


1982

Selected indices of iron and fitness status of women participating in an exercise clinic

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**SELECTED INDICES OF IRON AND FITNESS STATUS OF WOMEN
PARTICIPATING IN AN EXERCISE CLINIC**

Iowa State University

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Selected indices of iron and fitness status
of women participating in an exercise clinic

by

Mary Louise Meck Higgins

A Dissertation Submitted to the
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1982

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INTRODUCTION

Physical fitness for adults continues to be a popular pursuit. Why become a lunch-hour athlete? Subjectively, participants report a general sense of well-being, better work capacity, less fatigue, a calmer attitude, and more resistance to stress (Hanson and Nedde, 1974). Physical conditioning classes normally involve training the musculoskeletal system as well as the cardiovascular system, so as to improve the suitability of the bone and muscle for continual rhythmic endurance exercise. Physical benefits of a fitness program are improvement in cardiovascular endurance, joint mobility, and body mechanics (alignment, posture, balance, flexibility, and movement) and the release of musculoskeletal tensions that develop throughout the day (Kasch and Boyer, 1968).

Pseudoanemic hematological changes may occur during the initial stages of vigorous physical training, possibly because of an increased rate of red cell destruction or increased plasma volume, or both (Puhl and Runyan, 1980a; Puhl et al., 1981; Radomski et al., 1980; Yoshimura et al., 1980). This "sports anemia" effect is characterized by values for hgb and RBC concentrations and hct that, although decreased, stay within normal ranges and return to pretraining levels after 2 or 3 weeks (Yoshimura et al., 1980).

Iron stores are marginal or absent in many women due to low iron intakes and high iron needs to compensate for menstrual losses (Bowering et al., 1976; Cook and Finch, 1979; J. Cook et al., 1976). If physical training increases iron needs, especially to compensate for increased

erythropoiesis at the beginning of conditioning, as has been suggested (Buskirk and Haymes, 1972; De Wijn et al., 1971; Ehn et al., 1980; Puhl and Runyan, 1980a; Puhl et al., 1981; Ruckman and Sherman, 1981; Strauzenberg et al., 1979), then an exercise program could increase the incidence of iron deficiency among those female participants who have inadequate iron reserves. Inasmuch as iron balance is precarious for many women, they have received surprisingly scant attention as subjects regarding training-induced anemia. Most of the researchers who have studied iron-related effects of exercise among females have assessed blood variables only before and after training, thereby precluding any determination of the transient sports anemia effect.

A limited number of studies involving women subjects has been reported where blood sampling intervals were frequent during the training period (Frederickson, 1980; Puhl and Runyan, 1980a, b; Puhl et al., 1981; Horiguchi et al., 1975, as cited by Yoshimura et al., 1980). However, a search of the literature yielded no information regarding studies investigating sports anemia in women older than college age. Therefore, the present study was designed to determine whether moderate exercise would cause sports anemia to develop in untrained women. Because vigorous athletic training is not typical for most women, it was appropriate to examine hematological effects of low-intensity training under the hypothesis that an older, more sedentary group of women might respond to a moderate conditioning program in a manner similar to the previously studied younger (i.e., high school- and college-age) women who underwent strenuous aerobic training.

A second objective of this study was to estimate from food diaries selected nutrient intakes of the participants. Assessment of iron intakes was of particular interest. Protein intakes also were of interest because a relationship between protein nutriture and the development of sports anemia has been proposed (Yoshimura et al., 1980). A final major purpose of this study was to compare selected cardio-respiratory and body composition measures before and after the physical fitness program. The Iowa State University Committee on Use of Human Subjects in Research approved this project.

REVIEW OF LITERATURE

Selected literature reports relating to iron and fitness status are included in this review. Data regarding women have been emphasized. The first section covers iron balance of women, assessment of iron status, and effects of exercise on hematological status. The section on fitness status reviews effects of aerobic training with regard to several body composition and cardiorespiratory variables.

Iron Status

Iron plays a central role in tissue respiration since it is essential to hemoglobin (hgb), which transports oxygen to the cells, and to many enzymes which catalyze oxidative-reductive reactions inside the cells. Iron is also involved in porphyrin metabolism, collagen synthesis, lymphocyte and granulocyte function, somatic and neural tissue growth, and neurotransmitter synthesis and catabolism (Leibel et al., 1979). Normally, 70% of body iron is in hgb, 4% is in myoglobin, less than 1% is in iron-containing enzymes, and the remainder is in storage or transport for use in hgb synthesis (Oski, 1979).

Definition of iron deficiency

Iron deficiency, the most common nutritional deficiency, is defined as a reduction or disappearance of the physiologic body stores of iron, regardless of whether this reduction is accompanied by anemia (Mertz, 1980). Three stages of iron deficiency may be considered, although there are not always clear cut distinctions between them:

1. prelatent deficiency, where there is no clinical evidence of deficiency, nor any detectable disturbance in biochemical function, but the individual is less able to meet any increased demand for iron or withstand iron deprivation;
2. latent deficiency or sideropenia, where there is biochemical or clinical abnormality, or both, but no anemia; and
3. overt deficiency, where iron deficiency is sufficiently severe to produce anemia (Baker and DeMaeyer, 1979).

Anemia is commonly defined by using an arbitrary cutoff standard in conjunction with the frequency distribution of values for seemingly healthy individuals. In 1968, the World Health Organization (WHO) proposed that for adult nonpregnant females, the hgb concentration below which anemia is likely to be present (in populations living at sea level) is 12 g/dl blood (Baker and DeMaeyer, 1979). New values for 25-35 year old white females define "low" (15th percentile) and "deficient" (5th percentile) hgb levels as 12.7 and 12.0 g/dl, respectively, and are based on a national sample of 1469 women in that age group (Garn et al., 1981b). Values for other iron parameters recommended as indicating probable iron deficiency are: <15% saturation for transferrin, >100 µg/dl RBCs for free erythrocyte porphyrin (FEP; see Appendix A), and <12 µg/l blood for ferritin (J. Cook et al., 1976).

Effects of iron deficiency

Deleterious effects of iron deficiency include decreased growth, gastrointestinal problems, skin and mucous membrane lesions, behavior alterations, decreased immunological status, possibly increased susceptibility to respiratory infections in infants, and numerous

biochemical abnormalities (Baker and DeMaeyer, 1979; Oski, 1979). In pregnancy, increased incidence of fetal deaths, short gestation lengths, low birth weights, and medical abnormalities are associated with both high and low hgb and hematocrit (hct) values (Garn et al., 1981a). However, the cause(s) are not known.

Finch et al. (1976) reported that iron-deficient rats have a decreased running ability, possibly due to changes in skeletal muscle concentration of α -glycerophosphate dehydrogenase. These authors suggest that skeletal muscle α -glycerophosphate dehydrogenase is an iron metalloprotein with an important role in energy formation through its involvement with the regeneration of NAD^+ , necessary for glycolysis, and the oxidation of α -glycerophosphate. Similar changes in the concentration of this enzyme in response to the animal's iron status were not found in cardiac muscle. Impaired cytochrome-dependent mitochondrial function may also occur in iron-deficient rats (Siimes et al., 1980). Surplus iron given to normal rats provided no advantage with regard to running ability (Finch et al., 1976).

When iron deficiency is severe enough to cause anemia, physical work capacity is often concomitantly impaired. Decrements in physical performance, as gauged by an exhaustive run, are proportionally related to hgb concentration and hct in anemic rats and humans (Edgerton et al., 1972; Ekblom et al., 1972; Gardner et al., 1977; Ohira et al., 1981; Woodson et al., 1978). However, improvement in work capacity with iron treatment cannot be totally accounted for by an elevation in hgb concentration (Charlton et al., 1977; Edgerton et al., 1972; Ohira et al.,

1979). Furthermore, when hgb concentration and hct are within normal ranges, no correlation was observed between hematological status and competitive performance (Runyan and Puhl, 1980). Iron treatment improved the level of voluntary physical activity and worker productivity in anemic Sri Lankan tea pickers (Edgerton et al., 1979) and the work output, disease morbidity, and submaximal step-test performance in anemic Indonesian rubber tappers (Basta et al., 1979). Similarly, an iron-supplemented group of healthy elderly men and women had a greater increase in work capacity due to physical training than did a placebo-treated control group (Ericsson, 1970).

Iron balance of women

Nutritional balance is normally maintained when the amount of a nutrient absorbed from the diet equals the amount lost from the body. Since iron is recycled within the body and excretion of iron is limited, the major regulation of iron repletion is accomplished by keeping absorptive processes attuned to bodily requirements. Iron losses and requirements, dietary iron and its absorption, and iron stores are discussed in this section.

Iron losses Normal adults have similar basal iron losses. However, since most cells contain iron somewhat in proportion to the quantity of iron in body stores, daily obligatory cell loss of anemic individuals represents reduced iron losses compared to those of iron-replete persons (Conrad and Barton, 1981).

Iron in intestinal secretions (exfoliation of mucosa, bile and blood), urine, and desquamated epithelium and sweat amounts to losses

of approximately 14 μg iron/day/kg body weight or 0.8 mg iron/day for the average woman (INACG, 1977). Sweat is usually considered to be a negligible route of iron loss, regardless of environmental temperature or the amount of sweating (Baker and DeMaeyer, 1979; Bowering et al., 1976). Measurement of sweat is difficult and complicated by contamination from dermal cells and the small amounts of iron to be quantified. Volume of sweat increases in hot weather but its iron concentration decreases (Bowering et al., 1976). In cases of profound sweating, daily iron losses of 0.4 to 1.0 mg may occur via sweat (Consolazio et al., 1963; Ehn et al., 1980). Further research in this area, including comparisons of the sweating patterns of men versus women, seems warranted.

Women of childbearing age incur additional iron losses due to menstruation. Average losses in 459 normal women participating in 19 studies were 8-38 mg iron per period, with a range of individual values from 0.3 to 110 mg (Bowering et al., 1976). Individual women usually are constant in menstrual losses regardless of parity, recency of childbirth, or age (Hallberg et al., 1966), although some women's losses vary considerably more than others. Frequency distributions of losses are skewed. Median losses represent 0.5 mg/day when averaged over one month, but 10% of women have menstrual iron losses exceeding a daily average of 1.4 mg (Baker and DeMaeyer, 1979). Oral contraceptive steroids reduce menstrual flow in approximately two-thirds of users (Burton, 1967). However, no data were found regarding the magnitude of the reduction in monthly blood losses.

Other sources of iron loss include blood donation, which amounts to 250 mg iron for each 500 ml blood given; pregnancy, which has a net iron cost ranging from 1.5 to 3.6 mg/day (the average cost being 2.4 mg daily) (AMA, 1968); and episodes of bleeding from injury, surgery, or other causes.

Iron requirements Recommendations regarding the amount of iron that must be absorbed to meet iron requirements vary. To provide the iron needs of 50, 75, or 90% of menstruating nonpregnant women, 1.4, 1.8, or 2.2 mg iron, respectively, must be absorbed daily (Hallberg, 1981). WHO recommends 2.8 mg as the required amount of absorbable iron for this group, so that 95% will maintain iron homeostasis (Baker and DeMaeyer, 1979). In addition, every 500 ml unit of blood donated imposes an additional 0.7 mg/day requirement if the iron loss is to be replaced over a one year period (AMA, 1968).

Iron absorption It is difficult to convert absorptive requirements into requirements for food iron since iron absorption is a complex process that is influenced by many factors. Intraluminal, mucosal, and corporeal factors affecting iron absorption have recently been reviewed by Conrad and Barton (1981).

The amount of iron absorbed from a meal is dependent, in part, upon the individual's iron status. Enhanced iron absorption continues in iron-deficient subjects long after hgb is restored to normal levels and persists until body iron stores become normally replete (Conrad and Barton, 1981). The maximum absorption to be expected from an average diet by iron-depleted, but not anemic, subjects is approximately 15%

(Monsen et al., 1967). Recommendations for food iron have generally been set assuming an average 10% absorption of total dietary iron.

Food iron basically can be considered as two independent pools: a heme iron pool and a nonheme iron pool. Approximately 50-60% of the iron in beef, lamb, and chicken, and 30-40% of the iron in pork, liver, and fish, is in the form of heme (Cook and Monsen, 1976). Absorption of heme iron from a usual American diet ranges from 15, 23, 28 and 35% in subjects with 1000, 500, 250 and 0 mg iron stores, respectively (Monsen et al., 1978). The factor of 0.23 (corresponding to 500 mg stores) is suggested for most calculations in estimating the percentage of dietary heme iron absorption (Monsen et al., 1978).

The nonheme iron pool consists of the portion of iron in animal tissues which is not in heme in addition to the iron in all other foods, including eggs, dairy products, vegetables, and grains. Absorption of nonheme iron is influenced by the presence of other foods. Both a "meat factor" and ascorbic acid enhance the availability of nonheme iron. Persons with 500 mg iron stores absorb 3 to 8% of nonheme iron, depending on the quantity of animal tissue or ascorbic acid, or both, that is present in the meal (Monsen and Balintfy, 1982). A distinction must be made between animal tissues and animal proteins, since some sources of the latter, i.e., dairy products and eggs, are not equivalent to animal tissues in enhancing iron absorption (Cook and Monsen, 1976). Some of the substances that decrease nonheme iron absorption are phytates, oxalates, lactoferrin, antacids, EDTA, phosphovitin, and tannic acid. Nonheme iron makes a major contribution to the total amount of absorbable

dietary iron because even though the percentage absorbed is considerably lower than that of heme iron, the quantity eaten is higher. Nonheme iron amounts to 85-90% of the total iron intake in most western diets (Bull and Buss, 1980; Hallberg, 1981).

The quantity of absorbable iron in a meal or snack may be estimated as follows. Compute the amounts of (1) total iron, (2) heme iron (a heme iron factor of 0.4 of total iron in all animal tissues may be used), (3) nonheme iron, (4) ascorbic acid, and (5) meat, poultry and fish. Multiply (2) and (3) by the appropriate factors, as given by Monsen and Balintfy (1982), and sum the products. A different method for estimating absorbable dietary iron precludes knowing the amounts of ascorbic acid and animal tissue eaten at each meal. An assumption is made that all nonheme food iron is eaten in medium-availability meals (absorption factor = 0.05). Bull and Buss (1980) used this method to estimate heme and nonheme iron in British diets using data reporting national average intakes of food groups. They used two heme iron factors, 0.4 or 0.6, depending upon the type of meat eaten, and the mean absorption of dietary heme iron was taken to be 23%. The calculated amount of absorbable iron was 0.78 mg/day, or 7.1% of the total intake of 10.95 mg/day. This value is less than the 10% factor which is often assumed (NAS, 1980).

Iron intake Intakes of food iron by adult women usually fall below the recommended (NAS, 1980) allowance of 18 mg per day. Average intakes usually do not exceed 13 mg/day (Table 1). Iron consumption is positively correlated with energy intake (Bingham et al., 1981;

Table 1. Food iron intakes of adult women

Reference	Sample Size	Women's Age (years)	Mean Iron Intake (\pm SD) (mg/day)	Iron Nutrient Density ^a
Abraham et al., 1977 ^b	1,243	20-24	10.01	5.92
	1,896	25-34	10.33	6.31
Bingham et al., 1981	10	20-29	12.3 (3.4)	5.63
R. Cook et al., 1976	27	-	10.2	5.79
Driskell et al., 1979	53	19-22	11.3 (5.1)	6.33
Holden et al., 1979	11	15-51	12.7	6.35
Hudiburgh and Milner, 1979	28	19-37	12.5	-
Jakobovits et al., 1977	195	college age	12.4 (4.4)	6.42
Lenner et al., 1977	336	38	12 (0.3)	7.36
Monsen et al., 1967	13	19-37	9.9 (2.4)	6.19
Puhl and Runyan, 1980a	19	19-21	11.5 (0.9)	6.04
Science and Education Administration, 1980 ^c	949	23-34	10.7	6.9
	942	35-50	10.7	7.6
White, 1968	227 (literature)	21-49	11.7	-
Wirth et al., 1978	17	19-23	6.5	-

^aIron nutrient density (mg iron/1000 kcal) was calculated by dividing the mean iron intake (mg/day) by mean caloric intake (kcal/day) and multiplying by 1000.

^bHealth and Nutrition Examination Survey, 1971-72.

^cNationwide Food Consumption Survey, 1977-78, preliminary results.

Elsborg et al., 1979; Yearick et al., 1980). Dietary iron nutrient densities (mg iron/1000 kcals) for women, calculated from literature reports, range from 5.6 to 7.6 (Table 1), as compared with a recommended range of 7.5 to 11.2 (using values of 18 mg iron and 1600-2400 kcals; NAS, 1980). The diets of both men and women have similar iron densities, but the recommended density for men is low (i.e., 3.7). Thus, women are in an unfortunate situation, since their caloric consumption is lower than that of men while their iron requirements are greater. Average energy intake in the U.S. is 2514 versus 1582 kcals/day for males (n = 3023) and females (n = 5450), respectively, age 23-50 years (Abraham et al., 1977; Science and Education Administration, 1980).

Iron stores Not surprisingly, normal iron stores are greater in men than in women of childbearing age (1000 vs. 300 mg, respectively) (Conrad and Barton, 1981). Iron deficiency to one extent or another is prevalent among adult women. The frequency distribution of iron stores, as determined by serum ferritin concentration, among 426 women in the U.S. was as follows: 10% had iron deficits of 240 mg while 40, 60, 76, 84 and 91% had stores less than 150, 300, 450, 600 and 750 mg, respectively (Cook and Finch, 1979). Median stores in this group were 220 mg iron. Estimates of hemosiderin indicated that from 67 to 75% of women have scant or absent iron stores (Bowering et al., 1976; Monsen et al., 1967; Scott and Pritchard, 1967). Other studies have shown that 5 to 20% of menstruating women are iron deficient based on biochemical findings, while frank anemia occurs in 4 to 9% (Abraham et al., 1974; J. Cook et al., 1976; Fairhurst et al., 1977).

Negative iron stores are defined as iron that must be replaced in circulating red cells before iron reserves can reaccumulate (Cook and Finch, 1979).

As expected, iron stores are correlated with blood losses. Serum ferritin concentrations in blood donors decrease as the frequency of donation increases (Simon et al., 1981). However, no effect was observed on hct, hgb, or transferrin saturation values. Menstruating women may not be able to donate more than once or twice yearly without causing an appreciable increase in the incidence of iron depletion. The proportion of iron-deficient women at each donation frequency decreased among those who used dietary iron supplements compared with nonusers. Similarly, heavy menstrual blood losses (>60 ml/period) correlate with lower hematological values (Hallberg et al., 1966) and women with regular menstrual patterns tended to have lower transferrin saturation and higher total iron binding capacity values than did those who menstruated irregularly (Plowman and McSwegin, 1980). A relationship between iron intake and hematological parameters has been found only sometimes (Hudiburgh and Milner, 1979; Wirth et al., 1978; Yearick et al., 1980), possibly because the range of values was too small to detect statistically significant correlations.

Assessment of iron status

No single iron parameter monitors the entire spectrum of iron status. The most sensitive method for detecting mild iron depletion is by radioactive iron absorption studies, which show abnormalities before changes can be detected biochemically (Conrad and Barton, 1981).

Assessment of storage iron, erythrocyte iron, transport iron, and FEP is useful for detecting conditions of iron repletion, severe negative iron stores, mild iron deficits, and absent but not yet negative iron reserves, respectively. Only when ≥ 2 parameters fall within the iron deficiency range can the cause of anemia reasonably be attributed to iron deficiency (Cook and Finch, 1979).

Storage iron Histological assessment of bone marrow hemosiderin is considered to give the definitive indicator of iron stores. Unfortunately, this technique is expensive and not suitable for screening large groups of people.

In relatively iron-replete populations, serum ferritin may be the most useful and sensitive parameter of iron status. Ferritin is the major intracellular iron storage protein and reflects the size of total body iron stores as measured by quantitative phlebotomy or by iron absorption. Between 8 and 21 mg of stored iron (mean, 9.9 mg) are represented by 1 $\mu\text{g/l}$ serum ferritin. Once iron stores are exhausted, however, ferritin levels fall below 12 $\mu\text{g/l}$ and no longer reflect the true deficit in body iron (Cook and Finch, 1979). Ferritin measurement, by radioimmunoassay, is technically difficult, time consuming and expensive, but provides a sensitive, noninvasive, quantitative, and reproducible estimate of iron status. Serum ferritin levels rise in a few days after the start of iron therapy and also increase with chronic and acute inflammation. Thus, if a subject takes supplemental iron or has an infection, it would be possible to miss the diagnosis of a deficiency (Thomas et al., 1977). Since serum ferritin is only a measure of iron

stores, it is less suited to detect rapid changes in the balance between supply and demand of iron to the erythron compared with FEP (Koller et al., 1978).

Erythrocyte iron Hgb and hct tests are important, reliable, and inexpensive methods for detecting severe iron depletion. Hgb is preferred over hct as a screening test for anemia because the method for its determination relies less on human judgement and skill and because hgb concentration falls with iron depletion more rapidly than does hct (Graitcer et al., 1981). About 150 mg body iron is represented by 1 g/dl circulating hgb in a 70 kg adult woman (Cook and Finch, 1979). Neither hgb nor hct is a sensitive measure of mild degrees of deficiency and there is a wide scatter of normal values. Hct, or packed cell volume, is overestimated 3 to 4% by centrifugation methods because of plasma trapped between the cells (Fortney and Senay, 1979; Novosadova, 1977; Van Beaumont et al., 1972; Ward, 1979). Venous hct may be corrected to whole-body hct using factors of 0.91, 0.92, or 0.93 (Dill et al., 1974; Fortney and Senay, 1979; Novosadova, 1977; Van Beaumont et al., 1972).

Mean cell volume (MCV), red blood cell (RBC) concentration, mean cell hgb (MCH), and mean cell hgb concentration (MCHC) values that are derived from electronic devices are dependable, widely unappreciated, and useful for diagnostic purposes (Crosby, 1979). Decreased MCV (microcytosis), RBC concentrations, MCH (hypochromia), and MCHC (hypochromasia) occur in iron-deficiency anemia.

Blood values for red cell parameters for normal women are as follows (Ward, 1979):

Parameter	Mean (and Range)
hgb	14 (2) g hgb/dl blood
hct	42 (5) ml RBCs/dl blood
MCV	90 (9) cu. microns/cell
RBC concentration	4.8 (0.6) $\times 10^6$ cells/ μ l blood
MCH	29 (2) pg/cell
MCHC	34 (2) g/dl RBCs

Oral contraceptive steroids usually do not affect hgb or hct values in users compared with nonusers (Driskell et al., 1979; Hudiburgh and Milner, 1979; Mardell et al., 1969). However, one report was found where users had increased hcts, but similar hgb concentrations and MCHCs compared with nonusers (Burton, 1967).

Transport iron Precision of estimates of iron stores in sideropenia, when stores are absent but not yet negative, is enhanced by measuring transferrin saturation and FEP (Dagg et al., 1966). Cook and Finch (1979) interpreted elevated FEP concentrations as indicating absent iron stores, while abnormal values for both FEP and transferrin saturation were taken to represent an iron deficit of 150 mg.

Transferrin is the major serum iron transport protein. Transferrin saturation is the ratio of serum iron to total iron binding capacity (TIBC). Since serum iron levels fall and TIBC increases with iron deficiency, transferrin saturation is a more sensitive parameter than is either serum iron or TIBC alone. Transferrin saturation is subject to wide diurnal variations in normal subjects and fluctuates suddenly with mild infection, bleeding, and changes in diet (Langer et al., 1972; McLaren et al., 1975). Its response with iron treatment in

deficient subjects is variable (Thomas et al., 1977). Both serum iron and TIBC values may rise in women using oral contraceptive steroids (Burton, 1967; Mardell et al., 1969), perhaps partly due to a response to circulating progesterone. However, users in another study had serum iron concentrations similar to nonusers (Wirth et al., 1978). The assay for transferrin saturation is time consuming, influenced by iron contaminants, and hard to quantitate on small blood samples (Cook and Finch, 1979; Thomas et al., 1977).

Free erythrocyte porphyrin Elevated FEP concentrations indicate abnormalities in heme synthesis (see Appendix A). Levels increase linearly with a decrease in bone marrow iron stores (McLaren et al., 1975). FEP concentration appears to be the best index of the adequacy of iron supply because it integrates both iron supply and iron demand (erythropoietin stimulation and marrow requirements) (Langer et al., 1972). Values correlated better with iron supply than did hct, hgb, serum iron, or transferrin saturation (Dine, 1980; Koller et al., 1978; Stockman et al., 1975). Also, the ratio of protoporphyrin to heme showed better correlation with hct, hgb, serum iron, transferrin saturation, and serum ferritin than did any other single parameter (Labbe et al., 1979). A steady response in FEP concentration occurs with iron treatment of deficient patients. Levels are elevated within a week after iron supplies become deficient and require about a month to fall, with values normalizing in several months, long after red cell indices have returned to normal (Langer et al., 1972; Thomas et al., 1977).

The major disadvantage of using FEP concentration to assess iron deficiency is lack of specificity. Lead poisoning, erythropoietic protoporphyria, malignancy, liver disease, and chronic infection as well as conditions associated with hypoferrremia cause elevated FEP levels. Values are not increased with pernicious anemia, anemias of acute infection, or thalassemia. No consistent variation has been shown related to sex (Dine, 1980; Koller et al., 1978; Labbe et al., 1979; McLaren et al., 1975), use of oral contraceptives (Buchet et al., 1978), the menstrual cycle (Cartwright et al., 1948), or age (J. Cook et al., 1976). FEP concentrations are not subject to daily fluctuations and sudden changes for intra-individual measurements (Cartwright et al., 1948; Piomelli et al., 1976). Periods of increased erythropoiesis or reticulocytosis, which may occur after recent blood loss (e.g., blood donation), do not increase FEP levels provided that the iron supply is adequate, as reflected in $\geq 15\%$ transferrin saturation (Langer et al., 1972). Simplified fluorometric micromethods have made FEP measurement better suited for routine clinical use and for screening purposes (see Appendices A and B).

The normal sequence of events that occurs with a gradual depletion of body iron stores is summarized in Table 2. Cutoff standards are not universally agreed upon. No distinct separation exists between normal and deficient values. FEP values shown in Table 2 are discussed in Appendix A; other values are those suggested by Conrad and Barton (1981) and Cook and Finch (1979).

Table 2. The normal sequence of events that occur with a gradual depletion of body iron status^a

Iron Parameter	Iron Status						
	Normal		Depleted	Sideropenic		Anemic	
	Good	Suboptimal	Prelatent Deficiency	Latent Deficiency	Deficient Erythropoiesis	Early Deficiency Anemia	Late Deficiency Anemia
iron stores ^b (mg)	600	200	120	0	-150	-300	-600
stainable bone marrow iron	+ 2	+ 1	0	0	0	0	0
serum ferritin (µg/l blood)	60	20	< 12	< 12	< 12	< 12	< 12
FEP (µg/dl RBCs)	75	75	75	100	>100	>160	>160
transferrin saturation (%)	35	35	35	20	< 16	< 16	< 16
hemoglobin (g/dl blood)	14	14	14	14	13	< 12	< 12
MCV (cu. microns/cell)	90	90	90	90	88	86	< 82
MCHC (g/dl RBCs)	33	33	33	33	33	31	< 28

^aAdapted from Conrad and Barton (1981), Cook and Finch (1979), and Appendix A.

^bNegative iron stores indicate the amount of iron that must be replaced in circulating red cells before iron reserves can reaccumulate.

Exercise and hematological status

Hematological variables are affected by short-term exercise and long-term training or conditioning programs. Transient effects of exercise on blood parameters may be divided into those which are acute, lasting only during and immediately following an exercise bout, and those which are temporary, occurring within the first week or two of physical training, i.e., sports anemia. Chronic effects of training on hematological status have generally not been conclusively determined. Implications of these effects are discussed following the sections documenting which changes occur.

Acute effects of exercise Hemoconcentration may occur during exercise due to sweating and movement of water from the blood into extravascular spaces. Hgb and RBC concentrations as well as hct increase in response to the decrease in blood volume (specifically, plasma volume) (Karvonen and Saarela, 1976; Van Beaumont et al., 1972; Wilkerson et al., 1977). MCHC, however, does not vary (Novosadova, 1977; Wilkerson et al., 1977). Both intermittent and continuous types of exercise can cause hemoconcentration (Roitman and Brewer, 1973), as can maximal and prolonged submaximal exercise, but prolonged mild exercise may not induce changes (Novosadova, 1977). After the exercise session, intake of liquids and shift of fluids dilute the blood, causing hematological indices to normalize.

After long races, hematuria, myoglobinuria, and myoglobinemia have been reported in some male runners, possibly due to repeated trauma to the posterior bladder wall against the prostatic base (Dressendorfer et al., 1980; Schiff et al., 1978; Siegel et al., 1979).

Sports anemia Changes in iron-related blood variables may occur during the initial stages of strenuous physical conditioning or training. The temporary reduction of hgb and RBC concentrations and hct has been termed "sports anemia" (Yoshimura, 1970). If the individual continues training, within 2 or 3 weeks these hematological indices normalize. Sports anemia is normocytic and normochromic and is almost always associated with values for blood parameters that, although decreased, are within normal ranges. Possible causes of sports anemia will be considered following a discussion of its occurrence among training men and women.

A close correlation exists between the development of sports anemia and the intensity and duration of physical activity required of the trainee (Yoshimura et al., 1980). However, the condition has also been observed during sustained repetitive work levels involving 35% maximal $\dot{V}O_2$ (Radomski et al., 1980). The initial physical fitness level of the subject is another important variable. Well-conditioned individuals are less likely to develop sports anemia than are those having low fitness when both groups undergo similar training programs. Unfortunately, many studies regarding changes in hematology during training do not provide the necessary data to indicate sports anemia. The transitory nature of the phenomenon mandates that several blood samples be taken during the initial days or weeks of training. If sampling intervals are too infrequent, as when pre- and post-conditioning data are compared, the development of sports anemia cannot be ascertained since adaptation would have already occurred.

Most of the research on sports anemia has involved the use of men or laboratory animals as subjects. The condition has developed among

men during periods of a sudden increase in physical activity, including events such as a 312 mile, 20-day road race (Dressendorfer et al., 1981), a 4- or 5-day, 21 hours/day army combat course (Lindemann et al., 1978), a 4 hour/day Marine Corps training program (Rasch et al., 1969), a 6-day, 35 km/day march at 35% maximal $\dot{V}O_2$ (Radomski et al., 1980), rugby training for 4 to 4.5 hours/day, and bicycle ergometer training for 2 hours/day at a relative metabolic rate (RMR, the ratio of energy requirement of the work to basal metabolism) of 7 (Yoshimura et al., 1980). A sports anemia effect in high school and collegiate women has been demonstrated during training periods for basketball (Yoshimura et al., 1980) and running (Frederickson, 1980; Puhl and Runyan, 1980a; Puhl et al., 1981). The phenomenon did not occur in a group of already highly-trained collegiate women runners who increased their weekly training distance from 40 or 50 up to 50 or 60 miles (Puhl and Runyan, 1980b), nor in two groups of male runners, one of which was physically active but untrained as runners and the second of which was composed of recreational distance runners, who ran 4 miles at 75% maximal $\dot{V}O_2$ every other day (Hanson et al., 1978).

A variety of blood parameters in addition to hgb and RBC concentrations and hct have been studied during the initial stages of increased physical training. Results are often at variance, possibly due to sex, training intensity, or blood sampling interval differences between studies. Red cell indices (MCH, MCHC, and MCV) temporarily decreased or remained unchanged in male trainees (Dressendorfer et al., 1981; Radomski et al., 1980), but MCV increased in females (Puhl and Runyan,

1980a; Puhl et al., 1981). Serum iron levels rose with training in men (Dressendorfer et al., 1981), but fell in women (Frederickson, 1980). Osmotic fragility decreased temporarily among men and women (Puhl et al., 1981; Yoshimura et al., 1980). Reticulocyte counts remained unchanged in several studies involving both men and women (Hanson et al., 1978; Puhl et al., 1981; Rasch et al., 1969), but increased in another study (Yoshimura et al., 1980). Researchers using male trainees found that total body hgb decreased (Yoshimura et al., 1980), haptoglobin concentrations decreased (Hanson et al., 1978; Karvonen and Saarela, 1976; Lindemann et al., 1978), bilirubin levels increased (Lindemann et al., 1978), and erythropoietic activity increased (Lindemann et al., 1978). In a group of women runners, FEP concentrations initially declined with training, then rose (Frederickson, 1980).

Interpretation of these findings has resulted in the advancement of several possible hypotheses to explain the sports anemia effect. One mechanism may be hemodilution. However, changes in plasma volume can only partially account for the many observed changes which occur. The best explanation seems to be an increased rate of RBC destruction at the onset of strenuous activity. According to this hypothesis, an initial preferential destruction of more fragile cells is followed, after a short delay, by a compensatory increase in hematopoiesis. The large young cells generated have increased resistance to hemolysis. Perhaps also, the larger cells remaining after the destruction of smaller, older cells are more resistant to hemolysis.

Factors which might cause an increase in hemolysis during exercise have been reviewed by Yoshimura et al. (1980). Physical trauma may weaken the structural integrity of the RBC as a result of mechanical wearing of the membrane because of rapid circulation through narrow capillaries, mechanical trauma to the hands or feet, or compression by muscular activity. Another contributing factor may be chemical stress, including increased acidosis during exercise, or a hemolytic factor, lysolecithin, which is released from the spleen in response to adrenalin secretion. A third proposed factor relates to protein nutriture, possibly through its effects on osmotic or chemical resistance of the RBC membrane, or both, and on hematopoietic function. Yoshimura et al. (1980) postulated that hemolysis is an adaptation to hard muscular exercise, whereby the hgb freed from the destroyed erythrocyte is utilized for myoglobin accumulation in skeletal muscle. However, if dietary protein is sufficient (i.e., 1 g/kg body weight/day, 60% of which is animal protein, or 2 g/kg body weight/day, 30% of which is animal protein), the incidence of sports anemia is decreased or, at least, the changes in hematological indices are lessened and recovery is hastened (Yoshimura et al., 1980). The necessity for increased protein intakes in athletes has not been widely accepted (ADA, 1980; Buskirk, 1981; Darling et al., 1944; Durnin, 1978; Rasch et al., 1969). Current dietary allowances for protein are set at 0.8 g/kg body weight/day for adults with no increment believed necessary for work or training (NAS, 1980). Typically, protein intakes in the U.S. exceed the recommended allowance, with customary diets providing 10 to 15% of calories

from protein (ADA, 1980). Two national surveys found that the mean protein consumption of adult women was approximately 65 g or 1 g/kg body weight/day (Abraham et al., 1977; Science and Education Administration, 1980).

Chronic effects of training Comparisons of the hematological status of elite athletes and highly-trained individuals, especially long-distance runners, with sedentary groups are abundant in the literature, but findings are conflicting. The preponderance of evidence suggests that physical training results in a small increase in plasma volume, i.e., hemodilution (Brotherhood et al., 1975; Dill et al., 1974; Kjellberg et al., 1949; Oscai et al., 1968; Scheuer and Tipton, 1977; Yoshimura et al., 1980), as well as increased amounts of total body hgb (Brotherhood et al., 1975; Dill et al., 1974; Kjellberg et al., 1949; Yoshimura et al., 1980). Effects on hgb concentration are unclear, with increases, decreases, and no change being reported for highly trained men and women (Brotherhood et al., 1975; Bunch, 1980; Clement et al., 1977; De Wijn et al., 1971; Dill et al., 1974; Ehn et al., 1980; Kjellberg et al., 1949; Runyan and Fuhl, 1980; Scheuer and Tipton, 1977; Yoshimura et al., 1980). Hgb concentration and blood volume may be independently controlled (Brotherhood et al., 1975).

Hct was increased in a group of male athletes compared with sedentary controls (Yoshimura et al., 1980), but values were normal in other studies involving both males and females (Brotherhood et al., 1975; De Wijn et al., 1971; Runyan and Puhl, 1980). One-half of the six men studied by Bunch (1980) had a low hct. Effects on RBC concentration

are also equivocal. Red cell counts in male athletes may be high (Yoshimura et al., 1980) or unchanged (Brotherhood et al., 1975). Women runners had RBC counts which were below normal mean values (Runyan and Puhl, 1980). One report of red cell indices in highly-trained athletes was found; MCV, MCH, and MCHC for this group of women were above normal mean values (Runyan and Puhl, 1980).

Serum iron levels were decreased in one group of male athletes (Brotherhood et al., 1975), but were normal in other studies involving men and women (De Wijn et al., 1971; Ehn et al., 1980). Percent transferrin saturation and TIBC were normal in male elite athletes (Brotherhood et al., 1975; De Wijn et al., 1971), but abnormal in females (De Wijn et al., 1971). Based on both hgb concentration and transferrin saturation, 2 to 3% of Dutch Olympic athletes had iron-deficiency anemia, 3 to 5% had iron depletion, and 5 to 15% had latent iron deficiency. In all cases, prevalence of deficiency was greater among the female athletes (De Wijn et al., 1971).

Alterations in hematological status resulting from conditioning programs for nonathletes or from increased training during the competitive season for participants in various sports also may be considered. Again, however, inconsistent findings have been reported with regard to changes due to training programs lasting from 6 weeks to 4 months. Pre- and post-training comparisons in several studies using female subjects revealed no change (Kilbom, 1971) or an increase (Akgun et al., 1974; Fortney and Senay, 1979; Holmgren et al., 1960) in plasma volume; a decrease (Holmgren et al., 1960; Kilbom, 1971), increase (Puhl and

Runyan, 1980b), or no change (Akgun et al., 1974; Cooter and Mowbray, 1978; Kilbom, 1971; Puhl and Runyan, 1980a; Puhl et al., 1981; Wirth et al., 1974) in hgb concentration or hct; decreases (Frederickson, 1980; Kilbom, 1971) or no change (Cooter and Mowbray, 1978; Kilbom, 1971; Puhl and Runyan, 1980b; Wirth et al., 1978) in serum iron values; and increased FEP levels (Frederickson, 1980; J. L. Puhl and W. S. Runyan, Depts. of Physical Education and Food and Nutrition, Iowa State University, unpublished data, 1982).

Implications for athletes An increased iron need for athletes, especially at the beginning of conditioning, has been suggested (Buskirk and Haymes, 1972; De Wijn et al., 1971; Strauzenberg et al., 1979). Exercise seems to alter iron distribution and/or iron reutilization, absorption, or excretion (Ruckman and Sherman, 1981) and increase body iron turnover. Factors which may contribute to elevated iron needs during exercise include increased iron elimination (via sweat or urinary losses) and higher iron requirements (to increase the total amount of circulating hgb, compensate for an initial high rate of RBC destruction, form more cytochrome and myoglobin, satisfy an increased uptake of iron by muscle, increase levels of transport iron, and/or replace storage iron used for the return of hematopoietic balance). Menstruating women athletes have additional iron requirements because of monthly blood losses.

There is no doubt that some athletes suffer from a certain degree of iron deficiency. Male long-distance runners with no signs of bleeding, hemolysis, or increased excretion of hgb via the urine had

absent or only traces of bone marrow iron, normal to low iron absorption, and increased iron elimination corresponding to 2 mg daily (Ehn et al., 1980). Similar results were found in rats trained to swim, where decreases occurred in tissue (cardiac, hepatic and splenic) iron, apparent absorption of iron, and iron stores compared with values for nonexercised animals (Ruckman and Sherman, 1981). As has been discussed in a previous section, iron balance is marginal in many women. The additional stress that strenuous training seemingly imposes on body iron stores could precipitate iron deficiency among these women. Indeed, unfavorable iron status is not uncommon among elite female athletes (Clement and Asmundson, 1979; Clement et al., 1977; De Wijn et al., 1971).

Although a greater iron need for athletes seems probable, iron supplementation during training generally does not improve hematological parameters in men or women (Brotherhood et al., 1975; Cooter and Mowbray, 1978; Pate et al., 1978; Weswig and Winkler, 1974). Supplementation was beneficial in one study, where serum iron and hgb concentration were higher in iron- and ascorbic acid-supplemented women runners after 12 weeks of training compared with runners receiving only additional ascorbic acid (Plowman and McSwegin, 1980). Iron supplements also resulted in increased serum iron concentrations in another study, but did not prevent the development of sports anemia (Yoshimura et al., 1980).

Deleterious effects of iron deficiency on the running ability of rats and on physical work capacity were mentioned at the beginning of

this review. The extent to which sports anemia and other effects of exercise on hematological status affect physical performance is unclear. Dressendorfer et al. (1981) reported no impairment of long-distance running performance as a result of sports anemia. Of course, performance is affected by many variables, both physiological and psychological. While it is possible that aerobic capacity would be reduced, the problem appears to be one of small magnitude in this respect. Compensatory changes, such as an increase in 2,3-diphosphoglycerate concentration or higher P_{50} (arterial oxygen tension when 50% of the hgb is saturated), may occur with athletic training to increase tissue oxygen delivery (Scheuer and Tipton, 1977; Smalley, 1979; Uddin et al., 1978).

Physical Fitness Status

A limited review of the extensive topic of fitness status is discussed in this section. Effects of aerobic physical conditioning that are emphasized concern changes in physiological variables related to body composition, cardiorespiratory measures, flexibility, and grip strength. Evidence indicates that men and women respond in a similar manner to endurance training (American College Sports Medicine, 1978).

Body composition

The assessment and prediction of body composition have generated much research effort. Many biochemical, densitometric, and anthropometric methods exist for determining body composition. Some problems involved with the measurement and prediction of body composition were reviewed by Katch and Katch (1980). Factors which can influence body

composition include sex, age, inherited body type, physical activity, weight and health status, and diet. In general, women have more body fat than do men, and body fat increases as a person ages, even if body weight remains constant. Some athletes have higher than normal weight for their height yet carry little extra fat, while some of their sedentary counterparts have an excessive accumulation of fat without being overweight.

Body dimensions Body weight (BW) and waist, hip, and thigh girths are considered in this section with regard to the influence of fitness programs. Height, of course, also is a body dimension but is not affected by activity level.

Approximately 19% of adult women in the U.S. have excessive BW such that they may be classified as obese (Abraham et al., 1975). Total BW is a function of caloric intake and energy expenditure (both voluntary and involuntary). The relationship between these variables is not always clear cut. Mayer et al. (1956) reported that caloric intake increased with both low and high levels of activity. Consequently, BW was higher among individuals in the sedentary range, then decreased and plateaued as the amount of habitual physical activity increased. Leaner subjects reportedly engage in more vigorous leisure time physical activity ($RMR \geq 6$) than do their fatter peers (Blair et al., 1981). The leaner women dieters had an increased energy expenditure of about 190 kcals/day, the equivalent of 20 pounds of body fat/year. However, compensatory increases in caloric consumption may occur as a result of increased energy expenditure (Gwinup, 1975). A strong positive

correlation was found between caloric intake and general activity level by Epstein et al. (1978). These investigators reported no relationship between activity level and BW, however. In other cases, too, participation in a training program has not affected BW (Daniels et al., 1979; Frederickson, 1980; Getchell and Moore, 1975; Gulyas, 1973; Hanson and Nedde, 1974; Kilbom, 1971; Moody et al., 1972; Noland and Kearney, 1978; Pollock et al., 1969; White and Young, 1978). At variance with these data, BW is often reduced as a result of physical conditioning (American College Sports Medicine, 1978; Massicotte et al., 1979; Puhl and Runyan, 1980a; Rhodes and Dunwoody, 1980; Weltman et al., 1980; Wilmore et al., 1970). Two reports were found where BW increased during training (Patton et al., 1980; Smith and Stransky, 1976). The composition of BW changes is discussed in a separate section.

Waist, hip, and thigh girths are not frequently reported in the literature. Available evidence shows no effect of a fitness program on these body dimensions (Moody et al., 1972; Noland and Kearney, 1978; White and Young, 1978). "Spot reduction" through specific exercises for the abdomen, hips, and thighs is not possible. Subjects performing calisthenics showed results similar to those occurring in participants of a general exercise program which involved walking, jogging, biking, or bench stepping (Noland and Kearney, 1978).

Skinfold thickness Thickness of the subcutaneous fat layer may be estimated using a calibrated skinfold caliper. The skinfold is the double layer of skin and subcutaneous fat pinched between the thumb and forefinger. The number and location of sites chosen for measurement

vary widely between studies, and reproducibility of results may not be constant between individuals taking the measurements. Practice and attention to technique increase precision. Guidelines have been established to aid standardization of skinfold measurements (NRC, 1956).

Thigh fatfolds were not significantly affected by training, in contrast with suprailiac skinfolds, which decreased approximately 15 to 30% (Noland and Kearney, 1978; White and Young, 1978). The sum of skinfolds measured at multiple sites declined 16 to 22% as a result of conditioning programs (Getchell and Moore, 1975; Moody et al., 1972).

Predicted body composition In the last 30 years, at least 100 prediction methods have been proposed to evaluate the fat and lean components of the body (Katch and Katch, 1980). Age- and sex-specific regression equations predict body fat (BF), body density, or lean body mass using anthropometric data such as skinfolds, girths, diameters, height, and weight. This approach is more accurate than predictions based on a single body measurement. However, researchers disagree as to which combination of variables best reflects body composition. Also, the predictive validity of these equations (cross-validation) is poor when applied to an independent, although seemingly similar, group of subjects.

Predicted density, obtained via one of the proposed regression equations (e.g., Pollock et al., 1975), can be used to estimate percentage BF. If a fat-free body is assumed to have a constant density of 1.1 g/cubic cm, to which is added fat with a density of 0.9 g/cubic cm, the formula for percentage BF ($\pm 4\%$ BW) is $100 \times \left(\frac{4.95}{\text{density}} - 4.5 \right)$

(Siri, 1961). Other equations also have been developed based on different models and assumptions. Total amount of BF may be calculated as $\frac{BW \times \% BF}{100}$. The difference between BW and total amount of BF represents lean body mass. Density and lean body mass are positively correlated with each other and inversely related to BF.

According to Katch and Katch (1980), equations for predicting body composition may be inadequate for determining changes resulting from some experimental treatment, since the reliability of the change scores is low and valid equations are not available for use in the pre- to post-test situation. Nevertheless, these equations are frequently used to assess whether body composition changes occur as a result of a conditioning program. While there is a certain hazard in doing this, generalizations which are drawn from the data can be modified in the future if necessary.

Research with both animals and humans has shown that exercise often decreases BF, even in the absence of changes in BW. Changes in BF are related to initial values, i.e., the higher the total amount of BF, the greater the reduction due to training (American College Sports Medicine, 1978). Percentage BF decreased 3 to 13% among participants of some training programs (Daniels et al., 1979; Frederickson, 1980; Gulyas, 1973; Massicotte et al., 1969; Patton et al., 1980; Puhl and Runyan, 1980a), while statistically significant changes were not found in other studies (Brown et al., 1980; Moody et al., 1972; Noland and Kearney, 1978; Smith and Stransky, 1976). Middle- versus college-age women lost a significant percentage of BF in one study (White and Young, 1978),

possibly because their initial values were higher than those of the younger group.

Cardiorespiratory variables

Adaptations in the cardiovascular system due to physical training have been reviewed by Scheuer and Tipton (1977). The response to training of resting heart rate (HR), blood pressure, vital capacity, and forced expiratory volume taken for 1 second (FEV_1), as well as sub-maximal exercise recovery HR, is reviewed in this section.

The training effect achieved by a program of physical conditioning is inversely related to the initial level of fitness. Persons with low fitness can get a training effect with a sustained training HR as low as 110-120 beats per minute (American College Sports Medicine, 1978). The single most objective indication of a training effect is an increase in maximal $\dot{V}O_2$. However, maximal $\dot{V}O_2$ values may be influenced by other factors, such as leg fatigue or lack of motivation.

Chronic exercise results in bradycardia (Scheuer and Tipton, 1977). Short-term conditioning programs may also decrease resting HR (Akgun et al, 1974; Wilmore et al., 1970). However, in other cases, pre- and post-training measures of resting HR were not statistically different (Edwards, 1971; Fringer and Stull, 1974; Getchell and Moore, 1975; Smith and Stransky, 1976). Age was a factor in one study. Resting HR of a group of college-age women did not change, while that of the middle-age trainees declined (White and Young, 1978). A program of physical training also lowers HR in response to submaximal exercise

(Getchell and Moore, 1975; Kilbom, 1971; Massicotte et al., 1979; Puhl and Runyan, 1980a; Rhodes and Dunwoody, 1980; Smith and Stransky, 1976; White and Young, 1978).

Training seems to lower blood pressure responses in older, less fit, or hypertensive subjects, but its influence may not be observed in younger or active individuals (Scheuer and Tipton, 1977). Evidence of decreased resting blood pressure (either systolic or diastolic, or both) has been reported by Brown et al. (1974), Kilbom (1971), and Wilmore et al. (1970), while other investigators have found no change as a result of training (Akgun et al., 1974; Flint et al., 1974; Getchell and Moore, 1975; White and Young, 1978).

The response of pulmonary vital capacity and FEV_1 to training has not been extensively studied. Vital capacity and FEV_1 were similar before and after training in several studies (Akgun et al., 1974; Getchell and Moore, 1975; Massicotte et al., 1979). In other instances, however, vital capacity has increased with training (Rhodes and Dunwoody, 1980; Wilmore et al., 1970). Middle-age women trainees showed no change in vital capacity while their college-age counterparts had improved values (White and Young, 1978).

Flexibility and grip strength

Rhodes and Dunwoody (1980) reported improved flexibility, including back and shoulder extension and trunk flexion, among the participants of a fitness program. However, their subjects (70 men) showed no increase in hand grip strength.

PROCEDURES

Subjects

Twenty healthy Caucasian women served as subjects (mean age, 31.6 yrs; range, 22-45 yrs). All were enrolled in the Iowa State University Exercise Clinic. The study was conducted in accordance with the regulations for protection of human subjects and informed consents were obtained. Women ≥ 35 yrs received a physical examination, graded exercise test, and permission to participate from a physician. Prior to enrollment in the exercise clinic, the subjects were generally inactive, although some women occasionally engaged in moderate physical activity such as bicycling or jogging.

Information about blood losses was obtained from each subject by questionnaire (see Appendix C). All subjects had regular menstrual patterns (mean duration, 4.9 days; mean interval, 28.2 days). Among the 20 women, 2 used oral contraceptives, 2 donated blood within 6 months from the start of the study, and none had a child < 2 yrs old.

Nutrient intakes were estimated from the subjects' food intake diaries (see Appendix C) by using a computer program based on data in USDA Handbook No. 8 (Watt and Merrill, 1963) and product label information. Absorbable iron was estimated using the method of Bull and Buss (1980). Diary entries were made every Monday, Thursday, and Saturday throughout the study.

Training Program

The conditioning program consisted of 9 weeks of 3 hourly sessions/week. Each subject had at least 50% attendance (mean, 70%). The women

performed individually-prescribed muscular strength, muscular endurance, and flexibility exercises between intervals of walking/jogging. The distance covered during the latter showed a general increase over time (Table 3; $p < 0.02$).

Table 3. Mileage walked/jogged per exercise session per person

	Week of Conditioning					
	1	2	3	5	7	9
\bar{x}	1.1	1.4	1.5	1.8	1.8	1.8
SD	0.5	0.6	0.7	0.9	0.9	0.8

Measurements

Fitness level was assessed during weeks 1 and 9. Parameters recorded were: body weight (BW) and height, resting HR and blood pressure, sit-reach trunk forward flexion, forced vital capacity and 1-sec forced expiratory volume (FEV) (using a Collins spirometer), post-exercise HR (taken from 5 to 65 seconds after a 3-minute step test, 24 steps/min, 12-inch bench; Kasch and Boyer, 1968), and fitness level (based on post-exercise HR response; Kasch and Boyer, 1968). Pre- and post-conditioning anthropometric measurements were taken by the same experienced individual. Skinfold fat (thigh, suprailiac, and axilla) was estimated following standard procedures using Lange calipers. Girths (waist, hips, and thigh) were measured with a steel anthropometric tape. The equations used to predict density and body fat (BF) are those reported by Pollock et al. (1975):

$$\text{density} = 1.0852 - (0.0008 X_1) - (0.0011 X_2)$$

(used for women <35 yrs), or

$$\text{density} = 1.0754 - (0.0012 X_3) - (0.0007 X_2)$$

(used for women ≥ 35 yrs),

where X_1 = suprailiac skinfold, mm; X_2 = thigh skinfold, mm; X_3 = axilla skinfold, mm.

$$\% \text{ BF} = 100 \times (4.95/\text{density} - 4.5).$$

$$\text{lean body mass} = \text{BW} - (\text{BW} \times \% \text{BF}/100).$$

Iron status was assessed using selected indices of erythrocyte iron (hct, hgb and RBC concentrations, MCV, MCH, and MCHC) as well as FEP concentration. The latter is an index of uncompleted heme synthesis that can detect rapid changes in the balance between supply and demand of iron for erythropoiesis (J. Cook et al., 1976; Koller et al., 1978; Langer et al., 1972; McLaren et al., 1975; Thomas et al., 1977). Finger-puncture blood samples (warm-hand) were obtained in duplicate during weeks 1, 2, 3, 5, 7, and 9 of conditioning. Hgb concentration was measured spectrophotometrically on an Ames/BMI blood analyzer by the cyanomethemoglobin method. Hct was measured using a micromethod and was not corrected for trapped plasma or for the ratio between whole-body and venous hct. RBC concentration and MCV were determined with a Coulter Electronics Model B Counter and a Model H Particle Size Distribution Plotter calibrated and monitored with Coulter standards. Standard formulae were used to calculate MCH and MCHC. FEP concentration was determined for 13 of the 20 subjects using a modification of the methods of Piomelli et al. (1976) and Chisholm and Brown (1975). Paper discs (PKU cards, Sigma Co., St. Louis, MO) spotted with 20 μ l of blood were

torn into 4 pieces, then shaken vigorously 30 min in 0.3 ml of a celite/saline/sterox suspension. Two ml of ethyl acetate/acetic acid (3:1) were used to extract porphyrins. After addition of 2 ml of 1.5 N HCl, the solvent phase was aspirated. Fluorescence of the porphyrin-containing extract was measured at 605 nm with an Aminco-Bowman ratio spectrophotofluorometer that was equipped with a 1P21 photomultiplier tube, zeroed with a disc-reagent blank, and calibrated at 595 nm with a 2 ng/ml coproporphyrin III solution (Porphyrin Products, Logan, UT). FEP concentrations were calculated from a regression equation based on the fluorescence of 7 volumes (ranging from 2.5 to 25.0 μ l) from each of 8 batches of protoporphyrin IX standard solutions (Porphyrin Products) that were spotted onto discs and processed in triplicate in a manner identical to the blood samples. All samples from one person were assayed in the same batch to minimize effects of procedural variability. (The day-to-day coefficient of variation was 6% for FEP analyses on quality-control blood samples; see Appendix B.)

Statistical Analysis

Differences between pre- and post-conditioning variables were evaluated using a paired student's t-test. Analysis of variance was used to compare blood values over time. The relationship between dietary, fitness, and blood variables was examined using a multiple correlation analysis. Statistical significance was accepted at the $p < 0.05$ level. Mean (\pm SD) values are reported.

RESULTS

Nutrient Intakes

Estimated nutrient intakes and percentages of age- and sex-specific recommended dietary allowances (RDAs; NAS, 1980) that were consumed daily by the women are shown in Table 4. Values are based on an average of 23 food diaries per subject and do not include amounts consumed in dietary supplements. Average daily dietary intakes in the U.S.A. for women age 23-50 yrs are 1582 kcals, 65 g (or 1 g/kg BW) protein, and 10 to 10.7 mg iron (Abraham et al., 1977; Science and Education Administration, 1980). Comparison of our findings with these national surveys shows good agreement (Table 4). Seven of the 20 women took vitamin and iron supplements. Levels of food iron and vitamin intakes were not statistically correlated with supplement usage, indicating that dietary supplements were not necessarily consumed by those subjects eating the poorest-quality diets.

Some of the women expressed a desire to lose weight during their participation in the exercise clinic and, indeed, the mean value for BW fell slightly, i.e., 0.5 kg, during conditioning (Table 5). Calories were distributed among protein (15%), fat (39%), carbohydrate (44%), and alcohol (2%). Caloric intake was positively correlated with total iron intake ($r = 0.73$, $p < 0.001$). RDAs for iron are set assuming that 10% of total food iron is available for absorption (NAS, 1980). Our data for absorbable iron indicate that the subjects had 16% of food iron available (Table 4). Therefore, only 11.25 mg of total food iron would be needed to provide the group with the recommended amount of 1.8 mg

Table 4. Estimated daily food intakes of selected nutrients (dietary supplements excluded)

	Energy		Protein		Total Iron	Absorbable Iron
	kcal/s	kcal/s/kg BW	g	g/kg BW	mg	mg
\bar{x}	1727	28.6	66	1.10	10.09	1.60
SD	354	7.0	13	0.27	2.08	0.64
RDA ^a	2000 ^b	---	44	0.8	18 ^c	1.8
% of RDA Consumed	86	---	149	138	56	89

^aRecommended dietary allowance (NAS, 1980).

^bRecommended range is 1600 - 2400.

^cAssumes an average availability of 10% of food iron.

Table 5. Physical and performance characteristics of subjects

Characteristic	n	Pre-Conditioning		Post-Conditioning		Change %	p <
		\bar{x}	SD	\bar{x}	SD		
height, cm	20	165.0	4.5	165.0	4.5	0	NS
weight, kg	20	60.7	8.6	60.2	8.6	- 0.8	NS
density, g/ml	16	1.033	0.011	1.038	0.010	+ 0.5	0.001
body fat, %	16	29.1	5.7	26.3	4.8	- 8.8	0.01
body fat, kg	16	18.1	6.0	16.2	4.8	- 9.7	0.01
lean body mass, kg	16	43.1	4.2	44.4	5.2	+ 3.0	0.03
thigh skinfold, mm	16	31.2	6.9	28.5	6.9	- 8.7	0.001
suprailiac skinfold, mm	9	18.1	8.1	17.7	8.7	- 2.3	NS
axilla skinfold, mm	7	20.4	5.7	16.7	5.0	-18.2	NS
waist girth, cm	12	74.7	8.3	73.8	8.1	- 1.0	NS
hip girth, cm	12	98.7	8.0	98.9	7.6	+ 0.4	NS
thigh girth, cm	12	57.7	6.2	57.5	5.8	- 0.2	NS
forced vital capacity, ml	16	3406	319	3506	284	+ 3.2	0.05
FEV, %	16	86.4	10.7	83.9	8.0	- 2.2	NS
resting systolic BP, mm Hg	15	107.1	10.8	107.7	12.0	+ 0.6	NS
resting diastolic BP, mm Hg	15	71.1	6.6	70.8	9.3	- 0.4	NS
resting HR, bpm	16	77.3	10.5	70.3	11.2	- 8.1	0.04
post-exercise HR, bpm	16	120.3	18.5	102.4	18.9	-14.5	0.0001
fitness level (very poor = 6)	16	4.3	1.7	2.8	1.6	---	0.0001
sit-reach forward flexion, cm	15	9.2	8.6	13.3	5.2	+44.6	0.01

absorbable iron; 89% of this amount was consumed on the average. High-quality protein was eaten (61% was from animal products) and intake exceeded the RDA.

Fitness Indices

Values for physical and performance characteristics of the subjects before and after conditioning are presented in Table 5. Paired t-test comparisons showed that training altered body composition even in the absence of a significant decline in BW. Body density and lean mass increased, while BF decreased. Percentage BF was inversely correlated with the mileage covered per exercise session ($r = -0.37$, $p < 0.05$) but not with caloric intake. Pre- and post-conditioning skinfold and girth values were statistically similar, with the exception of decreased thigh skinfolds.

Several cardiorespiratory variables improved during the study. Forced vital capacity increased by 3% ($p < 0.05$). Bradycardia occurred at rest and after submaximal exercise (decreases were 8 and 14%, respectively), indicating that a training effect occurred despite the low intensity of aerobic training. Fitness level improved from category 4 ("fair") up to 2 ("good"). Trunk flexibility also improved significantly, from 9.2 cm before conditioning to 13.3 cm after conditioning. FEV and resting blood pressures showed no statistically significant changes.

Blood Variables

Values for blood variables remained statistically similar during the conditioning program (Table 6). Mean values for red cell parameters

Table 6. Mean (\pm SD) blood values during training

Variable	n	Week of Conditioning						Grand \bar{x}	Range of Individual Values
		1	2	3	5	7	9		
hematocrit, ml RBCs/dl blood	20	40.4 ± 2.0	39.5 ± 2.4	40.6 ± 1.6	40.1 ± 1.9	40.2 ± 2.2	40.0 ± 2.0	40.1 ± 2.0	34.9 - 44.6
hemoglobin, g/dl blood	20	12.99 ± 0.72	13.09 ± 0.65	13.32 ± 0.48	13.18 ± 0.61	13.19 ± 0.78	12.95 ± 0.71	13.12 ± 0.66	11.5 - 14.7
RBC, millions/ μ l blood	20	4.36 ± 0.22	4.32 ± 0.26	4.42 ± 0.30	4.33 ± 0.26	4.37 ± 0.34	4.31 ± 0.38	4.35 ± 0.30	3.64 - 5.35
MCV, cu. microns/cell	20	88.0 ± 5.0	88.1 ± 3.7	87.3 ± 2.8	86.8 ± 3.9	85.5 ± 3.0	85.9 ± 3.1	86.9 ± 3.7	76.9 - 98.5
MCH, pg/cell	20	29.86 ± 1.86	30.36 ± 1.97	30.23 ± 1.87	30.51 ± 1.63	30.38 ± 2.71	30.18 ± 1.87	30.25 ± 1.97	24.8 - 34.7
MCHC, g/dl RBCs	20	32.19 ± 1.57	33.18 ± 2.14	32.86 ± 0.91	32.88 ± 0.94	32.75 ± 1.34	32.40 ± 1.00	32.71 ± 1.40	28.8 - 37.8
FEP, μ g/dl blood	13	26.0 ± 5.8	25.5 ± 5.2	25.9 ± 7.1	26.6 ± 7.0	24.6 ± 6.8	25.9 ± 8.1	25.7 ± 6.5	14 - 42
FEP, μ g/dl RBCs	13	65.4 ± 15.5	65.9 ± 15.3	64.1 ± 18.6	66.8 ± 18.4	61.4 ± 17.7	65.1 ± 21.0	64.8 ± 17.4	35 - 111
FEP/hgb, μ g/g	13	1.99 ± 0.44	1.94 ± 0.41	1.94 ± 0.54	2.02 ± 0.56	1.87 ± 0.51	1.99 ± 0.62	1.96 ± 0.51	1.06 - 3.22

were within ranges considered to be normal for women (Ward, 1979). The mean FEP concentration was 64.8 $\mu\text{g}/\text{dl}$ RBCs, which falls within the 47-80 $\mu\text{g}/\text{dl}$ RBCs range reported in several other micromethod FEP studies involving almost 2000 seemingly healthy adults (Chisholm and Brown, 1975; J. Cook et al., 1976; McLaren et al., 1975; Piomelli, 1973; Schwartz, 1980; Stockman et al., 1975). The Center for Disease Control recommends that FEP results be expressed as $\mu\text{g}/\text{dl}$ blood (CDC, 1975, as cited by Chisholm and Brown, 1975). The FEP/hgb ratio is reportedly the more sensitive index of anemia (Piomelli et al., 1976).

Although mean values were within normal ranges, 11 of the 20 subjects had at least one below-normal value for RBC concentration during the study. A statistically significant positive correlation existed between RBC concentration and the mileage covered per exercise session ($r = 0.20$, $p < 0.03$). Similar associations occurred between hgb and FEP ($\mu\text{g}/\text{dl}$ blood) concentrations and MCH and the mileage covered per exercise session or total mileage run during all sessions ($r = 0.22$ to 0.43 , $p < 0.07$).

Blood donation was positively correlated with the FEP/hgb ratio ($r = 0.59$, $p < 0.05$) and inversely related to hct, MCV, and MCH ($r = -0.46$ to -0.59 , $p < 0.05$). Also, high FEP concentrations ($\mu\text{g}/\text{dl}$ RBCs) tended to be correlated with low intakes of absorbable food iron ($r = -0.54$, $p < 0.06$). Since iron-deficient erythropoiesis can cause FEP levels to increase, these data suggest that blood donation and low absorbable iron intakes are associated with suboptimal iron status.

DISCUSSION

In the present study, a low-intensity conditioning program resulted in improved fitness level. Body composition shifted towards decreased fat and increased muscle mass, flexibility improved, and bradycardia occurred at rest and after submaximal exercise (Table 5). Similar data have been reported in comparable studies involving post-college-age women (Getchell and Moore, 1975; Massicotte et al., 1979; White and Young, 1978).

Indices of red cell iron and of iron supply and demand did not change throughout 9 weeks of training. Since persons with low initial fitness levels are more likely to develop sports anemia than are well-conditioned individuals (Puhl and Runyan, 1980b; Yoshimura et al., 1980), it seemed possible that this transient condition would occur among our subjects. Women 18 to 45 yrs are more likely to have iron deficiency than are either men or teenage women (J. Cook et al., 1976). Our subjects had a history of iron losses through menstruation and, in some instances, blood donation and pregnancy (7 of the 20 women had given birth 1 to 4 times). Many women are in a borderline state of iron balance, presumably absorb iron at a maximal rate, and have little ability to compensate for additional iron requirements (J. Cook et al., 1976). Such women would have insufficient storage iron to meet demands for iron costs associated with training. Inasmuch as our subjects consumed a large amount (i.e., almost 90% of the RDA) of absorbable food iron (Table 4), perhaps they had better-than-average iron nutriture. Indeed, FEP concentrations were below the 100 $\mu\text{g}/\text{dI}$ RBC level that is

considered to be the upper limit for normal, iron-sufficient erythropoiesis (see Appendix A). An exception was one woman who had only slightly elevated values (Table 6). Although good iron status is desirable to compensate for increased iron demands, it nevertheless does not seem to prevent the development of sports anemia (Yamada et al., 1975, as cited by Yoshimura et al., 1980).

Differences in training intensity seem to be the most probable explanation for the conflicting data between studies. In instances where unfavorable changes in iron-related blood variables among young women occurred (Frederickson, 1980; Puhl and Runyan, 1980a; Puhl et al., 1981; Horiguchi et al., 1975, as cited by Yoshimura et al., 1980), subjects underwent more strenuous training compared to women in the present study. A relationship between the development of sports anemia and the intensity of physical activity has been noted (Yoshimura et al., 1980). Moderate rather than strenuous aerobic training, however, better represents the level of activity that large segments of post-college-age women undergo during conditioning.

Subjects' protein nutriture may be another reason for varying results between studies. The incidence of sports anemia is reportedly decreased among Japanese athletes if dietary protein is ≥ 1 g/kg BW/day, 60% of which is animal protein (Yoshimura et al., 1980). These amounts were consumed by participants in the present study (Table 4). The necessity for increased protein intakes by athletes has not been widely accepted, though (ADA, 1980; Buskirk, 1981; Durnin, 1978), and the RDA for adults is set at 0.8 g/kg BW/day with no increment believed necessary for work or training (NAS, 1980).

Results of the present study indicate no change in pre- versus post-training values for hgb concentration or hct. These findings are in agreement with those of several others using female subjects (Akgun et al., 1974; Cooter and Mowbray, 1978; Kilbom, 1971; Puhl and Runyan, 1980a; Puhl et al., 1981; Wirth et al., 1978).

In conclusion, a moderate program of aerobic training and conditioning exercises improved the fitness level and did not seem to affect the iron status of post-college-age menstruating women.

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APPENDIX A. REPORTED AND REFERENCE VALUES FOR
FREE ERYTHROCYTE PORPHYRIN (FEP)

Introduction

In the last decade, simplified micromethod assays have been developed for the measurement of FEP (Chisholm and Brown, 1975; Granick et al., 1972; Heller et al., 1971; Labbe et al., 1979; Piomelli, 1973; Piomelli et al., 1976), thereby increasing the frequency of its analysis. The "specificity" of assays for protoporphyrin IX (proto), which extract any porphyrin present, lies in the fact that with rare exception, proto from erythrocytes is the only porphyrin likely to be encountered in excess in human blood; $\geq 95\%$ of the extractable porphyrin in whole blood is proto associated with erythrocytes (Chisholm and Brown, 1975).

The final step in heme formation is the incorporation of iron into the proto. Normally, the rate of proto synthesis and the transfer of iron to its site of utilization are well-balanced, such that mature circulating erythrocytes contain only about 1 molecule of excess proto for each 30,000 molecules of heme formed (Labbe et al., 1979). This "obligatory" fraction of blood proto amounts to about 75% of mean normal values and is unrelated to either iron supply or red cell age (Langer et al., 1972). Heme synthesis decreases during iron deficiency because of insufficient substrate iron and possibly due to ferrochetalase inhibition in lead poisoning (Labbe et al., 1979). When heme synthesis cannot be completed, a relative excess of unutilized proto accumulates in the erythrocyte and is often described as "free". In fact, however, most proto is chelated with zinc and will exhibit fluorescence; proto bound

to iron is not fluorescent (Lamola and Yamane, 1974). Zinc-complexed proto has been shown to constitute about 90% of the total blood proto in clinically normal subjects, with only 10% being free (Schwartz et al., 1980). Zinc-proto may be bound to hemoglobin at unoccupied heme binding sites (Lamola et al., 1975). Thus, although the term "FEP" has long been used to describe the porphyrins extracted with acidic solvents, it is a misnomer. Usual methods for its assay actually measure any nonferrous porphyrins in the blood.

Reported FEP Concentrations

Reported FEP concentrations for normal and iron-deficient subjects are given in Tables A1 and A2, respectively. Micromethods generally give better recovery (>85%) and hence higher values than do macromethods (Chisholm and Brown, 1975; Heller et al., 1971; Piomelli, 1973; Tables A1 and A2). The customary unit ($\mu\text{g FEP/dl}$ packed RBCs) corrects for anemia, as does the ratio of FEP/hgb ($\mu\text{g FEP/dl blood} \div \text{g hgb/dl blood}$). Guidelines call for reporting results as $\mu\text{g FEP/dl}$ whole blood (Center for Disease Control, 1975, as cited by Chisholm and Brown, 1975). The latter units would be helpful for comparison of results in those instances where hematocrit data are not available to calculate results in the customary units (i.e., $\mu\text{g/dl RBCs}$), and to calculate the FEP/hgb ratio. Results reported as $\mu\text{moles FEP per dl RBCs}$ or whole blood can be converted to $\mu\text{g/dl}$ units ($1 \mu\text{mole FEP/dl} = 5.63 \mu\text{g/dl}$; McLaren et al., 1975).

Table A1. Reported FEP concentrations for seemingly healthy children and adults

Reference	Method	Subjects' Age	Sample Size	\bar{x} (\pm SD)	Range of Individual Values	
					$\mu\text{g FEP/dl RBCs}$	
Cartwright et al., 1948	macro	adult	64	31	13	-140
Dagg et al., 1966	macro	adult	30	15.5 (8.3)	6	- 35
Langer et al., 1972	macro	adult	20	39 (14)	16	- 67
Pagliardi et al., 1959	macro	adult	30	36.5	15	- 62
Chisholm and Brown, 1975	micro	adult	34	52 (14)	---	
J. Cook et al., 1976	micro	adult and children	1564	79.6	---	
Klopfenstein, 1982	micro	elderly	76	92 (25)	46	-160
Koller et al., 1978	micro	10-14 yrs	57	64 (13) ^a	<90 ^a	
McLaren et al., 1975	micro	adult	15	76 (24)	46	-141
Piomelli, 1973	micro	adult	48	47 (15)	22	- 87
Schwartz et al., 1980	micro	adult and children	several hundred	---	40	- 80
Stockman et al., 1975	micro	adult	70	50 (20)	8	-120

^aValue was converted from reported units of $\mu\text{moles FEP/dl RBCs}$ using formula: $1 \mu\text{mole FEP/dl} = 5.63 \mu\text{g FEP/dl}$.

Table A1. Continued

Reference	Method	Subjects' Age	Sample Size	\bar{x} (\pm SD)	Range of Individual Values
Walsh and Fredrickson, 1977	micro	adult	---	38	<55
present study	micro	adult	13	65 (17)	35 -111
<u>μg FEP/g hemoglobin</u>					
Klopfenstein, 1982	micro	elderly	73	2.73 (0.8)	1.4- 5.3
Piomelli et al., 1976	micro	1-6 yrs	100	2.27 (0.8)	---
		1-13 yrs	35	2.0	---
		adult	48	1.69 (0.7)	---
Thomas et al., 1977	micro	13 mos.	20	1.9 (0.4)	1.0- 2.7
present study	micro	adult	13	1.96 (0.5)	1.1- 3.2
<u>μg FEP/dl blood</u>					
Klopfenstein, 1982	micro	elderly	76	38.5 (10.1)	17 - 66
present study	micro	adult	13	25.7 (6.5)	14 - 42

Table A2. Reported FEP concentrations for iron-deficient subjects

Reference	Method	Subjects' Age	Sample Size	Iron Status	\bar{x} (\pm SD)	Range of Individual Values	
						$\mu\text{g FEP/dl RBCs}$	
Cartwright et al., 1948	macro	adult	13	anemia	207	99	-475
Dagg et al., 1966	macro	adult	15	sideropenia	93 (60)	15	-230
			27	anemia	159 (97)	28	-490
Langer et al., 1972	macro	adult	16	anemia and sideropenia	136	83	-457
		-	192 (literature)	-	201	---	---
Klopfenstein, 1982	micro	elderly	21	sideropenia	98 (42)	55	-255
Koller et al., 1978	micro	7-14 yrs	25	anemia and sideropenia	---	>101 ^a	
McLaren et al., 1975	micro	adult	14	anemia and sideropenia	175 (62)	---	
Stockman et al., 1975	micro	adult	52	anemia and sideropenia	167 (117)	18	-700
Walsh and Fredrickson, 1977	micro	adult	12	anemia	266	122	-548
						$\mu\text{g FEP/g hemoglobin}$	
Klopfenstein, 1982	micro	elderly	21	sideropenia	2.9 (1.4)	1.7-	8.3

Piomelli et al., 1976	micro	1-13 yrs	33	sideropenia	3.3-5.3	---
		1-13 yrs	51	anemia	5.4-9.9	---
Thomas et al., 1977	micro	14 mos.	20	anemia	10.4 (6.2)	4.5- 30.4
					<u>µg FEP/dl blood</u>	
Klopfenstein, 1982	micro	elderly	21	sideropenia	40.4 (12.3)	24 - 71

^aValue was converted from reported units of µmoles FEP/dl RBCs using formula: 1 µmole FEP/dl = 5.63 µg FEP/dl.

Reference Values

Several FEP concentrations, ranging from 30 to 160, have been suggested as arbitrary cut-off points to divide normal from abnormal values. Unfortunately, FEP units, supporting data, and instances where these standards apply are not always specified. FEP is normally measured in whole blood but results are expressed relative to packed RBCs (i.e., hematocrit). When results are reported only as μg FEP/dl, sometimes one cannot determine whether values are for volumes of blood or packed cells. The following discussion summarizes studies that provide supporting data for and against some of the standards suggested in the literature. The seemingly best FEP standards are presented in Table A3, and can be used to evaluate the implications for iron status that FEP results give. Caution is advised in the use of these data, since two or more iron parameter values should be outside normal ranges before the cause reasonably can be attributed to iron deficiency (Cook and Finch, 1979). Individual values for anemic and sideropenic patients often fall within "normal" ranges, and conditions other than iron deficiency can cause elevated FEP levels.

Data from several sources support the idea that the maximum value which can be regarded as indicating normal iron status for an individual is 100-110 μg /dl RBCs. First, McLaren et al. (1975) concluded that the upper limit of normal for patients with adequate bone marrow aspirate iron was 94 μg FEP/dl RBCs; 95% confidence interval levels were 80-110. Also, computer analysis of 4000 blood specimens assayed for FEP by Heller et al. (1971) indicated that the upper limit of normal was 100 μg /

Table A3. Proposed standards for interpreting FEP values with regard to iron status

FEP Unit	Iron Status Category ^a	
	Probably Normal	Probably Anemic
μg/dl RBCs	100 (maximum) 70 - 80 (typical)	>160
μg/dl blood	29 - 34 ^b (median)	> 60
FEP/Hgb ratio, $\frac{\mu\text{g/dl blood}}{\text{g/dl blood}}$	< 2.8 ^c	> 5.5

^aFEP values falling between those listed indicate probable sideropenia.

^b Assuming a \bar{x} hematocrit of 42%.

^c Assuming hemoglobin ≥ 12 g/dl.

dl RBCs. Thirdly, when 100 was used as the cut-off point for normality, 17.5% of 1564 adults and children could be classified as iron-deficient, which is similar to the percentage of subjects who had transferrin saturation <15% (14.2%) or serum ferritin levels <12 µg/dl blood (13.5%) (J. Cook et al., 1976). Fourth, two standard deviations above mean values that were reported by Chisholm and Brown (1975), Koller et al. (1978), Piomelli (1973), Stockman et al. (1975), and in the present study correspond to 77, 90, 80, 90, and 99 µg FEP/dl RBCs, respectively. By definition, an expected 95% of the subjects would have had concentrations below these levels.

Median values for groups of healthy people must be clarified since conflicting data have been reported. Comparison of 9 studies that involved almost 2000 seemingly healthy subjects and that determined FEP by micromethods (Table A1) shows that mean values range from 38 to 92 µg/dl RBCs, with the calculated average being 63. Similarly, Heller et al. (1971) reported a median value of 75 µg/dl RBCs for 4000 blood specimens. The mean value was 86 µg/dl RBCs and the range for individual values was 40-900. But subjects were not described, and since the maximum value was very high, an unknown percentage of people with health problems must have been included in the group. Based on the above data, 70-80 µg/dl RBCs are suggested (Table A3) as being representative values for normal FEP concentrations in groups of healthy subjects (but not as an upper limit cut-off point for normality).

Baker and De Maeyer (1979) credit the World Health Organization with setting >70 µg FEP/dl as an index suggesting probable iron

deficiency. Units were not given; most likely, $\mu\text{g}/\text{dl}$ RBCs was intended. However, if this level had been used as the cut-off point of normality for an individual value, approximately 75% of the seemingly healthy population ($n=1564$) studied by J. Cook et al. (1976) would have been classified as iron-deficient. A standard of $>70 \mu\text{g}/\text{dl}$ blood would have been too high, since only very anemic subjects would have blood values that high.

Units were not clearly identified by another group (Cook and Finch, 1979), who presented $30 \mu\text{g}/\text{dl}$ as the normal FEP level and $100 \mu\text{g}/\text{dl}$ as indicating iron deficiency. Most likely, $\mu\text{g}/\text{dl}$ RBCs was the intended unit for the value of 100. Supporting data for this standard come from Cook's own laboratory (1976), and also other groups, as was already discussed. If $100 \mu\text{g}/\text{dl}$ blood were to be considered as the upper limit cut-off point for normality, then values for μg FEP/dl RBCs would be extremely high (e.g., $270 \mu\text{g}/\text{dl}$ RBCs, assuming hematocrit = 37%). The highest value shown by J. Cook et al. (1976) was $120 \mu\text{g}/\text{dl}$ RBCs. Since $270 \mu\text{g}/\text{dl}$ RBCs is not a practical standard for dividing normal from abnormal FEP concentrations, the intended reference concentration must be $100 \mu\text{g}/\text{dl}$ RBCs.

In contrast, $\mu\text{g}/\text{dl}$ blood was probably the intended unit for the normal value of 30 (Cook and Finch, 1979). Results presented by Cook in a previous publication (J. Cook et al., 1976) form the basis of discussion in the paper by Cook and Finch (1979). Their 1976 data show that $<2\%$ of 1,564 subjects had FEP values $<50 \mu\text{g}/\text{dl}$ RBCs. Only 9% of their subjects were iron-deficient and 4% were anemic, so it seems clear that

30 $\mu\text{g}/\text{dl}$ RBCs cannot be considered a normal mean value. Further, only 2 of 6 studies by other research teams using micromethods reported an individual FEP value below 30 $\mu\text{g}/\text{dl}$ RBCs (Table A1).

The problem of different intended units for normal and iron-deficient standards also occurred in the review by Conrad and Barton (1981). These authors used 30 $\mu\text{g}/\text{dl}$ as the level for normal, iron depletion, and prelatent iron deficiency, 75 for latent iron deficiency, and >100 for iron-deficient erythropoiesis and anemia. It can only be assumed that they meant 30 $\mu\text{g}/\text{dl}$ blood and 75 or 100 $\mu\text{g}/\text{dl}$ RBCs.

Sideropenia probably increases group mean values to above 100 $\mu\text{g}/\text{dl}$ RBCs. A limited amount of data supports this standard. Minimum values of iron-deficient subjects in two studies (Koller et al., 1978; Walsh and Fredrickson, 1979) were 101 and 122. The mean in a group of 21 elderly men where 35% had hgb <14 g/dl (\bar{x} = 14.5) was 98 μg FEP/dl RBCs (Klopfenstein, 1982). The range for individual values of sideropenic or anemic patients varies considerably (Table A2). Contrary to criteria set forth by Piomelli et al. (1976), it is not justified to state an upper limit for FEP concentrations to discriminate between iron deficiency, lead intoxication, and erythropoietic protoporphyria (Koller et al., 1978).

Anemic patients had mean values of >160 $\mu\text{g}/\text{dl}$ RBCs in three studies (Table A2). This level is used by Piomelli et al. (1976) as a cut-off point for normality. While 160 $\mu\text{g}/\text{dl}$ RBCs is a useful minimum standard for anemia, this value is too high to be used as an expected value for normal subjects. FEP cut-off points for anemia used in Piomelli's

laboratory are: $>60 \mu\text{g FEP/dl blood}$ and $>5.5 \mu\text{g FEP/g hgb}$ (Davidow et al., 1976; Piomelli et al., 1976). These standards are consistent with reported values (Table A2).

More data are needed to verify reference values for specific populations according to age, sex, and health status, although no consistent variation in FEP has been related to age or sex differences thus far.

APPENDIX B. MEASUREMENT OF FEP

Materials

A. Supplies

1. 100 ml volumetric flasks [washed withalconox detergent, rinsed with distilled water, soaked in concentrated nitric acid several hours, and rinsed with double-distilled (Millipore purification system) water]
2. Disposable chemically clean 13 x 100 mm borosilicate culture tubes (Fisher Scientific Co., 711 Forbes Ave., Pittsburgh, PA)
3. 20 μ l capillary tubes (Unopette, Becton-Dickinson Co., Curtin-Matheson Scientific, Inc., 1850 Greenleaf Ave., Elk Grove Village, IL)
4. Filter paper (PKU blood collection cards, No. 160-C, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO)
5. Disposable plastic tips for automatic pipettes
6. Disposable glass Pasteur pipettes
7. 10 ml T.D. glass pipette

B. Apparatus

1. Vortex (Buchler vortex-evaporator; holds 56 tubes)
2. Centrifuge
3. Automatic pipettes
 - a. Dispensette (Brinkmann Instruments, Ltd., Rexdale, Toronto, Canada)

- b. Gilson Pipetman, variable, 1000 μ l (Rainin Instrument Co., Inc., Mack Rd., Woburn, MA)
- c. Finnpiquette, variable, 5 μ l (Markson Scientific, Inc., Box 767, Del Mar, CA); used to deliver 2.5 μ l aliquots
- d. Drummond calibrated dilamatic microdispensers (10, 25, 50, 100 μ l) (Drummond Scientific Co., 500 Parkway, Broomall, PA)
5. Spectrophotometer (Gilford Instrument Lab., Inc., Oberlin, OH)
6. Aminco-Bowman ratio spectrophotofluorometer (SPF) equipped with a 1P21 photomultiplier tube, xenon lamp, ellipsoidal condensing system, and recorder (American Instrument Co., Silver Spring, MD)
7. SPF matched, chemically clean, four-sided cuvettes, fused silica, 5 ml volume (American Instrument Co.)

C. Reagents (all keep indefinitely)

1. Celite/sterox/saline suspension. Combine 4.5 g NaCl, 500 ml double-distilled water, 25 g celite, and 0.25 ml sterox SE (Harleco, Arthur M. Thomas Co., Vine St. at Third, P.O. Box 779, Philadelphia, PA). (Store in a dark bottle.)
2. Ethyl acetate/glacial acetic acid 3:1 (V:V) solution. Combine 750 ml ethyl acetate and 250 ml glacial acetic acid (ACS reagent-grades) in an aluminum foil-wrapped Dispensette calibrated to deliver 2-ml aliquots. (Check purity of ethyl acetate by combining 1 ml of protoporphyrin IX standard (500 ng/ml) with 9 ml HCl (reagent 3) or 9 ml sat. HCl (reagent 4)). If former

solution fluoresces more than the latter solution, use a different lot of ethyl acetate (Davidow et al., 1976).

3. 1.5 N HCl solution. To 124.2 ml 37% HCl (ACS reagent-grade; density = 1.00045), add double-distilled water to make 1 liter. (Store in Dispensette calibrated to deliver 2-ml aliquots.)
4. Saturated HCl solution. Combine 125 ml solvent (reagent 2) and 500 ml HCl (reagent 3) in a separatory funnel. Invert 1 min. Let stand until phases separate. Discard upper solvent phase. (Store in glass-stoppered aluminum foil-wrapped bottle.)

D. Standards (use within expiration date listed with each lot)

1. Coproporphyrin. Dilute copro III stock solution (500 ng/ml) (PFS-9, Porphyrin Products, P.O. Box 31, Logan, UT) to obtain a range of concentrations (0.5, 1.0, 1.5, and 2.0 ng/ml): work in a darkened room and use Drummond pipettes; deliver 100, 200, 300, or 400 μ l copro stock solutions (at room temperature) into 100 ml volumetric flasks; make to volume with sat. HCl (reagent 4), invert \geq 15 times, and keep in glass-stoppered, aluminum foil-wrapped flasks. (Store all copro solutions at 8°C.)
2. Protoporphyrin. Dissolve proto IX standard (5 μ g/tube) (PFS-9, Porphyrin Products, P.O. Box 31, Logan, UT) by adding 100 μ l "protosolv" (supplied with the standard) using a Drummond pipette and shaking it 10 min in a Buchler vortex-evaporator. Using a glass pipette, add 10 ml HCl (reagent 3) to the standard and vortex 15 sec. The resulting proto stock solution should

have a concentration of about 500 ng/ml. Concentrations of proto stock solutions can be verified after reconstitution by calculation using spectrophotometric analysis and the formula:

$$\text{FEP (ng/ml)} = \frac{\text{absorbance at 408 nm}}{\text{millimolar absorptivity} \times \text{formula weight}}$$

(Chisholm and Brown, 1975). The millimolar absorption coefficient (ϵ_{mm}) used in this study was 241 and the formula weight of proto IX was 562.27 (NAS-NRC, 1972). Good agreement (e.g., 492 ng/ml versus 500 ng/ml) was obtained between calculated and specified concentrations, respectively. Proto solutions should be handled in darkened rooms in aluminum foil-wrapped tubes and used <2 hrs after reconstitution.

In this study, a proto standard curve was constructed based on the fluorescence of 7 concentrations of proto standard and based on 180 proto disc extracts processed in 8 different batches. Reconstituted proto standard solution (500 ng/ml) was expelled into the bottom of a tube directly onto a blank, torn disc. Volumes of proto standard solutions used were 2.5, 5, 7, 10, 15, 20, and 25 μl (corresponding to 6.25, 12.5, 17.5, 25, 37.5, 50, and 62.5 μg FEP/dl blood, respectively, since 20 μl blood spots were used). Finn and Drummond micropipettes gave the best results of those pipettes tried. Proto standard discs were processed following the normal FEP assay steps. The slope of regression between fluorescence and FEP concentration was determined, using a programmable Wang calculator, to be:

$$\text{FEP } (\mu\text{g/dl blood}) = \frac{\text{RIU} + 1.507}{1.28} \quad (r = 0.95, \text{SEM} = 7.5) \quad .$$

Procedure

A. Blood collection

1. Sample blood. Using 20 μl capillary tubes, collect finger-prick blood samples (warm-hand) in duplicate or triplicate and deliver them onto a small area of filter paper. After air-drying them, place samples in a plastic bag and keep them in a closed box at room temperature (to avoid exposure to air and light, which reduces stability of FEP). Deterioration of samples was not evident over any of the storage periods used in the present study (up to 15 months).
2. Quality control blood. Use a K_3 EDTA-treated vacutainer to collect one large aliquot of venous blood, from which 20 μl aliquots should be spotted onto filter paper. Spot enough control blood discs to process 2 or 3 with each batch of proto standard discs or sample blood discs. EDTA anticoagulated blood remains stable for FEP 6 to 8 wks if stored at 8°C (Chisholm and Brown, 1975). Freezing can cause changes that interfere with proto extraction, so the blood should not be stored frozen, according to Labbe et al. (1979). Nevertheless, proto was reportedly stable in frozen blood when whole blood samples were measured immediately after thawing (Chisholm and Brown, 1975).

B. FEP assay steps

1. Cut blood-soaked and blank discs out of the filter paper cards using the same pair of scissors for all. This can be done in advance and discs can be stored in Ziploc bags along with an identification paper.
2. Perform assays in a darkened room and wrap tube racks in aluminum foil to minimize exposure to light. Process samples in batches of 20 to 30 tubes, including all of one person's samples in the same batch if possible. Two or 3 blank and 2 or 3 control blood discs should be run with each batch of proto standard or sample blood discs.
3. Set up the SPF (see section C). Label 2 sets of tubes. Tear discs into 4 separate pieces and drop into tubes.
4. Deliver 0.3 ml of the celite/sterox/saline suspension (reagent 1), using a 1 ml Gilson Pipetman, to the bottom of each tube. (Avoid contact with upper portion of tube, if possible.) Shake suspension frequently.
5. Shake tubes in Buchler vortex-evaporator 30 min on speed "5.5" (vigorously).
6. Add 2 ml 3:1 solvent (reagent 2) with Dispensette and shake tubes 20 sec.
7. Centrifuge tubes at approximately 1800 x g 3 min.
8. Decant extract into a clean tube by gradually inverting and draining tube for 10 sec, then drawing off any residual extract with a twisting motion. Discard emptied tube.

9. Add 2 ml 1.5 N HCl (reagent 3) with Dispensette and shake tubes 20 sec.
10. Centrifuge tubes at approximately 1800 x g 2 min.
11. Remove upper solvent phase by aspiration.
12. Transfer aliquot of lower acid phase into a cuvette using disposable Pasteur pipettes.
13. Read and record fluorescence (relative intensity units, RIU) after calibrating the SPF (see section D).
14. Calculate FEP concentration in sample blood using a regression equation calculated from proto standard curves.

C. Daily set up steps for spectrophotofluorometer (SPF)

1. Turn power and blower switch on.
2. Close PM shutter and slit slide (up positions).
3. Turn xenon lamp on. (Verify by checking for green light in cell compartment).
4. Turn microphotometer and recorder on.
5. Allow SPF to stabilize ≥ 15 min. (After several days of non-use, allow several hours.)
6. Set sensitivity to "zero adjust", vernier to "100", blank subtract to "10", response to "1", chopper to "on", HV control to "manual", and kilovolts to "700".
7. Turn zero adjust until meter reads zero.
8. Set sensitivity to "0.1" and turn blank subtract fine until meter reads zero. (Periodically readjust as required.)

9. Align graph paper on recorder, activate "hold", and set pen to "up". Disconnect x-axis input lead from terminal, adjust x-axis zero until pen is positioned at 200 nm line, lock zero, and reconnect input lead.
10. Set emission wavelength to 200 nm (always turn dial from higher to lower numbers), and monochromators to "slow" and "emission". If pen is not still at 200 nm, adjust emission 200 potentiometer with screwdriver. Repeat, with wavelength set at 800 nm, then 500 nm. If pen is not within 498-502 nm, repeat step 10.
11. Repeat step 10, using excitation wavelength settings.
12. Repeat step 9, using y-axis knobs and positioning pen to zero line.
13. Repeat step 8, if necessary.
14. If pen is not at zero line, adjust y-axis zero to position pen, then lock zero.
15. Always keep PM shutter and slit slide closed (up positions) between measurements. Remove samples from optical unit when possible to reduce photodecomposition of FEP.
16. RIU equivalents (when vernier is constant) are:

<u>Sensitivity setting</u>	<u>Which meter to read</u>	<u>RIU</u>
0.1	upper	100
0.3	lower	10
1.0	upper	10
3.0	lower	1
10.0	upper	1

D. Daily calibration of SPF

1. Set up SPF (see section C).
2. Check that slit widths (4 mm slit = 22 nm spectral bandpass) are as follows:
 - a. 4 mm (slit) at excitation monochromator exit slit 2
 - b. 4 mm (slit) at emission monochromator entrance slit 3
 - c. 4 mm (dial) at excitation monochromator entrance slit 1
3. Set excitation wavelength to 405 nm, emission wavelength to 595 nm, sensitivity to 0.1, vernier to 100, response to 4, and monochromator to emission.
4. Turn blank subtract fine until meter reads zero.
5. Insert blank disc extract into optical unit. Open slit slide and shutter (down positions). Record meter reading. Place the blank disc extract with the lowest fluorescence into the optical unit and turn blank subtract fine until meter reads zero.
6. Insert 2 ng/ml copro solution into optical unit. Open shutter and slit slide. Record meter reading. Adjust vernier until meter reads 100.
7. Record fluorescence of 0.5, 1, and 1.5 ng/ml copro solutions. Expect RIUs of 25, 50, and 75, respectively. Make fresh solutions or check for the source of error if unexpected values are obtained.
8. Repeat steps 4 and 5.
9. Set emission wavelength to 605 nm and rezero meter with blank disc extract. Read 2 ng/ml copro solution and record meter

reading. Use this lower RIU as the new calibrating RIU setting.

10. Record fluorescence of control blood disc extracts. In the present study, there was a range of only 10 RIUs in meter readings for control blood with all batches. If unexpected values are obtained, check for the source of error and consider that other samples processed in that batch may give false values.
11. Record fluorescence of sample blood or proto disc extracts. Rezero meter with blank disc extract and readjust vernier with 2 ng/ml copro solution to calibration setting (step 9) between sets of readings; actual frequency is conveniently determined by the number of cuvettes being used.
12. Aspirate cuvettes (except blank disc extract and 2 ng/ml copro solution) and rinse with double-distilled water, then acetone, between readings of extracts from different sources.

E. Results of tests of the FEP procedure

1. The celite/saline/sterox suspension caused increased blank fluorescence compared to extracts without it.
2. Disc extracts had increased blank fluorescence compared to extracts without a disc.
3. Torn discs adhered to the bottom of tubes better and obstructed less solvent than did whole discs, thus giving higher fluorescence yields.

4. Spectrophotometric determination of the concentration of proto and copro standard solutions (500 ng/ml) was not considered essential since standard solutions were diluted considerably before actually being used. Dilute concentrations could not be assayed spectrophotometrically.
5. An extraction solvent mixture of 3:1 gave higher fluorescence yield compared to a 4:1 mixture, in accordance with results of Chisholm and Brown (1975).
6. A combination extraction and wash step (step 6, section B) gave higher fluorescence compared to separate steps (adding 1 ml of reagent 2, centrifuging, decanting, washing with 1 ml ethyl acetate, and decanting again), in contrast to results of Chisholm and Brown (1975).
7. Procedures that use 1 ml acid (Chisholm and Brown, 1975; Granick et al., 1972) were not suitable since we needed >1 ml aliquots for our cuvettes.
8. Unspotted fresh blood gave higher fluorescence yield than did fresh blood spotted onto discs.
9. A 30 min shaking time of blood discs (step 5) gave higher fluorescence yield than did a 15 min shaking time for blood discs older than 6 months. For fresh blood discs, there was no advantage to the longer shaking period.
10. Quality control blood discs processed in 21 batches (along with both proto discs and sample blood discs) showed that the coefficient of variation (CV) in the present study was 6%. This

CV includes both within-day and day-to-day variation. These results compare favorably with other studies, where the CV was 2.7-5% (Chisholm and Brown, 1975) and 15% (McLaren et al., 1975).

APPENDIX C. SUBJECTS' RECORDS

Informed Consent

Purpose

The purpose of this research is to compare several indices of iron-deficiency anemia over time in adult women participating in a moderate exercise program, and to estimate from food diaries nutrient intakes of the participants.

Procedures

You will be asked to sign this form and fill out an information sheet. You may refuse to answer questions if you desire.

Blood samples will be taken by finger puncture 6 times throughout the study. The amount of blood collected is about 10 drops. This will feel like a good pin prick would. You will be asked not to exercise immediately before the blood sample is taken.

You will be asked to record the foods you eat (type and amount) 3 days each week for 9 weeks. Forms will be provided to you for this purpose.

You will be asked to take the exercise clinic's fitness test at the beginning and the end of the study.

You will be asked to complete the exercise clinic's activity records each day you attend the clinic.

Benefits

At the end of the study, you will be told the results of blood analyses (including your hematocrit, hemoglobin, and red blood cell levels) and you will receive a nutrient analysis of your diet. Also you will have helped us gain a better understanding of the effects of exercise on various blood and dietary variables.

All data will remain confidential. No individual's name will be used in describing the data. You may ask questions or discontinue participation at any time if you so desire. However, we do encourage you to follow through so that our data are meaningful.

I have read the above statement and voluntarily agree to participate.

Name _____

Date _____

Information Sheet

1. GENERAL

Date _____

Name _____

Birth date _____ Age _____

Height _____ Weight _____

2. BLOOD LOSSES

Do you menstruate? Yes _____ No _____

What is the usual time span (in days) between your menstrual cycles? _____

How many days does your "period" usually last? _____

Are you currently taking oral contraceptives? Yes _____ No _____

Are you pregnant? Yes _____ No _____

Are you lactating? Yes _____ No _____

How many children have you borne? _____

Have you donated blood in the past two years? Yes _____ No _____

If yes, when? _____

Do you plan to donate blood in the next two months? Yes _____ No _____

3. DIETARY HABITS

Do you take vitamin or mineral supplements? Yes _____ No _____

If yes, specify kind and amount _____

Has your diet changed recently? Yes _____ No _____

If yes, specify _____

Do you intend to vary your normal eating pattern in the next two months?

Yes _____ No _____

If yes, specify _____

Year _____ (1-4)

RDA Group _____

FOOD RECORD

Name _____

ID# _____ (5-8)

_____ (35-36)

Date of Record _____ Day of Week _____

(22-23) Day Code	(24) Meal Code	(25-30) Food Code	(31-34) Size Code	Food Item and Description/Ingredients	Amount Eaten	Time of Day

91

Instructions for Food Intake Records

1. PLEASE EAT AS YOU DO ORDINARILY

This is a very important point because we are interested in your usual food habits and eating pattern. 92

2. Write down everything you eat and drink using the food intake record forms provided. Write down your name, date, and day of week on each sheet. Use a separate line for each food item you eat and as many sheets as you need for each day. Record time of day when meal or snack was eaten.

3. Describe foods eaten as accurately as you can. Use brand names to clarify descriptions. Indicate method of preparation. For example:

Fruit juice: Orange, grape, tomato, grapefruit, V-8, Hi-C, Tang; fresh, frozen and reconstituted, canned, reconstituted powder

Cereal: Oatmeal, Wheaties; cooked, dry; with sugar added (granulated or brown); with whole milk;

Bread or toast: White, whole wheat, cracked wheat, rye; homemade; dry; with butter, margarine, grape jelly

Milk: Whole, skim, 2 percent, reconstituted non-fat dry milk; chocolate.

Coffee or tea: Black, with cream (half and half, Coffee Mate) or sugar.

Mixed Dishes and Drinks: Give name of dish or drink and list the amount of ingredients consumed. If you give the recipe, indicate how much it makes. Be specific (if condensed soup, was it diluted with milk or water, etc.)

Fruits and vegetables: Raw or fresh, canned, frozen; plain, with butter, margarine, white or cheese sauce, sugar

Meats and fish: Fried, breaded, broiled, baked; trimmed of separable fat; cod, haddock, etc.

4. Estimate amounts eaten as carefully as you can and record amounts in household measures as suggested below:

Household amounts

Beverages (fruit juice, milk, tea, coffee, soft drinks, liquor, etc.)

Measuring cups or ounces

Breakfast cereals, cooked vegetables, canned fruits, gravies, sauces, ice cream, nuts, snack foods

Measuring cups or tablespoons

Meats, cheeses, cake, pizza

Ounces or inches
Example: 4" x 2" x 1/2"

Foods in small amounts (margarine, butter, sugar, grated foods, cream, etc.)

Teaspoons or tablespoons
1 pat butter = 1 teaspoon

Bread, packaged luncheon meats

Slices

Rolls, cookies, crackers, fresh fruits, boiled potatoes, etc.

Small, medium, large or inches

5. If you begin using a vitamin or mineral supplement during this study, please specify the kind and amount on your food intake record.

6. Please keep a food diary every Mon., Thurs., and Sat. throughout the study. If you have questions, feel free to call me. Mary Higgins, 294-8499 or 232-7246.

Year _____ (1-4)

RDA Group _____

FOOD RECORD

Name Mary Higgins

ID# _____ (5-8)

_____ (35-36)

Date of Record 9-11-80 Day of Week Thurs.SAMPLE

(22-23) Day Code	(24) Meal Code	(25-30) Food Code	(31-34) Size Code	Food Item and Description/Ingredients	Amount Eaten	Time of Day
				reconstituted frozen orange juice	4 oz.	7:00 am
				cooked oatmeal	1 c.	
				with brown sugar	1 t.	
				+ whole milk	1/4 c.	
				cracked wheat toast	1 slice	
				with margarine	1 t.	
				+ grape jelly	1 t.	
				black coffee	2 c.	
93				coffee with whole milk (1T.)	1 c.	10:15
				cracked wheat bread	2 slices	noon
				chicken salad	1/2 c.	
				(cubed chicken 1 c.		
				diced celery 1/2 c.		
				pickle relish 1 T.		
				Miracle Whip 1 T.)		
				margarine on bread	1 T.	
				head lettuce leaves	2	
				banana	1 medium	
				skim milk	1 c.	
				light beer	12 oz.	5:30

Year _____ (1-4)

RDA Group _____

FOOD RECORD

Name Mary Higgins

ID# _____ (5-8)

_____ (35-36)

Date of Record 9-11-80 Day of Week Thurs.SAMPLE

(22-23) Day Code	(24) Meal Code	(25-30) Food Code	(31-34) Size Code	Food Item and Description/Ingredients	Amount Eaten	Time of Day
				cheddar cheese	1 oz.	5:30
				saltine crackers (2" x 2")	4	
				grilled pork chop, 1" thick	6 oz.	7:00
				+ trimmed of fat		
				with barbeque sauce	3 T.	
				canned peas	1/2 c.	
				with margarine	1/2 t.	
				cole slaw	3/4 c.	
94				(shredded cabbage, 2 c. shredded carrots 3 T. real mayonnaise 3 T.)		
				powdered reconstituted lemonade	1 c.	
				diet coke	12 oz.	10:00 p.m.
				homemade peanut butter cookie (3")	2	
				popcorn with butter	2 c.	

IOWA DEPARTMENT OF PUBLIC SAFETY

Name	_____			Sex: M F
	95			
Date	_____	_____	_____	_____
Time	_____	_____	_____	_____
Test Administrator	_____	_____	_____	_____
I. General Description				
Age (yrs., mos.)	_____	_____	_____	_____
Height	_____	_____	_____	_____
Weight	_____	_____	_____	_____
Temperature	_____	_____	_____	_____
II. Resting Cardiovascular				
Heart Rate (bpm)	_____	_____	_____	_____
Blood Pressure (S/D)	_____	_____	_____	_____
III. Muscular Strength/Endurance				
Grip (kg.)	_____	_____	_____	_____
Sit-ups (1 Min.)	_____	_____	_____	_____
Pushups	_____	_____	_____	_____
IV. Flexibility				
Sit and Reach	_____	_____	_____	_____
V. Respiratory				
Vital Capacity	_____	_____	_____	_____
FEV ₁	_____	_____	_____	_____
FEV ₁ ₃	_____	_____	_____	_____
VI. Reaction and Movement Time				
Reaction Time	L S	L S	L S	L S
Trial 1-5				
Average	—	—	—	—
Movement Time	L S	L S	L S	L S
Trial 1-5				
Average	—	—	—	—

VII. Girth Measurements

Waist	_____	_____	_____	_____
Hips	_____	_____	_____	_____
Thigh	_____	96	_____	_____

VIII. Skinfolds (mm.)

--Females (Ages 20-35)

Suprailiac (mm.)	_____	_____	_____	_____
Thigh (mm.)	_____	_____	_____	_____
Density	_____	_____	_____	_____
% Body Fat	_____	_____	_____	_____

--Females (Age 35+)

Axilla (mm.)	_____	_____	_____	_____
Thigh (mm.)	_____	_____	_____	_____
Density	_____	_____	_____	_____
% Body Fat	_____	_____	_____	_____

--Males (Age 20-35)

Chest (mm.)	_____	_____	_____	_____
Subscapular (mm.)	_____	_____	_____	_____
Thigh (mm.)	_____	_____	_____	_____
Density	_____	_____	_____	_____
% Body Fat	_____	_____	_____	_____

IX. YMCA Bicycle Test (Submaximal)

Work Load _____ Seat Height _____ R/M _____

Min.

1	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____
5	_____	_____	_____	_____
6	_____	_____	_____	_____
7	_____	_____	_____	_____

ml/kg. Min.

Fitness Level	_____	_____	_____	_____
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X. KASCH 3 Minute Recovery Heart Rate Step Test

1 Minute Recovery H.R.	_____	_____	_____	_____
Fitness Level	_____	_____	_____	_____

Time _____	#	1st			2nd			3rd			4th			5th			6th			7th			8th			9th			10th		
		M	W	F	M	W	F	M	W	F	M	W	F	M	W	F	M	W	F	M	W	F	M	W	F	M	W	F	M	W	F
NOTE																															
Limitations:																															
Heart Rate Range																															
Target HR \pm 6																															
Body Weight (lbs)																															
Warm-up Exercises: 10X or H 20s, 2X																															
Ankle Flex, Ext, Rotation																															
Groin Stretch, Hurdler																															
Toe Touch, Side Bends																															
Trunk Rotation, Twists																															
Arm Circles, Neck Rotation																															
Fwd, Back, Shoulder Shrugs																															
Wall Stretch A + B (H 20s, 2X)																															
Program:																															
Walk/Jog/Bicycle (Laps)																															
Walk/Jog/Bicycle (Mins)																															
Heart Rate (b/min)																															
Bent Leg Sit-ups																															
Quadriceps Stretch (H 30s, 1X)																															
Push-ups																															
Arm Curls (20X)																															
R + L Leg Ab/Adduction (H 3s)																															
Doggie Exercise (10X)																															
Walk/Jog/Bicycle (Laps)																															
Walk/Jog/Bicycle (Mins)																															
Heart Rate (b/min)																															
Heads Up (H 3,5,7,10s,10X)																															
Super Man/Woman (H 3,5,7,10s,10X)																															
Flutter Kick (H 3s)																															
Flat Back																															
Single Leg Pull (2X, H 20s)																															
Double Leg Pull (1X, H 30s)																															
Chorus Line (2X, H 10,20s)																															
Total Laps (Jog/Walk)																															
Swimming (Min)																															
Swim HR (b/min)																															
Weight Training																															
Key H=Hold, S=Sec, X=Rep																															