subjects of similar age. Because nutrient needs tend to parallel the velocity of growth, current research in adolescents should be directed toward determining requirements based on levels of growth (70).

According to the 1980 Food and Nutrition Board (81), the

recommended energy intake for adolescents, 11-14 yrs. old, is 60 kcal/kg body weight for males and 50 kcal/ kg body weight for females. Adolescents, 15-18 yrs. of age, have lower energy needs (45 kcal/kg body weight, on the average for males and 35 kcal/kg body weight for females) than younger children based on kcal/kg body weight. The results from this study are compatable with these recommendations since there was a trend for the older subjects in each category to have smaller energy intakes per kg. body weight than the younger subjects for each group. The male and female runners had greater values for kcal/kg body weight than the intake recommended by the RDA, for Phases II and III. The MC had energy intakes similar to the 60 kcal/kg body weight recommendation (for 11-14 yrs. old) in Phase II but a lower energy intake, by 20%, in Phase III. These subjects were approximately a year older in Phase III from Phase II (because all but one subject participated in both phases of the study), which could be why they had lower energy needs. The female control group had energy intakes which were less than the recommended intake in both phases. Routine participation in a sport increases the energy needs of a moderately active male or female two to three fold or more (133). Hence, the athletes generally need higher intakes of kilocalories than their control peers (134). Runners had greater energy intakes (whether expressed as kcal/kg body weight or %RDA for kcal) than controls of each gender in both phases of the study. These results were similar to the data reported in several studies (9,10,56,86) comparing the energy intake for athletes vs. non-athletes.

Increased energy needs associated with physical activity can be met by a diet containing 50-55% of the kilocalories from

carbohydrates, 10-20% from protein and 30-35% from fat (133). The distribution of kcal of the diets for the male and female runners in Phases II and III fell into these percent ranges for energy from carbohydrate, protein, and fat. MC had a similar percent distribution of kcal. as did MR and FR. FC consumed a lower energy intake, which was low in carbohydrate, because they consumed an adequate mean protein intake. Runners consumed greater protein intakes than controls for each gender. An increase in energy intake is associated with an increase in intake of other nutrients (134).

In several nutritional surveys (3,4,5,6,24) adolescents were reported to have inadequate intakes of dietary iron. The data on nutriture of the adolescent population has shown that intakes of iron were lower than intakes of any other nutrient (19). Because dairy foods, which are low in iron, form a significant amount of many teenagers' daily intake and because many foods popular with adolescents contain little iron, it is possible that adolescents' average daily intake of iron is less than the norm (40). Milk, soda pop, and pizza were popular snacks for the subjects in this study, all being poor sources of iron (116). In Phases II and III, all groups had mean intakes which were less than 100% of the RDA for iron. According to the U.S. Department of Health, Education, and Welfare dietary intake findings from the Health and Nutritional Examination survey of 1971-74 (5), mean intakes of dietary iron were 73.2% and 57.7% of the RDA for male and female adolescents, 10-14 yrs., respectively. In Phases II and III, MR, FR, and MC had mean %RDA values for iron intake in this study which were greater, while FC had a mean %RDA value which was comparable to values found in NHANES II.

In general, males had higher dietary iron intakes than females, and runners had higher dietry iron intakes than controls. Since all groups had similar concentrations of dietary iron per 1000 kcal (mean intake was 5-6 mg. dietary iron/1000 kcal for each group), the differences in the dietary iron intake between groups were mainly due to the differences in energy intake. Male subjects consumed more kcal. than the female subjects did, just as runners consumed more kcal than the controls subjects. Subjects with low dietary iron consumption (less than 9 mg/day) also had low energy intakes. There was one male runner in Phase II who had a low dietary iron intake, even though he had over 100% of the RDA for kcal. His diet consisted of little meat, and was high in carbohydrates. He fulfilled most of his nutrient requirements by taking supplements. There were two subjects, a male runner and a male control, in Phase II who had high dietary iron intakes; that is, greater than 27 mg./day. Each subject's diet consisted of an iron-fortified cereal every day for breakfast and for snacks and at least two servings of meat per day. In Phase III, there was a different male runner and a female runner who had high dietary iron intakes. Each subject had energy intakes much greater than 100% of the RDA for kcal. The male runner consumed at least two servings of meat per day and the female runner consumed a highly iron-fortified cereal every day for breakfast.

According th the U.S. Department of Agriculture survey of 1977-78 (6), 36% of male and 64% of female adolescents had less than 70% the RDA of 18 mg. of dietary iron intake per day. The percent of MR and MC with inadequate dietary iron intakes were similar to the values reported in the survey study. On the other hand, more FC and fewer FR

had inadequate intakes as compared to the values reported in the survey study. Many adolescent girls become weight-conscious as their bodies mature and decide to follow weight-reduction diets at this time, reducing the chances for an adequate iron intake (40). This could be true for the FC but not for the FR. Female runners usually had more than adequate energy intakes, consuming greater than 100% of the RDA for kcal, resulting in sufficient dietary iron intakes.

Several studies have been conducted (135,136,137), concerning the accuracy of values reported for the food and nutrient intake, obtained from dietary records as compared to the actual intake. Three-day dietary records, with the aid of measuring devices to quantify food intakes, were used in this study. These were effective methods in reducing experimental error for calculating nutrient intake. There is no ideal method of collecting dietary information. Rather, there may be "preferred" methods for particular uses (138). A three-day dietary record was the most feasible method of data collection from the adolescent population in the context of this study. A dietary record of four or more days may have been too complicated for this age group to complete, and may have resulted in a lower overall quality of the record. Since nutritional data have been collected for the past three years, dietary intakes could be compared for subjects for each year they participated.

IRON SUPPLEMENTATION

Optimal nutrition is nessary for the development and maintenance of top physical performance. Sports competitors often experiment with fad diets, protein supplements and megavitamin therapy in order to achieve the 'competitive edge'. However, there is no conclusive

evidence that such regimens improve performance (139). Iron supplementation of athletes with anemia increases their physical performance. Plowman et. al. (14) administered oral iron therapy and ascorbate to 11 high school and collegiate female cross country runners throughout their 12 week season. Seven other female runners received only an ascorbate supplement and served as a control group. The groups had similar hemoglobin values before treatment, which were on the low side of normal; 27% of the subjects were iron deficient as indicated by transferrin saturation values less than 15%. After treatment, the runners receiving iron had significantly greater hemoglobin values. However, contrasting results to Plowman's et. al. study have been reported (12,13,14). Similar values for hematological and iron status measurements were observed for athletes taking iron supplements as compared to those who did not. In Phases II and III, subjects taking a supplement (containing iron and vitamin C) had similar hemoglobin, hematocrit, and plasma iron values (plasma iron values in Phase III only) as those subjects who took nothing for each group of male and female runners and controls. Even when sample size of the supplement user vs. non-user groups were increased (by combining the runner and control groups of each gender) there still were no significant differences observed. The hematological values of the subjects were in the normal range; a noninvasive procedure to increase hemoglobin and hematocrit concentration above the normal range has not been found (85). The subjects in Plowman's study had low hemoglobin values, and therefore, the iron supplement could be improving their values to normal.

In Phase III, there was a trend for the supplement users of each

group to have higher VO_2 Max values than non-users. Weswig et. al. (13) compared performance capability in addition to hematological and iron status between iron-supplemented and non-supplemented college varsity swimmers. No differences were reported between the supplemented and the non-supplemented groups for hematological and iron status values, but the measures of physical work capacity and performance capability were greater in the iron-supplemented group. Results from Weswig's et. al. study were similar to the trend found in this study. But it would be premature to conclude from these studies that iron supplementation should be given to athletes as a way of improving performance capabilities. More definitive research must be done in this area before such a recommendation can be made. In this study, iron supplemented subjects may have had greater VO_2 Max levels than nonsupplemented subjects, even before they began using the supplement. Because this trend was not observed in Phase II suggests that it may have been an incidental relationship in Phase III. A better design of study would be to to use the same group of runners and compare the hematological, iron nutriture, and VO_2 Max values of this group betweem two seasons; taking a placebo for one season and taking an iron supplement for the following season. Eight subjects had taken an iron supplement in one phase but not in the other. There was a significant increase in hemoglobin and hematocrit values for subjects in the phase when they took the iron supplement, but there were no differences in the VO_2 Max values between phases. This supports the possibility that the trend observed, that VO_2 Max values were greater in supplemented than non-supplemented subjects, was indeed incidental. Four of the six subjects, who had hemoglobin and

hematocrit values which had increased when taking the supplement, were in the peak of their growth spurt. The increase in hemoglobin and hematocrit values may have been caused by blood volume expansion occuring during the maturation process. One of the other two subjects - who had completed their growth spurt - had a low normal hemoglobin values and both of these subjects consumed less than 100% of the RDA for iron. Therefore, the iron supplement could have been needed to improve their iron status and return their hemoglobin values to normal.

The question of iron supplementation of the diet has aroused considerable controversy during recent years. The evidence in the literature thus far suggests that even mild or moderate iron deficiency may be disadvantageous to a population. On the contrary, it has been well established that excess iron will produce tissue damage in individuals who are predisposed to iron overloading. Prevalence of hemachromatosis is unknown and the addition of excess iron to the diet might make it a more prevalent disease with an earlier age of onset (40). Iron overload increases the risk for infection and neoplasia by serving as an essential nutrient for bacteria, fungi, protozoa or neoplasia cells (139). Thus, a change in dietary patterns would be more appropriate by eating a wide variety of foods, including those foods which are rich sources in bioavailable iron.

CASE STUDY REPORTS

One subject in Phase II and two subjects in Phase III had low hematological values, indicating iron deficiency anemia. In Phase II, a 15 yrs. old, male runner had hemoglobin and hematocrit values much

below normal. His RBC, MCV, and MCH, were also below normal, indicating a microcytic, hypochromic anemia - charcteristic of iron deficiency. His MCHC value was not low, but this measurement is the least sensitive of the indices in the diagnosis of iron deficiency (9). This subject was completing his growth spurt during Phase II and had completed it by Phase III. He competed in middle- and long-distance races, but had stopped running and participated as a control subject in Phase III. His hemoglobin, hematocrit, RBC, MCV, and MCH values all increased from Phase II and were in the normal range in Phase III. Based on the high prevalence of iron deficiency reported among runners, one would be tempted to conclude that this subject improved in Phase III because he had stopped running. Since he had an adequate dietary intake of iron and energy in both phases, his iron deficiency was not caused by an inadequate diet. In Ehn's et. al. investigation (10), elite male distance runners with latent iron deficiency absorbed a smaller percent of a labled iron dose than the control group of nonrunners, having similar iron status. Magnusson et. al. (56) measured and compared the level of iron absorption between an iron replete group and a latent iron deficient group (based on serum ferritin values less than 25 ug/l and transferrin saturation less than 15%) of elite male runners; they found no difference between the groups. The subject in this study could have had a low level of dietary iron absorption, combined with an increased loss of iron though sweat and urine caused by running (48,53), resulting in iron deficiency. Generally, with an excessive loss of iron from the body or prolonged intervals of dimished absorption of iron, iron depletion would progress to iron-deficiency anemia after body storage sites were

depleted with iron, inhibiting hemoglobin synthesis. To confirm the possibility of a decreased level of iron absorption due to running, an iron absorption test would have to be conducted on this subject before and after he had stopped running. Another reason this subject may have been iron deficient could be due to an increased need for iron for blood volume expansion and increase in lean body mass associated with puberty, because he was completing his growth spurt in Phase II. The growing adolescent must absorb an average of 0.5 mg. of iron daily, in excess of body losses, to maintain this concentration and attain a body iron content of about 4 g. as an adult (20). Plasma volume expansion occurs in 1-3 days while an increase in red blood cell mass, to compensate for this expansion takes 3-4 weeks (124). The hematological values for this subject may have been measured before red cell mass had increased to compensate for plasma volume expansion.

The male subject, with low hematological values in Phase III, competed in road races during both phases. He had not begun his growth spurt. He had low hemoglobin, hematocrit, and RBC values. His MCV and MCH values were in the low normal range. The subject's plasma iron value was normal and did not indicate iron deficiency erythropoiesis. Although plasma iron values for a group of individuals should become abnormal at an earlier stage of iron deficiency than do the hemoglobin and MCV, the usefulness of this value as the sole criterion of iron deficiency in individual patients is decreased by its poor reproducibility. The wide variations that have been noted in plasma iron is partly due to diurnal, dietary, and biological factors (41). This subject consumed over 100% of the RDA

for iron in both phases. His energy intake increased by 30% and his height by 4% from Phase II to Phase III. These increases were signs that he would be beginning his growth spurt soon. His height measured in Phase IV indicated that he did begin his growth spurt at that time. Endurance training has been reported (125,126) to be accompanied by increases in plasma volume and in total body hemoglobin. A decrease in the subject's hemoglobin concentration could have resulted because the increase in plasma volume (due to running or initial maturational changes) did not match proportionally to the elevation of the red blood cell mass and total hemoglobin. The decrease in hematological values could also have been caused by a transient decrease associated with sports anemia (85). He was tested in September, coinciding with the beginnning of the cross country season. Heavy training may have caused a transient anemia in this subject during the initial few weeks of exercise.

The third subject with a low hemoglobin value in Phase III, was a runner in Phase II but a control in Phase III. This subject was a female and was in the peak of her growth spurt in both phases. She also had low hematocrit and RBC values, though her plasma iron, MCV, and MCH values were normal. Though she had stopped running, she remained physically fit by participating in other sports, as seen by her VO_2 Max increasing by 11%. Her dietary iron and energy intakes increased by 18% and 30%, respectively, and she took an iron supplement in Phase III. Hence, the decrease in hematological values in Phase III were not caused by an inadequate intake. This subject's hematological values could have decreased because of a plasma volume expansion, not accompanied with a proportional increase in red blood

cell mass, caused by an increased level of activity or maturational development.

IRON BIOAVAILABILITY

Iron absorption from foods depends not only upon the amount of iron supplied, but by the nature of that iron and the composition of the meal in which it is consumed (16). Total iron intake, therefore, provides only a rough approximation of the amount available for absorption (17). There are two kinds of iron compounds in the diet with respect to the mechanism of absorption; heme iron, derived from hemoglobin and myoglobin from meat, fish, and poultry, and non-heme iron, derived mainly from cereals, fruits, and vegetables (16). A greater percentage of heme iron is generally absorbed than non-heme iron, although heme iron makes up a relatively minor part of total iron intake (88). Even in diets with a high meat content, it accounts for only 10-15% of total iron intake (85). MR had 10% of their total dietary iron intake in the form of heme. The other groups had lower amounts. The amount of meat in a meal is the only bioavailability factor known to influence absorption of heme iron. Absorption of iron from non-heme iron is influenced by enhancing and inhibiting factors (93).

It is estimated that 1.8 mg. of iron must be absorbed to meet the needs of adolescents (1). The current Recommended Dietary Allowance for food iron intake was thus established at 18 mg./day (for females, 11-50 yrs. and males, 11-18 yrs. old), assuming an absorption of 10% (81). However, if the average western diet contains 6 mg. dietary iron per 1000 kcal, one would have to consume at least 3000 kcal to meet this requirement with ordinary foods (17). Male runners had the

highest energy intake of all groups, which was over 100% of the RDA for kcal, but still did not supply 100% of the RDA for dietary iron. Because iron absorption varies so markedly according to the diet, recommended dietary allowances for iron merely provide rough guidelines (1).

Iron is the first biologically essential mineral for which a schema has been proposed which considers the interaction of food factors on absorbability (18). The main factors that influence the absorption of iron from the diet are; the amounts of heme and non-heme iron, the content of the dietary factors influenceing iron bioavailability, and the iron status of the subjects (16). Monsen (18) developed a model to calculate the amount of absorbable iron in a diet based on; the level of iron stores of the individual, the amount of heme iron present in the meal, and the amount of enhancing factors, meat and ascorbic acid, present in the meal. Percentage absorption of iron - both heme and non-heme - will be influenced by iron status in an inverse logarithmic function (17). During rapid growth, the adolescent must absorb an average of 0.5 mg. of iron daily in excess of body losses (body losses are approximately 1-2 mg.iron), which is an amount similar to that of an adult with low iron stores (20). In this study, if the subjects were predicted to have no body iron stores, they would have diets containing enough bioavailable iron that could be absorbed to meet their needs during this period of rapid growth. Since all but three subjects had normal hematological values in both phases, it seems unlikely that they had empty iron storage sites. If subjects were assumed to have approximately 250 mg. of iron in their storage sites, only the male subjects would have intakes

containing enough bioavailable iron to be absorbed to meet their bodies' needs. The females would have intakes with only enough bioavailable iron to replace their daily body losses. At the 500 mg iron storage level, none of the groups had enough bioavailable iron in their intakes to replace daily losses. In adult men, about 95% of the iron required for the production of red blood cells was recycled from the breakdown of senescent red cells and only 5% comes from dietary sources. In contrast, a child, due to his rapid growth, was estimated to derive much less red cell iron from senescent red cells and required a greater percent from the diet (1). The increased rate of erythropoiesis, accompanying blood volume expansion during adolescence, enhances the absorption of iron (124). Because of this increased need for iron from dietary sources, adolescents have an enhanced absorption rate for iron, most likely corresponding to the percent calculated to be absorbed for an iron storage level between 0 and 250 mg. A bone marrow hemosiderin or a serum ferritin analysis should be conducted to assess the level of the subjects' iron stores to measure the accuracy of this estimation. The main objective of Monsen's method was to classify the quality of iron in a meal as having high, medium, or low availability. It is suggested that a reference level for body iron stores be used for all subjects in order to compare the quality of iron content for each group's diet (18). The 250 mg. iron storage level seems to be most appropriate for this group of adolescents, and therefore, was selected as a basis of comparison among groups.

Male runners absorbed about 30% more dietary iron than did FR, although their %RDA for dietary iron was only 19% greater. MR

consumed more animal protein, vitamin C, and had a greater percent of their dietary iron intake in the form of heme. Since these factors enhance the absorption of iron, the quality of diets of MR - regarding the bioavailability of iron - was better than that of FR. MR absorbed a greater percent of total dietary iron intake than did FR and could be considered to have a diet of greater iron bioavailability. MC absorbed about 30% more dietary iron than did FC; their %RDA for dietary iron intake was also 30% greater than that of FC. MC consumed more animal protein and vitamin C than FC. Thus, the quality of their diet, as it relates to iron absorption factors, was better than that of FC. A possible explanation for the similarities in percent differences between the MC and FC for the amount of dietary iron absorbed as calculated by Monsen's model and for the %RDA was because the female subjects had a total dietary iron intake much lower than that of the male subjects. Thus, iron intake was the limiting factor. While increased amounts of iron are absorbed from larger levels of iron present in the meal, the percentage absorbed progressively decreases (20).

Based on the recommended 1.8 mg. of iron needed to be absorbed to meet the body's needs, the quality and amount of iron in the male subjects' diets were sufficient. On the contrary, the female subjects had an insufficient amount of iron in their diets which were not greatly improved by the quality - since both the %RDA of 18 mg. and percent absorbed of 1.8 mg. were low. The quality of the diets for MR, MC, and FC allowed them to meet their bodies' needs for iron. This could be seen because each group had greater percent values for iron absorption (at 250 mg. level of iron stores) than they did for

the %RDA. The quality of the diets for FR did not improve the level of iron absorption because they had a lower percent value for iron absorbed than they did for the %RDA. Even though FR had a larger amount of iron in their diets than did FC, they had similar intakes of animal protein and a lower percent of dietary iron in the form of heme. FR had a greater %RDA for iron, by 21%, than FC but their percent absorption of 1.8 mg. of iron was only 8% greater than that of the controls.

The percentages of subjects consuming less than 67% of the RDA for iron were similar to the percentages of subjects absorbing less than 67% of the recommended 1.8 mg. of iron (at the 250 mg. iron storage level; 1.2 mg. of iron) in all groups. Hemoglobin values were not more closely related to percent of the recommended 1.8 mg. of iron absorbed, as compared to %RDA for iron, because no significant correlations were observed among these parameters in any group. Thus, the adequacy of the adolescents' diets, in terms of iron nutriture, could be predicted with similar accuracy by using %RDA for iron, as compared to percent of iron absorbed, as calculated by Monsen's model.

Few studies have reported dietary fiber intakes, partly because there is no generally accepted definition and standard of measurement for dietary fiber. Western diets are characterized by low fiber refined cereal grains and low intakes of whole grains, legumes, fruits, and vegetables. Intakes of fiber for adolescents have been reported at 10.6-12.2 g./day (140). Male subjects had dietary fiber intakes greater than this level, probably due to high consumption of fruits and vegetables, as indicated by large Vitamin C intakes. FR had dietary fiber intakes in this range while FC consumed slightly

less. Though MC had larger quantities of enhancing factors than FC, they also had a significantly greater dietary fiber intake, decreasing the quality of their diets, in terms of iron bioavailability. Widdowson and McCance (103) found that iron balances in subjects were more positive when bread in their diets was white rather than brown, even though brown bread supplied 50% more iron than did white bread. High levels of iron intake may prevent adverse effects of fiber on iron balance. Kelsay (103) found no effect of fiber from fruits and vegetables on iron balance when the iron intake was high. Though MC had more fiber in their diets than FC, they also had greater amounts of iron and vitamin C to compensate for any losses in bioavailability.

Monsen and Cook (104) reported a significant reduction in non-heme iron absorption in the presence of calcium and phosphate salts as compared to absorption in their absence. This was true regardless of whether the calcium and phosphorus were added as a combined salt or as two separate salts added simultaneoulsly. On the other hand, the addition of either the calcium or phosphate salt alone had no singificant effect on non-heme iron absorption. The phosphate was more efficiently precipated by iron in the presence of calcium salts. Because most subjects had adequate intakes of milk and cheese, all but FC had mean intakes greater than 100% of the RDA for calcium and phosphorus. Ascorbic acid has been shown to reverse the inhibitory effect of substances such as tea, calcium, and phosphate, the degree of reversal of inhibition being directly proportional to the quantity of ascorbic acid in the meal (96). Thus, the high consumption of vitamin C for all subjects would aid in overcoming any inhibition due to the calcium and phosphate salts present in the meal. The tannates

in tea and polyphenols in coffee, have also been demonstrated to inhibit absorption of nonheme iron by 50% and 33%, respectively (84). No subjects regularly drank coffee and only one subject, a FC, drank tea, on a daily basis. She consumed 77% of the RDA for iron but only absorbed 66% of 1.8 mg. (1.2 mg.) of iron (as calculated by Monsen's method) for the 250 mg. iron storage level. This low dietary iron absorption level and daily consumption of tea, decreasing absorption even more, may have been one reason why her hematological values were low normal, with hemoglobin and hematocrit values of 12.3 g/dl and 37.2%, respectively.

Nutrition may be improved by increasing either the amount of iron in the diet or its availability. Monsen's method of determining the adequacy of diets with respect to iron would be considered useful in the effort to improve the iron nutriture of an individual, but not in predicting the exact amount of iron absorbed from the diet. Because iron is found in modest supply in the diet - in a form not readily absorbed - one could improve the adequacy of her/his intake by; increasing the content of vitamin C and/or meat/fish/poultry to enhance the absorption of non-heme iron, decreasing the ingestion of inhibiting agents, and improving the food choice to increase ingestion of dietary iron with greater bioavailability.

CONCLUSIONS

This investigation provided evidence that the incidence of iron deficieny anemia was not more prevalent among a population of elite adolescent runners than nonrunners. The effects of rapid growth and developmental changes were presumably more influential on hematological parameters and maximal oxygen consumption than running. However, the effects of rapid growth and developmental changes on hematological parameters could not be determined from the present data because of the small sample size when subjects were classified into growth spurt categories.

Specifically, the following conclusions can be drawn:

1. Elite male and female runners were not at risk of iron deficiency anemia as indicated by hematological parameters.
2. Significant positive correlations of gain in height with hemoglobin and hematocrit were found in MR but not in MC, FR, or FC.
3. Hematological values were similar among growth spurt categories of each group, except for hematocrit values in FC. FC in their peak growth spurt had lower hematocrit values than FC in their pre and post growth spurt.
4. In general, there were no significant correlations observed between VO_2 Max with hemoglobin, hematocrit, and plasma iron values in any group in either phase, except for a significant positive

correlation of VO_2 Max with hemoglobin values in FR, and a significant inverse correlation of these parameters in FC, observed only in Phase III.

5. Significant inverse correlations were observed of lactate levels with plasma iron values in MR and with hematocrit values in FC. No significant correlations were observed for these parameters in FR or MC.

6. Male and female runners consumed more energy, protein, and iron than their respective control groups.

7. Hematological parameters were similar between iron supplemented and nonsupplemented subjects of each group.

8. Low hemoglobin and hematocrit values observed in three subjects were presumably related to an increased level of activity or to maturational development. The three subjects participated in both phases of the study and their hematlogical parameters were low in only one phase of the study.

9. Male subjects tended to have diets of higher quality - in terms of iron bioavailability - than female subjects due to a greater consumption of enhancing factors, meat and vitamin C.

LIMITATIONS OF THE STUDY AND RECOMMENDATIONS FOR FUTURE RESEARCH

1. Subjects in this study were mostly caucasian (except for 2 hispanic children) and most were from families of high socio-economic status. Thus, this group was not representative for adolscents of the whole population. This fact should be taken into consideration when results from this study are compared to those of studies investigating groups of different backgrounds. A more representative sample would consist of subjects from all socioenonomic levels and race.

2. Differences in hematological status among growth spurt categories of runners and controls may be more apparent with larger sample sizes. Because there was a trend for post growth spurt runners to have lower hemoglobin and plasma iron values than post growth spurt controls; all subjects should be investigated in Phase IV of the study to observe if this trend continues. In Phase IV, more subjects would have completed their growth spurt, therfore, increasing the sample size of the post growth spurt categories.

3. Determination of hemoglobin and hematocrit can only detect the final stage of iron deficiency, 'iron deficiency anemia'. Plasma iron values can detect the second stage of iron deficiency, 'iron deficiency erythropoiesis', although biological variation exists in this measurement. Hence, total iron binding capacity and percent transferrin saturation values should be measured on subjects, since

these measurements can also detect iron deficiency erythropoiesis. However, in a relatively iron replete population, the serum ferritin value (which can detect the first and earliest stage of iron deficiency, 'latent iron deficiency') is the most sensitive measurement to assess body iron stores. Most investigators reported higher incidents of latent iron deficiency among runners as compared to nonrunners, as determined by serum ferritin measurements. Serum ferritin measurements on subjects in this study could be used: to assess differences in levels of iron stores between runner and controls in relation to developmental age; to compare quantitative changes in growth, physical capacity and anaerobic metabolism with iron storage levels; and to evaluate the amount of iron absorbed from the diet, as calculated by Monsen's model, according to subjects' correct iron storage level.

APPENDICES

APPENDIX A.

NHANES II (25) HEMATOLOGICAL AND IRON NUTRITURE MEDIAN AND 95% RANGE VALUES FOR ADOLESCENTS, 9 - 17 YEARS OF AGE

AGE	HB.	HCT.	RBC	MCV	MCH	MCHC
years	g/dl	%	10^{12}/l	fl	pg	ug/dl
			MALES AND FEMALES			
9-11	13.3	38.4	4.51	84	28.7	34.5
	11.4-14.8	34-43	3.9-5.1	76-94	26-32	32-37
			MALES			
12-14	14.0	40.5	4.71	85	29.1	34.4
	12.0-16.0	35-45	4.1-5.2	77-94	26-32	32-37
15-17	14.9	43.0	4.92	87	29.9	34.4
	13.1-16.5	37-48	4.2-5.6	79-95	27-32	32-36
			FEMALES			
12-14	13.4	39.0	4.47	86	29.4	34.1
	11.5-15.0	34-44	3.8-5.0	73-95	26-32	32-36
15-17	13.5	39.5	4.47	88	30	33.9
	11.7-15.4	34-44	3.8-5.0	78-98	26-34	32-36

AGE	TIBC	SERUM	TRANSFERRIN IRON SATURATION	ERYTHROCYTE PROTOPORPHYRIN
years	ug/dl	ug/dl	%	g/dl of RBC
			MALES AND FEMALES	
9-11	377	90	25	53
	299-472	41-151	11-43	35-82
			MALES	
12-14	391	95	24	50
	313-497	34-167	10-42	33-80
15-17	385	106	28	46
	295-490	54-196	15-50	30-69
			FEMALES	
12-14	388	96	25	52
	301-493	39-175	10-47	32-85
15-17	387	100	25	51
	304-502	40-189	11-48	33-71

APPENDIX B

MONSEN'S MODEL (18); A METHOD TO CALCULATE PERCENT OF ABSORBABLE IRON IN A MEAL

ASSUMPTIONS AND CALCULATIONS:

1. The model is based on the assumptions that dietary iron is mostly found as either heme or nonheme iron and that nonheme iron is absorbed at a very low level unless enhancing factors are present in the meal.

2. For each snack or meal, the amounts of total iron, heme iron, nonheme iron, and enhancing factors are calculated.

3. Heme iron is assumed to equal to 40% of the total iron found in meat, fish, and poultry (MFP).

4. The absorbable amount of heme-iron is assumed to be 35%, 28% and 23% for the reference individual. This is based on a logarithmic relationship between body iron stores of 0 mg., 250 mg. and 500 mg., respectively, and absorption rate.

5. Nonheme iron is absorbed at a lower rate ranging from 5 to 20%, 4 to 12% and 3 to 8% corresponding to body iron stores of 0 mg., 250 mg., and 500 mg. respectively.

6. The rate of nonheme absorption is regulated by the quantity of enhancing factors present: milligrams of ascorbic acid plus the grams of cooked meat (1.3 g. raw meat is equivalent to 1 g. cooked meat).

7. These enhancing factors bear a logarithmic relationship to the bioavailability of nonheme iron.

8. When no enhancing factors are present, the lowest percent of each iron storage level will be absorbed by the reference individual.

9. When enhancing factors exceed 75 units, the greatest percentage of each iron storage level will be absorbed by the reference individual. It is anticipated that the rate of absorption would diminish at this point, particularly for the individual with 500 mg. body iron stores.

APPENDIX B

MONSEN'S MODEL (18); A METHOD TO CALCULATE PERCENT OF ABSORBABLE IRON IN A MEAL

The equations are as follows:

Enhancing Factors (EF) = mg. of ascorbic acid + g. of MFP

According to the sum of enhancing factors, the percent of iron absorbed (%) for each iron storage level will be calculated as follows:

500 mg. Storage Level

$(EF) < 75$; $(\%) = 3 + 8.93\log_n(EF+100)/100.$
$(EF) > 75$; $(\%) = 8$
$(EF) = 0$; $(\%) = 3$

250 mg. Storage Level

$(EF) < 75$; $(\%) = 4 + 14.3\log_n(EF+100)/100.$
$(EF) > 75$; $(\%) = 12$
$(EF) = 0$; $(\%) = 4$

0 mg. Storage Level

$(EF) < 75$; $(\%) = 5 + 26.8\log_n(EF+100)/100$
$(EF) > 75$; $(\%) = 20$
$(EF) = 0$; $(\%) = 5$

An illustration of the estimated percentages associated with each category of iron status, as calculated by this model, of the two extremes and the average level of availability of iron in a meal are displayed in the table on the following page.

APPENDIX B

MONSEN'S MODEL (18); A METHOD TO CALCULATE PERCENT OF ABSORBABLE IRON IN A MEAL

Table B1. FACTORS FOR ESTIMATING PERCENT ABSORPTION OF DIETARY IRON AT EACH IRON STORAGE LEVEL OF 0 MG., 250 MG., AND 500 MG.

	IRON STORAGE LEVELS		
	0 mg.	250 mg.	500[a] mg.
I. Heme Iron	35%	28%	23%
II. Nonheme Iron			
A. Lowest Availability Meal	5%	4%	3%
MFP[b] + Ascorbic Acid = 0			
B. Medium Availability Meal	10%	7%	5%
MFP + Ascorbic Acid = 1 - 75			
C. Highest Availability Meal	20%	12%	8%
MFP + Ascorbic Acid > 75			

a The factors for 500 mg. iron stores are suggested for most dietary calculations.
b MFP; meat, fish, or poultry measured as lean, raw weight.

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