THE EFFECTS OF MODERATE EXERCISE ON DIETARY INTAKE, IRON STATUS, AND CARDIOVASCULAR ENDURANCE OF 56- TO 67-YEAR-OLD WOMEN.

by

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(ABSTRACT)

The purpose of this study was to determine the effects of moderate exercise on iron status, dietary intake and cardiovascular fitness in 56- to 67-year-old women. Women 56- to 67-years-old were randomly assigned to two groups: exercise (n=8) or non-exercise (n=9) groups. Women in the exercise group cycled on an ergometer three days/week, 30 minutes/session at 70-75% maximal heart rate for 10 consecutive weeks. At weeks 0 and 11, submaximal treadmill stress tests were obtained to determine cardiovascular fitness level. Venous blood samples were also obtained at weeks 0 and 11 to determine serum ferritin, transferrin saturation, serum iron, total iron-binding capacity, hematocrit and hemoglobin concentrations. Three-day dietary and activity records were obtained from each subject at weeks 0 and 10. Using paired t-tests for statistical analysis, the data indicated a significant increase in time to reach 70% maximum heart rate ($p<0.001$), a significant decrease in average heart rate/grade ($p<0.05$) and a significant increase in hemoglobin concentrations ($p<0.01$) in the exercise group at week 11. Student

t-tests indicated significant differences between the groups in hemoglobin concentration at week 0 ($p<0.005$) and hematocrit concentration at weeks 0 and 11 ($p<0.05$). The exercise group had significantly lower vitamin C and monounsaturated fatty acid intake than the non-exercise group at week 0 ($p<0.05$). No other significant differences in nutrient intakes were observed between or within the two groups. Caloric intakes varied widely between the two groups, ranging from 1223.0 $\pm$ 248.4 to 1533.3 $\pm$ 480.8 kcal at weeks 0 and 10 in the exercise group; 1270.1 $\pm$ 376.3 to 1348.7 $\pm$ 334.8 kcal in the non-exercise group. Intakes of zinc were less than 70% of the 1989 RDA in both groups, ranging from 56.9 $\pm$ 14.6 to 66.6 $\pm$ 16.3 percent. The results indicate that moderate exercise does not significantly change the dietary intake and all parameters of iron status but enhances the cardiovascular fitness level in 56- to 67-year-old women.

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CORRECTION: Figures 11, 12 and 14 have the wrong unit (ml/dl) for serum iron. The unit for serum iron should read mcg/dl.

CHAPTER 1

INTRODUCTION

An estimated 30 million Americans are now 65 years of age or older, representing approximately 12% of the total population [Van Camp and Boyer, 1989]. The population of older Americans is expected to increase by 20% in the next decade [Bethesda Conference 18, 1987]. Efforts to prevent the development of pathophysiological changes and/or promote desirable health status in the elderly individuals requires attention to possible physiological and pathophysiological changes with age. Especially important are nonpharmacological therapies, including diet, exercise and health-promoting lifestyles.

Elderly Americans today have led a more sedentary and well-fed lifestyle than ever before in history [Evans and Meredith, 1989]. A study by Sallis et al. [1985] concluded that only 8% of men and 2% of women 50- to 64-years-old participated in regular vigorous exercise. The authors noted that the high number of inactive elderly individuals may lead to increased body weight. Approximately 20% of the male and 40% of the female population are obese [US Department of Health, Education and Welfare, 1972]. Obesity, which is most prevalent in persons aged 40-60, is in itself a health hazard and further discourages exercise because of the greater effort of breathing and moving [Whipp and Davis, 1984].

Age affects the aerobic power (ml/kg/min) and maximal heart rate in elderly women when compared to women in their twenties. A study by Plowman et al. [1979] observed a decrease in aerobic power in the elderly women while women in their twenties maintained a constant level of maximum oxygen consumption (VO2 max). Maximum heart rate (max HR) also decreases with age, remains relatively steady during the middle years and then gradually declines at a faster rate in the 50- and 60-year-old groups. Compared with younger individuals, an older individual's cardiovascular system responds to submaximal exercise, resulting in decreased heart rate and improved aerobic capacity [Van Camp and Boyer, 1989].

The effect of moderate exercise on the micronutrient status, especially on the iron status in older women, has received little attention. Some researchers have reported a decline in erythrocyte count, hemoglobin concentration and hematocrit value with aging [Kelly and Munan, 1977], low intake of dietary iron [US Department of Health, Education and Welfare, 1972], decreased iron absorption [Freiman et al, 1963; Jacobs and Owen, 1969] and less efficient erythropoiesis [Marx, 1979] in the elderly. Aerobic exercise training induces an increase in red blood cell concentration and therefore may increase hemoglobin, hematocrit and red blood cell volume [Gabaree, 1989]. Ericsson [1979] concluded that in apparently healthy people aged 58-71 years the increase in physical work capacity during moderate training is

related to the availability of iron.

It is unknown whether exercise training affects various components of iron metabolism in elderly women. The question of whether iron requirements of physically active elderly women differ from inactive elderly women is also unknown. Therefore, the objectives of this study are:

1. To examine the changes in dietary intake with moderate aerobic exercise in women 56- to 67-years-old;
2. To investigate whether iron status in 56- to 67-year-olds is affected by regular exercise; and
3. To examine the changes in cardiovascular fitness with regular exercise.

CHAPTER II

REVIEW OF LITERATURE

Nutrient Intake of the Elderly.

The Recommended Daily Allowances (RDA) are guidelines for dietary and essential nutrients considered "to meet the known nutritional needs of practically all healthy persons" [Committee on Dietary Allowances of the Food and Nutrition Board, 1980]. The National Health and Nutrition Examination Survey (HANES III) obtained 24-hour recalls from individuals 50 years of age and older and observed inadequate dietary intake. Inadequate intake was found in folacin; vitamins D, B6 and B12; zinc; magnesium and calcium. With the expected increasing number of people aged 65 years and older, anticipated to increase to more than 22% of the United States population by the year 2050 [Evans and Meredith, 1989] (Figure 1), much research on the effect of nutrition on the health of the elderly population is needed [Schneider et al., 1986]. Estimates of the elderly who have nutritionally inadequate diets range from 10% to 90% [Wilson, 1981].

The RDA guidelines apply to the general healthy population (Table 1), not accounting for varying socioeconomic status, environmental conditions and physiological disabilities such as obesity, arthritis, heart conditions, hypertension and cancer, which may lead to loss of appetite [Siegel, 1972]. Many females live alone in their households; approximately one of three

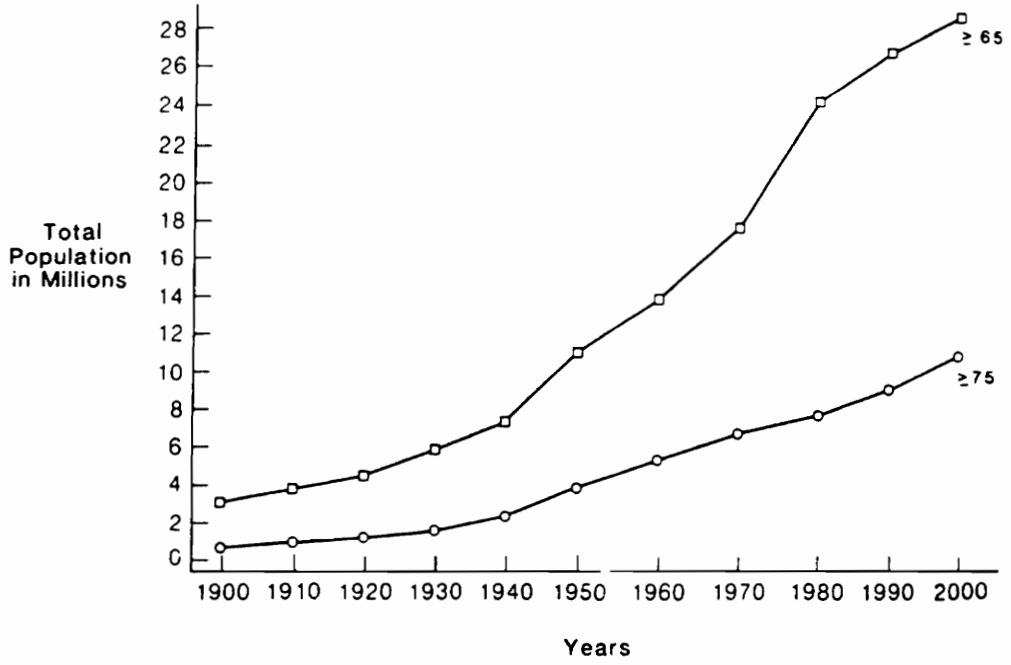


Figure 1 Actual or expected prevalence rate of people over 65 ([]) and 75 (o) years of age in the United States from 1900 to 2000 [Leon, 1987].

Table 1 Recommended Daily Allowances (RDA) for women 51+ years old [Committee on Dietary Allowances and Food and Nutrition Board, 1989].

Nutrient	Dietary Intake
Protein (g)	50.0 *
Vitamin A (mcg RE)	800.0
Vitamin D (mcg)	5.0
Vitamin E (mg alpha-TE)	8.0
Vitamin K (mcg)	65.0
Vitamin C (mg)	60.0
Thiamin (mg)	1.0
Riboflavin (mg)	1.2
Niacin (mg NE)	13.0
Vitamin B6 (mg)	1.6 **
Folate (mcg)	180.0 **
Vitamin B12 (mcg)	2.0
Calcium (mg)	800.0
Phosphorus (mg)	800.0
Magnesium (mg)	280.0 **
Iron (mg)	10.0
Zinc (mg)	12.0 **
Iodine (mcg)	150.0
Selenium (mcg)	55.0

* Higher than 1980 RDA, Ninth edition.
** Lower than 1980 RDA, Ninth edition.

females 65 years of age and older are married. Older individuals living alone are often not interested in eating alone [Blumberg, 1986]. Loneliness may result in decreased appetite and apathy towards eating, leading to poor nutritional intake [Exton-Smith, 1980; Gibbs and Turner, 1986]. Financial restriction of the elderly can also contribute to inadequate food consumption. Poverty is a common cause of poor nutrition among Americans over 65 years of age. In 1972, 42.3% of individuals 65 years of age and older had incomes less than poverty level [Siegel, 1972]; in 1980, data indicate that 16% of Americans 65 years of age and older had incomes in the poverty level [Ryan and Bower, 1989]. Ryan and Bower [1989] analyzed the relationship of socioeconomic status and nutritional intake of 268 individuals, 55 years of age or older. Intakes of calcium, iron, vitamin A and vitamin B6 were less than 67% of the RDA in 89% of the subjects (Table 2). A significant positive relationship between dietary intake and socioeconomic status was observed ($p<0.002$). Other studies utilizing large samples observed similar findings [Norton and Wozny, 1984; Learner and Kivett, 1981; Davis et al., 1985].

Physiological parameters such as obesity and physical impairment have a great effect on the well-being and longevity of the elderly population. The Ten State Nutrition Survey [US Department HEW, 1972] found 20% males and 40% females obese. Whipp and Davis [1984] found obesity to be prevalent in individuals 40- to 60-years-old. In addition to being a risk

Table 2 Nutrient intake and socioeconomic status of 268 elderly persons as percentage of 1980 RDA [Ryan and Bower, 1989]

A. General Nutrient and Socioeconomic Status

1. Nutrient

Nutrient	Average, % RDA	Range, % RDA
Calcium	76.0 ± 62.4	3-627
Iron	115.3 ± 72.3	11-400
Vitamin A	132.4 ± 145.2	1-775
Vitamin B6	38.7 ± 37.0	0-252

2. Nutrient Intake

Nutrient Intake	no.	%
High	29	11
Low	239	89

3. Socioeconomic Status

Socioeconomic Status		
High	157	59
Low	111	41

B. Relationship Between Nutrient and Socioeconomic Status

	Socioeconomic Status	
Nutrient Intake	Low	High
High	3%	8%
Low	39%	50%

factor in cardiovascular disease (CVD) and diabetes [Keys et al., 1972; Rimm et al., 1972; Van Itallie, 1979; Van Itallie, 1977], obesity is also a discouragement to exercise due to the greater difficulty in breathing, walking and other physical activities [Whipp and Davis, 1984].

The Ten State Nutrition Survey [US Department of HEW, 1972] and the Health and Nutrition Survey [Dresser et al., 1979; Abraham et al, 1979, Abraham at al., 1977] observed that individuals fifty years of age and older consumed less calories than younger adults. Energy intake decreases with age because basal metabolic rate and physical activity decrease [McGandy et al., 1966]. Several surveys in the US observed that caloric intake of the women 65 years of age and older was about 1400 kcal/day (23 kcal/kg/day); in the elderly men, 1870 kcal/day (27 kcal/kg/day) [Brown et al., 1977; McGrandy et al., 1966; Dibble et al., 1967]. In a study conducted by Debry et al. [1977], the average energy intake of elderly women was less than 1500 kcal/day. Thirty-three percent of the women were classified as "inactive"; 12% "moderately active"; and 9% "very active". The Health and Nutrition Examination Surveys I and II (HANES I and II) [US Department HEW, 1979] determined the energy intake of females aged 55-64 years of age to be 1382 kcal and 1401 kcal, respectively; 2076 kcal and 2071 kcal per day for men, respectively (Table 3). The energy intake for both females and males gradually decreased from adolescence to 64 years of age.

Table 3 Comparison of mean energy and iron intakes for A) females and B) males reported in HANES I and II [Department of HEW, 1979]

A) Mean energy and iron intakes for females.

Hanes I 1971-1974				Hanes 2 1976-1980			
Age	Energy	Iron		Age	Energy	Iron	
yr	*kcal*	*mg*	*mg/1000 kcal*	*yr*	*kcal*	*mg*	*mg/1000 kcal*
				6-11 (mo)	991	12.9	13.0
1	1207	7.2	6.0				
2-3	1412	7.2	5.1	1-2	1262	8.5	6.7
4-5	1628	8.4	5.2	3-5	1508	9.5	6.3
6-7	1829	9.6	5.2	6-8	1807	10.8	6.0
8-9	1864	9.8	5.2				
10-11	2023	10.3	5.1	9-11	1857	11.4	6.2
12-14	1932	10.4	5.4	12-14	1813	10.8	5.9
15-17	1756	9.5	5.4	15-17	1731	9.9	5.7
18-19	1739	10.0	5.8				
20-24	1691	10.0	5.9	18-24	1687	10.6	6.3
25-34	1638	10.3	6.3	25-34	1643	10.9	6.6
35-44	1558	10.4	6.7	35-44	1579	11.2	7.1
45-54	1533	10.6	6.9	45-54	1439	10.4	7.3
55-64	1382	9.8	7.1	55-64	1401	10.7	7.6
>64	1307	9.2	7.0	65-74	1295	10.2	7.9

B) Mean energy and iron intakes for males.

Hanes I 1971-1974				Hanes 2 1976-1980			
Age	Energy	Iron		Age	Energy	Iron	
yr	*kcal*	*mg*	*mg/1000 kcal*	*yr*	*kcal*	*mg*	*mg/1000 kcal*
				6-11 (mo)	1001	12.8	12.8
1	1316	7.5	5.7				
2-3	1563	8.2	5.3	1-2	1311	8.7	6.6
4-5	1826	9.4	5.2	3-5	1628	10.5	6.5
6-7	2061	11.2	5.4	6-8	1981	12.5	6.3
8-9	2173	11.3	5.2				
10-11	2261	12.7	5.6	9-11	2183	14.4	6.6
12-14	2519	13.6	5.4	12-14	2430	15.9	6.5
15-17	2981	16.3	5.5	15-17	2817	17.4	6.2
18-19	2949	16.4	5.6				
20-24	2888	16.5	5.7	18-24	3040	17.8	5.8
25-34	2739	16.7	6.1	25-34	2734	17.3	6.3
35-44	2554	15.9	6.2	35-44	2424	16.1	6.6
45-54	2301	14.5	6.4	45-54	2361	16.2	6.9
55-64	2076	13.7	6.6	55-64	2071	14.8	7.1
>64	1805	12.1	6.7	65-74	1828	14.1	7.7

The low energy intake was related to a more sedentary lifestyle.

Vegetarian dietary practices have been associated with many health benefits, including reduced blood pressure levels [Beilin et al., 1987], decreased serum total cholesterol [Sacks et al., 1986; Masarei et al., 1984], and decreased mortality from cardiovascular disease and various forms of cancer [Kahn et al., 1984; Position of the American Dietetic Association, 1988]. It is questionable whether a vegetarian diet would provide adequate nutrient intake for the elderly population. Nieman et al. [1989] compared the nutrient intake of vegetarian Seventh-Day Adventist elderly women (average age 72.2 years old) and nonvegetarian (average age 71.1 years old). Of the twenty-three lacto-ovo-vegetarians, 4% had inadequate intake of dietary iron; 4% of vitamin C; 48% of vitamin B12; 65% of folate; and 30% of calcium. Of the fourteen non-vegetarians, 7% had inadequate intakes of iron, 0% of vitamin C; 36% of vitamin B12; 71% of folate, and 50% of calcium. On the basis of group means, 67% of the RDA was met for all nutrients except for zinc and vitamin D in both groups with the addition of vitamin B6, folate and vitamin E in the non-vegetarians. This study observed that the vegetarian diet was associated with improved nutrient intake compared to the non-vegetarian diet.

Healthy persons absorb approximately 5% to 10% of dietary iron; iron-deficient individuals absorb approximately 10-20% (Shils and Young, 1988]. The Subcommittee on Iron [1979] observed

that adult males in developed countries should absorb 6% dietary iron and adult females about 12%. The maximum amount of iron absorption expected in the United States is 1) 2 mg in normal adults and 2) 6 mg in iron-deficient adults. The current Recommended Daily Allowance for dietary iron intake for females 51+ years old is 10 mg/d, assuming an absorption of 10% [RDA, 1989] (Table 1). The Ten State Nutrition Survey [US Department of HEW Center for Disease Control, 1972] observed mean dietary iron intake for elderly male and female greater than fifty-nine years of age; the intakes of low income females tend to be below the RDA (Table 4). Low mean iron intakes were associated with lower mean energy intake.

Low energy intake may also be associated with low iron intake. The typical American diet normally supplies 6 mg iron/1000 kcal. Diets providing 1400 kcal/day or less will supply approximately 9 mg of iron which is below the RDA for women 51 years of age and older. In endurance-trained middle-aged men, the energy needs were a linear function of hours spent exercising/week in the same way as for young men [Meredith et al., 1987]. This suggests that better nutrition, together with improvements in physical endurance and strength, may be achieved by increasing physical activity,

The amount of iron potentially available from foods depends on the nature of iron, the level of iron supplied and the compositon of the meal [Hallberg and Bjorn-Rasmussen, 1972;

Table 4 Comparison of iron intakes of different income groups [US Department of HEW for Disease Control, 1972].

Group	Dietary Iron	
	mg	mg/1000 kcal
1. All male	13.1	6.7
Low income white male	12.1	6.3
Low income black male	10.6	7.1
2. All female	9.6	6.8
Low income white female	9.5	6.6
Low income black female	7.8	6.7

Hallberg et al., 1974; Layrisse et al., 1974). Layrisse et al. [1968] found different levels of iron absorption among dietary heme iron compared to dietary nonheme iron. Heme iron, found primarily in animal products, is composed of ferrous or ferric iron in the center of a porphyrin ring [Pike and Brown, 1986; Monsen, 1988]. It comes from hemoglobin which contains four porphyrin units bound to the protein globin and myoglobin which contain only one ferrous porphyrin group per molecule (Figure 2). The rate of absorption is inversely related to the level of stored iron, ranging from 15% to 35%. Heme appears to be absorbed intact as iron porphyrin complex into the intestinal mucosal cell and is not dependent on intraluminal factors such as ascorbic acid, fiber and minerals. The absorbed heme iron is then released and enters the body iron pool, demonstrating an efficient process [Pike and Brown, 1986; Monsen, 1988].

Nonheme iron, found primarily in plant products, has a lower rate of absorption, ranging from 2% to 20%. The absorption rate of nonheme iron is dependent on two factors: 1) other concomitantly ingested dietary components which the nonheme iron would come in contact with in the gastrointestinal tract; and 2) the amount of iron stores. Upon digestion, the dietary nonheme iron is released in the stomach and forms a chelate complex (with ascorbic acid, amino acids or other intermediary products of digestion) which then releases the iron at the gut wall. Other studies have also observed the enchancing effect of ascorbic acid

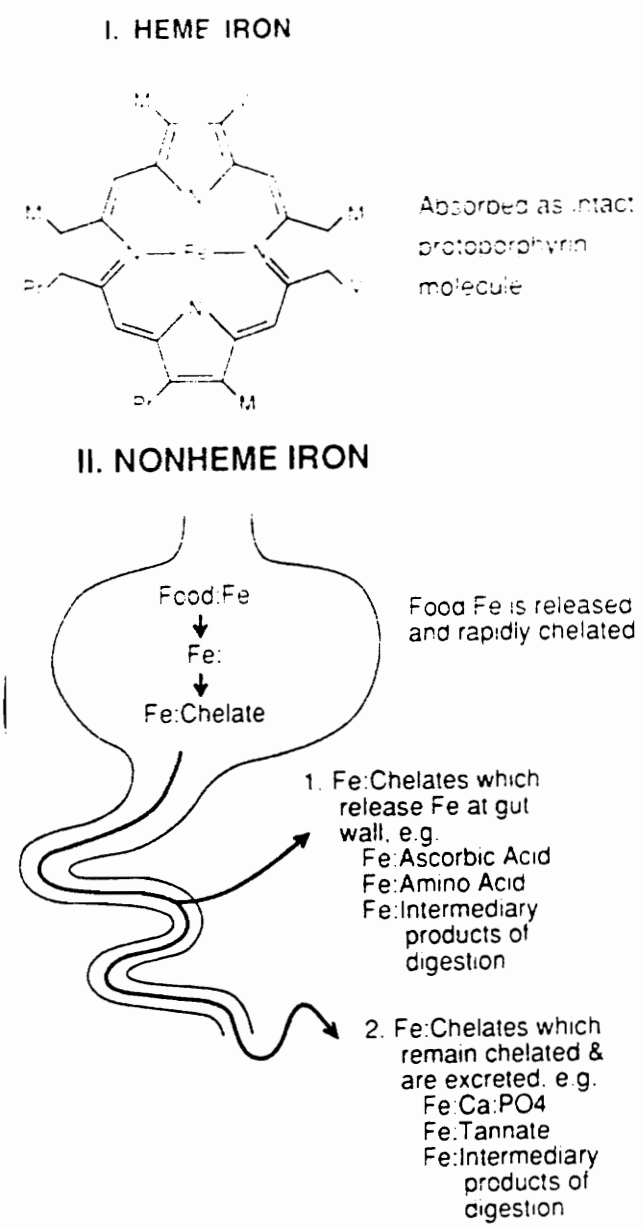


Figure 2 Schematic diagram of the absorption of the two forms of food iron: Heme and nonheme [Monsen, 1988].

on nonheme iron absorption [Cook and Monsen, 1977]. If the nonheme iron forms a chelate with calcium phosphate, magnesium supplements, phosphate salts, ethylene-diaminetetracetic acid (EDTA), tannin, or antacids, the chelate complex remains strongly bonded and insoluble, and may be excreted, decreasing iron absorption and availabilty [Monsen and Cook, 1976; Cook and Monsen, 1976; US Department of Health and Human Services, 1989; Disler et al., 1975; Hegsted et al., 1949]. Phytic acid (phytate) is considered a potent inhibiter of iron absorption. Fiber has also been shown to have an inhibitory effect. Several investigators [Jenkins et al., 1975; Bjorn-Rasmussen, 1974; Foy et al., 1959]. have reported that the substitution of whole-wheat bread for white bread or the addition of increasing quantities of wheat bran to the diet will depress iron utilization. However, because wheat bran contains both fiber and phytate, it is hard to determine which is the critical factor in reducing iron utilization. Reinhold et al. [1975] reported that phytin-free fiber can reduce iron utilization in humans, suggesting that fiber binds iron and reduces its availability.

The two-pool intrinsic tag method (heme and nonheme) has yielded estimates of food iron absorption that closely agree with estimates based on normal body iron losses. Bjorn-Rasmussen et al. [1974] administered radioactive heme and nonheme iron to 32 young men for a 6-week period. The total daily intake of iron by these men was 17.4 mg, 1 mg in the form of heme. The total

absorption from this study averaged 1.25 mg/d (7.1%). The absorption of the nonheme iron averaged 5.3%, or 0.88 mg/d whereas the absorption of the heme iron averaged 37%, or 0.37 mg/d. Layrisse and Martinez [1972] also found a higher absorption rate from heme iron (27%) vs. nonheme iron (6%), underscoring the important contribution of heme iron to the diet. However, the absorption of nonheme iron is greatly enhanced when meat, fish, poultry and foods rich in vitamin C are consumed in the same meal [US Department of Health and Human Services, 1989]. Monsen [1989] substituted 100g beef for an equal amount of egg albumin in a test meal and observed that there was a five-fold increase in total iron absorption. Thus, meat is not only a source of heme iron, but is also an enhancer of the absorption of nonheme iron.

Iron, folate and vitamin B12 are involved in maintaining adequate iron storage in the internal system, thereby influencing the rate of iron absorption. The site of erythrocyte production (erythropoiesis) is the bone marrow. The erythrocytes are formed from the bone marow stem cells called erythrocyte progenitors which contain no hemoglobin but do contain nuclei and are able to divide. After several divisions, immature erythrocytes emerge; as maturation continues, increased amounts of hemoglobin accumulate within the erythrocytes and the nuclei become progressively smaller. The mature erythrocytes leave the bone marrow and enter the general circulation for the 120 day lifespan. This maturation and growth cycle of the erythrocytes

requires specific nutrients, primarily iron, folate and vitamin B12.

Approximately seventy percent of the total blood iron is in the form of hemoglobin. As erythrocytes are destroyed, most of the released iron from the hemoglobin are returned to the liver, spleen and bone marrow to be released again for hemoglobin synthesis. Folic acid is necessary for normal erythrocyte multiplication and maturation [Luciano et al., 1983]. The Canadian Nutrition Survey [1977] indicated that the average daily folate intake of men and women sixty-five years of age or older was 151 mcg and 130 mcg, respectively, both below the 1989 RDA. A study by Garry et al. [1982] surveyed 270 healthy elderly volunteers and calculated the average folate intake from three-day dietary records. Approximately 40% received less than 200 mcg folate/day; approximately 20% received less than 150 mcg folate/day, falling below 1989 the RDA values (Figure 3). The Ten-State Survey [US Department HEW, 1972] did not observe a depletion in folic acid to be a significant factor in the hemoglobin level in women older than fifty-nine years of age (Figure 4).

Normal growth of erythrocytes also necessitates approximately one-millionth gram per day of vitamin B12, allowing the final maturation of erythrocytes. Because B12 is not synthesized in the body it is necessary to consume adequate dietary intake. Dietary deficiency of vitamin B12 leads to the

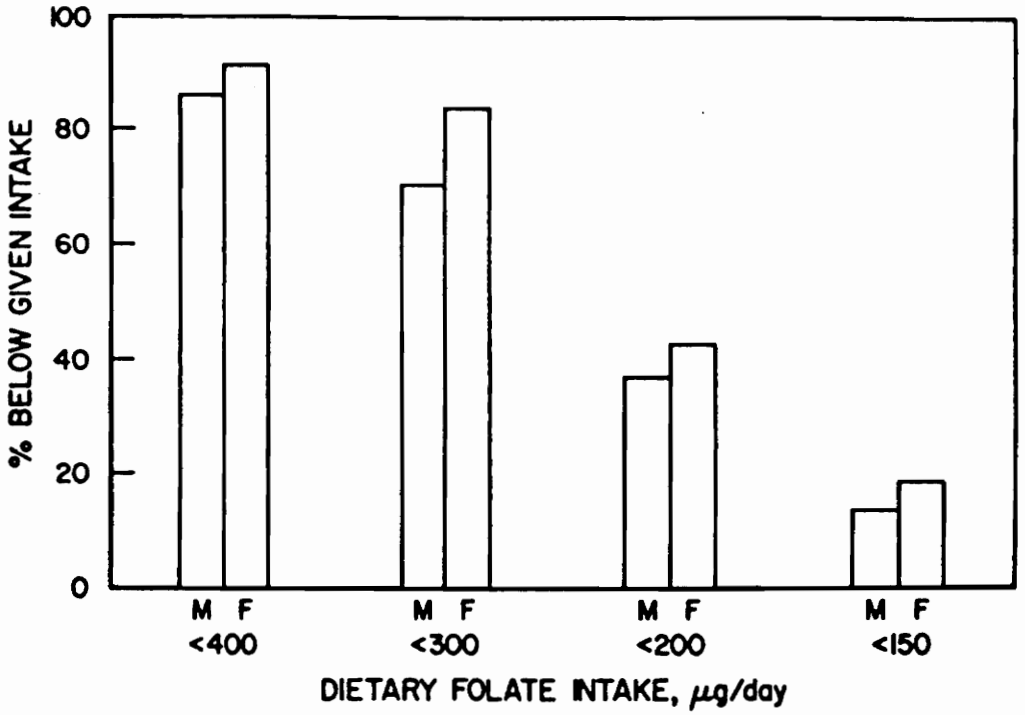


Figure 3 Folate intake from dietary sources for 125 elderly men and 145 elderly women [Garry et al., 1982].

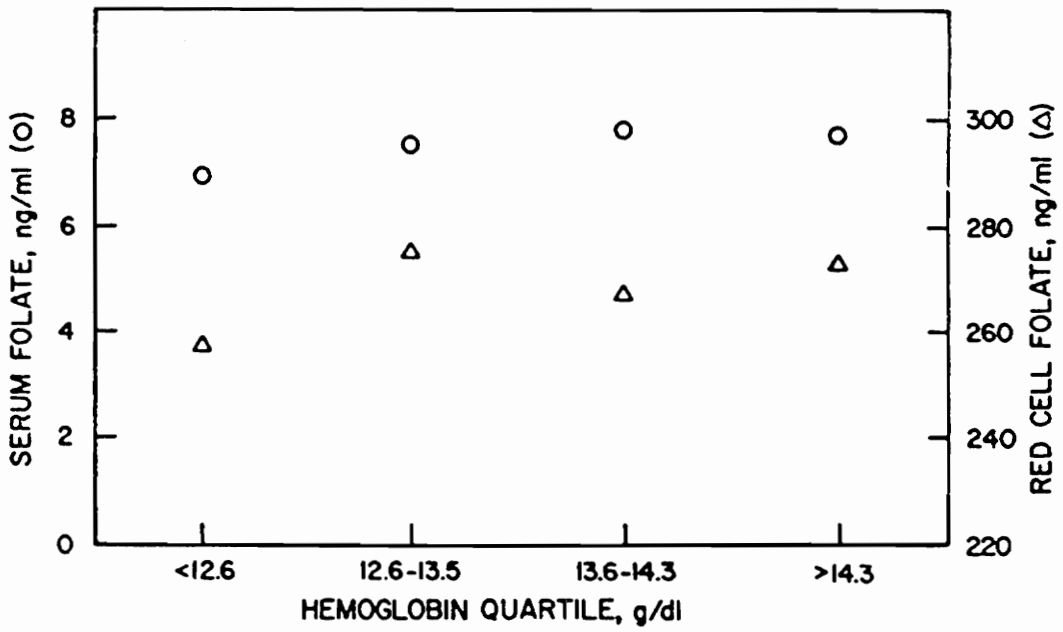


Figure 4 Circulating folate concentrations related to hemoglobin concentration for women age >59 years in the Ten State Nutriton Survey [Department HEW, 1972].

failure of erythrocyte proliferation and maturation, otherwise known as pernicious anemia. Low serum B12 has been reported in the elderly without evidence of anemia [Thompson et al, 1987].

Changes in the Digestive Tract with Aging.

Changes in nutrient digestion, absorption, metabolism and excretion in the elderly population are often linked to pathogenic or socioeconomic factors. The elderly population are more susceptible to malnourishment due to several diseases of the digestive system (Table 5). Much research has been focused on common disorders, such as dysphagia, gastrointestinal bleeding, defecation, abdominal pain and jaundice [Roe, 1987]; however, more attention and further research needs to be directed to normal physiological morphology of the gastrointestinal tract with aging.

One of the most important changes in aging is the loss of teeth [Kenney, 1982; Bowman and Rosenberg, 1983]. Half of Americans have lost all their teeth by age 65. By age 75, the proportion increased to two-thirds [Buss, 1978]. Smith [1979] conducted a survey of 254 elderly individuals and reported that only ten percent wore full dentures; 30 percent had difficulty chewing. Problems with poor-fitting dentures in the elderly may lead to restriction of dietary intake to only soft foods and liquids and avoidance of solid foods. Such diets may contain limited amounts of several micronutrients, especially minerals

Table 5 Diseases of the gastrointestinal tract of the elderly [Roe, 1987].

Location	Disease
Mouth	Nutritional stomatitis
	Candidiasis
	Leukoplakia
	Cancer
Salivary Glands	Mixed salivary tumors
	Cancer
Pharynx	Pharyngitis
	Pharyngeal paralysis
	Cancer
Esophagus	Esophageal varices
	Achalasia
	Stricture
	Progressive systemic sclerosis
	Cancer
Stomach	Hiatus hernia
	Gastric ulcer
	Acute/Chronic gastritis
	Zollinger-Ellison syndrome
	Cancer
Duodenum	Duodenal ulcer
Exocrine pancreas	Acute/Chronic pancreatitis
	Cancer
Small intestine	Tropical sprue
	Acute enteritis
	Progressive systemic sclerosis
	Amlyloidosis
	Gluten-sensitive enteropathy
	Whipple's disease
	Crohn's disease
	Malignant lymphomas
	Carcinoid tumors
Large intestine	Crohn's disease
	Ulcerative colitis
	Diverticulosis; diverticulitis
	Appendicitis
	Ischemic colitis
	Colonic polyposis
	Cancer of colon or rectum

and water-soluble vitamins.

Changes in the salivary gland morphology and function is uncertain. Kenney [1982] observed a decrease in saliva after age fifty; Schiffman [1977] associated a decrease in saliva with aging with the dehydration and thinning of the gum tissue and shrinking of the connective tissue of the mouth. Baum [1981], however, did not observe any difference in the stimulated parotid salivary flow rate in post-menopausal women.

Abnormalities in the gastroesophageal reflux have been detected in asymptomatic elderly individuals, particularly in individuals older than seventy years [Kahn et al., 1977; Kenney, 1982]. The peristaltic wave in the esophagus is not initiated by every swallow and the lower esophageal sphincter fails to relax with the arrival of each wave [Kenney, 1982]. Thus, the lower esophagus shows ring-life contractions that are nonperistaltic. This results in delayed entrance of food into the stomach, giving the illusion of substernal fullness and decreasing the amount of food consumed.

Studies of gastric mucosa of the healthy asymptomatic elderly population is another area which needs more research. Khanna et al. [1988] analyzed qualitative morphological changes in the gastric wall in aged laboratory mice. The study indicated an increase in the volume of the gastric mucosa late in the life-span (34 months), due to physiological rather than pathological factors. However, Holler [1952] and Zhulkova and Smolyanski

[1973] noted reduced thickness of the gastric mucosa and a decrease in gastric glands with aging. Palmer [1954] and Henning et al. [1957] did not observe age-related mucosal atrophy, atrophic gastritis or any generalized mucosal disease. Possible atrophy of the stomach should be further investigated because this may affect the concentration of the gastric secretions.

Kenney [1982] observed a decrease in the volume of gastric secretion after age forty. Blackman et al. [1970] observed a forty percent decrease in acid secretion between ages forty-five and sixty-five. Studies have shown significantly decreased secretion (decreased volume and concentration) of hydrochloric acid (leading to achlorhydria), intrinsic factor and pepsin [Bhanthumnavin and Schuster, 1977; Giannella et al., 1973; Fikry, 1965; Kenney, 1982; Steinberg and Toskes, 1978]. Kenney [1982] observed that pepsin secretion diminishes between forty and sixty years of age and then remains constant. These changes could result in decreased digestion and/or absorption of iron, calcium, vitamin B12, folate and protein.

Kenney [1982] did not observe a change in the volume nor the bicarbonate content of pancreatic juice secreted in response to intravenous secretion in the elderly. Snook [1974; 1975] observed significant and permanent reductions in pancreatic digestive enzyme levels in aged rats, yet no reduction in the digestion of dietary nitrogen, carbohydrates and protein.

There is no evidence that hepatic bile secretion is affected

by age but cholelithiasis is very common in the elderly. Approximately ten percent of men and twenty percent of women older than fifty-five years of age in the US are diagnosed with cholelithiasis [Kenney, 1982]. Studies have also indicated the ratio of liver weight to body weight decreases after age fifty, yet there are no age-related change in liver function in persons wtih histologically normal livers [Kampmann et al., 1975; Bhanthumnavin and Schuster, 1977].

A ten percent reduction in the serum albumin concentrations of adults older than eighty years of age was noted; however, these lower concentrations did not present any disorder known to affect albumin metabolism [Greenblatt, 1979].

Iron Deficiency Anemia.

The iron status of individuals has been the subject of considerable debate. Iron deficiency in trained athletes has been considered to be greater than in sedentary individuals [Blum, 1986]. In addition to iron-poor diets, iron deficiency may be partially attributed to impaired absorption, repeated pregnancies, chronic blood loss (e.g., hiatal hernia and gastrointestinal bleeding; in women of child-bearing age; and severe menstrual loss) [Shils and Young, 1988]. The body has three unique mechanisms for maintaining iron balance and preventing the development of iron deficiency [Hallberg, 1985]: 1) the continuous reutilization of iron from cells catabolized in the body; 2) the presence of specific storage protein, ferritin,

which makes it possible to store iron in the body to meet excessive iron demands as in late pregnancy; and 3) the homeostatic regulation of iron absorption: an increased iron absorption in the presence of iron deficiency and a decreased iron absorption in states of iron overload.

Iron deficiency exists in three stages: 1) prelatent iron deficiency or iron depletion; 2) latent iron deficiency; and 3) iron deficiency anemia [Haymes, 1987] (Table 6). The mildest form, prelatent iron deficiency, is characterized by decreased or absent storage iron. Serum ferritin measurements are often used to detect this state; the senstitive immunoradiometric assay (IRA) is often used to reflect the iron stores [Siimes et al., 1974; US Department of Health, Education and Welfare, 1972; Sturgeon and Shoden, 1971].

The latent iron deficiency state ensues after iron stores are depleted. It is characterized by reduced serum iron concentration, elevated transferrin and a decreased concentration of transferrin saturation. Although circulating hemoglobin concentrations remain within the normal range, hemoglobin synthesis is impaired by the lack of iron, causing erythrocyte protoporphyrin concentrations to increase to levels greater than 70 mcg/dl.

Overt iron deficiency anemia is reached when the restrictive hemoglobin synthesis contributes to a measurable decrease in the concentration of circulating hemoglobin or hematocrit. This

Table 6 Stages of iron depletion in progressive stages of iron deficiency [US Department of Health and Human Services, 1989].

Stage	Descriptive Term	Laboratory Assessments
First	Depleted iron stores	Serum ferritin level
Second	Iron deficiency (without anemia)	Transferrin saturation
		Erythrocyte protoporphyrin
Third	Iron deficiency anemia	Hemoglobin
		Mean corpuscular volume (MCV)

Table 7 Results of lab tests indicating stages of iron deficiency [Herbert, 1987].

Blood Sample	Normal	Prelatent Iron Defic.	Latent Iron Defic.	Iron Defic. Anemia
Hemoglobin (mg/dl)	>12	>12	>12	<12
Hematocrit (%)	>37	>37	>37	<37
TIBC (mcg/dl)	330±30	360	390	410
Plasma Iron (mcg/dl)	115±50	115	<60	<40
Plasma Ferritin (mcg/l)	100±60	20	12	<12
Transferrin Saturation (%)	35±15	30	<15	<10
Protoporphyrin	30	30	100	200
Red Blood Cells	normal	normal	normal	abnormal pale

level of iron-deficiency occurs with diminished production of iron-proteins which serve known physiological functions (hemoglobin, myoglobin and iron-containing enzymes).

The Second National Health and Nutrition Examination Survey (NHANES II) provided adequate data on which to base estimates of the prevalence of iron deficiency anemia [Dallman et al., 1984], defined in terms of the percentage of hemoglobin values <95% reference range for age and sex. Anemia can also be viewed in terms of the depression of hemoglobin concentration by the presence of common abnormalities such as iron deficiency or inflammation, even if that depression occurs within the "normal" reference range. With the increasing age in the adult there is a slight rise in the hemoglobin concentration in females and a substantial decline in males. The relative occurrence of a low hemoglobin concentration due to anemia and inflammatory disease was highest in infants aged 1-2 years (6.8%); declined in children aged 3-5 years (5.3%); 6-8 years (5.5%); and declined in preadolescents aged 9-11 years (3.1%). Iron deficiency anemia peaked in males aged 12-14 years (6.3%) and in females aged 15-17 years (4.6%). Between 18-24 years of age, values were low in both males (1.4%) and females (2.2%). However, the values in males remained low until 55-64 years (2.1%) and 65-75 years (2.6%). Young females had values near 4% which declined at age 65-74 years (2.4%).

Dietary iron deficiency in adults progresses at a slow rate

making it difficult to observe the three sequential stages. Researchers examining the stages of iron deficiency in rats observed an overlap of the stages during gradual progression of dietary iron deficiency [Dallman et al., 1982]. The presence of such overlap may prove pertinent to the interpretation of laboratory tests used in the diagnosis of iron deficiency.

Siimes et al. [1980] examined the interrelationships among iron stores, serum iron, hemoglobin, myoglobin and cytochrome c under conditions of iron deficiency which did not interfere with normal growth of rats. Male Sprague-Dawley weanling rats were fed diets containing 7, 10, 13, 17, 25, 40, 50, 75, 125, 250 or 500 mg ferric citrate/kg body weight for three weeks. Results showed that dietary levels of 7 and 10 mg ferric citrate/kg body weight resulted in a gradual decline in hematocrit values and the diets containing <25 mg/kg resulted in a decreased concentration of hemoglobin and cytochrome c. Myoglobin values were depressed ($p<0.05$) with dietary iron intakes less than 10 mg/kg. These findings indicate that iron restriction first depletes iron stores, as evident in the low myoglobin levels, followed by a reduction in transferrin saturation and hemoglobin production.

Hematological Indices of Iron Status.

Serum Ferritin. Body iron in excess of that required for hemoglobin formation is stored in tissues as ferritin. Approximately one-third of the total body iron is stored in the

liver; one-third in the bone marrow; and the remaining one-third in the spleen, muscle, etc. Studies indicated that in the normal subject, serum ferritin is directly proportional to body iron stores [Saarine and Siimes, 1978; Siimes et al., 1974]. Ferritin is a specialized tissue protein formed from the combination of iron chelate and apoferritin. It is a complex molecule, 480,000 M.W., consisting of an iron core surrounded by 20 or 24 spherical peptide subunits. Ferritin is readily mobilized by the body when needed [Beck, 1973]. Serum ferritin is produced by the endoplasmic reticulum of the reticulendothelial cells and hepatocytes [Puro et al., 1971].

Since serum ferritin reflects iron storage status, substantial differences may be anticipated depending on age, sex, socioeconomic status or the geographic area of the survey. In a study conducted by Qvist et al. [1980], varying serum ferritin values were observed in the different age groups and between the males and females. The researchers observed a mean of 166 mcg/l serum ferritin in 31 men, 73 years of age. This value was similar to that of men age 18 to 55 years of age. A mean of 161 mcg/l in 22 females, 73 years of age was observed, significantly higher than that of twenty females, aged 18-45 years (mean serum ferritin value 47 mcg/l) and four females, aged 46-55 years (mean serum ferritin value 74 mcg/l). Luxton et al. [1977] and Valberg et al. [1976] observed similar results. In the males, no age difference in serum ferritin was observed. Cook et al. [1974]

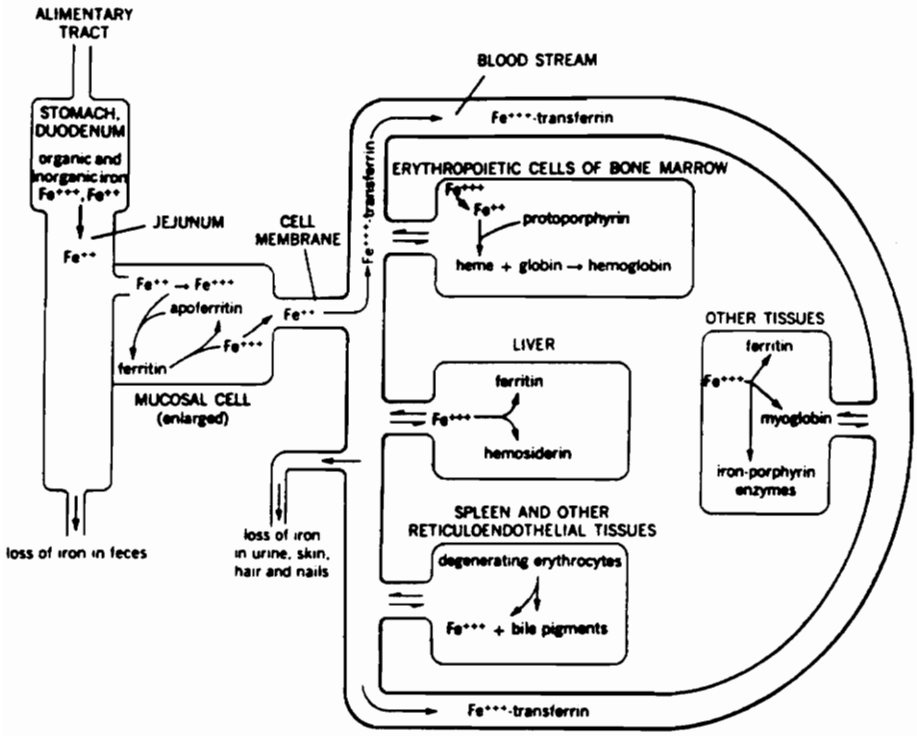


Figure 5 Scheme of iron metabolism [Beck, 1971].

observed a high correlation between the serum ferritin level and age in 83 healthy men, aged 18-35 years old.

Loria et al. [1980] observed different values in the eighty people above 60 years of age. The elderly women had statistically lower mean serum ferritin levels (149.2 $\pm$ 39.5 mcg/l) compared to the elderly men (170.8 $\pm$ 52.6 mcg/l). The male control group, aged 20-50 years old, had a lower average than the elderly men (150.1 $\pm$84.2 mcg/l), and the female control group had a lower value than the elderly women (78.2 $\pm$ 42.5 mcg/l). Cook et al. [1976] and Loria et al. [1979] noted that in men, serum ferritin rises slowly from late adolescence to middle-age whereas in females the increase does not begin until the time of menopause. Loria et al. [1980] observed that the higher serum ferritin levels may gradually increase upon reaching 70 years of age, still questioning whether it is the result of normal physiological events in the elderly.

A disadvantage of the serum ferritin assay is that when iron deficiency and inflammatory disease coexist, the serum ferritin concentration may be within the normal range. An important advantage of the serum ferritin is its relative stability with repeated measurements [Cook, 1982]. Cook et al. [1974] analyzed serum ferritin at weeks 0 and 2, observing correlation coefficients of 0.88. The serum ferritin and iron status were measured by radioiron absorption, indicating that serum ferritin is a useful survey tool for the initial assessment and

prospective monitoring of the iron stores in the normal population.

Serum Iron. The average serum iron concentration is 125 mcg/100 ml in men and 100 mcg/100 ml in females [Jacobs, 1974]. Although some studies indicate that the serum iron concentration does not change until the storage iron is completely depleted [Cook, 1982], others indicate that the measurement of serum iron concentration is subject to many variables, such as contamination of reagents with small amounts of iron, turbidity and entrapment of iron in plasma during precipitation which may introduce substantial error into the results [Fairbanks and Beutler, 1977]. In addition, physiologically, serum iron concentration has a diurnal rhythm; it decreases in late afternoon, reaching a nadir near 9:00 p.m. and increases to its maximum between 7:00 a.m. and 10:00 a.m.. Pilon et al. [1981] observed 28% day-to-day variability in the serum iron measurements in the same subject.

Total-Iron Binding Capacity (TIBC). TIBC may increase as iron stores are depleted [Ballas, 1979; Weinfield, 1964]; however, it is less sensitive than serum ferritin to changes in the iron stores. Cook et al. [1974] observed a relatively low correlation of TIBC with serum ferritin ($r=-0.23$, $p<0.05$) and a low correlation of TIBC with iron absorption ($r=0.20$, $p>0.05$). The normal iron binding capacity of plasma is 300-400 micrograms per 100 ml [Beck, 1973].

Hematocrit. Hematocrit is a measure of the volume of red

blood cells (RBC) per unit volume of blood. Because RBC remains confined in the intravascular compartment, the size of the circulating RBC mass may be altered by variations in the rate of RBC production as long as RBCs are destroyed at a fixed rate. Plasma volume is regulated by homeostatic mechanisms sensitive to pressure, osmolarity and flow rate, the plasma volume changes occur rapidly because of the ease with which fluid shifts between the intravascular and extravascular compartment.

The hematocrit of circulating blood varies in different parts of the vascular tree. True plasma volume exceeds the plasma volume calculated from the measurement of the red cell mass and venous hematocrit. These considerations imply that elevation of the venous hematocrit can result from an increase in the red cell mass or a decrease in plasma volume. These two conditions cannot be distinguished unless the red cell mass is measured.

Hemoglobin. Hemoglobin is a complex protein macromolecule composed of four subunits of polypeptide chains. Each subunit contains an iron-containing heme group to which oxygen may bind. Hemoglobin molecules are contained within the red blood cell for transport throughout the body. The average mature RBC is approximately 8 microns in diameter and 2 microns in thickness and biconcave in shape. This shape increases the surface area exposed to the surrounding milieu. The RBC can readily change shape to facilitate its passage through the fine capillary network. Normal RBC for adult man is approximately

5,400,000 (± 600,000) cells/cubic millimeter of blood; for adult women, approximately 5,600,000 (± 500,000) per cubic millimeter.

Hemoglobin is synthesized within the immature RBC, thus requiring the cell to have an adequate supply of iron and protein present. Hemoglobin accounts for 85% of the essential iron in mature males and 60% in females [Subcommitte on Iron, 1979]. The primary role of hemoglobin is to transport oxygen from the lungs to the respiring tissue. Each hemoglobin binds to four oxygen molecules. Therefore, the hemoglobin concentration in normal humans depends on ambient oxygen tension, hemoglobin affinity for oxygen and circulating testosterone levels [Adamson and Finch, 1975].

Iron Status of the Elderly.

The potential for trace element malnutrition (i.e., iron) appears to be considerable. Lack of dietary iron is identified as the most prevalent nutritional deficiency in society [Preliminary Findings, 1974]. Availability of iron depends on three factors: 1) amount of heme and nonheme iron; 2) presence of other dietary factors influencing the bioavailability of iron; and 3) the iron status of the individual [Nordstrom, 1982]. The favorable influence of meat on dietary iron absorption has been demonstrated. An elderly individual who may change foods in order to decrease fat intake may inadvertently omit nutritious

foods which supply a substantial proportion of one or more essential nutrients [Kohrs, 1982]. Although decreased iron absorption in the elderly has been reported [Freiman et al., 1963; Jacobs and Owen, 1969], Marx [1979] found equally increased iron absorption in both the young and old patients with iron deficiency. However, the elderly subjects utilized the absorbed iron less effectively, suggesting a less efficient erythropoiesis in old age.

The liver contains approximately 33% of the total body iron store [Bothwell et al., 1979]. Loh and Chang [1980] measured the hepatic iron concentration of men and women of various ages. In the men, the liver concentration remained low until puberty, progressively rose until the age range of 16-25 years, and remained unchanged until age seventy-nine. Among the women, the hepatic iron concentration remained low until menopause, followed by a gradual increase. Charlton et al. [1979] and Gautier et al. [1980] reported similar findings (Figure 6).

The Ten-State Nutrition Survey [US Department of Health, Education and Welfare, 1972] indicated deficient hemoglobin in approximately 20% of the elderly subjects. Serum iron and transferrin saturation were also in the lower margin (Table 8).

Other studies also indicated a normal decline in erythrocyte count, hemoglobin and hematocrit concentration with aging [Kelly and Munan, 1977; Baisden, 1978; Htoo et al., 1979]; however, other studies have reported no changes in the hemoglobin and

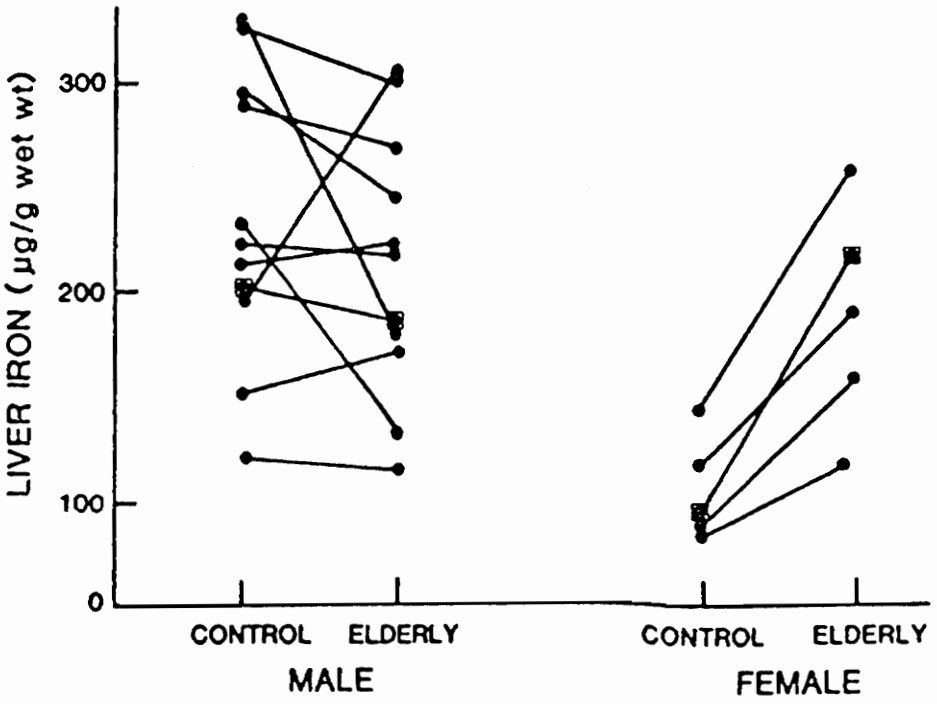


Figure 6 The relationship between age and liver iron concentration. Measurements from the US indicated by open blocks. The age range of the elderly population was 61-93 years for males and 46-93 years for females [Charlton et al., 1979; Gautier et al., 1980].

Table 8 Anemia and Iron Status of Adults 59+ Years of Age
[US Department of Health, Education and Welfare, 1972]

	n	Mean Hgb g/100 ml	Mean Iron Intake mg/d	% subjects w/ low values in blood: Hgb	% subjects w/ low values in blood: Serum Iron
1. Ten-State Nutrition Survey					
A. Males, low-income					
Black	308	13.3	10.6	50.3	42.8
White	281	14.5	12.2	22.8	28.6
B. Females, low income					
Black	502	12.5	7.8	26.7	42.8
White	347	13.4	9.5	9.5	32.2
2. Missouri Nutrition Survey					
White males	53	14.6	15.0	20.5	33.4
White females	66	13.7	10.5	11.0	14.9
3. Nutrition Program Participants in Missouri					
White males	97	15.1	12.7	18.0	8.0
White females	223	14.0	11.1	9.0	4.0

* Low values for males: Hgb <14.0 g/100 ml
Serum Iron <60 microg/100 ml

Low values for females: Hgb <12.0 g/100 ml
Serum iron <40 microg/100 ml

hematocrit levels with aging except from pathogenic association [Griffiths et al., 1970; Marx, 1979]. Several studies observed a decrease in hemoglobin in men beginning at age sixty; little change was observed in women before the age of eighty-five (Figure 7) [Hawkins et al, 1954; Gillum and Morgan, 1955; Veller, 1967].

Qvist et al. [1980] and Loria et al. [1979] found a higher value of serum ferritin in the elderly women compared to the controls. Qvist et al. [1980] analyzed the serum ferritin level in fifty-four healthy men and women aged 73 years and correlated serum ferritin with their iron intakes. Because serum ferritin concentration correlates with body iron stores in normal subjects and in patients with iron deficiency, low serum ferritin values seem to be specific for iron deficiency [Addison et al., 1972; Cook et al., 1974; Kalmin et al., 1978]. Subjects consisted of thirty-one males and twenty-three females whose results were compared to those of twenty-three males and twenty-four healthy females aged eighteen to fifty-five years old. The serum ferritin level in the elderly men were in the same range as those of the younger men, with a geometric mean of 166 mcg/l. The values of the elderly women were significantly higher ($p<0.001$) compared to the results of the younger women, with a geometric range of 161 mcg/l (Figure 8, A). No low serum ferritin values were found in the group of fifty-four elderly subjects. A high significant correlation was observed between the serum ferritin concentration

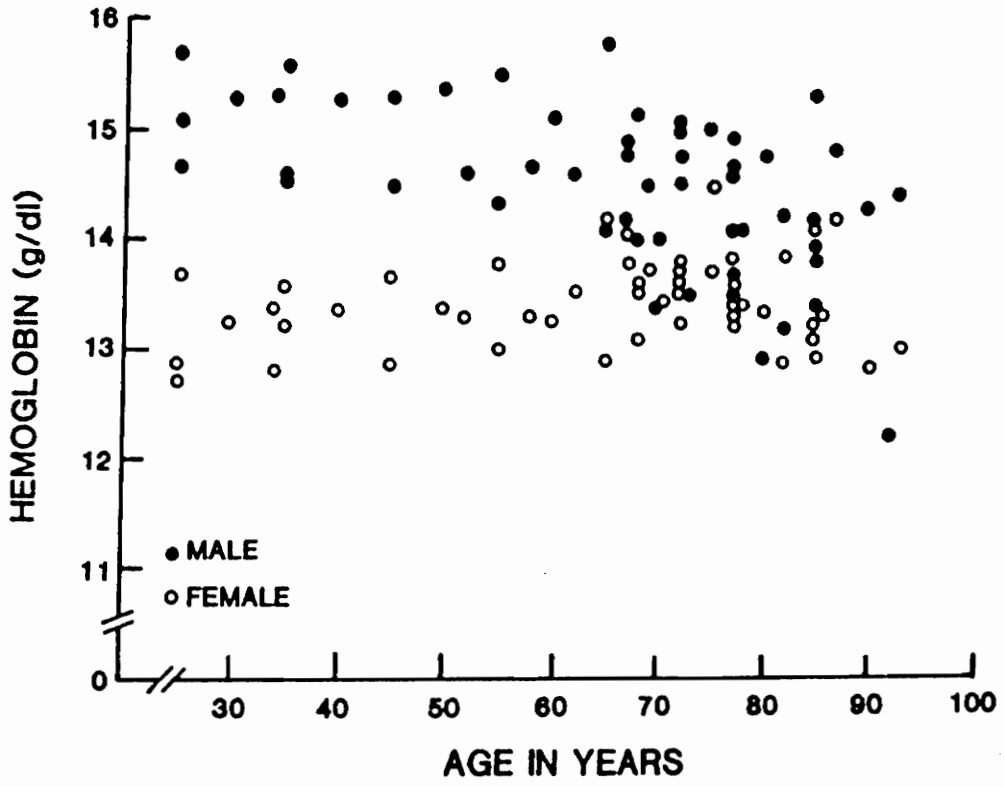
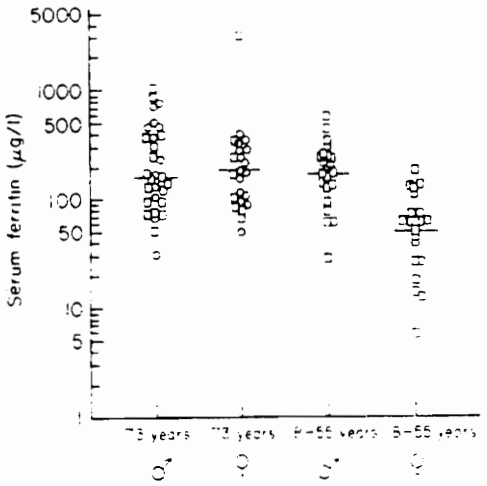
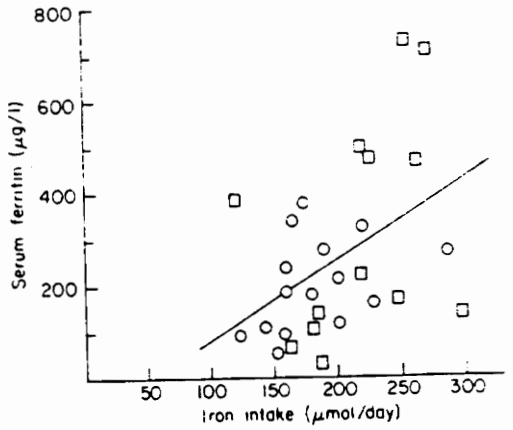


Figure 7 Effect of hemoglobin concentration with age [Hawkins et al., 1954; Gillum and Morgan, 1955; Veller, 1967].



A) Serum ferritin levels in elderly males and females and in male and female controls. Geometric means are indicated by horizontal lines. Open circles represent participants in a study of food intake.



B). Relationship between serum ferritin and food iron intakes in twenty-eight elderly subjects. Open squares represent males, open circles represent females; $r=0.44$, $p<0.01$.

Figure 8 Serum ferritin levels in elderly and control groups and relationship between serum ferritin and food iron intake in eldelry subjects [Qvist et al., 1980].

and the food iron intake ($r=0.44$, $p<0.01$) (Figure 8,B). Loria et al. [1979] analyzed the serum ferritin level of individuals above 60 years of age and compared the results to individuals 20 to 50 years of age. A wide variance in the serum ferritin level was observed in the elderly (Table 9); however, the elderly women had a higher serum ferritin level and lower standard deviation than the younger subjects. Cook et al. [1976] also observed an increase in the serum ferritin levels with increasing age (Figure 9).

Iron and Exercise. The response of the physically active person to endurance training programs is greatly influenced by diet. In addition to adequate dietary carbohydrate and fat for energy metabolism, sufficient dietary iron and adequate serum hemoglobin concentration may also affect the physical performance. It has been proposed that iron deficiency among highly trained athletes ("sports anemia") is greater compared to sedentary individuals [Plowman and McSwegin, 1981; Parr et al., 1984]. Several studies have shown that exercise may compromise iron status [Bottiger et al., 1971; Kilbom, 1971], while other studies indicate no effect [Wirth et al., 1978; Cooter and Mowbray, 1978]. A study by O'Toole et al [1989] and Ehn et al. [1989] found good iron profiles (serum ferritin, serum iron, TIBC, trans saturation and hemoglobin) in intense physical activity. Dufaux et al. [1981], however, found negative levels in serum ferritin, serum iron and haptoglobin but higher serum transferrin levels in distance

Table 9 Distribution of serum ferritin (mcg/l) in elderly women compared to younger adult women [Loria et al., 1979].

	A) Age 60+		B) Age 20-50	
Group	N	Serum Ferritin Range	N	Serum Ferritin Range
I	6	23-52	14	15-48
II	29	58-191	31	56-179
III	11	209-246	--	--
IV	4	495-1270	--	--
Mean	46	149.2	45	78.2
SD		± 39.5		± 42.5

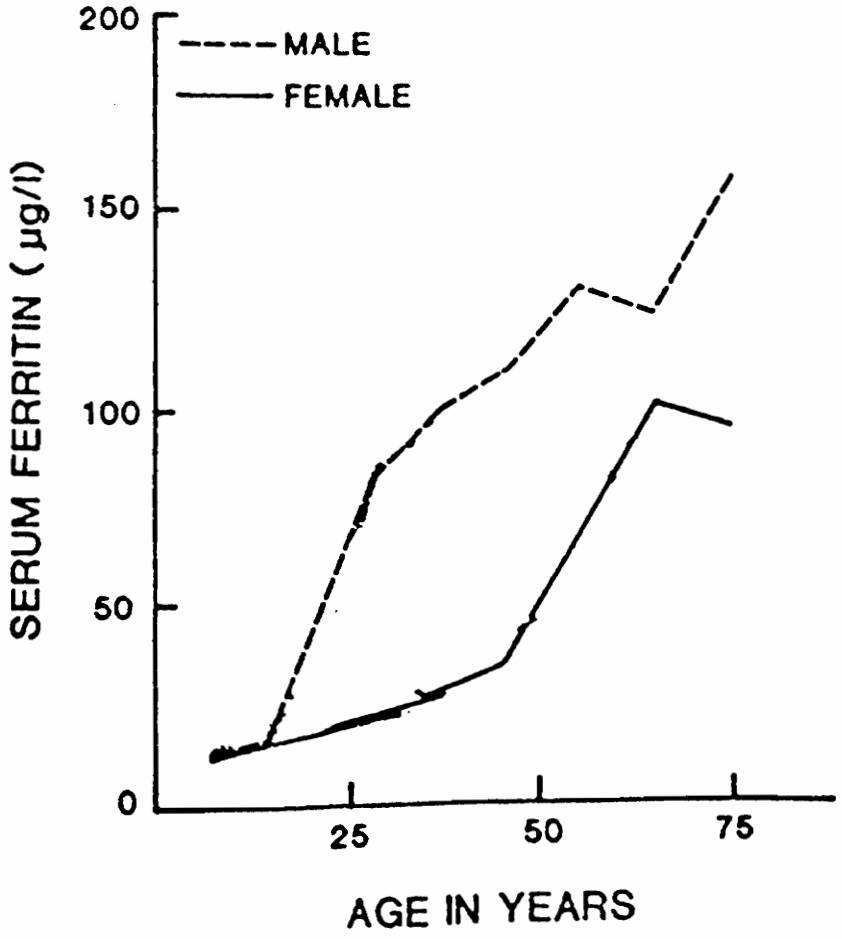


Figure 9 Effect of age on serum ferritin concentration [Cook et al., 1976].

runners than control rowers and cyclists. Clement and Asmundson [1982] had similar findings to those of Dufaux et al. [1981] in endurance runners.

Hemoglobin, in specific, has a significant effect on physical work capacity. It has been hypothesized [Gaehtgens et al., 1979; Stone et al., 1968] that the inconsistent effects of altered hemoglobin may be due to a nonlinear (inverted U) relation between oxygen delivery and hematocrit. According to this hypothesis, VO2 max is predicted to increase following elevation of hemoglobin and hematocrit if hematocrit is below the optimal hematocrit level for oxygen delivery but will decrease if hematocrit is above this level. The decrease in oxygen delivery in this state results from decreased cardiac output due to increased blood viscosity and peripheral resistance. Gardner et al. [1977] found a close relationship between work performance capacity and hemoblogin concentration (Figure 10), consistent with several previous reports in rats and humans [Ericsson, 1970; Gardner et al., 1975; Basta and Churchill, 1974; Beutler et al., 1960; Anderson and Barkve, 1979; Davies et al., 1973; Bowering and Norton, 1981]. Total blood volume has been shown to increase with physical training (Table 10) [Brotherhood et al., 1975; Kjellberg et al., 1950]. However, the hemoglobin concentration and hematocrit remain relatively stable so that oxygen delivery is not impaired by the potential for increased viscosity, which might be expected with the increased total hemoglobin.

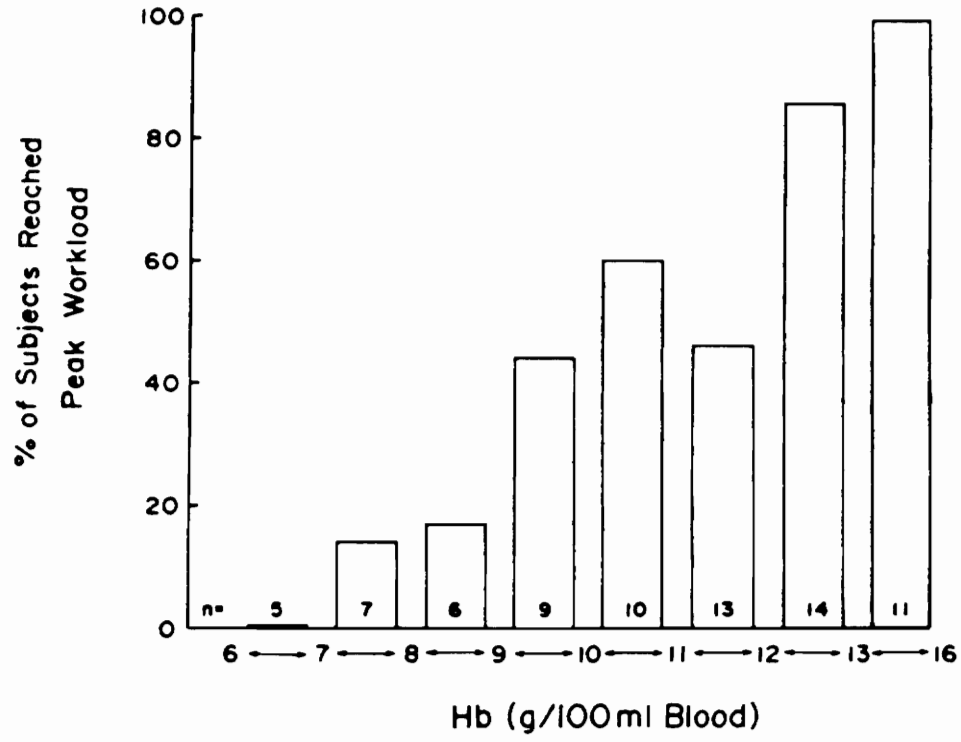


Figure 10 Percentage of subjects in each hemoglobin group who reached the maximum work load of 6.36 km/hr and 20% grade [Gardner et al., 1977].

Table 10 Amount of hemoglobin and blood volume of physically trained and not specially trained women [Kjellberg et al., 1950].

Group	Age	n	kg BW	Hgb (gr)	Hgb/kg BW	Rel. Hgb	Blood Vol. (l)
Untrained	37.6	92	65.5	555	0.86 + 0.013	88	4.070
Trained	26.0	8	64.0	800	1.25 + 0.050	93	5.670

Blood volume regulation is a dynamic process--the hydrostatic and osmotic pressure balance across the capillary walls may be altered with disturbance by physical activity [Kaltreider and Meneely, 1940]. At rest, the muscles receive approximately 20% of the total blood flow; during exercise, they receive approximately 85% of the cardiac output [Brooks and Fahey, 1985]. Two mechanisms which produce the increase in blood volume during endurance training consist of: 1) large increases in plasma renin and anti-diuretic hormone (ADH) during exercise which increases the retention of sodium and water by the kidneys; and 2) increases in plasma protein, mainly albumin, which increases the osmolality of the blood [Brooks and Fahey, 1985]. Thus, the increase in blood volume facilitates venous return of blood to the heart which enhances cardiac output to the skeletal muscles.

Exercise and the Elderly.

In the past few decades the population of older adults has increased. The Council on Scientific Affairs [1984] and studies by Jamy [1980] indicate that the baby boom of the past is being replaced by senior citizens. Diseases, such as coronary heart disease (CHD) are responsible for approximately 555,000 deaths/year [Slayton, 1989]. Risk factors in the development of CHD include the following [Pollock et al., 1978; Holloszy, 1983; Smith et al., 1988]:

		Relative High	Level of Risk Very High
1)	Blood pressure		
	Systolic	150-160	>170
	Diastolic	94-100	>106
2)	Cigarettes/day	30-40	>50
3)	Cholesterol	260-280	>300
4)	Triglycerides	200	>300
5)	Glucose	120-130	>140
6)	% Body fat		
	Male	25	>30
	Females	33	>40
7)	Stress/tension	constant	
8)	Phys. act. >6/minute		
	(5 METS) per week	80-60	<30
9)	EKG abnormality	1	>2
10)	Family history heart attack	3	>4
* 11)	Age	50	>60

The last two factors, family history and age are irreversible; the preceding factors, however, are reversible primarily through appropriate physical activity and diet. Exercise decreases the risk of premature mortality, maintains physical well-being and may increase longevity [Hodgson et al., 1977; Holloszy, 1983].

Lakatta [1979] reports that up to 60% of people between 50-80 years of age may have CHD with inactivity being a major contributing factor. Several studies indicate that regular aerobic exercise improves cardiovascular performance, and improves functional capacity [Blumenthal et al.. 1982; Morey et al., 1989]. Many studies on the physiological aspects of exercise performance have been conducted using elite athletes or middle-aged volunteers. It is questionable whether exercise has beneficial effects on the elderly population as well. Because the aging years (65+ years) are associated with a reduction in physiological and cardiovascular functions and because exercise

generally improves cardiovascular function, it is necessary to implement safe standards of exercise guidelines to promote health and wellbeing among the elderly.

To prescribe a healthy and positive exercise program, several parameters need to be evaluated. The basis for selection of an elderly individual to participate in an exercise program may be better defined by focusing on individuals most likely to benefit from such a program [Council of Scientific Affairs, 1981]. A patient recovering from myocardial infarction, cardiac surgery or who has angina pectoris is usually counselled to increase his/her physical activity in small progressions from a period of a few weeks to months. A formal exercise testing and training program would allow the selection of safe levels of activity and a more rapid recovery for those patients [Council on Scientific Affairs, 1981].

In most cases, the "healthy" elderly population ("healthy" pertaining to those individuals with no other risk factors) has the capability to participate fully or at least to some extent in a structured and progressive exercise endurance program and should be encouraged to do so [Lampman, 1987]. Four factors determine the selection of elderly individuals to participate in certain exercise programs: 1) medical history; 2) physical examination; 3) laboratory tests; and 4) exercise test. The combination of test results determine if the elderly individual has any additional risk factor indicating a need to initiate

further examination or diagnosis, whether additional precautions need to be taken in the exercise program and if exercise should or should not be advised.

The medical history includes several questions concerning peripheral vascular disease; family history of coronary disease and congenital heart disease; caffeine, alcohol and tobacco intake; an exercise history; the type of activity, frequency, duration and intensity. Several cardiac complications are contraindications to participating in exercise programs. These include recent acute myocardial infarction, recent coronary bypass or other cardiac surgery, unstable chest discomfort, complex dysrhythmia, uncompensated congestive heart failure, third degree heart block, and uncontrolled hypertension [ACSM, 1986].

Weight and height are also recorded during physical examination. Common orthopedic problems such as arthritis among the elderly population should also be recorded. Information on acute illness, significant non-cardiac problems which may affect exercise testing, pulse rate and regularity, and blood pressure, xanthoma and xanthelasma needs to be obtained prior an acceptable exercise prescription [Bortz, 1982; ACSM, 1986].

Laboratory tests may incidate whether additional risk factors are present. The total cholesterol and the ratio of total cholesterol to high density lipoprotein are determined as well as the level of triglycerides and blood glucose. Chest x-rays

should also be done which may provide further needed information on the elderly individual.

The principle use of the exercise stress test is to measure endurance fitness, to diagnose CHD and to classify any impairment in cardiac patients as well as the current cardiovascular status [Brooks et al., 1985; Lampman, 1987]. It is valuable because it provides a method of objectively measuring fitness in a safe environment. The American College of Sports Medicine (ACSM) [1986] recommends that a physician be present during the maximal exercise stress test for healthy individuals aged 45+; for high risk asymptomatic and symptomatic individuals aged 35+, and for any individual with a disease. For submaximal testing, it is not recommended that a physician be present for healthy individuals aged 45+; however a physician should be present during the submaximal testing of higher risk asymptomatic and symptomatic individuals aged 35+ and also with any individual with a disease. In short, because being greater than 60 years old is a risk factor, and a physician should be present for all testing except for the submaximal testing of a healthy elderly individual.

There are several indicators of an abnormal exercise test in which the exercise should be stopped [ACSM, 1986; Lampman, 1987]:

1) ST depression >/= 2mm
2) Ventricular tachycardia
3) Exercise induced left or right bundle branch block
4) Light headedness, confusion, ataxia, nausea of signs of severe peripheral circulatory insufficiency
5) Dramatic rise in blood pressure

6) Multifocal PVC's
7) Chest discomfort
8) R on T PVCs
9) Atrial tachycardia or fibrillation

If the elderly person was undiagnosed, he or she will need further medical examination to clarify the abnormal response. The exercise prescription should not be administered until his/her condition is confirmed, in case exercise is not advisable.

The exercise test for the elderly is of great diagnostic value. Martinez-Caro et al. (1984) administered a stress test to 197 elderly volunteers over 65 years of age. Their findings indicate that the stress test is a very feasible diagnostic method of determining cardiovascular status in the elderly. Subjects were divided into four groups: 1) Healthy (N=42); 2) Hypertensive (HBP) (N=20); 3) Mitral valve disease (MVD) (N=10); and 4) Coronary heart disease (CHD) (N=125). The test was administered on an electric cycloergometer with regular increase of load of 30W every 3 minutes except the MVD group, whose load was increased 15W. Martinez-Caro et al. observed that 69% of the subjects in the stress test did not reach their maximum heart rate. Several factors (Table 11) that contributed to the early termination of the stress test included: frequent arrhythmias during exercise, expecially in hypertensive subjects [Fleg, 1982]; claudication and quadricep weariness, considered to be a partial and sometimes a total impediment in physical activities;

Table 11 Factors contributing to early termination of the stress test on elderly subjects [Martinez-Caro, 1984].

Termination:	Healthy	HBP	MVP	CHD
Maximum HR	22	5	8	67
Exhaustion	4	3	-	5
Leg Fatigue	8	4	1	8
VPB Arrhythmias	6	6	3	27
APB Arrhythmias	5	4	-	9
Claudication	4	4	-	11
Dyspnea	1	1	-	
Chest Pain	1	-	-	22
Severe Symptoms	-	3	1	6
Technical Problems*	2	-	-	2
Total	42	20	20	125

* Patients could not adapt to the cycloergometer.

and use of beta-blockers. These factors preclude the extrapolation of the heart rate for determinating the intensity of stress [Kostis, 1982].

The primary benefit of an exercise program with the elderly population is the positive effect on the cardiovascular system. Ordinarily, the maximum oxygen consumption (VO2 max) is a good indicator of cardiovascular functional capacity which declines with age at 0.5-1% per year [Bortz, 1982, Morey et al., 1989; Smith et al., 1988]. The working capacity also decreases 24-30% and the cardiac output decreases 30%. The decline in cardiac output in the elderly population is attributed to the decrease in stroke volume, as the resting heart rate does not change with age [Brandfonbrener, 1955; Lakatta, 1979]. Respiratory capacity decreases 40-50% and muscle mass decreases 25-30% [Physician and Sports Medicine, 1983].

Yerg, Seals, Hagberg and Holloszy (1985) looked at the effect of endurance exercise training on the ventilatory function in eleven sedentary elderly volunteers. VO2 max, maximum achievable ventilation during exercise (Ve max), maximum voluntary ventilation (MVV) and HR were obtained. The average age of the subjects was 63 $\pm$ 2 years old, ranging from sixty-one to sixty-seven years. The subjects were given a stress test prior to the exercise training program. This involved 6 months of low intensity training consisting of moderately vigorous walking several times per week. The subjects averaged 4.6 $\pm$ 0.4

walks/week of 27 ± 1 minutes. Average heart rate during exercise was 107 ± 2 beats/minute. The low intensity exercise program was followed by 6 months of higher intensity training consisting of 30-45 minutes of endurance exercise (cycling, graded treadmill walking or jogging) at 75-85% heart rate reserve at least 3 times/week for an average of 3.6 ± 0.2 sessions/week. Stress tests were given at the end of the one-year training program.

The group demonstrated significant differences in the VO2 max, Ve max, Ve/VO2 and HR (Table 12). The VO2 max and Ve max were significantly higher and the HR significantly lower after the exercise program compared to the intial values. Heath, Hagberg, Ehsani and Holloszy [1981] concluded that healthy men in their fifties who exercise vigorously on a regular basis have a VO2 max 20-30% higher than that of young, sedentary men. Middle-aged and old-master athletes who train for competition in middle- and long-distance running have a VO2 max 50% or higher than that of ex-athletes of the same age who have stopped training. Robinson et al. [1976] stated that Ve max is reduced during exercise in proportion to the decline in VO2 max that occurs with increasing age. However, these subjects' Ve max increased with training in proportion to the increase in VO2 max (29% vs. 25%, respectively). This indicates that healthy elderly individuals who exercise regularly utilize the ventilatory system and increase aerobic capacity to a greater extent than do the sedentary. No significant changes were observed MVV and Ve/CO2.

Table 12 Ventilatory capacity of elderly before and after twelve-months of exercise [Yerg et al., 1985]

	Before Ex. Program	After Ex. Program	P
VO2 max (l/min)	1.91 ± 0.43	2.39 ± 0.55	p<0.005
VO2 max (ml/kg/min)	25.70 ± 4.90	32.90 ± 7.60	p<0.005
Ve max (l/min)	66.70 ± 18.6	86.10 ± 17.4	p<0.005
Ve/VO2	27.70 ± 2.60	25.10 ± 2.80	p<0.005
Ve/VCO2	28.50 ± 3.40	27.40 ± 4.30	NS
MVV, l/min	130.40 ± 33.3	130.00 ± 32.5	NS
HR, bpm	141.00 ± 18.0	125.00 ± 13.0	p<0.025

A more prolonged and intense training may elicit an increase in MVV.

A supervised exercise program is recommended for high risk patients who have been medically cleared by the several tests such as laboratory tests, medical history and stress tests [ACSM, 1986]. With the change from sedentary to active lifestyle, an unsupervised program for the elderly may be a risk factor, exemplified by the risk of unexpected mortality [Thompson et al., 1982]. An alert and active exercise specialist will notice any symptoms and provide fast and safe assistance when needed. If the participant is a cardiorespiratory patient, the exercise program should be medically supervised in case any complications occur [ACSM, 1986].

In addition, high environmental temperature is another hazard which can causes a high mortality rate for individuals older than 60 years of age [Ellis, 1973]. The inability to sweat sufficiently and the benefit from evaporative cooling may be a factor in the mortality rate [Ellis et al., 1976]. It would be more reassuring if the supervisor of the exercise program was present to ensure that the exercise was conducted in a comfortable environment.

One aspect of physical activity which needs to be further developed in the exercise program for the elderly population is the attitude of the elderly towards exercise. Sidney and Shephard (1976) analyzed the attitudes of elderly men and women

towards health and physical activity as seen before and after 3 months of supervised endurance training. Fourteen men and 28 women volunteered for one-hour of physical training classes for 4 days/week for 14 continuous weeks. Exercise consisted of walking and the majority of subjects developed warm and sincere feelings for the exercise leader. Six test were given pre- and post-testing: 1) Cornell Medical Index Health Questionnaire; 2) Manifest Anxiety Scale; 3) Life Satisfaction Index; 4) two assessments of body image; 5) Inventory of Attitudes to Physical Activity; and 6) General Information and Health Habit Questionaire.

There were significant changes in the questionnaire response post-training. Approximately 83% of the subjects reported improvements in well-being and in the perceived body image. The two common responses regarding motivation towards exercise were: 1) improved fitness and health; and 2) exercise instruction on how to exercise safely with opportunities for the measurement of physical fitness. Positive response towards exercise was also found by Hanson (1974) and Massie (1971).

Blumenthal, Schocken, Needels and Hindle (1982) also reported on the psychological effects of physical conditioning on the elderly. Twenty-four subjects, average age of 69.3 years, volunteered for an 11-week conditioning program. Exercise consisted of 30 minutes/session on a stationary cycle 3 times/week. The results from their questionnaire are shown in

Table 13.

Although longer exercise time and an increase in exercise workloads were achieved during the 11 week period, approximately 60% of the subjects saw no changes in physical and psychological well-being from exercise. Approximately 40% of the subjects indicated that they felt healthier, more satisfied, self confident and in a better mood from exercise. Barry et al. (1966) also found no changes in personality, cognition or motivation with training, although there were remarkable increases in the physical performance.

Exercise should have improved the physiological and psychological status of the individual. However, the individual's indifferent perception of exercise (Table 13) may indicate that some adjustment or improvement to the exercise program is needed. Older subjects may require longer conditioning periods to achieve psychological and physiological benefits. When an elderly individual is persuaded to exercise, he is making a significant change in the life-style pattern, transforming from a sedentary to an active individual [Holloszy, 1983]. Participation in sports and recreational activities with other elderly individuals in a leisurely atmosphere may motivate elderly individuals to adapt to exercise in a more positive manner. Rewards for meeting goals may improve compliance. For example, encouraging feedback from the personal physician and from the exercise specialist can improve compliance among the elderly.

Table 13 Responses to psychological questionnaire post-training [Blumenthal, 1982].

	Better	Same
Health	33.3%	55.6%
Sleep	25.9	52.9
Fatigue	25.9	51.9
Achievement	40.7	40.7
Satisfaction	51.8	33.3
Mood	40.7	48.1
Self Confidence	44.4	44.4

Guidelines for Adult Fitness in the Elderly Population

Information regarding the effect of exercise on the aging process is very difficult to obtain, particularly in humans. Although the reversible risk factors responsible for the incidence of CHD cause a high percentage of mortality, small amount of experimental data are available suggesting that regular performance of exercise may improve these factors and prevent CHD [Huttunen et al., 1979; Saltin et al., 1979]. If physical activity and diet could be monitored in healthy young and elderly subjects to analyze if a true decrease in CHD did occur, more people would be motivated to follow the recommended patterns of living.

A change in the living habits, involving regular participation in exercise, cannot be viewed lightly in terms of the potential hazards of unsupervised exercise for elderly individuals who have been sedentary for years. It is best advised to have a supervisor present so the exercise prescription results in a safe and rewarding environment.

As previously stated, the elderly individual's perception of exercise needs to be taken into consideration in planning the structure of the exercise program. Being aware of the true benefits of exercise on the physiological and biological functions may results in a more optimistic view and dedication towards the physical activities.

Controlled longitudinal studies comparing middle-aged and

elderly participants engaging in regularly performed exercise and consuming modified diets may indicate definite benefits pertaining to cardiovascular function and VO% max, lean body mass, and bone mass. As previously stated, data obtained from healthy elderly individuals with appropriate health and living conditions is difficult to obtain but would give more concrete results.

Exercise has a positive effect on the cardiovascular system in the elderly, but because of the increased risk, caution is required. As previously mentioned, the medical history should be obtained and physical exam, laboratory test and stress test should conducted be taken before an exercise program is prescribed.

Great care should be taken in prescribing the frequency, intensity, duration and type of exercise. The exercise should be performed at from 3-5 times/week [Lampman, 1987]. This allows enhancement of the muscular and cardiovascular function.

Exercise for most healthy elderly is performed at low to moderate intensity, with emphasis placed on the duration of effort rather than intensity level [Gorman et al., 1988]. Lower to moderate intensity activity of longer duration is recommended for non-athletic adults because of the potential hazards and compliance problems associated with high intensity activity. Nineteen percent of adults 65+ years old have some activity limitation due to impairment of the spine, back, hips and lower

extremities [US Department of Commerce, 1987].

The intensity of the exercise may be prescribed by target heart rate, Borg Rating of Perceived Exertion (RPE) or by functional capacity (METs) [ACSM, 1986]. The heart rate during training should range between 65-75% of the maximum heart rate achieved during exercise testing [Lampman, 1987]. Cardiac patients should have a target heart rate that also considers symptoms and any signficant EKG abnormalities. The RPE to graded exercise correlates highly with VO2, heart rate and ventilation. With the Borg scale, a perceived exertion rating of 12-13 corresponds to approximately 60% maximum heart rate; a rating of 15 corresponds to approximately 90% maximum heart rate. The RPE can be used in conjunction with heart rate prescription methods. When the participant being tested has developed familiarity with the heart rate-RPE relationship, the heart rate can be monitored less frequently and the RPE used the as primary method for regulating exercise intensity [ACSM, 1986]. The intensity may also be prescribed by 50% to 85% of the individual's functional capacity. Sixty percent to 70% of maximal METs is an appropriate average intensity. Heath et al. [1981] determined that cardiac rehabilitative patients should train at 50-75% maximal VO2. Considering the error which occurs when predicting the maximum heart rate of the elderly, it is optimal to prescribe exercise with a heart rate that corresponds to a VO2 of 50-75%, and/or and RPE of 12-16.

The exercise session should last 30-60 minutes [Lampman, 1987], 30-45 minutes for cardiac patients [Heath et al., 1981]. Regardless of the duration of the exercise, it should always include appropriate warm-up and cool-down [Heath et al, 1981; Lampman, 1987]. The duration of exercise is dependent on the intensity of the activity, thus lower intensity activity should be conducted over a longer period of time [ACSM, 1986].

Exercise should be aerobic and should use large muscle groups [Heath et al., 1981; Lampman, 1987]. In a sedentary person, the exercise should be one that minimizes soft tissue injuries [Brooks et al., 1985]. Activities best suited for the elderly population involve rhythmic and dynamic motion of large muscle groups, such as walking, dancing, swimming, jogging and cycling. Walking places minimum stress on the heart and improves musculoskeletal function, mental outlook and directly benefits the cardiovascular system [Council on Scientific Affairs, 1984]. The ergometer cycle also minimizes the stress and the workload can be easily regulated to the desired intensity [Sheldahl, 1986].

Isotonic exercises, such as jogging, are recommended because they increase the heart rate and cardiac output, improve cardiovascular fitness and maintain flexibility of the trunk and extremities [Council on Scientific Affairs, 1984].

Weight training with light weights is an excellent method to strengthen musculoskeletal muscles [Lampman, 1987]. Light dumb bells, weighing 5-20% of an individual's body weight, can be used

by the patient so that at least 8-15 repetitions are executed. Some studies, however, indicate that isometric exercises such as weight lifting is not advantageous for the elderly individual because it is not improving the cardiovascular function but is increasing the systolic blood pressure [Council on Scientific Affairs, 1984].

The Council on Scientific Affairs [1984] recommends that physicians make sure that elderly patients are aware of the physiological and psychological benifits of exercise, maintain an active interest in their patient's exercise program with appropriate follow-up exams and encourage their patients to maintain the exercise program as a lifetime commitment. If the guidelines and recommendations are followed, a safe, rewarding and suitable exercise program can be prescribed for the appropriate elderly population.

CHAPTER 3

METHOD AND MATERIALS

Subject and Recruitment Procedures.

Twenty healthy Caucasian women, age 56-67 years old, were recruited from the Blacksburg, Christiansburg and Radford areas, Christiansburg and Radford each located within twenty-five miles of Blacksburg. The research protocol was approved by the Institutional Review Board (IRB) for Research Involving Human Subjects. Subjects were recruited by placing flyers in strategic locations (Appendix A) on VPI&SU campus and in the community. Subjects were also recruited from the Seniority Program at the Montgomery Regional Hospital, Blacksburg, VA.

To be included in the study, subjects had to meet the following criteria: be healthy and free from any illness, disease or physical limitation and have a physical examination by personal physician (subjects were reimbursed $50.00). The study protocol was explained to all the subjects and each signed a written consent form prior to participating in the study. The subject's personal physician also signed the informed consent form (Appendix D). This was done to ensure their physician's knowledge of the experimental protocol, potential risk to the subjects and the subject's intent to participate in the study. Each subject received an honorarium in the form of a catered luncheon.

Dietary Assessment.

Each subject completed a three-day dietary record (Appendix E) the week prior to the study (Week 0) and then during the final week of the study (Week 10). The time, specific food, drink and quantity of food and drink consumed were recorded. Dietary analysis was conducted using the USDA's Dietary Analysis Program (DAP) for the Personal Computer (1988). Additional data bases to estimate vitamin, mineral and kilo-caloric intakes were derived from the updated Agriculture Handbook No. 8, Agriculture Research Division, United States Department of Agriculture (1984). Dietary adequacy was assessed using the 1989 RDA [Committee on Dietary Allowance and Food and Nutrition Board, 1989].

Energy Expenditure in Normal Activities.

In addition to the three-day dietary records, each subject also recorded normal activities for three days (Appendix F). Activity data were collected in week 0 and 10 on the same day during which dietary intake data were also collected. Energy expenditure was calculated using the energy costs of activities and the basal energy expenditure equation [Zeman and Ney, 1988; Harris and Benedict, 1919].

Body Fat Composition.

At week 11, skinfolds were obtained from three sites: triceps, thigh and suprailium, using a Lange caliper (Cambridge Scientific Industries, Inc.). Body fat percentage was calculated

using the Siri equation [Jackson et al., 1980].

Submaximal Exercise Stress Test.

A submaximal stress test was performed on each subject one week before (week 0) and one week after (week 11) the study. The purpose of the stress test is to 1) measure the physiological response to a standard bout of exercise that exceeds the resting requirement; and 2) provide electrocardiogram (ECG) observations to detect meaningful coronary insufficiency [ACSM, 1986; Brooks, 1985; McArdle, 1986). The submaximal work levels (70% maximal HR) allows work to be increased in small increments until ischemic manifestations such as anginal pain or ST segment deviations, if any, are recorded.

The submaximal stress test was conducted at the Cardio-Pulmonary Unit (CPU), Montgomery Regional Hospital, Blacksburg, VA, performed by Cam McLaughlin, BS Biology, Associate Respiratory Therapy, Director of Cardio-Pulmonary; Nancy Schuessler, BS Respiratory Therapy, Assistant Director of Cardio-Pulmonary; and Karen Burress, RN, Cardiac-Rehabilitation. On the day of the stress test, each subject was instructed to report to the CPU approximately twenty minutes prior to the test. After arriving at the CPU, each subject signed a consent form (Appendix G and H), was instructed on the submaximal stress test procedure and was appropriately prepared for the test. Electrodes were placed on each subject with the 12-leads to observe

electrocardiogram strips on the Quinton 2000 (Quinton Instrument Co., Seattle, WA). Energy expenditure was measured on the Gould 2900 Energy Expenditure Unit (Gould Products, Cardiopulmonary Products Division, Dayton, OH) with an IBM computer and printer (IBM, Armonk, NY). The Quinton 55 Treadmill (Quinton Instrument Co., Seattle, WA) was used throughout the submaximal stress test.

Flaky dry skin and oil were removed from ten locations on each subject using an abrasive pad. This was done to give more accurate readings. The 10 electrode pads with conductive jelly were placed on the following ten locations:

1) Lead 1--right arm; bony nonmuscular region;
2) Lead 2--left arm; bony nonmuscular region;
3) Lead 3--right beltline region, down from midclavicular region;
4) VI-fourth intercostal, to the right of the sternum;
5) V2-fourth intercostal, to the left of the sternum;
6) V3--Between V2 and V4;
7) V4--fifth intercostal, down the midclavicular region;
8) V5--Between V4 and V6;
9) V6--Horizontal to V4, dow the midaxillary region; and
10) ground lead--left beltline region, down from midclavicular region.

An ace bandage was wrapped around each subject to hold the electrodes in place.

ECG tracings were observed on the Quinton 2000 ECG. Each subject was asked: 1) to stand and breath normally for 30 seconds; 2) to sit and hyperventilate for approximately 15 seconds; and 3) to continue to hyperventilate and remain in the supine position for approximately 15 seconds.

Each subject walked heel-toe and had a long enough stride to

position the foot near the front part of the machine, thereby keeping a fairly erect posture and maintaining minimal pressure on the arms. A chair was located proximal to the treadmill for cool down and/or emergency stop.

Each subject was fitted with a Rudolf face mask (Appendix J) and asked to breath to get baseline values of resting heart rate and blood pressure.

The modified Balke method (Appendix K) was used, maintaining 2 miles/hour during the submaxmaxal stress test with increasing grade at successive two-minute intervals. During the first 2 minutes, the treadmill was set at 180 degrees (flat). Blood pressure and heart rate were recorded every 90 seconds (2 minutes stage time). Each subject used the Borg Scale (Appendix L) to indicate her perceived level of exertion. When each subject reached 70% of her predicted maximum heart rate, the speed of the treadmill was lowered to a gradual stop. Each subject then sat on a chair during the recovery period. Blood pressure and heart rate were recorded every two minutes until baseline levels were observed. The test was then considered complete.

Subjects were randomly separated into two groups, exercise (Ex) or non-exercise (NE) (Appendix M), based on the time to reach 70% predicted maximum heart rate. At week 0, the subjects assigned to one group had approximately the same duration on the stress test to reach 70% max heart rate. Because several of the subjects assigned to the exercise group were going to be out of

town during the study, reassignments to groups were made. Therefore, baseline time to reach 70% maximum heart rate was not the same between the two groups (Appendix W).

Blood Collection Procedure.

Venous blood was withdrawn one week before the study (Week 0) and during the final week of the study (Week 10) Each subject fasted for eight hours prior to the day of blood collection. A signed consent form (Appendix O) was obtained from each subject. A Registered Medical Technologist withdrew 30 ml venous blood from each subject using three 10 ml mineral-free non-heparinized tubes. The blood samples were centrifuged at 5000 rpm for 20 minutes in the IEC DPR-6000 Centrifuge (Damon-IEC Division, International Equipment Company, Needham Heights, MA). The serum was removed with disposable polytheylene pipettes and stored in capped polyethylene 20 ml tubes at -20 ^{o}C until analysis. Height and weight of each subject were also recorded using the Detecto Weighing Scale (Detecto Company, NY). Subjects were given refreshments after blood collection.

Exercise Protocol.

The ergometer used for the ten-week exercise program was located at the Fitness Connection, Blacksburg, VA. Bruce Gehrig, MS Exercise Physiologist, Director of Fitness Connection, was the consultant during the program. The exercise prescription for each subject in the exercise group was based on age predicted maximum

heart rate. The target HR was calculated from a selected sub-maximal percentage (70%) of the maximum HR [Fox and Mathew, 1981]. The exercise protocol consisted of cycling three times per week for 40 minutes duration (5 minutes warm-up, 30 minutes cycling designated pace, 5 minutes cool-down), approximating 70-75% maximum HR:

Weeks 1-2 Training Period

Weeks 3-10 Exercise Period

1)	Warm up	5 minutes 0-35 watts
2)	Exercise	30 minutes at 70-75% max HR 50 watts
3)	Cool down	5+ minutes to baseline HR 0-35 watts

Each cycling session was monitored by Violet Woo; Dan Bluntzer, graduate student, exercise physiology; or Charles Bishop, senior, physical education. Heart rate and Borg Rating of Perceived Exertion were recorded at 0, 2, 3 minutes of warm up and cool down and at consistent five-minute intervals during the exercise. The cycling was performed on the Monarch 818E. After 5 minutes of warm-up, the tension was set at 50 watts and the subject maintained between 60-70 rpm for 30-minutes. At least 5-minutes cool-down was provided to achieve approximate baseline HR. A fan, music, water and towels were provided for each subject.

Biochemical Analysis.

Serum Ferritin, Serum Iron, TIBC, Iron Saturation. Frozen serum samples of the subjects obtained during Week 0 and Week 11 were analyzed by Roche Biomedical Laboratories, Inc., a subsidiary of Hoffmann-La Roche Inc., Burlington, NC.

Serum ferritin was determined using Irma-Count Ferritin with monoclonal anti-ferritin antibodies [Diagnostic Products Corporation] (Appendix S).

Serum iron and TIBC were assayed using the AM Blue 610 method [American Monitor Corporation, Indianapolis, Indiana] (Appendix T).

Iron saturation was determined using the following equation:

$$\frac{\text{Serum Iron}}{\text{TIBC}} \times 100 = \text{Transferrin Saturation (\%)}.$$

Hematocrit. Venous blood samples were immediately analyzed for hematocrit concentration (%) by microcapillary estimation in duplicates and the mean value was determined per subject and per group. Blood was drawn into 2 heparinized micro-hematocrit (capillary) tubes. Each tube was filled approximately 2/3 full and sealed with "seal-ease". The capillary tubes were placed in the micro-centrifuge, being careful so that the sealed ends of all tubes were touching the outside edge of the centrifuge. The centrifuge was closed, set at 3000 rpm for 10 minutes. The bottom of the meniscus of the plasma and the top of the packed red cells were read and expressed as percent of whole blood.

Hemoglobin. Venous blood samples were immediately analyzed for hemoglobin concentration (g/dl) by the cyanomethemoglobin method and read on a Bausch and Lomb Spectronic 21 in duplicates per subject. The mean value was determined per subject and per group. Five milliliters of cyanomethemoglobin reagent was pipetted into each test tube. Two hemocap tubes were filled with sample blood and carefully dropped into each of the two tubes containing the cyanomethemoblogin reagent. A stopper was placed on each hemocap tube and mixed to allow the blood to dissipate from the capillary tubes and then was allowed to stand at least 10 minutes at room temperature. The contents of the tubes were transferred to cuvettes and the absorbance was measured against the cyanomethemoglobin reagent at 540 nm.

Total Cholesterol and High-Density Cholesterol. Total cholesterol and HDL-C were determined using Sigma Diagnostic kits No. 352 and No. 352-3, respectively (Sigma Chemical Company, St. Louis, MO) (Appendix U).

Statistical Analysis.

Data were analyzed by the Statistical Analysis System (SAS). Student t-tests were used to compare variables between the exercise and the non-exercise groups. Paired t-tests were used to compare changes in the variables for within-group comparisons at weeks 0 and 11. Differences were considered significant at $p<0.05$.

CHAPTER III

RESULTS AND DISCUSSION

Subject Characteristics. Twenty Causcasian women volunteered for the study. Two women from the exercise group were excluded due to 1) daily intake of Geritol Multivitamins which contains 50 mg iron; and 2) medication which significantly increased the serum ferritin level >1000 mg. One subject from the non-exercise group was excluded due to the intake of Century-Vite Multivitamin Multimineral Supplement which contains 18 mg iron. Another woman from the non-exercise group was not included in the pre- and post-submaximal stress tests because stress tests results were unavailable; however, her data from the dietary, hematological and energy expenditure in daily activity were used in the analysis. Data were analyzed using eight subjects in the exercise group and nine subjects in the non-exercise group.

There was a significant difference between the average age of the subjects in the exercise group and those in the non-exercise group at weeks 0 and 11 ($p<0.02$) (Table 14). The magnitude of the difference in age was, however, small (<1 year). Although we were recruiting subjects between 56- to 60-years-old, one of the nine subjects in the non-exercise group was 67-years-old. Two of the eight subjects in the exercise group were <60-years-old (56 and 58-years-old).

There were no other significant differences in the

characteristics (Table 14). Body weights, heights and body mass index (BMI) were similar for both groups at weeks 0 and 11.

Similar characteristics have been reported in previous studies. For example, Ericsson [1970] reported age and height values for twenty elderly women with similar characteristics. His subjects consisted of two groups, experimental (n=10) and control (n=10). The experimental group had an average age of 64.7 years old, average height and weight of 162.3 $\pm$ 7 cm and 60.1 $\pm$ 7.3 kg, respectively. The control group had an average age, height and weight of 63 years old, 163.6 $\pm$ 5.4 cm, and 66.8 $\pm$ 8.8 kg, respectively. Yerg et al. [1985] conducted a study with elderly women, 61-67 years of age, and provided no other characteristics. Garry et al. [1982] analyzed the nutrient status of elderly women 60 years of age and older, who had an average BMI of 23.7 $\pm$ 3.4 kg/m^2.

Body fat, total cholesterol and HDL-C measures were obtained at week 11. There were, however, no significant differences between the two groups in their cholesterol profile or in their body composition. Body mass index was calculated as weight in kg/height in m^2. There was no differences in BMI between the groups at weeks 0 and 10. A BMI of 27.5 or greater for women is an indication of obesity [National Institute of Health Consensus Development Conference Statement, 1985]. Using this criteria, none of the subjects are considered obese but are within the normal BMI range. Moderate exercise did not alter body weight

Table 14 Subject Characteristics*

	Week 0		Week 11	
	Ex.	Non-Ex.	Ex.	Non-Ex.
Age, years	60.8 ± 2.5 [a]	63.7 ± 1.7 [a]	61.0 ± 2.4 [b]	63.9 ± 1.8 [b]
Body weight, kg	62.7 ± 3.7	58.9 ± 13.2	62.9 ± 3.5	59.3 ± 13.1
Height, cm	161.3 ± 7.3	162.4 ± 5.7	161.3 ± 7.3	162.4 ± 5.7
Body fat, %	**	**	24.8 ± 4.5	23.4 ± 6.6
Body mass index, kg/m^2	23.9 ± 2.5	22.3 ± 5.4	24.0 ± 2.6	22.5 ± 5.3
Total cholesterol, mg/dl	**	**	228.1 ± 42.2	193.6 ± 24.3
HDL-C, mg/dl	**	**	65.6 ± 17.3	58.1 ± 14.3
Total cholesterol/HDL-C	**	**	3.7 ± 0.8	3.5 ± 1.0

* = $\bar{x} \pm SD$

** = Value only obtained in week 11.

a,b = $p<0.02$

and body mass index.

Due to the availability of volunteer subjects within Blacksburg area, the recruitment of the subjects was focused on the immediate area. If the recruitment had been conducted in a more metropolitan area or in a larger geographical region, a more diverse subject group may have been available. The majority of the subjects were retired. One subject was currently employed by VPI&SU as a secretary; another subject was also employed by VPI&SU as well as a self-employeed editor; and several subjects did volunteer hospice work.

Dietary Analysis. The three-day dietary records were analyzed to determine if energy, iron, vitamin B6, vitamin C, fiber and folate intakes were signficantly different within or between the groups at weeks 0 and 10. Dietary intake of carbohydrate, protein, fat, zinc and calcium were also observed (Tables 15, 16, 17).

There were no significant changes in the energy consumption due to the ten-week submaximal exercise program. Exercise has been reported to increase [Thompson and Blanton, 1987], have no effect on [Thompson et al., 1982] or decrease food intake [Wilmore, 1983]. Mayer et al. [1954] reported that in female rats, food intake first decreased and then increased proportionately with increasing energy expenditure. Thompson et al. [1988] analyzed the effect of low-intensity exercise (LIE)

Table 15 Average Caloric and Nutrient Intakes Among Exercise and Non-Exercise Groups: Percentage of 1989 RDA [Committee on Dietary Allowances and Food and Nutrition Board, 1989].

	Week 0		Week 10	
	Exercise	Non-Exercise	Exercise	Non-Exercise
Kcal	1223.0 ± 248.4	1533.3 ± 480.8	1270.1 ± 376.3	1348.7 ± 334.8
CHO, % kcal	52.8 ± 7.0	50.7 ± 5.5	48.4 ± 6.1	51.0 ± 3.1
Protein, % kcal	16.8 ± 6.9	17.4 ± 3.1	18.6 ± 5.3	17.5 ± 2.7
Fat, % kcal	31.7 ± 9.3	29.3 ± 4.5	31.9 ± 8.5	30.2 ± 5.1
Fiber, g	14.4 ± 5.6	16.4 ± 5.7	17.9 ± 6.3	14.8 ± 5.6
Iron, %	95.5 ± 25.4	114.1 ± 43.5	117.8 ± 48.6	105.4 ± 33.1
Zinc, %	56.9 ± 14.6	66.6 ± 16.3	63.5 ± 24.9	65.7 ± 18.7
Vitamin C, %	160.3 ± 33.7[a]	232.0 ± 60.1[a]	227.0 ± 112.3	213.1 ± 67.1
Vitamin B6, %	88.0 ± 26.1	106.3 ± 34.1	98.0 ± 53.0	89.8 ± 34.9
Folate, %	123.3 ± 19.0	121.0 ± 31.5	162.3 ± 78.2	124.4 ± 54.3
Calcium, %	85.0 ± 30.9	84.1 ± 32.6	85.9 ± 39.4	85.2 ± 35.3

% = Percentage of Recommended Daily Allowances [1989]

a = $p<0.02$

Table 16 Nutrient Intakes of Exercise Group.

	Week 0		Week 10	
	Amount	% 1989 RDA	Amount	% 1989 RDA
Protein (g)	51.5 ± 21.9	103.0 ± 43.9	60.6 ± 26.6	121.1 ± 53.2
Vitamin A (mcg RE)	7125.0 ± 4921.1	178.1 ± 123.0	8035.0 ± 3704.8	200.9 ± 92.6
Vitamin E (mg alpha-TE)	5.8 ± 1.8	71.9 ± 22.9	7.2 ± 4.5	89.5 ± 56.9
Vitamin C (mg)	96.2 ± 20.0	160.3 ± 33.7	136.2 ± 67.4	227.0 ± 112.3
Thiamin (mg)	1.0 ± 0.1	102.5 ± 13.6	1.3 ± 0.6	127.6 ± 59.3
Riboflavin (mg)	1.3 ± 0.3	109.3 ± 27.5	1.5 ± 0.6	124.3 ± 52.5
Niacin (mg NE)	15.6 ± 8.4	120.0 ± 64.5	16.7 ± 8.4	128.5 ± 64.5
Vitamin B6 (mg)	1.4 ± 0.4	88.0 ± 26.1	1.6 ± 0.8	98.0 ± 53.0
Folate (mcg)	221.9 ± 34.2	123.3 ± 19.0	292.1 ± 140.8	162.3 ± 78.2
Vitamin B12 (mcg)	2.7 ± 1.2	136.0 ± 58.7	3.4 ± 2.1	170.9 ± 106.1
Calcium (mg)	680.0 ± 247.4	85.0 ± 30.9	687.0 ± 315.5	85.96 ± 39.4
Phosphorus (mg)	936.0 ± 312.1	117.0 ± 39.0	1032.0 ± 413.4	129.0 ± 51.7
Magnesium (mg)	248.2 ± 77.4	88.6 ± 27.6	284.6 ± 98.1	101.6 ± 35.0
Iron (mg)	9.6 ± 2.5	95.5 ± 25.4	11.8 ± 4.9	117.8 ± 48.6
Zinc (mg)	6.8 ± 1.8	56.9 ± 14.6	7.6 ± 3.0	63.5 ± 24.9

Table 17 Nutrient Intakes of Non-Exercise Group.

	Week 0		Week 10	
	Amount	% 1989 RDA	Amount	% 1989 RDA
Protein (g)	65.2 ± 18.2	103.0 ± 43.9	58.0 ± 12.9	130.4 ± 36.4
Vitamin A (mcg RE)	9991.1 ± 7604.9	249.8 ± 190.1	9560.0 ± 6758.6	239.0 ± 169.0
Vitamin E (mg alpha-TE)	7.2 ± 3.3	90.4 ± 40.7	6.2 ± 3.8	77.5 ± 47.1
Vitamin C (mg)	139.2 ± 36.0	232.0 ± 60.1	127.9 ± 40.6	213.1 ± 67.7
Thiamin (mg)	1.3 ± 0.4	128.3 ± 40.2	1.2 ± 0.4	122.9 ± 38.8
Riboflavin (mg)	1.3 ± 0.3	128.3 ± 46.7	1.4 ± 0.5	120.4 ± 40.0
Niacin (mg NE)	18.2 ± 6.6	140.0 ± 50.4	15.8 ± 5.3	121.3 ± 40.9
Vitamin B6 (mg)	1.7 ± 0.5	106.3 ± 34.1	1.4 ± 0.6	89.8 ± 34.9
Folate (mcg)	217.8 ± 56.7	121.0 ± 31.5	224.0 ± 97.8	124.4 ± 54.3
Vitamin B12 (mcg)	2.9 ± 1.4	145.7 ± 67.9	3.4 ± 1.2	167.9 ± 58.9
Calcium (mg)	672.9 ± 260.8	84.1 ± 32.6	681.8 ± 282.1	85.2 ± 35.3
Phosphorus (mg)	1035.6 ± 320.2	129.4 ± 40.0	958.2 ± 246.6	119.8 ± 30.8
Magnesium (mg)	284.7 ± 94.6	101.7 ± 33.8	241.7 ± 65.9	86.3 ± 23.5
Iron (mg)	11.4 ± 4.4	114.1 ± 43.5	10.5 ± 3.3	105.4 ± 33.1
Zinc (mg)	8.0 ± 2.0	66.6 ± 16.3	7.9 ± 2.2	65.7 ± 18.7

and high-intensity exercise (HIE) set at 35 and 68% maximal oxygen intake, respectively. Total energy expenditure for exercise sessions was set at 4.1 kcal/kg body weight. Results indicate that only the HIE group suppressed hunger immediately post-exercise. Maxwell [1985] reported hunger suppression for up to 2 hours post-exercise and concluded that there are interacting effects of exercise intensity and total energy expenditure. A higher intensity and duration of exercise may, therefore, affect caloric intake.

Dietary intake of iron in both the exercise and non-exercise groups met the 1989 RDA of 10 mg iron/day at weeks 0 and 10 ($\pm$ SD). HANES I [US Department HEW, 1974] observed that the average dietary iron intake for women aged 55-64 was 9.8 mg/day and for women older than 64 years of age, 9.2 mg/day. HANES II, however, had higher values, meeting the RDA. Women aged 55-64 averaged 10.7 mg dietary iron/day and women older than 64 years of age averaged 10.2 mg iron/day. In this study, although energy intake was low, ranging between 1223 to 1533 kcal/day, dietary iron intake was adequate, ranging from 9.6-11.8 mg/day. An examination of the dietary intake data revealed that the major sources of iron in the diets of these women were chicken, pork, turkey, spaghetti, and vegetables (lettuce, broccoli, cauliflower).

The intake of dietary iron in the exercise group was higher, but not significantly higher, at week 10 (11.8 mg or 117.8% RDA)

compared to week 0 (9.6 mg or 95.5% RDA). The Health and Nutrition Examination Surveys I and II [US Department of Health, Education and Welfare, 1979] demonstrated that the mean dietary iron intake of elderly Americans aged 65-74 years of age is adequate (12.9 mg, 14.5 mg, respectively). However, up until now, research on the effect of moderate exercise on dietary intake of elderly women is not existent.

A study by Clement and Asmundson [1982] observed that 90% of the seventeen female endurance runners, age 21-22 years, had a mean dietary iron intake of only 89% RDA (of 14 mg iron/day). Low iron stores usually lead to increased absorption. Ehn et al. [1980], however, used ^{59}Fe sulphate and found absorption to be 16.4% in elite male distance runners who were iron deficient, compared to 30% absorption in the control (sedentary) group who were also iron deficient. The combination of inadequte iron absorption plus increased iron loss (urine, stool, sloughed skin, menstrual cycle) may explain why 80% of the female subjects in the study [Clement and Asmundson, 1982] were in the latent iron deficiency stage. Approximately eight-two percent of the subjects had serum ferritin levels below 25 ng/100 ml, 36.4% had serum iron levels below 65 mcg/100 ml and 54.6% had transferrin saturation levels below 21%.

Snyder et al. [1989] also observed low intake of dietary iron in female runners. Nine runners (n=9), average age 37.8 years, consumed a diet from the four basic food groups, including red

meat (RM). The second experimental group (n=9), average age 39.2 years, consumed a modifed vegetarian diet (MV) including milk, eggs, fish, poultry and very little (<100 g/week) or no red meat. The RM group consumed 14.0 ± 2.2 mg iron/day and the MV group consumed 14.7 ±2.0 mg/day, each below the RDA of 18 mg/day.

Although the RM and MV did not have significantly different dietary iron intake nor VO2 max (50.6 ±2.0 ml/kg/min, 49.5 ± 2.2 ml/kg/min, respectively), serum ferritin (19.8 ± 4.2 ng/100 ml, 7.4 ± 1.4 ng/100 ml, respectively) and TIBC (327.2 ± 9.6 mcg/100 ml, 366.5 ± 12.2 mcg/100 ml, respectively) were significantly different between the two groups. This may be attributed to the source of iron. The RM group had a significantly higher ($p<0.05$) intake of heme iron (1.2 ± 0.2 mg) compared to the MV group (0.2 ± 0.1). There was no significant difference in the nonheme iron intake between the RM group (13.0 ± 2.3 mg) and the MV group (14.3 ± 2.0 mg).

Dietary intake of vitamin B6 was fairly adequate among the women in the present study. At week 0 the exercise group consumed 1.4 ± 0.4 mg vitamin B6/day (88.0 ± 26.1% RDA); the non-exercise group consumed 1.7 ± 0.5 mg vitamin B6/day (106.3 ± 34% RDA). At week 11, the exercise group consumed 1.6 ± 0.8 mg vitamin B6/day (98.0 ± 53% RDA) and the non-exercise group consumed 1.4 ± 0.6 mg vitamin B6/day (89.8 ± 34.9% RDA). Had the data been assessed with the 1980 RDA, the subjects would have had inadequate intake of vitamin B6 because the 1980 RDA was 2.0 mg

vitamin B6/day, compared to the 1989 RDA of 1.6 mg/day. A study by Manore et al. [1990] observed that 26% of 198 elderly subjects consumed less than 66% of the 1989 RDA for vitamin B6, plant foods being the primary source (1.06 mg vitamin B6/day) and animal sources providing only 0.48 mg vitamin B6/day.

Garry et al. [1982] also observed low intakes of vitamin B6 in the elderly population. For their study, 138 men and 166 women 60 years of age and older recorded 3-day dietary records. Ninety-four percent of the men and 97% of the women received less than 100% of the 1980 RDA of B6; 83% men and 86% women received less than 75% the of 1980 RDA; and 54% men and 61% women received less than 50%. Ryan et al. [1989] analyzed the 24-hour dietary recall of 268 elderly individuals older than 55 years of age. The average intake of vitamin B6 was 38.7 $\pm$ 37.0% of the 1980 RDA, ranging from 0 to 252 mg. Guilland et al. [1984] observed that 50% of their institutionalized subjects had dietary intakes below 50% of the 1980 RDA for vitamin B6. Vir and Love [1978] observed 32 hospitalized females who were not in an acute stage of illness but had been in the geriatric unit for a minimum of 3 months. The average intake of vitmain B6 was 0.8 $\pm$ 0.19 mg/day. None of the subjects in the present study had a daily intake greater than 2 mg of vitamin B6/day; 3 ranged between 1-2 mg of vitamin B6/day; 29 showed values less than 1 mg of vitamin B6/day; and 8 consumed 0.66 mg of vitamin B6 intake/day. Other studies indicate that 50-90% of the elderly have dietary intakes

of vitamin B6 below the 1980 RDA [Driskell, 1978; Hampton et al. 1977; Vir and Love, 1978].

In the present study, dietary intake of folic acid was adequate. At week 0, the exercise group consumed 221.9 $\pm$ 34.3 mcg folate/day (123.3 $\pm$ 19.0% RDA); the non-exercise group consumed 217.8 $\pm$ 56.7 mcg folate/day (121 $\pm$ 31.5% RDA). At week 11, the exercise group consumed 292.1 $\pm$ 140.7 mcg folate/day (162.3 $\pm$ 78.2% RDA); and the non-exercise group consumed 224.0 $\pm$ 97.8 mcg folate/day (124.4 $\pm$ 54.3% RDA). The 1980 RDA for folate was 400 mcg, compared to the 1989 RDA value of 180 mcg/day. If the results were assessed with the 1980 RDA, the subjects would have had inadequate intakes of folate.

Garry et al. [1982] found poor intake of folic acid in elderly women. One-hundred sixty-six women, aged 60 years and older, volunteered to keep an accurate 3-day dietary record. Results indicate that 91% had dietary intake of folate less than 100% of the 1980 RDA; 84% less than 75% of the 1980 RDA; and 43% less of the 1980 RDA. The Nutrition Canada Survey [1977] found average daily folate intakes of men and women age 65 years and older to be 151 mg and 130 mcg, respectively. MacLeod et al. [1974] found daily intakes of 50 to 150 mcg folate/day, below the 1980 and 1989 RDA. Rosenberg et al. [1982], however observed dietary intake of folate to average between 184-250 mcg folate/day in elderly Florida residents.

There was a significant difference ($p<0.02$) in the dietary

intake of vitamin C between the exercise and non-exercise group at week 0. The RDA for women aged 51+ is 60 mg [Committee on Dietary Allowance and Food and Nutrition Board, 1989]. In this study, the subjects demonstrated an approximate 2.0-fold increase in dietary ascorbic acid intake. Other studies [Cook and Monsen, 1977; Brise and Hallberg, 1962; Bothwell and Finch, 1962] have shown average dietary intake of ascorbic acid to be 280 mg/day, approximately five-times the RDA [Cook and Monsen, 1977]. Previous studies [Brise and Hallberg, 1962; Bothwell and Finch, 1962] have shown that dietary intakes of 200 mg have an enhancing effect on ferrous sulfate absorption. Smaller amounts can increase the absorption of nonheme iron in foods. Layrisse et al. [1974] demonstrated a 5-fold increase in iron absorption from maize when taken with 70 mg ascorbic acid. Cook and Monsen [1977] analyzed the affect of ascorbic acid on iron absorption in standard (STD) or semisynthetic meals (SS), containing 4.1 mg iron, 202 mg calcium, 414 mg phosphorus with varying amounts of ascorbic acid added. Results indicated that the iron absorption from the SS meal, containing no ascorbic acid, averaged less than 1%. The ratio of iron absorption of the SS meal, with 100 mg ascorbic acid, to the SS meal, without ascorbic acid, was 4.15. The mean absorption from the STD meal of 4.05% increased to 6.78% when the meal had 100 mg of ascorbic acid added.

In this study, most of the ascorbic acid was consumed in the morning with breakfast (orange juice) or in the afternoon for a

snack (fresh tangerine), with some contribution from fresh vegetables at lunch and dinner (tomatoes, broccoli). Thus, the ascorbic acid was taken simultaneously with foods rich in dietary iron. The potential effect of ascorbic acid on iron absorption depends on the pattern of intake, relative to food intake [Cook and Monsen, 1977]. If a vitamin C-rich source was only taken as a single dose at breakfast, that normally provided 0.2 mg iron, iron absorption would increase on the average from 1.0 mg to 1.86 mg. If the vitamin C-rich source was taken in divided doses with each meal, iron absorption would increase more than 3-fold [Cook and Monsen, 1977].

The calcium intake of both groups was below the RDA of 800 mg calcium/day. At weeks 0 and 10, the exercise group averaged 680.0 $\pm$ 247.4 mg/day (85.0 $\pm$ 30.9% RDA) and 687.0 $\pm$ 282.2 mg/day (85.9 $\pm$ 39.4% RDA), respectively; the non-exercise group averaged 672.9 $\pm$ 260.8 mg/day (84.1 $\pm$ 32.6% RDA) and 681.8 $\pm$ 282.1 mg/day (85.2 $\pm$ 35.3% RDA), respectively. Garry et al. [1982] observed similar calcium intakes in elderly women. Of the 166 women age 60 years and above, 75% had dietary calcium intakes less than 100% of the RDA; 43% below 75% RDA; and 12% below 50% RDA. Ryan et al. [1989] observed all 268 volunteer subjects, aged 55 years and above, to have inadequate intakes of calcium. Average intake was 76.0 $\pm$ 62.4% RDA, ranging from 0 mg to 627 mg calcium/day. Other studies also indicated low calcium intake in the elderly population [Andres et al., 1989; Nieman et al., 1989; Abraham, 1977].

The dietary intake of zinc was also below the 1989 RDA. At weeks 0 and 10, the exercise group averaged 6.8 ± 1.8 mg zinc/day (56.9 ± 14.6% RDA) and 7.6 ± 3.0 mg/day (63.5 ± 24.9% RDA), respectively; the non-exercise group averaged 8.0 ± 2.0 mg zinc/day (56.9 ± 14.6% RDA) and 7.9 ± 2.2 mg/day (65.7 ± 18.7% RDA), respectively. Many elderly adults are believed to consume marginal amounts of zinc and, therefore, are at risk for zinc deficiency [Greger and Sciscoe, 1977; Greger, 1977]. In this study, most of the dietary zinc was obtained from pork and fish. The Nationwide Food Consumption Survey 1977-78 [1989] noted dietary zinc intake in elderly females aged 65-74 and 75+ years to be 7.6 mg and 7.0 mg, respectivley. The Health and Nutrition Examination Survey No. 2 [US DHEW, 1981] also observed inadequate zinc intakes in females 55-64 years of age (8.2 mg/day) and females 65-74 years of age (7.2 mg zinc/day). Garry et al. [1982] also observed low intake of zinc in elderly women. Ninety-eight percent of the 166 elderly women studied consumed less than 100% of the 1980 RDA; 88% less than 75% of the 1980 RDA; and 47% less than 50% of the 1980 RDA.

In summary, women in the present study had adequate intakes of the essential nutrients. However, dietary intakes of vitamin E, calcium and zinc were low, suggesting that nutrition education on the dietary sources of these nutrients is needed.

Iron Status. Results from the biochemical assays are shown in

Table 18 and Figures 11 and 12. All of the hematological parameters at weeks 0 and 11 were within the normal range in both groups. There were no significant differences between or within the exercise and non-exercise groups in the serum ferritin, transferrin saturation, serum iron, and TIBC analysis. There was a significant difference in the hematocrit concentration between the two groups at weeks 0 and 11 ($p<0.05$). Paired t-test indicated a significant increase in hemoglobin concentration in the exercise group at week 11 ($p<0.05$).

Although no significant differences were found, the serum ferritin level increased in the exercise and non-exercise groups at week 11 (Figure 13). Qvist et al. [1980] and Loria et al. [1979] observed a higher value of serum ferritin in the elderly women compared to younger women (Figure 8), but the iron status of the elderly population with exercise has not received attention. A decrease in iron reserves has been reported in athletes [Clement and Asmundson, 1982; Clement and Saechuk, 1984; Magnusson et al., 1984]. O'Toole et al. [1989], however, observed high levels of serum ferritin in ultraendurance triathletes. The mean serum ferritin levels were 95.2 ng/ml for men (n=31), 29 ng/ml for women less than 45 years old (n=15) and 57.5 ng/ml for women 45 years of age or older (n=4), each group higher than 12 ng serum ferritin/dl. Serum ferritin level higher than 12 ng/fl is an indication of abnormal status. Dufaux et al. [1981] observed significantly higher ($p<0.01$) ferritin levels in elite

Table 18 Hematological Parameters* of Subjects and Stages of Iron Deficiency.

A. Hematological parameters of subjects.

	Week 0		Week 11	
	Exercise	Non-Ex.	Exercise	Non-Ex.
Serum Ferritin, ng/dl	71.6 ± 44.8	100.4 ± 54.7	85.4 ± 52.0	120.7 ± 70.8
Trans Saturation, %	27.6 ± 11.8	34.7 ± 9.9	34.1 ± 16.7	34.6 ± 19.1
Serum Iron, mcg/dl	91.6 ± 25.9	111.1 ± 26.2	109.6 ± 44.4	100.9 ± 39.4
TIBC, mcg/dl	354.7 ± 103.1	321.3 ± 42.7 [a]	348.4 ± 85.5 [b]	306.7 ± 46.8 [b]
Hematocrit, %	41.9 ± 3.3 [c,d]	45.6 ± 2.4 [c]	42.0 ± 2.4 [d]	44.7 ± 1.0
Hemoglobin, mg/dl	14.6 ± 1.3	16.4 ± 0.9	15.1 ± 1.2	16.0 ± 0.6

* = $\bar{x}$ ± SD
a,b,d = $p<0.05$
c = $p<0.005$

B. Hematological parameters of iron deficiency [Herbert, 1987].

	Normal	Prelatent Iron Deficiency	Latent Iron Deficiency	Iron Deficiency Anemia
Serum Ferritin, mcg/dl	100 ± 60	20	12	<12
Trans Saturation, %	35 ± 15	30	<15	<10
Serum Iron, mcg/dl	115 ± 50	115	<60	<40
TIBC, mcg/dl	330 ± 30	360	390	410
Hematocrit, %	>37	>37	>37	<37
Hemoglobin, mg/dl	>12	>12	>12	<12

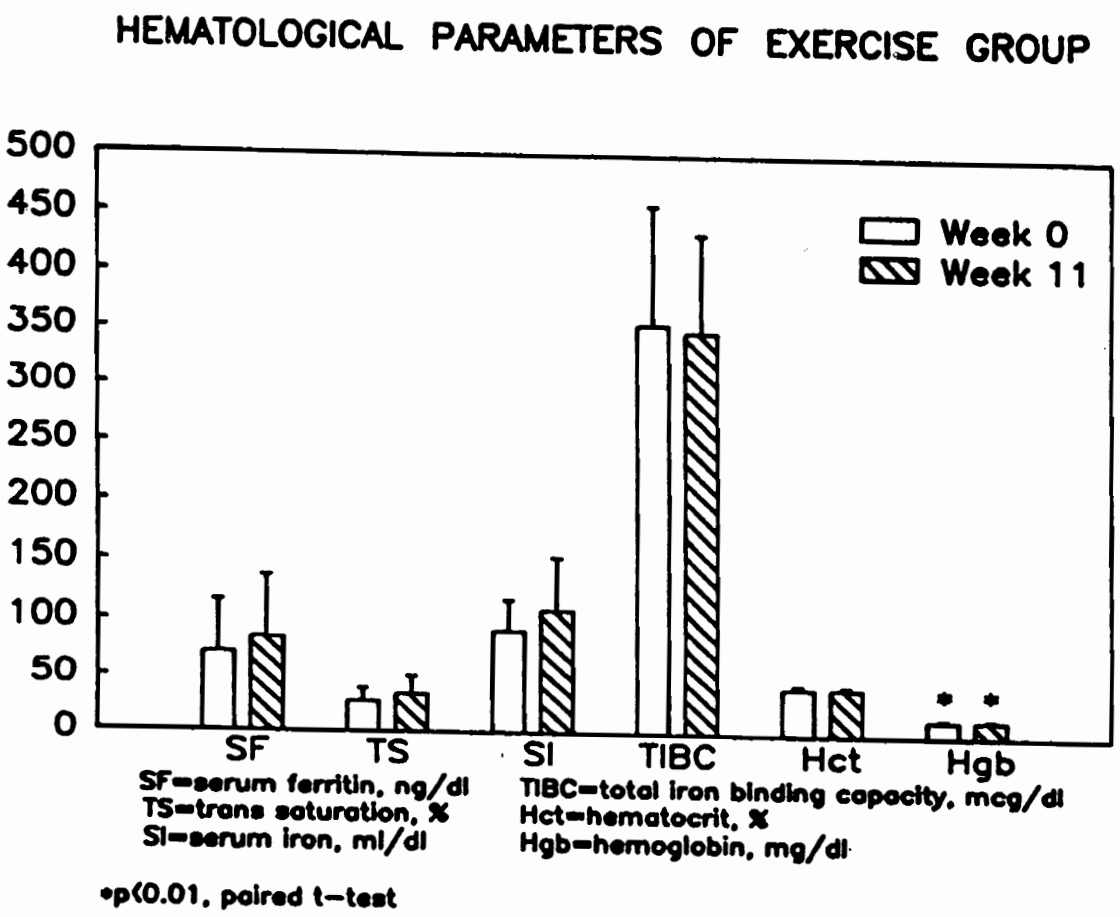


Figure 11 Hematological parameters of exercise group.

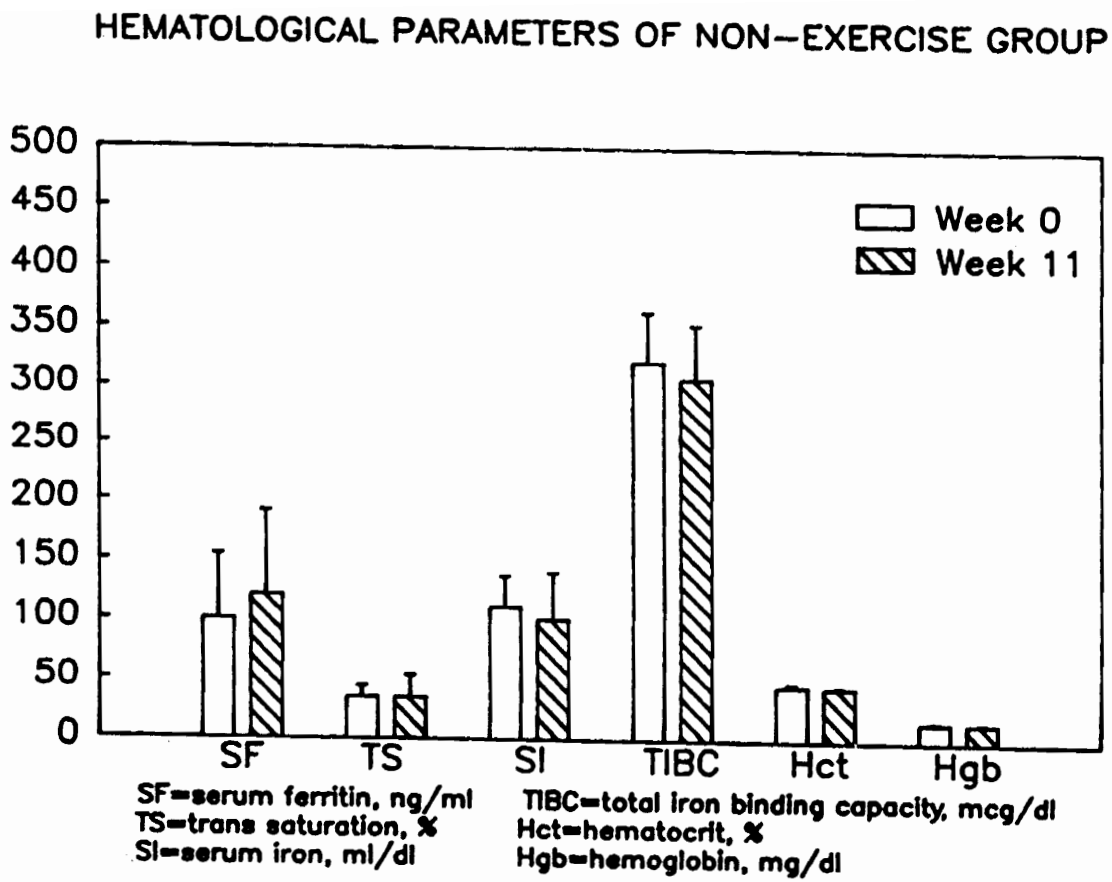


Figure 12 Hematological parameters of non-exercise group.

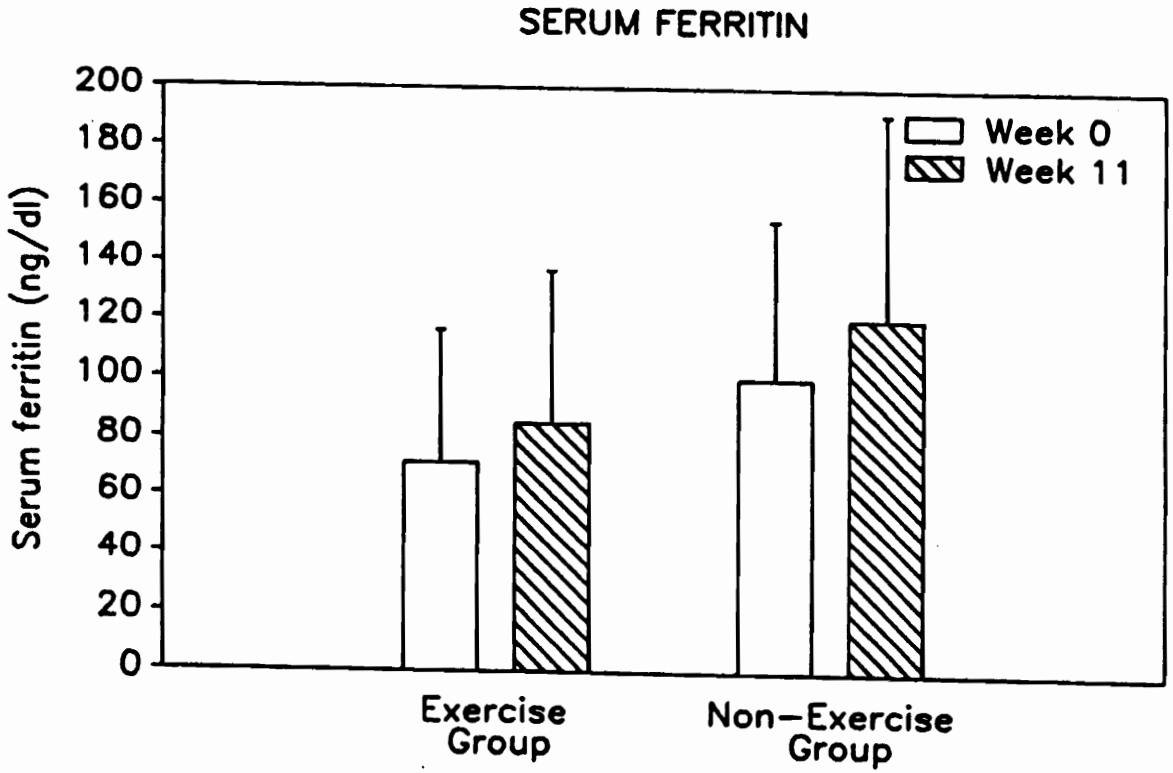


Figure 13 Serum ferritin level in exercise and non-exercise groups.

rowers than in the controls.

The iron transport parameters (serum iron, TIBC and transferrin saturation) usually change as storage iron is depleted, suggesting latent iron deficiency. In latent iron deficiency, serum iron and transferrin saturation decrease while TIBC increases. In the present study, serum iron, TIBC and transferrin saturation for both groups were within the normal range at weeks 0 and 11 (Figures 14, 15, 16; Tables 7,18). The exercise group in this study had desirable results; the serum iron and transferrin saturation increased and the TIBC decreased at week 11. The non-exercise group, however, had a decrease in serum iron, an increase in transferrin saturation and an increase in TIBC at week 11, but results from the paired t-tests indicate no significant differences at weeks 0 and 11.

Snyder et al. [1989] reported similar results in their study which determined the iron status among female runners. Nine females, average age 37.8 $\pm$ 1.3 years, and who ran approximately 32 miles/week volunteered for the study. Serum iron, TIBC and transferrin saturation among the runners, who were following a modified vegetarian diet, were 81.9 $\pm$ 8.2 mcg/100 ml, 366.5 $\pm$ 12.2 mcg /dl, and 22.4 % transferrin saturation, respectively; values were 89.8 $\pm$ 12.4 mcg/100 ml, 327.2 $\pm$ 9.6 mcg/dl and 28.0 $\pm$ 4.4 percent, respectively, in non-vegetarian runners. All iron status indicators in both groups were within normal limits. Adequate transport iron parameters were also found by Brotherhood

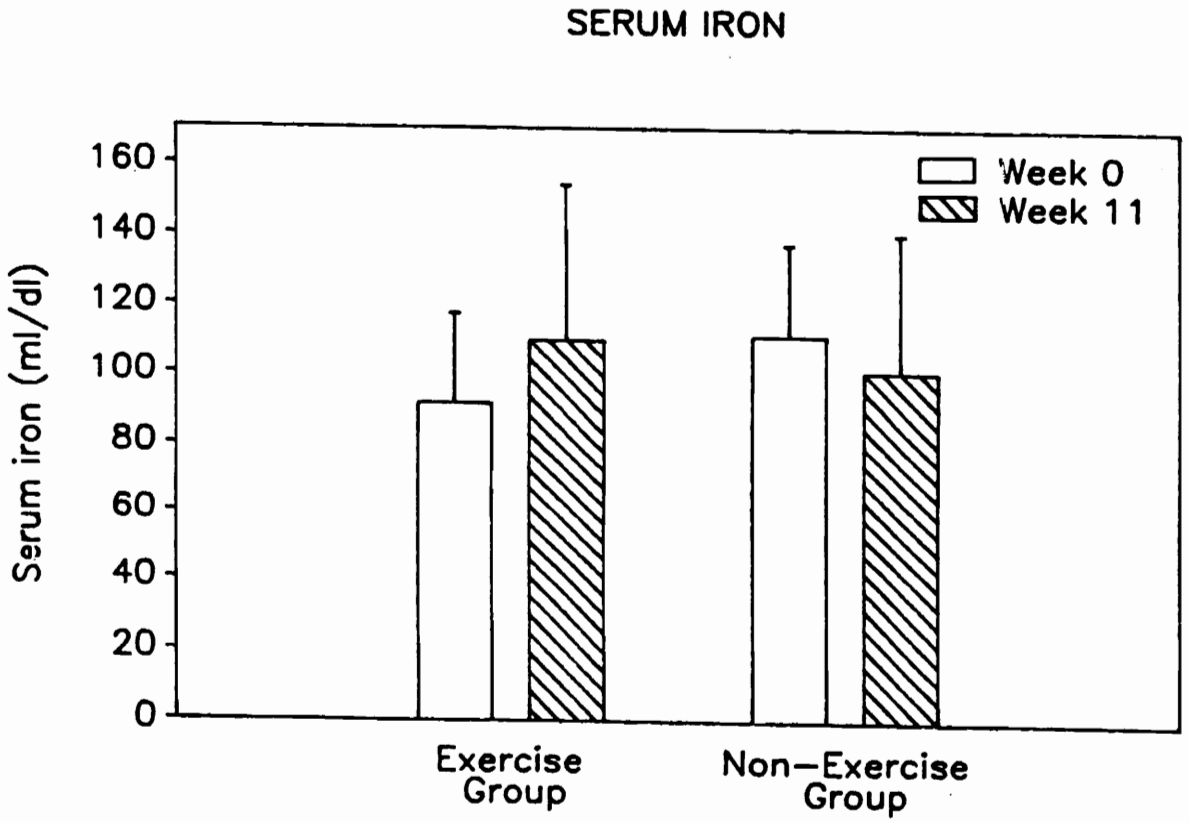


Figure 14 Serum iron levels in exercise and non-exercise groups.

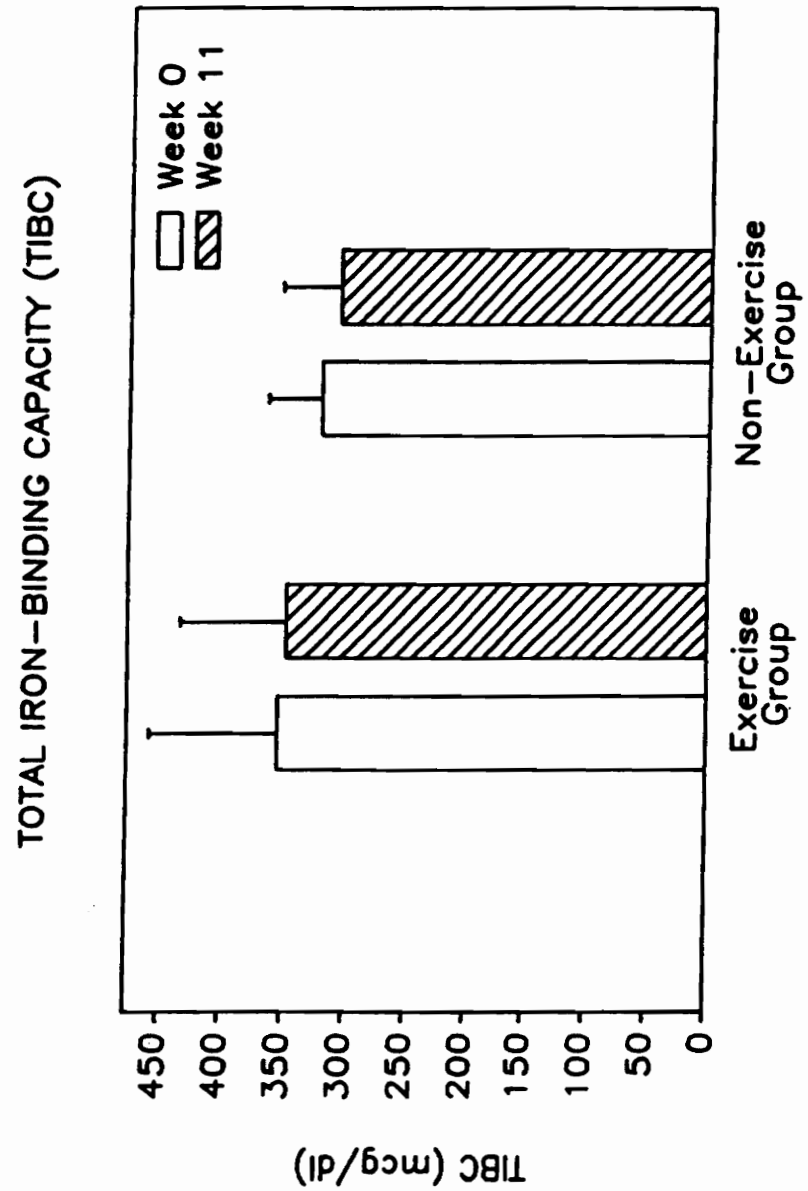


Figure 15 TIBC levels in exercise and non-exercise groups.

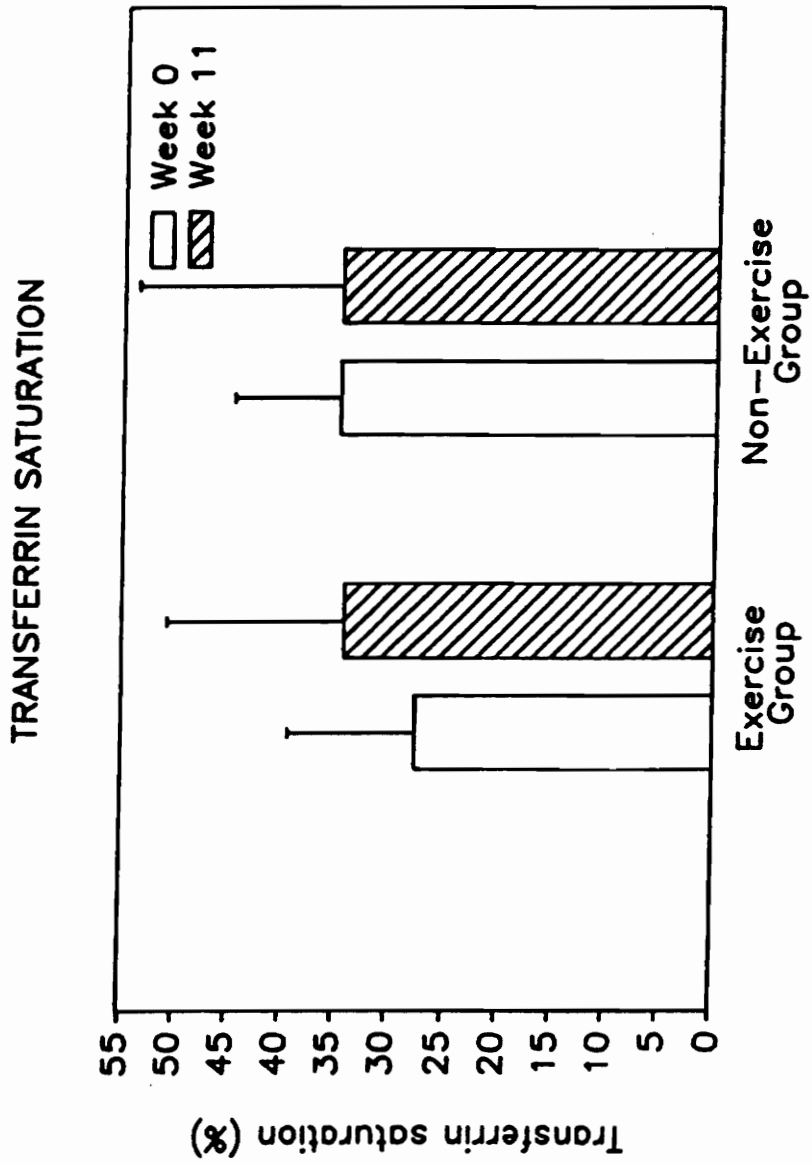


Figure 16 Transferrin saturation in exercise and non-exercise groups.

et al. [1975] in middle- and long-distance runners. Average values of 18.65 ± 5.73 mcmol/l for serum iron and 66.91 ± 8.63 mcmol/l for TIBC were within normal values (12-30 mcmol/l, 52-73 mcmol/l, respectively). Percent transferrin saturation was within the standard range of 20-50%.

Studies by Ericsson [1979] and Blum et al. [1986] also indicate maintenance of iron transport parameters with exercise. Ericsson [1979] analyzed the iron status in ten women, between the age of 57-71 years, before and after the physical work test. This test was performed on a cycle ergometer with increasing values for physical work capacity (W_{130}, W_{150}, W_{170}). The investigator observed that there was no difference in before and after treatment in the serum iron levels (106.6 ± 17.1 mg%, 101.7 ± 23.7 mg%, respectively); in the TIBC levels (341.3 ± 44.1 mg%, 333.3 ± 45.5 mg%, respectively); and transferrin saturation (31.5 ± 5.2%, 30.5 ± 6.4%, respectively). Blum et al. [1986] studied the iron status in women, aged 22-51 years, who attended a fitness-type aerobic class for thirteen weeks; they observed no signficant differences at weeks 0 and 13 in plasma iron (114.0 ± 8.6 mcg/dl, 117.1 ± 9.7 mcg/dl, respectively), TIBC (386.8 ± 14.2 mcg/dl, 378.2 ± 15.7 mcg/dl, respectively) and transferrin saturation (30.1 ± 2.8%, 27.8 ± 2.5%, respectively). However, in their study, serum ferritin levels were lower after thirteen weeks of exercise compared to baseline. In the present study, no significant differences were observed in serum ferritin level

between the two groups. There were, however, wide variations in serum ferritin among the women.

The final stage of iron deficiency is associated with a significant decrease in circulating hemoglobin and hematocrit. Suboptimal hemoglobin values have been observed among athletes [Stewart et al., 1972; Yoshimura, 1970; Frederickson et al., 1983; Selby et al., 1986]. In this study, the exercise group had a significantly lower hematocrit level than the non-exercise group ($p<0.05$) at weeks 0 and 11 (Figure 17). At week 0, the exercise group had a significantly lower hemoglobin level ($p<0.005$) than the exercise group, but at week 11 the exercise group had a significantly higher hemoglobin concentration compared to week 0 ($p<0.05$) (Figure 18). It is likely that the exercise training induced an increase in the red blood cell concentration and thereby increased hemoglobin concentration [Gabaree, 1989]. Aerobic exercise training also enhances the delivery of oxygen to the exercising muscle.

Blum et al. [1986] observed higher hemoglobin concentrations at week 6 (15.0 ± 0.2 g/dl) of their 13 week study; however, hemoglobin levels were similar to baseline values at week 13 (14.1 ± 0.2 g/dl). The hematocrit concentration at week 0 ($41,6 \pm 0.4$ percent) did not change at week 6 (42.1 ± 0.5) but significantly decreased and returned to initial values at week 13 (41.0 ± 0.6). The authors indicated that the elevated hemoglobin levels at week 6 are possibly due to the stress of exercise

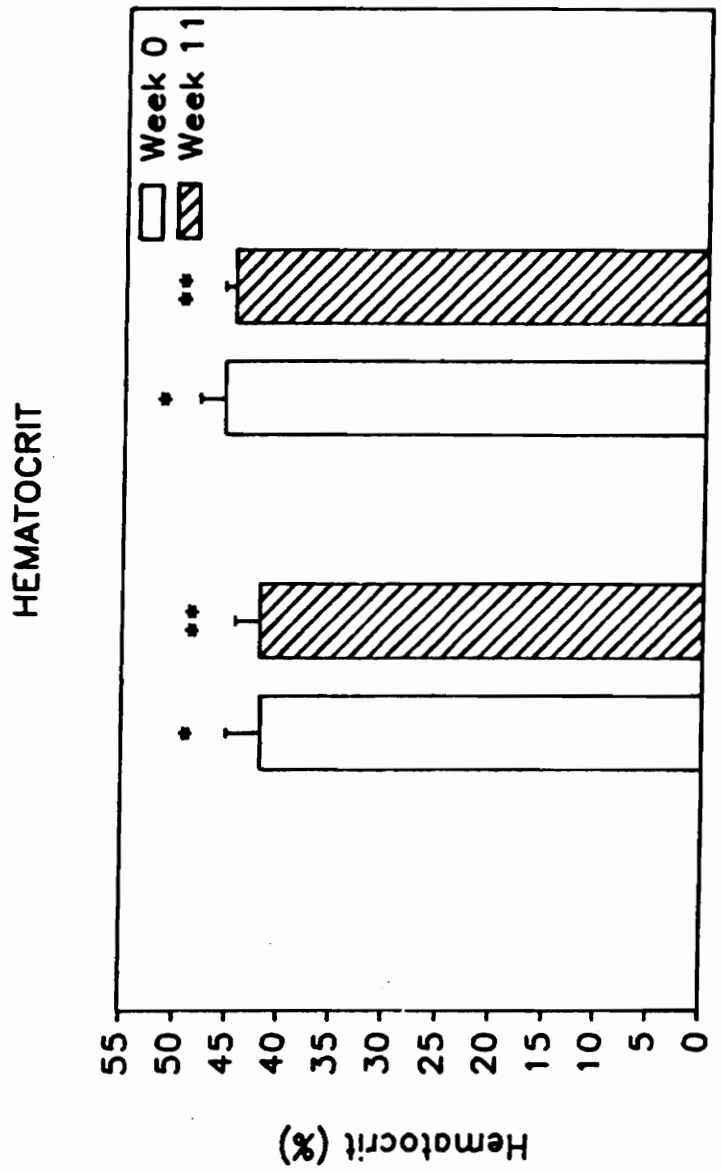


Figure 17 Hematocrit levels in exercise and non-exercise groups.

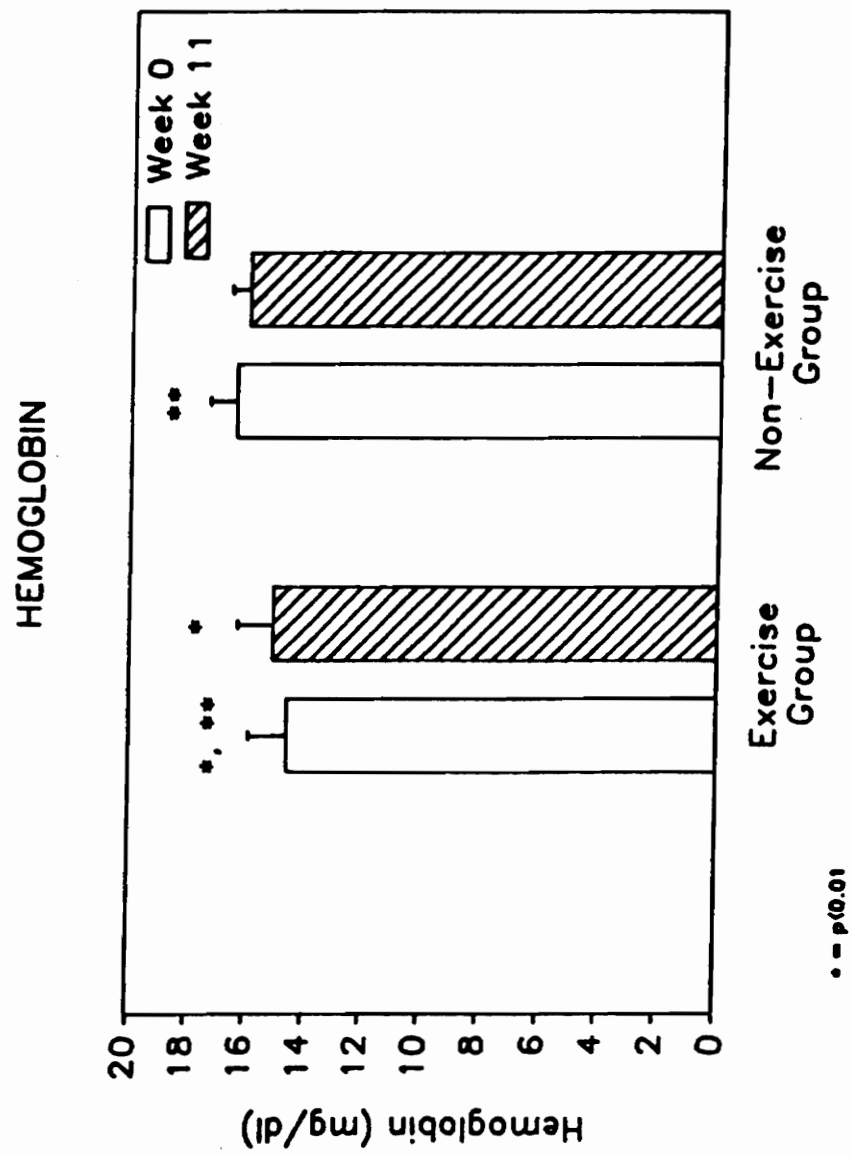


Figure 18 Hemoglobin levels in exercise and non-exercise groups.

stimulating an increase in the production of red blood cells and a concomitant increase in hemoglobin concentration.

The results of the present study are similar to those of Newhouse et al. [1989] and Balaban et al. [1989]. Newhouse et al. [1989] studied the iron status in forty females between the ages of 18 and 40 years. Hemoglobin concentration was determined pre- and post- eight weeks of moderate exercise, indicating no change post-exercise. Hemoglobin level at week 8 (13.1 $\pm$ 0.5 g/dl) was essentially the same as the hemoglobin level at week 0 (13.0 $\pm$ 0.6 g/dl), both values remaining within the normal limits. Balaban et al. [1989] observed higher hemoglobin levels in female long-distance runners (29 $\pm$ 5 years old) (13.3 $\pm$ 1.5 g/dl) compared to female non-runners (30 $\pm$ 6 years old) (12.8 $\pm$ 0.7 g/dl). Clement and Asmundson [1982] also noted normal hemoglobin (13.3 $\pm$ 0.9 g/100 ml) and hematocrit (37.6 $\pm$ 3.0%) levels in seventeen female endurance runners. Similar findings were reported by Loria et al. [1979].

In this study, the normal hematological parameters of the exercise and non-exercise groups at weeks 0 and 11 suggest that participation in the exercise program had no significant influence on the iron status of the elderly women. Because anemia in the elderly population is common [Hyams, 1985], it is unknown whether this is a result of disease with age or a normal physiological consequence of aging [Walsh, 1984]. In this present study, there were no significant changes in the iron status

except for the significant increase in the hemoglobin level in the exercise group. Future studies should consider the following factors: 1) longer duration of moderate exercise program; 2) complete assessment of subjects pre-, mid- and post-treatment; 3) larger number of participants in each group; 4) greater control of diet; 5) exclusion of all supplements; and 6) greater control of subject's cardiovascular fitness levels prior to participation.

Energy Expenditure in Normal Activity. There were no significant differences between or within the exercise and non-exercise groups in the energy expenditure in normal activities at weeks 0 and 10. At weeks 0 and 10, the exercise group averaged 1554.9 $\pm$ 64.5 kcal/day and 1563.2 $\pm$ 29.2 kcal/day, respectively; the non-exercise group averaged 1496.4 $\pm$ 162.2 kcal/day and 1487.4 $\pm$ 153.8 kcal/day, respectively.

In the exercise group, the average energy expenditure is greater than the average energy intake at weeks 0 and 10 (Table 19). Although not significantly different, both the energy expenditure and intake are higher in week 10 then week 0 for the exercise group. In the non-exercise group, the average energy expenditure is less than the average energy intake at week 0 and is greater than the average energy intake at week 10.

Several studies [Uauy et al., 1978; Gersovitz et al., 1982] of elderly women indicate that energy intakes show a tendency to decrease with age. Activity accounts for most of the difference

Table 19 Energy Expenditure and Intake

	Week 0	Week 10
1. Exercise Group		
Energy Expend.	1554.9 ± 64.5	1563.2 ± 29.2
Energy Intake	1223.0 ± 248.4	1270.1 ± 376.3
2. Non-Exercise Group		
Energy Expend.	1496.4 ± 162.2	1487.4 ± 153.8
Energy Intake	1533.3 ± 480.8	1348.7 ± 334.8

in the energy needs of individuals [Evans and Meredith, 1989]. For the elderly, an increase in energy expenditure with the intention of increasing energy and nutrient intakes may be achieved by participating in light to moderate exercise, allowing 4 to 5 kcal/min energy expenditure [Calloway and Zanni, 1980; Passmore and Durnin, 1955]. Meredith et al. [1987] observed that in endurance-trained middle-aged men, energy needs were a linear function of hours spent exercising per week in the same way as for young men. Debry et al. [1977] observed that in elderly women, energy intakes below 1500 kcal/day were found in 33% of those classified as "inactive" and only 9% of the "very active" women.

In summary, increased physical activity in the elderly is associated with an increase in energy intake and a favorable effect on health. However, education on the duration, frequency and intensity of the exercise as well as the source of energy (carbohydrate vs. fat vs. protein) is much needed.

Cardiovascular Endurance. An exercise stress test was conducted on each participating subject at weeks 0 and 11 1) to assess the safety of exercise prior to starting the exercise program; and 2) to assess the cardiopulmonary functional capacity of apparently healthy individuals [ACSM, 1986].

The average HR per grade (average HR per 2 minute intervals) is listed in Table 20 and shown in Figures 19 and 20. There was

Table 20 Average HR per Grade in Submaximal Stress Test *

	Week 0				Week 11			
Grade	n	Exercise	n	Non-Ex.	n	Exercise	n	Non-Ex.
1	8	103.8 ± 10.4 [a]	8	100.1 ± 8.4 [b]	8	94.8 ± 10.6 [a]	8	95.8 ± 6.0
2	7	113.0 ± 7.8 [c]	7	103.6 ± 6.5	8	102.8 ± 9.6 [c]	8	103.0 ± 8.6
3	2	119.0 ± 9.9 [d]	7	109.4 ± 6.8	8	111.3 ± 9.7 [d]	8	109.6 ± 8.5
4	1	117.0 ± 0.0 [e]	4	114.5 ± 4.2	5	113.0 ± 8.0 [e]	5	110.0 ± 1.4
5	0	0	1	119.0 ± 0.0	2	111.0 ± 9.9 [f]	4	117.3 ± 1.5
6	0	0	0	0	1	106.0 ± 0.0	0	0

* = $\bar{x} \pm SD$

a-e = $p<0.05$

f = Subject was only at 60% maximum HR at 12 minutes and requested the testing be ceased because she was becoming "bored".

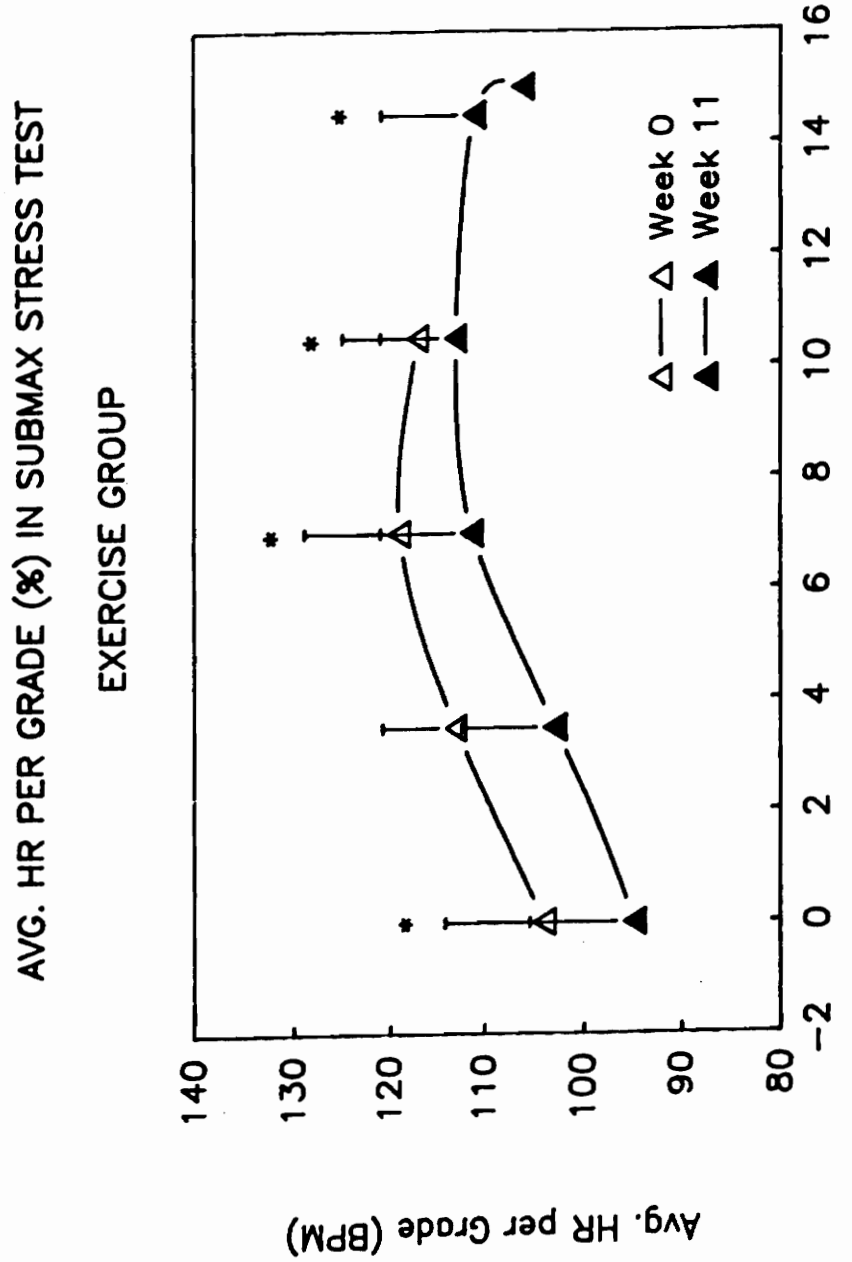


Figure 19 Average HR per grade of exercise group in submax stress test.

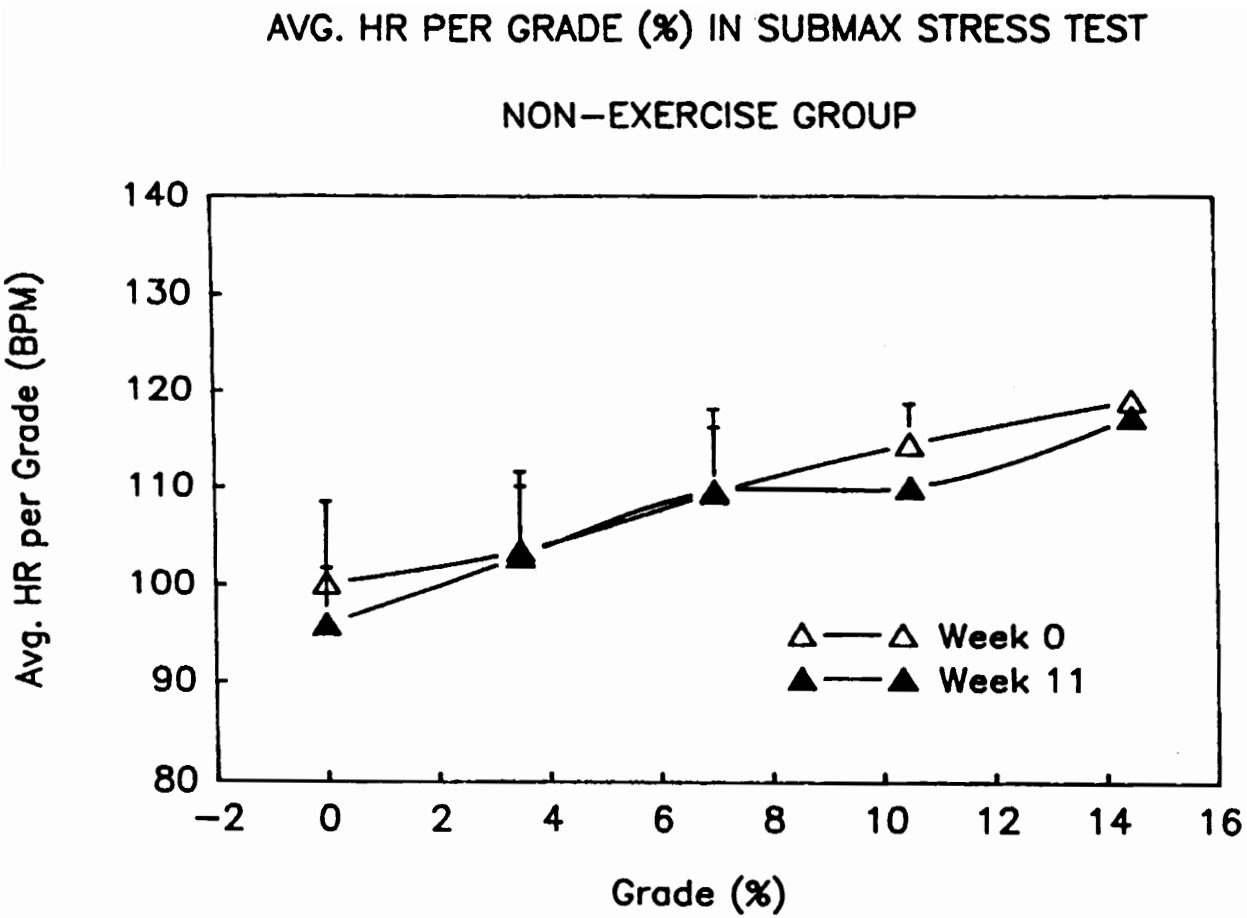


Figure 20 Avg. HR per grade of non-exercise group in submax stress test.

a steady increase in heart rate as the incline and duration of the submaximal stress test increased. Comparing the exercise group to the non-exercise group, there was a significant difference at grade 2 at week 0 ($p<0.05$). At week 11, a paired t-test indicated the exercise group had a significant decrease in HR at grade 1, 3, 4 and 5 ($p<0.05$). One subject from the exercise group reached grade 6 and only reached 60% maximum HR at 12 minutes. The non-exercise group displayed no significant differences in heart rate at week 11.

Our findings are similar to those Kilbom [1971] and Yerg et al. [1985]. Kilbom [1971] analyzed the effect of submaximal physical activity on female subjects 51-64 years of age. They participated in a 7-week physical training program, conducted intermittently two to three times a week on ergometers, work load set to 70% of the maximal aerobic capacity. The author observed significantly lower heart rate (6-14 beats/minute) at submaximal loads after training than before training ($p<0.01$).

Yerg et al. [1985] also observed a decrease in HR in elderly individuals after a twelve month training program. Heart rate was significantly lower ($p<0.025$) after the training program (125 ± 13) compared to before (141 ± 18).

In the present study, baseline values (week 0) of time to reach 70% max HR were significantly higher in the non-exercise group than in the exercise group ($p<0.05$). At week 0, the exercise group averaged 4.3 ± 1.6 minutes and the non-exercise group

averaged 6.5 ± 2.3 minutes. At week 11, time to reach 70% max HR was significantly greater ($p<0.001$) in the exercise group (8.0 ± 2.3 minutes) compared to week 0. The non-exercise group averaged 8.1 ± 2.2 minutes at week 11 which was higher but not significantly higher than week 0 (Figure 21). No other significant differences in the submaximal stress test were noted.

Paired t-tests indicate the average VO2 (ml/kg BW) of the exercise group at week 11 was significantly lower at grades 1, 4 and 5 ($p<0.05$); VO2 values at grades 2 and 3 were also lower (Table 21). Paired t-tests indicate the non-exercise group had a significantly higher VO2 average at grade 5 ($p<0.05$) and had lower average VO2 values at grades 1, 2, 3 and 4. Table 22 shows the individual VO2 values at 70% maximum HR for each subject. Three of the subjects had an extremely high submaximal VO2 at week 0. No significant differences were observed between or within the two groups in VO2 at 70% maximum HR.

Many studies have observed a gradual decrease in the functional capactiy and VO2 max with aging [Astrand et al., 1973; Dehn and Bruce, 1972]. Dehn and Bruce [1972] found a significantly lower VO2 max in sedentary men 50 to 69 years of age compared to physically active men who ran at least three miles/week). The sedentary men, age 50-59 years, had an average VO2 max (ml/kg/min) of 31.15 ± 4.67; active men had an average value of 37.67 ± 4.52. The sedentary men, age 60-69 years, had an average of 28.76 ± 3.09; the active men had an average of

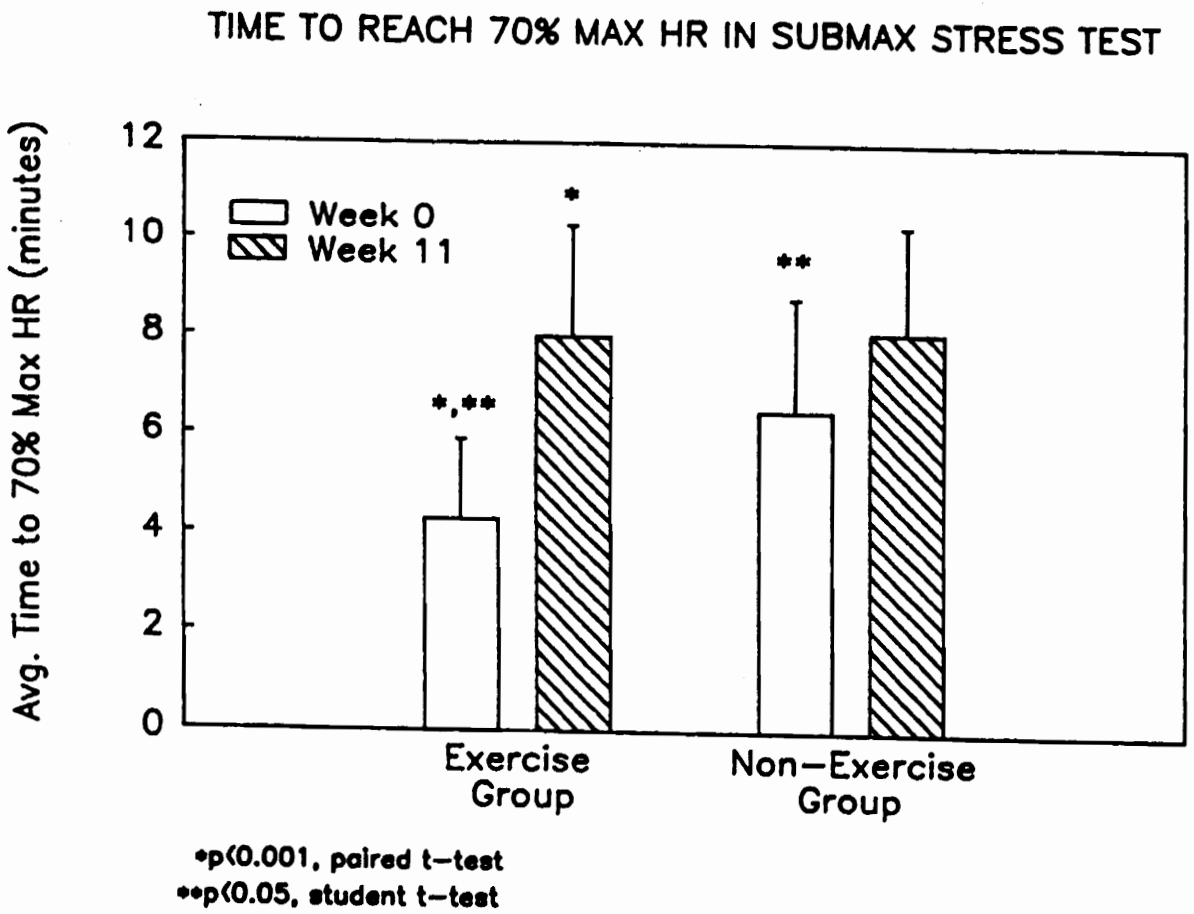


Figure 21 Time to reach 70% max HR in submax stress test.

Table 21 VO2 (ml/kg BW) per Grade in Submaximal Stress Test.*

	Exercise Group				Non-Exercise Group			
Grade	n	Week 0	n	Week 11	n	Week 0	n	Week 11
1	8	10.9 ± 2.4 [a]	8	8.4 ± 1.7 [a]	8	11.9 ± 7.5	8	9.1 ± 1.7
2	7	16.3 ± 4.5	8	12.5 ± 1.8	7	16.4 ± 8.4	8	13.3 ± 1.7
3	2	21.7 ± 5.8	8	14.6 ± 2.1	7	17.8 ± 9.1	8	14.6 ± 1.8
4	1	19.0 ± 0.0 [b]	5	16.1 ± 2.4 [b]	4	21.2 ± 12.3	5	15.6 ± 1.4
5	0	0 [c]	2	18.9 ± 1.3 [c]	1	9.8 ± 0.0 [d]	4	16.7 ± 0.9 [d]
6	0	0	1	20.2 ± 0.0 [e]	0	0	0	0

* = $\bar{x} \pm SD$

a-d = $p<0.05$

e = Subject was at 60% maximum HR.

Table 22 Individual VO2 (ml/kg BW) at 70% Maximum Heart Rate

A) Exercise group

	VO2 (ml/kg BW)	
Subject	Week 0	Week 11
1	15.28	17.15
2	21.75	13.81
3	22.56	19.61
4	16.07	18.36
5	13.15	13.72
6	18.42	19.78
7	27.34 *	12.64
8	17.60	18.73
**		
Average	19.0 ± 4.6	16.7 ± 2.9

B) Non-Exercise Group

	VO2 (ml/kg BW)	
Subject	Week 0	Week 11
1	12.72	18.49
2	15.04	18.36
3	18.23	14.95
4	36.32 *	18.53
5	8.93	19.09
6	32.12 *	16.99
7	7.07	19.65
8	10.91	17.63
**		
Average	17.7 ± 10.8	18.0 ± 1.5

* Extremely high VO2 value.
** No significant differences in student and paired t-tests.

31.29 $\pm$ 6.92.

Submaximal oxygen uptake in the elderly (compared to maximal VO2) at standardized workloads remains unchanged as a result of training [Barry et al., 1966; Benestad, 1965; Stamford, 1973]. A study by Seals et al. [1984] determined the effects of prolonged endurance training on submaximal oxygen consumption. Twenty-four healthy men and women participated in the study which involved six months of low intensity training, followed by six months of high intensity training. Results indicate that cardiac output was not significantly different after training at the same absolute work rates or at similar relative work rates compared with before training. Oxygen consumption (l/min) at the same absolute work rates was unaffected by training. Similar results were found by Yerg et al. [1985] who reported lower ventilatory equivalent for oxygen during submaximal exercise in elderly sedentary men (61 to 67 years old) who completed a twelve month endurance training program. The authors speculate that the subjects are less physiologically stressed after training.

A study by Montoye [1982] compared the oxygen uptake during submaximal treadmill exercise in men aged 10 to 34 years to older men aged 35 to 69 years. The results from his study indicate an increase in walking efficiency (decreased VO2) in the older compared to the younger group. Daniels and Oldridge [1971] and Daniels et al. [1978] had similar findings. These two studies compared boys 10 to 18 years of age and reported decreased oxygen

cost of running at 202 m/min with no change in VO2 max as the boys became older. This improved "efficiency", which they attributed mainly to growth, was responsible for improved performance in the 1- and 2-mile run. Robinson et al. [1973] also reported no change in submaximal exercise VO2 (ml/kg/min) when they tested 10 men, at ages 18 to 22, 41 to 44 and 50 to 54 years on treadmill walking exercises.

Although there were no significant differences within or between the exercise and non-exercise group in the VO2 level at 70% maximum heart rate, three of the values at week 0 were extremely high. This may have been due to gas leakage in the tube attatched to the Rudolf gas mask or mechanical error which may have altered the volume of air and the measurements of oxygen and carbon dioxide. An orientation of the stress test procedure and equipment may have been helpful because some of the subjects were unacquainted with the equipment (mask and electrodes).

In summary, the exercise group obtained significantly higher time ($p<0.001$) and significantly lower heart rate per grade ($p<0.05$) to reach 70% heart rate at week 11. There were no significant changes in time and heart rate in the non-exercise group. The exercise group had significantly lower VO2 values at grades 1, 4 and 5 ($p<0.05$). There were no significant differences between or within the two groups in the average VO2 at 70% maximum HR which may be due to the improved efficiency and decreased anxiety of the subjects. The data on the time and

heart rate per grade to reach 70% heart rate indicate improved cardiovascular endurance of the exercise group as a result of ten weeks of moderate exercise.

CHAPTER IV

SUMMARY AND CONCLUSION

The objectives of this study were to 1) examine the changes in dietary intake during moderate aerobic exercise in women 56- to 67-years old; 2) to investigate whether iron status is affected by regular exercise; and 3) to examine the changes in cardiovascular fitness with regular exercise. The subjects in the exercise (N=8) and non-exercise (N=9) groups had no significant differences in height, weight, body composition and body mass index. Neither group had an average BMI greater than 27.5. The exercise group was significantly lower in age ($p<0.02$), but only by a small magnitude (<1 year).

Dietary intakes of the subjects in both groups were adequate. The non-exercise group had a significantly higher intake of vitamin C ($p<0.02$) than the exercise group at week 0. No other significant differences were observed in energy and nutrient intakes between or within the exercise and non-exercise groups at week 0 and 10. One-hundred percent or more of the 1989 RDA of protein, vitamin A, vitamin C, thiamin, riboflavin, niacin, folate, vitamin B12 and phosphorus was consumed by both groups at weeks 0 and 10. Both groups consumed adequate amounts of vitamin B6, folate and iron, each contributing to the iron status. Dietary intakes of vitamin E averaged 71% to 90% of the 1990 RDA, calcium averaged approximately 90%, and zinc averaged 56% to 66%,

indicating that education on the importance of proper nutrition of essential nutrients needs attention. Dietary analysis of the two groups at weeks 0 and 10 also indicates that moderate aerobic exercise does not alter the dietary intake in the active group.

Hematological analysis indicated the exercise group had significantly lower hematocrit concentration than tbe non-exercise group at week 0 ($p<0.05$) and week 11 ($p<0.05$). The exercise group also had significantly lower hemoglobin concentration than the non-exercise group at week 0 ($p<0.01$). Paired t-tests indicate the exercise group had significantly increased the hemoglobin concentration at week 11 ($p<0.01$). This may be the result of increase in red blood cell concentration inducing the increase in hemoglobin concentration [Gabaree, 1989; Blum et al., 1986] or enhancing the delivery of oxygen to the exercising muscles [Gabareet, 1989]. No other significant differences were observed in the serum ferritin, serum iron, TIBC and transferrin saturation between or within the groups. These findings suggest that moderate exercise does not have a significant affect on the iron status of elderly women.

There were no significant differences between or within the groups in the energy expenditure during normal activities. However, energy utilization was greater than energy intakes for the exercise group at week 0 and 10 and for the non-exercise group at week 10. Participants in exercise programs realize the health benefits of activity but should be informed of the source of

energy (carbohydrates, protein and fat) to obtain the improvement in diet and cardiovascular fitness.

The average heart rate per grade in the submaximal stress test was significantly lower in the exercise group at week 11 at grades 1, 3, 4 and 5 ($p<0.05$). One subject from the exercise group who reached grade 6 at week 11 was only at 60% maximum heart rate. The exercise group also had a significant increase in time to reach 70% maximum HR at week 11 ($p<0.001$). The non-exercise group displayed no significant differences in time and heart rate at week 11. There were no significant differences in the VO2 per grade (ml/kg BW) or at VO2 (ml/kg BW) when 70% maximum HR was reached between or within the groups. The exercise group, however, had slightly lower VO2 values at week 11 compared to week 0 which may have been attributed to improved efficiency and decreased anxiety. The data from the ten-week moderate exercise protocol indicates the exercise group improved their cardiovascular endurance level. Because aerobic capacity declines with age, moderate exercise should be suggested to the elderly population with proper initial testing and adequate frequency, duration and intensity for individual levels..

In conclusion, results from this study indicate that moderate aerobic exercise did not have an adverse effect on energy and nutrient intakes or hematological parameters of iron status in elderly women. The ten-week exercise program, however, did significantly improve the cardiovascular endurance level of women

in the exercise group.

There are several suggestions for future research. A larger sample group may be desirable as well as a longer period of study. Although it would need more money and time, collecting identifical food for each subject and having food composites of one or three-day dietary intakes instead of dietary records may be more accurate. Similar baseline values of the exercise group may be obtained through a longer training period to see the effects of moderate exercise on cardiovascular endurance. An orientation and/or practice session with the equipment used for the stress test may be helpful to ease the anxiety of the subjects if they are unfamiliar with the equipment. This practice session and the training period will also allow the subjects in the exercise group become familiar with the Borg RPE which correlates with the VO2, heart rate and ventilation and came be used to prescribe the intensity of the exercise [ACSM, 1986]. Also, because some studies [Barry et al., 1965; Benestad, 1965; Stamford, 1973] indicate that the elderly subjects show no changes in submaximal oxygen uptake, a maximal stress test pre- and post-treatment may provide more information.

REFERENCES

Abraham S, Carroll MD, Dresser CM, Johnaon CL. Caloric and selected nutrient values for persons 1-74 years of age, United States, 1971-74. Vital and health statistics series II-no 209. Rockville, MD: National Center for Health Statistics, 1979 DHEW publication no (PHS) 79-1657..

Abraham S, Carroll MD, Dresser CM, Johnson CL. Dietary intake finds, United States, 1971-74. Vital and health statistics II-no 202. Rockville, MD: National Center for Health Statistics, 1977. DHEW publication no (HRA) 77-1647.

Adamson JW and Finch CA. Hemoglobin function, oxygen affinity, and erythropoietin. Ann Rev Physiol. 1975;37:351-69.

Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Liewellin P. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. J CLin Path. 1972;25:326.

American College of Sports Medicine. Guidelines for graded exercise and exercise prescription (3rd ed.). Philadelphia, PA: Lea and Febiger, 1986.

Anderson HT and Barkve H. Iron deficiency and muscular work performance. Scan J Clin Lab Invest Supple 25. 1970;25:114.

Andres R, Hallfrisch J. Nutrient recommendations needed for the older American. J Amer Diet Assoc. 199;9:1739-41.

Astrand I, Astrand PO, Hallback I, et al. Reduction in maximal oxygen uptake with age. J Appl Physiol. 1973;35:649-54.

Badenhop DT, Cleary PA, Schaal SF, Fox EL, Bartels RL. Physiological adjustments to higher- or lower- intensity exercise in elders. Med Sci Sports Ex. 1983;15:496-502.

Balaban EP, Cox JV, Snell P, Vaughan RH, Frenkel EP. The frequency of anemia and iron deficiency in the runner. Med Sci Sports Ex. 1989;21:643-48.

Ballas SK. Normal serum iron and elevated total iron binding capacity in iron deficient states. Am J Clin Pathol. 1979;71:401-3.

Barry AJ, Steinmetz JR, Page HF, Rodahl, K. The effect of physical conditioning in older subjects. II. Motor performance and cognitive function. J Geontol. 1966;21:192-99.

Basta SS and Churchill A. Iron deficiency anemia and the productivity of adult males in Indonesia. World Bank Staff Working Paper. 1974. Washington, DC:World Bank.

Baum BJ. Characteristics of participants in the oral physiology component of the Baltimore longitudinal study of aging. Comm Dent Oral Epidemiol. 1981;9:128-34.

Baum BJ. Evaluation of stimulated parotid saliva flow rate in different age groups. J Dent Res. 1981;60:1292.

Beck, WS. Iron metabolism and the hypochronic anemias. In Hematology (Ed. Ws Beck) p. 27-50, 1973. MIT press.

Beilin LJ, Armstron BK, Margetts BM, Rouse IL, Vandongen R. Vegetarian diet and blood pressure. Nephron (suppl). 1987;47:47.

Benestad AM. Trainability of old men. Acta Med Scand. 1965;20:938-47.

Bethesda Conference 18: Cardiovascular disease in the elderly. J Am Coll Cardiol. 1987;10:1A-87A.

Beutler E, Larsh S, Tanzi F. Iron enzymes in iron deficiency. VII. Oxygen consumption measurements in iron deficient subjects. Appl J Med Sci. 1960;239:759.

Bhanthumnavin K and Schuster MM. Aging and gastrointestinal function. In Handbook of Biology of Aging (Finch CE and Hayflick L, ed). 1977; Chapter 27. New York:Van Nostrand Reinhold.

Blackman AH, Lambert DL, Thayer WR, et al. Computed normal values for peak acid output based on age, sex and body weight. Am J Dig Dis. 1970;15:783.

Blum SM, Sherman AR, Boileau RA. The effects of fitness-type exercise on iron status in adult women. Am J Clin Nutr. 1986;43:456-63.

Blumenthal JA, Schocken DD, Needels TL, Hindle P. Psychological and physiological effects of physical conditioning on the elderly. J Psychosom Res. 1982;26:505-10.

Bjorn-Rasmussen E. Iron absorption from wheat bread: Influence of various amounts of bran. Nutr Metab. 1974;16:101-10.

Bjorn-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man: Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. J Clin Invest. 1974;53:247-55.

Blumberg JB. Nutrient requirements for the healthy elderly. Contemp Nutr. 1986;11:1.

Bortz WM. Disuse and aging. JAMA. 1982;248:1203-8.

Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron Metabolism in Man. Oxford:Blackwell Scientific Publications, 1979; pp 88-104.

Bothwell TH and Finch CA. Iron metabolism. London:Churchill, 1962.

Bottiger LE, Nyberg A, Astrand I, Astrand PO. Iron administration to healthy, physically active students. Nord Med. 1971;85:396-98.

Bowering J and Norton GF. Relationships between iron status and exercise in male and female growing rats. J Nutr. 1981;111:1648-57.

Bowman BB and Rosenberg IH. Digestive function and aging. Hum Nutr: Clin Nutr. 1983;37C:75-89.

Brandfonbrener M, Landome M, Shock N. Changes in cardiovascular output with age. Circulation. 1955;12:557-66.

Brise H and Hallberg L. Iron absorption studies II. Acta Med Scand (Suppl 376). 1962.

Brooks GA and Fahey TD. Coronary heart disease. Exercise Physiology--Human Bioenergetics and Its Applications. united States: MacMillan Publishing Co., 1985.

Brotherhood J, Brozovic B, Pugh LGC. Haematological status of middle- and long-distance runners. Clin Sci Mol Med. 1975;48:139-45.

Brown PT, Bergan JG, Parsons EP, Krol I. Dietary status of elderly people. J Am Diet Assoc. 1977;71:41-45.

Buss EW. How mind, body and environment influence nutrition in the elderly. Postgrad Med. 1978;63:118.

Calloway DH, Zanni E. Energy requirements and energy expenditure of elderly men. Am J Clin Nutr. 1980;33:2088-92.

Charlton RW, Hawkins DM, Mavor WO, Bothwell TH. Hepatic storage iron concentrations in different population groups. Am J Clin Nutr. 1970;23:358-71.

Clement DB, Asmundson RC: Nutritional intake and hematological parameters in endurance runners. Phys Sportsmed. 1982;10:37-43.

Clement DB, Sawchuk LL. Iron status and sports performance. Sports Med. 1984;1:65-74.

Committee on Dietary Allowances and Food and Nutrition Board. Recommended Dietary Allowances. Ninth Revised Edition, 1989. National Academy of Sciences, Washington, DC. 1989.

Cook JD. Clinical evaluation of iron deficiency. Semin in Hematol. 1982;19:3-18.

Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. Blood. 1976;48:449-55.

Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr. 1974;27:681.

Cook JD and Monsen ER. Food absorption in man. II. The effect of EDTA on absorption of dietary non-heme iron. Amer J Clin Nutr. 1976;29:614-20.

Cook JD and Monsen ER. Food iron absorption in human subjects. V. The effect of egg albumin and its amino acid components. Amer J Clin Nutr, 1979.

Cook JD and Monsen ER. Vitamin C, the common cold, and iron absorption. Am J Clin Nutr. 1977;30:235-41.

Cooter GR and Mowbray KW. Effects of iron supplementation and activity on serum iron depletion and hemoglobin levels in female athletes. Res Q. 1978;49:114-18.

Council on Scientific Affairs. Physician-supervised exercise programs in rehabilitation of patients with coronary heart disease. JAMA. 1981;245:1463-66.

Council on Scientific Affairs. Exercise programs for the elderly. JAMA. 1984;252:544-46.

Dallman PR, Refino C, Yland MJ. Sequence of development of iron deficiency in the rate. Am J Clin Nutr. 1982;35:671-77.

Dallman PR, Yip R, Johnson C. Prevalence and causes of anemia in the United States, 1976-1980. Amer J Clin Nutr. 1984;39:437-45.

Daniels J, Oldridge N. Changes in oxygen consumption of young boys during growth and running training. Med Sci Sports. 1971;3:161-65.

Daniels J, Oldridge N, Nagle F, White B. Differences and changes in VO2 among runners 10 to 18 years of age. Med Sci Sports. 1978;10:200-3.

Davies CTM, Chakweumeka AC, Van Haaren JP. Iron deficiency anemia; Its effect on maximum aerobic power and response to exercise in African males aged 17-40 years. Clin Sci. 1973;44:555.

Davis MA, Randall E, Forthober RN, Lee SE and Margon S. Living arrangements and dietary patterns of older adults in the United States. J Gerontol. 1985:40:434.

Debry G, Bleyer R, Maring JM. Nutrition in the elderly. J Hum Nutr. 1977;31:195-203.

Dehn MM, Bruce RA. Longitudinal variations in maximal oxygen intake with age and activity. J Appli Physiol. 1972;33:805-7.

Dibble MV, Brin M, Thiele VF, Peel A, Chen N, McMullen E. Evaluation of the nutritional status of eldelry subjects, with a comparison between fall and spring. J Am Geritr Soc. 1967;15:1031-61.

Disler PB, Lynch SR, Charlton RW, Torrance JD, Bothwell TH, walker RB, Mayet F. The effect of tea on iron absorption. Gut. 1975;16:193.

Dresser CM, Carrol MD, Abraham S. Food consumption profiles of white and black persons ages 1-74 years by age, United States 1971-74. Vital and health statistics series II-no 210. Rockville, MD: National Center for Health Statistics, 1979. DHEW publication no (PHS) 79-1658.

Driskell JA. The vitamin B6 status of the elderly. In: Human vitamin B6 requirements. Washington, DC: National Academy of Sciences, 1978:252-6.

Dufaux B, Hoederath A, Streitberger I, Hollman W, Assmann G. Serum ferritin, transferrin, haptoglobin, and iron in middle- and long-distance runners, elite rowers, and professional racing cyclists. Int J Sports Med. 1981;2:43-46.

Ehn L, Carlmark B, Hoglund S. Iron status in athletes involved in intense physical activity. Med Sci Sports Ex. 1980;21:61-64.

Ellis FP. Mortality from heat illness in the United States. A progress report. Archives of Science Physiology.1973:27:A573.

Ellis FP, Exton-Smith AN, Foster KG et al. Eccrine sweating and mortality during heat waves in very young and old persons. Isreal J Med Sci. 1976;12:815.

Ericsson P. The effect of iron supplementation on the physical work capacity in the elderly. Acta Med Scand. 1970;1:361-74.

Evans WJ and Meredith CN. Exercise and nutrition in the elderly. In Nutrition, Aging and the Elderly. (Munro HN and Danford DE, eds.) Plenum Publishing Corporation, 1989, pp89-126.

Exton-Smith, AN. Nutritional status: Diagnosis and prevention of malnutrition. In Metabolic Nutritional Disorders of the Elderly (Exton-Smith AN and Caird FI, eds.). 1980; Chicago:Year Book Medical Publishers.

Fairbanks VF, Beutler E. Erthyrocyte disorders--anemias related to disturbances of hemoglobin synthesis. In Hematology (Ed. Williams WJ et al.) 1977.

Fikry ME. Gastric secretory functions in the aged. Geront Clin (Basel), 1965;7:216.

Foy H, Kondi A, Austin WH. Effect of dietary phytate on fecal absorption of radioactive ferric chloride. Nature. 1959;183:691-92.

Frederickson LA, Puhl JL, Runyan. Effects of training on indices of iron status of young female cross-country runners. Med Sci Sports Ex. 1983;15:271-76.

Freiman HD, Tauber SA, Tuslky EG. Iron absorption in the healthy aged. Geriatrics, 1963;18:716-20.

Gaehtgens P, Kreutz F, Albrecht KH. Optimal hematocrit for canine skeletal muscle during rhythmic isotonic exercise. Eur J Appl Physiol. 1979;41:27-39.

Gardner GW, Edgerton VR, Barnard RJ, Bernauer EM. Cardiorespiratory hematological and physical performamce responses of anemic subjects to iron treatment. Am J Clin Nutr. 1975;28:982.

Gardner GW, Edgerton R, Senewiratne B, Barnard RJ, Ohira Y. Physical work capacity and metabolic stress in subjects with iron deficiency anemia. Am J Clin Nutr. 1977;30:910-17.

Gabaree CLV, Hemoglobin. Natl Strength Cond Assoc J. 1989;11:54-7.

Garry PJ, Goodwin JS, Hunt WX, Hooper EM, Leonard AG. Nutritional status in a healthy elderly population: dietary and supplemental intakes. Am J Clin Nutr. 1982;36:319-31.

Gautier de Defaix , Puente R, Vidal B, Perez B, Vidal H. Liver storage iron in normal population of Cuba. Am J Clin Nutr. 1980;33:133-6.

Gersovitz M, Motil K, Munro HN, Scrimshaw NS, Young VR. Human protein requirements: Assessment of the adequacy of the current recommended dietary allowance for dietary protein in elderly men and women. Am J CLin Nutr. 1982;35:6-14.

Giannella RA, Broitman SA, Zamcheck N. Influence of gastric acidity on bacterial and parasitic enteric infections. Ann Intern Med. 1973;78:271.

Gibbs SE and Turner HB. Social-psychological aspects of nutrition among the elderly. Part II. In Nutritional Aspects of Aging (Chen LH, ed);1986;Boca Raton, RL:CRC Press.

Gillum HL, Morgan AF. Nutritional status of the aging: I. Hemoglobin levels, packed cell volumes and sedimenation rates of 577 normal men and women over 50 years of age. J Nutr. 1955;55:265-88.

Gorman KM and Posner JD. Benefits of exercise in old age. Clinics in Ger Med. 1988;4:181-91.

Greenblatt DJ. Reduced serum albumin concentration in the elderly: a report from the Boston Collaborative Drug Surveillance Program. J Am Geriatr Soc. 1979;27:20.

Greger JL, Sciscoe BS. Zinc nutriture of elderly participants in an urban feeding program. J Am Diet Assoc. 1977;70:37-41.

Greger JL. Dietary intake and nutritional status in regard to zinc of institutionalized aged. J Gerontol. 1977;32:549-53.

Griffiths HJL, Nicholson WJ, O'Gorman PO. A haematological study of 500 eldelry females. Geront Clin. 1970;12:18.

Guilland JC, Bereksi-Reguig B, Lequeu B, Morreau D, Klepping J, Richard D. Evaluation of pyridoxine intake and pyridoxine status among aged institutionalized people. Int J Vitam Nutr Res. 1984;54:185-93.

Hallberg L and Bjorn-Rasmussen E. Determination of iron absorption from whole diet. Scand J Haematol. 1972;9:193.

Hallberg L, Garby L, Suwanik R, Bjorn-Rasmussen E. Iron absorption from Southeast Asian diets. Am J Clin Nutr. 1974;27:826.

Hampton DJ, Chrisley BM, Driskell JA. Vitamin B6 status of the elderly in Montgomery County, VA. Nutr Rep Int. 1977;16:743-50.

Hanson JS and Neede WH. Long-term physical training effect in sedentary females. J Applied Physiol. 1974;37:112-16.

Harris JA, Benedict FG. Biometric Studies of Basal Metabolism in Man. Washington:Carnegie Institute, 1919.

Hawkins WW, Speck E, Leonard VG. Variation of the hemoglobin level with age and sex. Blood. 1954;9:999-1007.

Haymes EM. Nutritional concerns: need for iron. Med Sci Sports Ex. 1987;19:S197-S200.

Heath GW, Hagberg JM, Ehsani AA, Holloszy JO. A physiological comparison of young and older endurance athletes. J Applied Physiol. 1981;51:634-40.

Hegsted DM, Finch CA, Kinney TD. The influence of diet on iron absorption. II. The interrelation of iron and phosphorus. J Exp Med. 1949;90:147-56.

Henning N, Heinkel K, Elster K. Ultersuchungen uber die Sogenannte Altergastritis". Schweiz Med Wochenschr. 1957;87:387-90.

Herbert V. Recommended dietary intakes (RDI) of iron in humans. Am J Clin Nutr. 1987;45:679-86.

Hodgson JL and Buskirk ER. Physical fitness and age, with emphasis on cardiovascular function in the elderly. J Amer Ger Soc. 1977;25:385-92.

Holler G. Magenerkrankungen im Loheren Alter mit besonderer Berucksichtigung der Diagnost des Karzinoms; krebsarzi. 1952;7:205-9.

Holloszy JO. Exercise, health and aging: a need for more information. Med Sci Sports Ex. 1983;15:1-5.

Htoo MH, Kofkoff RL, Freeman ML. Erythrocyte parameters in the elderly: An argument against new geriatric normal values. J Amer Ger Soc. 1979;27:547-51.

Huttunen JK, Lansimies E, Froelicher V. Effect of moderate physical exercise on serum high-density lipoproteins. Circulation. 1979;60:1220-29.

Hyams DE: The Blood. in Brocklehurst JC (ed): Testbook of Geriatric Medicine and Gerontology. Edinburgh, Churchill Livingston, 1985, p 848.

Ivy AC. Cowdry's Problems in Ageing (Landry AI, ed.). 1952;Baltimore, Williams and Wilkins Co.

Jackson A, Pollock M, Ward A. Generalized equations for predicting body density of women. Med Sci Sports Med. 1980;12:175-82.

Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Br Med J. 1972;4:206-8.

Jacobs A and Owen GM. The effect of age on iron absorption. J Gerontol 1969;24:95-6.

Jamy PP. Prescribing for the Eldelry. (1980). Littleton, Massachusettes: PSG Publishing Company, Inc. p. 1-11.

Jenkins DJA, Hill MS, Cummings JH. Effect of wheat fiber on blood lipids, fecal steroid excretion and serum iron. Amer J Clin Nutr. 1975;28:1408-11.

Kahn HA, Phillips RL, Snowdon DA, Choi W. Association between reported diet and all-cause mortality:Twenty-one-year follow up on 27,530 adult Seventh-Day Adventists. Am J Epidemiol. 1984;119:775.

Kalmin ND, Robson EB, Bettigole RE. Serum ferritin and marrow iron stores. NY State J Med. 1978;78:1052.

Kaltreider NL and Meneely GR. The effect of exercise on the volume of the blood. J Clin Invest. 1940;19:627-34.

Kampmann JP, Sinding J, Moller-Jorgensen I. Effect of age on liver function. Geriatr. 1975;30:91.

Kelly A and Munan L. Haematologic profile of natural populations: red cell parameters. Brit J Haematol. 1977;35:153.

Kenney RA. Physiology of Aging A Synposis. Year Book Medical Publishers, Inc. Chicago, 1982.

Khanna PB, Davies I, Faragher EB. Age-related changes in the stomach of the laboratory mouse: a quantitative morphological study. Age and Aging. 1988;17:257-64.

Khan TA, Shragge BW, Crispin JS, Lind JF. Esophageal motility in the elderly. Am J Dig Dis. 1977;22:1049.

Kilbom A. Physical training with submaximal intensities in women. Scan J Clin Lab Invest. 1971;28:141-61.

Kjellberg SR, Rudhe U, Sjostrand T. Increase of the amount of hemoglobin and blood volume in connection with physical training. Acta Physiol Scand. 1950;19:146-51.

Kohrs MB. A rational diet for the elderly. Amer J Clin Nutr. 1982;36:796-802.

Kostis JB, Moreyra AE, Amendo MT, Di Pietro J, Cosgrove N, Kuo PT. The effect of age on heart rate in subjects free of heart disease. Studies by ambulatory electrocardiography and maximal exercise stress test. Circulation. 1982;65:141-45.

Lakatta E. Alterations in the cardiovascular system that occur in advanced age. Fed Proc. 1979;38:163-67.

Lampman RM. Evaluating and prescribing exercise for elderly patients. Geriatrics. 1987;42:63-75.

Layrisse M and Martinez-Torres C. Model for measuring dietary absorption of heme iron: Test with a complete meal. Amer J Clin Nutr. 1972;25:401-11.

Layrisse M, Martinez-Torres C, Gonzalez. Measurement of the total daily dietary iron absorption by the extrinsic tag model. Am Clin Nutr. 1974;27:152.

Layrisse M, Martinez-Torres C, Roche M. Effect of interaction of various foods on iron absorption. Amer J Clin Nutr. 1968;21:1175-83.

Learner RM, Kivett VR. Discriminators of perceived dietary adequacy among the rural elderly. J Amer Diet Assoc. 1981;78:330.

Leon AS. Age and other predictors of coronary heart disease. Med Sci Sports Ex. 1987;19:159-67.

Loria A, Hershko C, Konijn AM. Serum ferritin concentration in an elderly population. J Gerontol. 1979;34:521-24.

Loh TT, Chang LL. Hepatic iron status in Malaysians and Singaporeans. Southeast Asian J Trop Med Pub Health. 1980;11:131-6.

Luciano DS, Vander AJ, Sherman JH. Blood. In Human Anatomy and Physiology Structure and Function.McGraw-Hill, New York;1978, pp.404-20.

Luxton AW, Walker WHC, Gauldie J, Ali MAM, Pelletier C. A radioimmunoassay for serum ferritin. Clin Chem. 1977;23:683.

MacLeod CC, Judge TG, Caird FI. Nutrition of the elderly at home. II Intakes of vitamins Age Aging. 1974;3:209-20.

Magnusson B, Hallberg L, Rossander L, et al. Iron metabolism and "sports anemia." I. A study of several iron parameters in elite runners with differences in iron status. Acta Med Scand. 1984;216:149-55.

Manire MA, Vaughan LA, Lehman WR. Contribution of various food groups to dietary vitamin B-6 intake in free-living, low-income elderly persons. J Amer Diet Assoc. 1990;90:830-34.

Martinez-Caro D, Allegria E, Lorente D, Azpilicueta J, Calaburg J, Ancin R. Diagnostic value of stress testing in the eldelry. European Heart J (Supplement E). 1984;5:63-67.

Marx JM. Normal iron absorption and decreased red cell iron uptake in the aged. Blood, 1979;53:204-11.

Maseri JRL, Rouse IL, Lynch WJ, Robertson K. Wandongen R, Beilin LJ. Effects of a lacto-ovo-vegetarian diet on serum concentrations of cholesterol, triglyceride, HDL-C, HDL$_2$, HDL$_3$, apoprotein-B, and Lp(a). Am J Clin Nutr. 1984;40:468.

Massie JF and Shephard RJ. Physiological and psychological effects of training. Med Sci in Sports. 1971;3:110-17.

Mayer J, Marshall NB, Vitale JJ, Christensen NH, Mashayekhi MB, Stare FJ. Exercise, food intake and body weight in normal rats and genetically obese adult mice. Am J Physiol. 1954;177:544-48.

McArdle WD, Katch FI, Katch VL. Exercise Physiology. Energy, Nutrition, and Human Performance. Lea and Febiger, Philadelphia. 1982.

McGandy RB, Barrows CH, Spanias A et al. Nutrient intakes and energy expenditure in men of different ages. J Gerontol, 1966;21:581.

Meredith CN, Zackin MJ, Frontera WR, Evans WJ. Body composition and aerobic capacity in endurance-trained men: Effects of age and habitual exercise. Med Sci Sports Ex. 1987;19:557-63.

Monsen ER. Iron nutrition and absorption: Dietary factors which impact iron bioavailability. Am Diet Assoc. 1988;88:786-90.

Monsen ER, Cook JD. Food absorption in human subjects. IV. The effects of calcium and phosphate on the absorption of nonheme iron. Amer J Clin Nutr. 1976;29:1142-48.

Montoye HJ. Age and oxygen utilization during submaximal treadmill exercise in males. J Geron. 1982;37:396-402.

Morey MC, Cowper PA, Feussner JR, DiPasquale RC, Crowley GM, Kitzman DW, Sullivan RJ. Evaluation of a supervised exercise program in a geriatric population. J Am Ger Soc. 1989.37:348-54.

National Institute of Health Consensus Development Conference Statement. Health Implications of Obesity. 1985; pp.1-16.

Nationwide food consumption survey 1977-78. Preliminary report no 2. Food and nutrient intakes of individuals in 1 day in the United States, Spring 1977, (1980) Washington, DC: USDA Science and Education Administration.

Newhouse J, Clement DB, Taunton JE, McKenzie DZ. The effects of prelatent/latent iron deficiency on physical work capacity. Med Sci Sports Ex. 1989;21:263-68.

Nieman DC, Underwood BC, Sherman KM, Arabatzis K, Barbosa JC, Johnson M, Shultz TD. Dietary status of Seventh-Day Adventist vegetarian and non-vegetarian elderly women. J Amer Diet Assoc. 1989;89:1763-69.

Nordstrom JW. Trace mineral nutrition in the elderly. Am J Clin Nutr. 192;36:795.

Norton L, Wozny MC. Residential location and nutritional adequacy among elderly adults. J Gerontol. 1984;39:592.

Nutrition Canada: Food Consumption Patterns Report. Ottawa, Canada: Department of National Health and Welfare, 1977.

O'Toole ML, Iwane H, Douglas PS, Applegate EA, Hiller DB. Iron status in ultraendurance triathletes. Phys Sports Med. 1989;17:90-102.

Palmer ED. Gastritis-a revaluation. Medicine. 1954;35:199-210.

Parr RB, Bachman LA, Moss RA. Iron deficiency in female athletes. Phys Sports Med. 1984;12:81-86.

Patrick JM, Bassey EJ, Fentem PH. The rising ventilatory cost of bicycle exercise in the seventh decade: a longitudinal study of nine healthy men. Clin Sci. 1983;65:521-26.

Passmore R, Durnin JV. Human energy expenditure. Phsiol Rev. 1955;35:801-40.

Physician and Sports Medicine. (No further information available. Referred to in Smith et al., 1988). 1983;11:92.

Pike RL and Brown ML. Nutrition: An Integrated Approach (Third edition). Wiley, New York. 1984.

Pilon VA, Howanitz JH et al. Day-to-day variation in serum ferritin concentration in healthy subjects. Clin Chem. 1981;27:78-82.

Plowman SA and McSwegin PC. The effects of iron supplementation on female cross country runners. J Sports Med. 1981;21:407-16.

Pollock ML, Wilmore JH, Fox SM. Health and Fitness Through Physical Activity. (1978) NY:John Wiley and Sons.

Position of the American Dietetic Association. Vegetarian diets. J Am Diet Assoc. 1988;88:351.

Preliminary Findings of the First Health and Nutrition Examination Study, US, 1971-72. dietary Intake and biochemical findings. HRA publication no. 74-1219-1, Washington, DC. National Center for Health Statistics, 1974.

Puro DG, Richter GW. Ferritin synthesis by free and membrane bound (poly) ribosomes of rat liver. Proc Soc Exp Med Biol. 1971;138:399-403.

Qvist I, Norden A, Olofsson T. Serum ferritin in the eldelry. Scan J Clin Lab Invest. 1980;40:609-13.

Reinhold JG, Ismail-Beigi F, Faradji B. Fibre vs. phytate as determinant of the availability of calcium, zinc and iron of breadstuffs. Nutr Rep Int. 1975;12:75-85.

Robinson S, Dill DB, Robinson RD, Taznkoff SP, Wagner JA. Physiological aging of champion runners. J Applied Physiol. 1976;41:46-51.

Robinson S, Dill DB, Ross JC, Robinson RD, Wagner JA, Tzankoff SP. Training and physiological aging in man. Fed Proceed. 1973;32:1628-34.

Roe DA. Geriatric Nutrition. Prentic Hall, Englewood Cliffs, NJ, 1987.

Rosenberg IH, Bowman BB, Cooper BA, Halsted CH, Lindenbaum J. Folate nutrition in the elderly. Am J Clin Ntr. 1982;36:1060-6.

Ryan VC and Bower ME. Relationship of socioeconomic status and living arrangements to nutritional intake of the older person. J Amer Diet Assoc. 1989;89:1805-7.

Saarinen IM, Siimes MA. Serum ferritin in assessment of iron nutrition in healthy infants. Acta Paediatr Scand. 1978;67:745-51.

Sacks FM, Handysides GH, Marais GE, Rosner B, Kass EH. Effects of a low-fat diet on plasma lipoprotein levels. Arch Intern Med. 1986;146:1573.

Sallis JF, Haskell WL, Wood PD, Fortman SP. Physical activity assessment methodology in the Five City project. Am J Epidemiol. 1985;121:91-106.

Saltin B, Lindgarde F, Houston M, Horlin R, Nygard E. Physical training and glucose tolerance in middle-aged men with chemical diabetes. Diabetes 28 (Supplement 1). 1979;30-32.

Schiffman SS. Food recognition by the elderly. J Gerontol. 1977;32:586-92.

Seals DR, Hagberg JM, Hurley BF, Ehsani AA, Holloszy JO. Endurance training in older men and women. Cardiovascular responses to exercise. J Appl Physiol. 1984;57:1024-29.

Selby GB, Eichner ER. Endurance swimming, intravascular hemolysis, anemia, and iron depletion. Am J Med. 1986;81:791-94.

Sheldahl LM Special ergometric techniques and weight reduction. Med Sci Sports Ex. 1986;18:25-29.

Shils ME, Young VR. Modern Nutrition in Health and Disease, Seventh edition. Lea and Fibiger, Philadelphia.1988.

Sidney KH and Shephard RJ. Attitues towards health and physical activity in the eldelry. Effects of a physical training program. Med Sci in Sports. 1976;8:246-52.

Siegel JS. Some demographic aspects of aging in the US. In: Epidemology of Aging. (Ostfield AM, Gibson DG, eds.) Bethesda, MD: National Institute of Health, 1972.

Siimes MA, Addiego JE, Dallman PR. Ferritin in serum: Diagnosis of iron deficiency and iron overload in infants and children. Blood. 1974;43:581-90.

Siimes MA, Refino C, Dallman PR. Manifestation of iron deficiency at various levels of dietary iron intake. Am J Clin Nutr. 1980;33:570-74.

Smith JM. Oral and dental discomfort-a necessary feature in old Age? Age Ageing. 1979;8:25.

Smith EL, Smith PE, Gilligan C. Diet, exercise and chronic disease patterns in older adults, Nutr Rev. 1988;46:52-61.

Snook JT. Adaptive and nonadaptive changes in digestive enzyme capacity influencing digestive function. Fed Proc. 1974;33:88.

Snook JT. Effect of age and long-term diet on exocrine pancrease of the rat. Am J Physiol. 1975;228:262.

Snyder Ac, Dvorak LL, Roepke JB. Influence of dietary iron source on measures of iron status among female runners. Med Sci Sports Ex. 1989;21:7-10.

Stamford BA. Effects of chronic institutionalization on the physical working capacity and trainability of geriatric men. J Gerontology. 1973;28:441-446.

Statland BE, Winkel P. Relationship of day-to-day variation of serum iron concentrations to iron-binding capacity in healthy young women. Am J Clin Pathol. 1977;67:84-90.

Steinberg WM and Toskes PP. A practical approach to evaluating maldigestion and malabsorption. Geriatrics. 1978;33:73-85.

Stewart GA, Steel JE, Tayne MB, Stewart MJ. Observations on the haematology and the iron and protein intake on Australian olympic athletes. Med J Aust. 1972;2:1339.

Stone HO, Thompson K, Schmidt-Nielsen K. Influence of erythrocytes on blood viscosity. J Appl Physiol. 1968:214:913-18.

Sturgeon P and Shoden A. Total liver storage iron in normal populations of the USA. Am J Clin Nutr. 1971;24:469-74.

Subcommittee on Iron; Committee on Medical and Biologic Errects of Environmental Pollutants. Division of Medical Sciences, Assembly of Life Sciences, National Research Council. Iron. University Park Press, Baltimore. 1979. pp. 81.

Thompson JD, Funk EJ, Carleton RA, Sturner WO. Incidence of death during jogging in Rhode Island from 1975-1980. JAMA. 1982;247:2535-38.

Thompson JK, Blanton P. Energy conservation and exercise dependence: a sympathetic arousal hypothesis. Med Sci Sports Ex. 1987;19:91-99.

Thompson JK, Jarvie GJ, Lahey BB, Cureton KJ. Exercise and obesity: etiology, physiology, and intervention. Psychol Bull. 1982;91:55-79.

Thompson WG, Babitz L, Cassino C, et al. Evaluation of current criteria used to measure vitamin B12 levels. Am J Med. 1987;82:291.

Thompason DA, Wolfe LA, Eikelboom R. Acute effects of exercise intensity on appetite in younge men. Med Sci Sports Ex. 1988;222-27.

Uauy R, Scrimshaw MS, Young VR. Human protein requirements: Nitrogen balance response to graded levels of egg protein in elderly men and women. Am J Clin Nutr. 1978;31:779-85.

US Department of Commerce. Statistical Abstract of the US in 1988. 1987. Bureau of Census.

US Department of Health and Human Services. Assessment of Iron Nutriture. In The United States An Update Report on Nutrition Monitoring. Hyattsville, MD, 1989.

US Department of Health, Education and Welfare. Public Health Service. Preliminary Findings of the First Health and Nutrition Examination Survey, United States, 1971-72. Dietary Intake and Biochemical Findings. DHEW Publ. (HRA)74-1219-1. Rockville, MD: US Department of Health, Education and Welfare, 1974. 183 pp.

US Department of Health, Education, and Welfare. Ten-State Nutrition Survey 1968-70. IV. Biochemical. DHEW Public No. (HSM) 72-8132. Atlanta, Georgia: US Department of Health, Education, and Welfare, Center for Disease Control, 1972, p.81, 197.

US DHEW. Health and nutrition examination survey no. 2. Hyattesville, MD: USPHS Division of Health Statistics, 1981.

Valberg LS, Sorbie J, Ludwig J, Pelletier O. Serum ferritin and the iron status of Canadians. Can Med Assoc J. 1976;114:417.

Van Camp SP, Boyer JL. Cardiovascular aspects of aging (Part 1 of 2). Phys Sports Med. 1989;17:121-30.

Veller OD. Studies on hemoglobin values in Norway. IX. Hemoglobin, hematocrit and MCHC values in old men and women. Acta Med Scand. 1967;182:681-9.

Vir SC, Love AHG. Vitamin B6 status of institutionalized and noninstitutionalized aged. Int J Vitam Nutr Res. 1977;47:364-72.

Walsh JR. Hematology, in Cassel CK, Walsh JR (ed): Geriatric Medicine. Vol I. NY, Springer-Verlag, 1984, p. 332.

Weinfeld A. Iron Stores. In: Hallberg L, Harwerth HG, Vanotti A, eds. Iron deficiency. NY:Academic Press, 1970:329-63.

Whipp BJ and Davis JA. The ventilatory stress of exercise in obesity. Am J Respir Dis. 1984;129:S90-S92.

Wilmore JH. Body composition in sports and exercise: directions for future research. Med Sci Sports Ex. 1983;15:21-31.

Wirth JC, Lohman TG, Avallone JP, Shire T Jr, Boileau RA. The effect of physical training on the serum iron levels of college-age women. Med Sci Sports. 1978;10:223-26.

Yerg JE, Seals DR, Hagberg JM, Holloszy JO. Effect of endurance exercise training on ventilatory function in older individuals. J Appl Physiol. 1985;58:791-94.

Yip R, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr. 1984;39:427-36.

Yoshimura H. Anemia during physical training (sports anemia). Nutr Rev. 1970;28:251.

Zeman FJ, Ney DM. Applications of Clinical Nutrition. Prentic Hall, Englewood CLiffs, NJ. 1988.

Zhukova NM, Smolyanski BL. Changes due to age of human gastric mucosa. Ark Anat Gistol Embriol. 1973;65:92-5.

Appendix A

Recruitment Notice

THE DEPARTMENT OF HUMAN NUTRITION AND FOODS IS LOOKING FOR VOLUNTEERS, 55 TO 70 YEARS TO PARTICIPATE IN A STUDY TO DETERMINE THE IRON NEEDS OF ADULTS.

IF YOU ARE INTERESTED, PLEASE CALL 703-961-5549 OR WRITE TO DR. CHARLOTTE PRATT, DEPARTMENT OF HUMAN NUTRITION AND FOODS, VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY, BLACKSBURG, VA 24061-0430. A HONORARIUM IS AVAILABLE FOR THOSE WHO SUCCESSFULLY COMPLETE THE STUDY.

Appendix B
Invitation to Participate

COLLEGE OF HUMAN RESOURCES

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061-0430

DEPARTMENT OF HUMAN NUTRITION AND FOODS

October 10, 1988

Dear

I am inviting you to participate in an iron and exercise study. Attached is a description of the study. If you have a choice as to which group you prefer to be in, or know of anyone who might be interested, please let me know.

I hope you will seriously consider participating in this study, as it will broaden our understanding of the iron needs of adults. Thank you.

Cordially,

Charlotte Pratt

Charlotte Pratt
Assistant Professor

- -

Please cut and drop in the mail to Charlotte Pratt, HNF 324 Wallace Hall.

Name: _____

_____ I am interested in participating in the study.

_____ I am not interested in participating.

Appendix C
Response to Participate

COLLEGE OF HUMAN RESOURCES

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061-0430

DEPARTMENT OF HUMAN NUTRITION AND FOODS

September 30, 1988

Dear

Thank you for indicating an interest in participating in the Iron study. Enclosed is a consent form which explains the study. Please sign the form to indicate your interest. It is important that you also obtain your physician's signature to certify your participation. I will reimburse you with the cost of your physician's visit - up to $50.00. The enclosed self-addressed, stamped envelope is for your use. Please be sure to include your social security number and address when mailing the form to us.

We are happy that you will participate in this study and will get back with you shortly.

Cordially,

Charlotte Pratt, Ph.D.,R.D.
(Principal Investigator - Iron Study)

lwv

Enclosure

Appendix D
Consent for Participation

Subject Code Number _____

CONSENT FOR PARTICIPATING
IN
IRON NUTRITION AND EXERCISE STUDY

Virginia Polytechnic Institute and State University

Purpose

The purpose of this study is to determine whether the iron needs of adults who are active are different from those of adults who are inactive.

Requirements

I am a healthy female/male, aged 55-65 years. My weight is within 20% of my ideal body weight. I do not have any illness, disease, physical limitation, or history of cardiovascular impairment. I do not have any injury that will hinder my exercise performance, or use any medications, vitamin or mineral supplements which would influence iron status.

Procedures

I will provide 3-day food intake and activity records at the beginning of the study. Data on iron, cholesterol, saturated fats and other nutrients intakes will be provided to me. I will also participate in an exercise test at the Human Performance Laboratory at Virginia Tech to determine my level of oxygen consumption, heart rate and exercise capacity. A submaximal exercise test will be performed. There will be four groups for the study. I will be randomly assigned to one of the following groups: 1) Exercise/no iron supplement, 2) Exercise/iron supplement, 3) No exercise/no iron supplement, 4) No exercise/ iron supplement. The iron supplement will consist of 10 mg of iron (100% RDA, commonly found in over-the-counter supplements). Participation in the non-supplemented group will be allowed only if I voluntarily abstain from iron supplementation. Those in the exercise group will exercise progressively up to 30 minutes per day/5 days per week for six weeks on a cycle ergometer. The location of the bicycle will be discussed with me. Blood samples will be taken by a qualified technician on the first week and at the end of six weeks to determine my iron status. I will provide 3-day food intake and activity records at the end of the study. My body weight will be recorded at the beginning and end of the study (6-8 weeks). I will check with a physician before participating. The cost of the physician's visit will be paid up to $50.00 per person by the investigators.

All information obtained in the study will be held strictly confidential and will be used for statistical purposes only.

Appendix D
Consent for Participation (continued)

Any inquiries I may have concerning the procedures utilized in this study will be answered at any time.

I understand the above and agree to participate in this study.

- -

Please cut and enclose in the self-addressed envelope.

_____ _____
Date Name

_____ _____
Date Physician

Principal Investigators: Dr. C. Pratt (961-5549)
Ms. Violet Woo (Graduate Student)

Chairman, Institutional Review Board of Research Involving Human Subjects:
Dr. Charles Waring (961-5283)

Comments or questions

Subject code number _____
(Iron study)

Appendix E
Dietary Record

Name: _____
Age: _____
Date: _____

DAY ONE

DIETARY INTAKE RECORD

Please express the amount of all food and all liquid consumed in lbs or cup or oz or g for each serving size.

Example: 9:30 am

1 poached egg
w/ 1/4 tsp. salt
1 slice whole wheat bread
w/ 1 tsp. margarine
1 tsp. grape jam
1/2 grapefruit
w/ 1 tsp. sugar

6 oz. orange juice
5 oz. decaff. coffee
w/ 1 tsp. sugar

Time	Food		Drink	
	Type	Amount	Type	Amount

Appendix F
Activity Record

Name: _____
Age: _____
Date: _____

DAY ONE

ACTIVITY RECORD

Please express the time from the start to completion of the activity on this day (24 hours).

Example: 12:30 p.m. - 1:30 p.m. Moderate Aerobics
10:00 p.m. - 7:00 a.m. Sleep

TIME SPENT	ACTIVITY

Appendix G
Consent for Exercise Testing

MONTGOMERY REGIONAL HOSPITAL
CARDIO-PULMONARY DEPARTMENT

CONSENT FOR EXERCISE TESTING

I hereby consent to voluntarily engage to an exercise test to determine the state of my heart and circulation. The information thus obtained will help to aid my physician in advising me as to the activities in which I may engage.

I have had an interview and have been examined by a physician to determine if I have any condition which would indicate that I should not engage in this test.

The test which I will undergo will be performed on a treadmill with the amount of effort increasing graudually. This increase in effort will continue until symptoms such as fatigue, shortness of breath, or chest discomfort may appear, which would indicate to me to stop.

During the performance of this test, a physician or his trained observer will keep under surveillance my pulse, blood pressure, and electrocardiogram.

There exists the possibility of certain changes occuring during the tests. They include abnormal blood pressure, fainting, disorders of heart beat, too rapid, too slow, or ineffective, and very rare instances of heart attack. Every effort will be made to minimize them by the preliminary examination and by observations during testing. I have been assured that emergency equipment and trained personnal are available to deal with unusual situations which may arise.

The information which is obtained will be treated as privileged and confidential and will not be released or revealed to any person without my expressed written consent. The information obtained, however, may be used for a statistical or scientific purpose with my right of privacy retained.

I have read the foregoing and I understand it and any questions which may have occured to me have been answered to my satisfication.

Patient

Date

Witness

Physician Supervising Test

Appendix H
Liability Release Form

[redacted]

MONTGOMERY
REGIONAL
HOSPITAL

I_____, AM RELEASING MONTGOMERY REGIONAL HOSPITAL AND ITS EMPLOYEES FROM ANY LIABILITY DURING THIS STRESS TEST. I HAVE BEEN INFORMED AS TO THE TESTING PROCEDURE AND COMPLICATIONS WHICH MIGHT ARISE BEFORE, DURING AND AFTER THE STRESS TEST. I REALIZE THAT MY PARTICIPATION IN THIS TEST IS STRICTLY VOLUNTARY.

SIGNATURE:_____DATE:_____

WITNESS:_____

Route 460 South
Blacksburg, Virginia 24060
Telephone (703) 951-1111

An Affiliate of
HEALTHTRUST
INC. The Hospital Company

Appendix I
Treadmill Test Worksheet

TREADMILL TEST WORKSHEET

NAME_____ DATE_____ AGE____ D.O.B._____

REFERRING PHYSICIAN_____ SUPERVISING PHYSICIAN_____

CLINICAL INFORMATION_____

CURRENT EXERCISE_____

CURRENT MEDICATIONS_____

90% MAX HR _____

SUPINE HR_____ SUPINE BP_____ 100% MAX HR _____

RESTING ECG_____

MIN/ STAGE	METS	SPEED MPH	GRADE %	HR	BP	ECG CHANGES	SIGNS & SYMPTOMS	R

EXERCISE

MIN RECOVERY	HR	BP	ECG CHANGES	SIGNS & SYMPTOMS
IMMEDIATE				
2 MIN				
4 MIN				
6 MIN				
8 MIN				
10 MIN				

RECOVERY

EXERCISE ECG INTERPRETATION:

POST-EXERCISE ECG INTERPRETATION:

CONCLUSIONS:

_____

ATTENDING PHYSICIAN

Appendix J
Rudolf Face Mask

MAKERS OF RESPIRATORY VALVES SINCE 1938
HANS RUDOLPH, inc.
7200 Wyandotte • Kansas City, Mo 64114 USA
Phone: 816-363-5522 Telex: 9102408293 Worldwide
Toll Free Within the USA: 1-800-346-3026, Ext. 1667

Data Sheet . . .

RUDOLPH FACE MASK
Series 7900

Our New Breathing Mask with inhalation / exhalation valve diaphragms is designed for exercise testing. It is especially useful with patients who have difficulty in using the more common mouthpiece, noseclip and our 2-Way Non-rebreathing valve set up.

This revolutionary **Rudolph Mask** provides an excellent solution to overcome the previous problems of face masks, i.e., facial sealing, slipping during exercise, achieving a minimum dead space, minimal inhalation / exhalation resistance to flow and capability of gas sampling.

The patient comfort afforded with this mask, which usually results in more accurate test results, is very important factor. For example, as compared to a mouthpiece setup, this mask eliminates saliva build up, dry mouth, and throat irritation. It allows communication between patient and technician.

For additional information on the effects of breathing patterns comparing the face mask with mouthpiece technique, refer to "Human breathing patterns on mouthpiece of face mask during air, CO_2 or Low O_2" by Judith Ann Hirsch and Beverly Bishop, Journal of Applied Physiology, Nov 1982-Vol. 53 No. 5, pages 1281-1290. The mask discussed in the article is not this **Rudolph Mask.**

Mask Face Piece** - Molded of tufel* silicone rubber which is durable, resilient, hypoallergenic (safe for all skin types), comfortable to facial skin, resistant to oxidation and chemical degradation.

- Double lip facial seal - conforms without creating pressure points.
- Chin nest prevents slipping, especially when perspiring during exercise testing.
- Elastic headband straps, easily adjusted for a comfortable four-point suspension fit with minimal tension to hold mask firmly in place.
- Tufel* outlasts theromplastic and organic rubber more than ten to one.

Three Sizes Available - For optimum fit and appropriate breathing parameters such as dead space and flow requirements.

- Cat #7900-L Large for Large Adult.
- Cat #7900-M Medium for Average Adult.
- Cat #7900-S Small for Small Adult and Pediatric.
- Refer to legends for typical mask dead space and resistance to flow.

Valving - Utilizes two inhalation ports and one exhalation port.

- Uses our patented, time proven low resistant molded silicone Spiral Diaphragms.
- Diaphragm sizes used in the inhalation / exhalation ports are shown in legend.
- All port tube adapters are for standard large 1 3/8" Bore Tubing.

Gas Sampling Ports

- One in mask body for breath by breath analysis.
- Hose barb is for 3/32" I.D. Tubing.

PRINTED IN U.S.A
Ref 691094-1 4/86

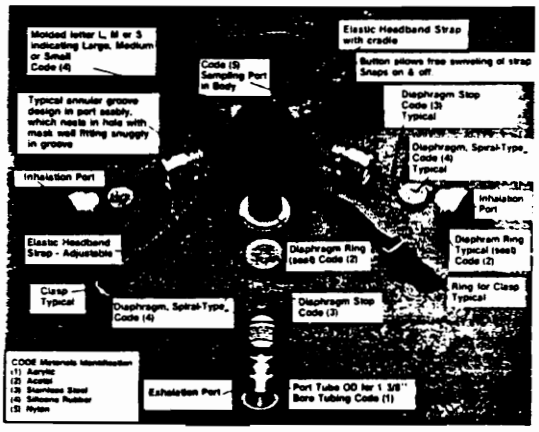
• Prices & Specifications subject to change without notice •

Appendix J
Rudolf Face Mask (continued)

LEGEND OF PERTINENT DATA

*ITEM	LARGE ADULT	AVERAGE ADULT	SMALL ADULT & PEDIATRIC	REQUIRED PER MASK
MODEL (order by #)	#7900-L	#7900-M	#7900-S	N/A
Letter on Mask noting size	"L" - Large	"M" - Medium	"S" - Small	N/A
Typical Patient Fit	Large Adult	Average Adult	Small Adult & Ped.	N/A
Typical Dead Space	195 cc	185 cc	145 cc	N/A
(Approximate dead space in mask when measured on average subject.)				
Weight	9 OZ	9 OZ	6 OZ	N/A
Replacement Parts (Order by #):				
Headband, Elastic Strap - **Includes cradle:**				
One Set	#7901	#7901	#7901	One Set
Cap for Sampling Port	#1911	#1911	#1911	ONE
Exhalation Port (One):				
Diaphragm A	#2708	#2708	#2608	ONE
Diaphragm Stop D	#5703	#5703	#5603	ONE
Inhalation Ports (Two):				
Diaphragm B	#2608	#2608	#2608	TWO
Diaphragm Stop C	#5603	#5603	#5603	TWO

*ITEM - Refer to expanded illustration for identification



EXPANDED ILLUSTRATION

INSIDE VIEW OF SILICONE RUBBER FACE PIECE

Resistance to Flow - mm H_2O Pressure				
LPM	#7900 L & M		#7900 S	
	INH.	EXH.	INH.	EXH.
100	4	7	5	8
200	9	12	13	26
300	17	19	23	53
400	29	28	40	87
500	44	37	58	130
600	62	48	80	180
800	106	68	132	270

Maintenance, Cleaning, Inspection -

- The port assemblies are easily dissassembled and reassembled:
  To remove valve port assemblies: Grip mask firmly with fingers on inside and thumb on exterior next to port. Hold port with other hand and apply a force 90° to side of mask. The mask wall, which is sandwiched in the ports groove, will slip free from groove.
  To replace: Grip firmly as in disassembly and manipulate groove of port into its respective hole in mask wall. Once port assembly is in mask, rotating it is difficult. This procedure is typical for all three ports on each size mask.
  Each individual port assembly can be disassembled by unscrewing mating parts, for cleaning and parts replacement.
- Do Not Autoclave. We recommend ETO or cold sterilization. Follow manufacturers instructions. Avoid alcohol on the transparent acrylic parts.
- Inspect the mask before usage for cleanliness, diaphragm in proper position for flow direction and sampling ports capped if not used.
- Beards are limiting factors and will possibly result in leakage.

* Tufel is a registered trademark of General Electric, Silcone Division.
** Silicone Rubber Mask Face Piece is the product of Survivair. A division of U.S.D. Corp. A subsidiary of Liquid Air Corp. of North America
Some statements reflect information from their product literature "Blue 1 TM Air Purifying Respirators."

Appendix K

High Tolerance Protocol

Stage	MET	Speed	Grade (%)	Duration (min.)
1	2.5	2.0	0	2:00
2	3.5	2.0	3.5	2:00
3	4.4	2.0	7.0	2:00
4	5.4	2.0	10.5	2:00
5	6.5	2.0	14.5	2:00
6	7.6	2.3	15.0	2:00
7	8.7	2.7	15.0	2:00
8	9.7	3.0	15.0	2:00

Appendix L

Borg Perceived Exertion Scale

RPE	
6	
7	Very, very light
8	
9	Very light
10	
11	Fairly light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	

Appendix M
Notification of Group--Non-Exercise Group

COLLEGE OF HUMAN RESOURCES

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061-0430

DEPARTMENT OF HUMAN NUTRITION AND FOODS

January 12, 1989

Dear

Subject: Iron study - Non-exercise group.

Happy New Year! We had just finished with the sub-max stress test for everyone. The results are on file at the Cardiopulmonary Unit of Montgomery Regional Hospital. You may ask your physician to request a copy of the results by calling 953-5158.

We have randomly assigned you to the Non-exercise group. This means that you can continue with your normal level of activity and you will not be requested to participate in any structured activity. We will, however, be collecting fasting blood samples for iron analysis the week of January 23, 1989 in Room 301 Wallace Hall between 7 a.m. - 9 a.m. Violet will call you to schedule a time with you.

We hope you are as motivated as we are and that you are looking forward to the study. Your contribution will certainly broaden our knowledge of the iron needs for the adult.

Thank you and we look forward to seeing you shortly.

Sincerely,

Violet Woo
Graduate Student

Charlotte Pratt
Assistant Professor

Appendix M
Notification of Group--Exercise Group

COLLEGE OF HUMAN RESOURCES

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061-0430

DEPARTMENT OF HUMAN NUTRITION AND FOODS

January 16, 1989

Dear

Subject: Iron study - exercise group.

Happy New Year! We had just finished with the sub-max stress test for everyone. The results are on file at the Cardiopulmonary Unit of Montgomery Regional Hospital. You may ask your physician to request a copy of the results by calling 953-5158.

We have randomly assigned you to the exercise group. The exercise bicycle is located at the Fitness Connection, 1101 N. Main Street. The exercise frequency is 3 days/week, 30 mins/session for 8 weeks. Please choose 3 days and indicate your preferred times on the attached schedule. Mail the schedule in the enclosed self-addressed envelope.

We will be collecting fasting blood samples for iron analysis the week of January 23, 1989 in Room 301 Wallace Hall between 7 a.m. - 9 a.m. Violet will call you to schedule a time with you.

We hope you are as motivated as we are and that you are looking forward to the study. Your contribution will certainly broaden our knowledge of the iron needs for the adult

Thank you and we look forward to seeing you shortly.

Sincerely,

Violet Woo
Graduate Student

Charlotte Pratt
Assistant Professor

Appendix N
Exercise Schedule

Exercise Schedule begins January 23, 1989 at the Fitness Connection, 1101 N. Main Street. Please choose three days and one convenient time slot per day.

Mondays	6:00 a.m. - 8:00 a.m.	_____
	11:30 a.m. - 3:30 p.m.	_____
	7:00 p.m. - 9:00 p.m.	_____
Tuesdays	6:00 a.m. - 8:00 a.m.	_____
	11:00 a.m. - 3:30 p.m.	_____
Wednesdays	6:00 a.m. - 8:00 a.m.	_____
	11:30 a.m. - 3:30 p.m.	_____
	7:00 p.m. - 9:00 p.m.	_____
Thursdays	6:00 a.m. - 8:00 a.m.	_____
	1:30 p.m. - 3:30 p.m.	_____
Fridays	6:00 a.m. - 8:00 a.m.	_____
	11:00 a.m. - 4:30 p.m.	_____
	7:00 p.m. - 9:00 p.m.	_____
Saturdays	Noon - 6:30 p.m.	_____
Sundays	1:00 p.m. - 3:30 p.m.	_____

Appendix O
Consent to Donate Blood

CONSENT FORM

The undersigned consents to donate blood for the following study:

IRON STUDY

I have received an explanation of the study.

A sample of blood, approximately _____ milliliters, will be taken.

All information obtained in the study will be held strictly confidential and will be used for statistical purposed only.

_____ _____

Date **Signature of Participant**

Appendix P
Height/Weight Chart

HEIGHT AND WEIGHT
(PRE-STUDY)

	HEIGHT	WEIGHT	FAST
1. Ann Tatem			
2. Betty Bell			
3. Margaret Cecchini			
4. Frances Knowles			
5. Mary Holliman			
6. Beth Oehring			
7. Jane Wentworth			
8. Anita McDowell			
9. Rita Collins			
10. Margaret Lambert			
11. Thelma Moses			
12. Hilda Daily			
13. Emily Collins			
14. Lucille Calhoun			
15. Shirley Farrier			
16. Carolyn Michaels			
17. Madeleine Hylton			
18. Kathleen Clark			
19. Ann Hayman			
20. Peggy Wright			

Appendix Q
Cycling Data Worksheet (Exercise Group)

CYCLING DATA FOR 8 WEEK EXERCISE

WATTS	Minutes	HR	RPE	RPM
	0			
Time	2			Date
	3			
	5			
	5			
	5			
	5			
	5			
	5			
	3			
	2			
	0			

WATTS	Minutes	HR	RPE	RPM
	0			
Time	2			Date
	3			
	5			
	5			
	5			
	5			
	5			
	5			
	3			
	2			
	0			

Appendix R
Predicted Heart Rate for Age (Robinson)

PREDICTED HEART RATES FOR AGE - ROBINSON

AGE	100%	85%	70%	AGE	100%	85%	70%
15	205	174	144	47	173	147	121
16	204	173	143	48	172	146	120
17	203	173	142	49	171	145	120
18	202	172	141	50	170	145	119
19	201	171	141	51	169	144	118
20	200	170	140	52	168	143	118
21	199	169	139	53	167	142	117
22	198	168	139	54	166	141	116
23	197	167	138	55	165	140	116
24	196	167	137	56	164	139	115
25	195	166	137	57	163	139	114
26	194	165	136	58	162	138	113
27	193	164	135	59	161	137	113
28	192	163	134	60	160	136	112
29	191	162	134	61	159	135	111
30	190	162	133	62	158	134	111
31	189	161	132	63	157	133	110
32	188	160	132	64	156	133	109
33	187	159	131	65	155	132	108
34	186	158	130	66	154	131	108
35	185	157	130	67	153	130	107
36	184	156	129	68	152	129	106
37	183	156	128	69	151	128	106
38	182	155	127	70	150	128	105
39	181	154	127	71	149	127	104
40	180	153	126	72	148	126	104
41	179	152	125	73	147	125	103
42	178	151	124	74	146	124	102
43	177	150	124	75	145	123	102
44	176	150	123	76	144	122	101
45	175	149	123	77	143	122	100
46	174	148	122	78	142	121	99

Appendix S
Irma-Count Ferritin

IRMA-Count

FERRITIN

With Monoclonal Anti-Ferritin Antibodies

IMPORTANT NOTICE

IRMA-Count Ferritin October 24, 1989

Please note that the B calibrator (FER4006) contains 5 ng/ml as stated in the package insert. Also, with FER3-9006 calibrators, the CON6 lot 010 control ranges for IRMA-Count Ferritin are as follows:

Tabulated in ng/ml:

Level	Mean	SD	2SD Range
4	57	7.6	42 - 72
5	108	7.6	93 - 123
6	341	28.3	284 - 398

Expiration Date: SEP 30 92

DPC
Diagnostic Products Corporation

Appendix S
Irma-Count Ferritin (continued)

IRMA-Count Ferritin

Immunometric Assay Procedure

All components must be at normal room temperature before use.

1 Label fourteen Ligand-Coated Tubes A (nonspecific binding) and B through G ("maximum binding") in duplicate. Label additional ligand-coated tubes, also in duplicate, for controls and patient samples.

If Total Counts tubes are required for data reduction, label two plain (uncoated) 12×75mm tubes T (total counts) in duplicate, and set them aside until step 8.

Calibrator	Ferritin ng/ml
T*	—
A(NSB)	0
B	5
C	25
D	100
E	200
F	500
G("MB")	2000

* optional

2 Pipet **50 µl** of each calibrator, control and patient sample into the tubes prepared.

Pipet directly to the bottom. All samples with Ferritin levels greater than 2000 ng/ml should be diluted in the kit's zero calibrator. Use a disposable-tip micropipet, changing the tip between samples, to avoid errors due to carry-over. Do not use positive-displacement pipets or automatic pipetter-diluters.

3 Add **100 µl** of ligand-labeled Monoclonal Anti-Ferritin to all tubes except the T tubes. Shake the rack.

Pipet directly to the bottom. A repeating dispenser (Nichiryo or equivalent) is recommended for this step and for the addition of Anti-Ligand at step 5 and tracer at step 8.

4 Incubate for **15 minutes** at room temperature.

5 Add **25 µl** of Anti-Ligand (BLUE) to all tubes except the T tubes.

Pipet directly to the reaction mixture. In dispensing the Anti-Ligand, be careful to avoid carry-over contamination, and make sure that all components of the reaction mixture are in solution.

6 Shake for **15 minutes** on a rack shaker.

7 Decant thoroughly. Add **2 ml** of Buffered Wash Solution to each tube. Wait 1 to 2 minutes, then decant thoroughly.

Removing all visible moisture will greatly enhance precision. After the wash, using a foam decanting rack, decant the contents of all tubes and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

8 Add **100 µl** of Monoclonal [^{125}I] Anti-Ferritin Tracer to every tube.

Pipet directly to the bottom. Set the (optional) T tubes aside for counting (at step 11); they require no further processing.

9 Shake for **15 minutes** on a rack shaker.

10 Decant thoroughly. Add **2 ml** of Buffered Wash Solution to each tube (except the T tubes). Wait 1 to 2 minutes, then decant thoroughly. Again add **2 ml** of Buffered Wash Solution, wait 1 to 2 minutes, and decant thoroughly.

Removing all visible moisture will greatly enhance precision. After the second wash, decant the contents of all tubes (except the T tubes), using a foam decanting rack and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

11 Count for 1 minute in a gamma counter.

In multi-head gamma counters, the (optional) Total Counts tubes should be separated from the remaining assay tubes by at least one space, to minimize the possibility of spillover.

Appendix S
Irma-Count Ferritin (continued)

Calculation of Results and Quality Control

To calculate ferritin concentrations from a log-log representation of the calibration curve, first correct the counts per minute (CPM) of each pair of tubes by subtracting the average CPM of the nonspecific binding tubes (calibrator A):

Net Counts = Average CPM *minus* Average NSB CPM

Then determine the binding ($\%B/B_{2000}$, here called "%B/MB") of each pair of tubes as a percent of "maximum binding," with the NSB-corrected counts of the highest calibrator (calibrator G) taken as 100%:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net MB Counts}} \times 100$$

Using the log-log graph paper supplied with this kit, plot Percent Bound versus Concentration for each of the nonzero calibrators B through G, and draw a curve approximating the path of these six points. (Connect the calibration points with arcs or straight line segments. Do not attempt to fit a single straight line to the data.) Ferritin concentrations for controls and unknowns within range of the nonzero calibrators may then be estimated from the standard curve by interpolation.

Comments: Although other approaches are acceptable, data reduction by the method just described has certain advantages from the standpoint of Quality Control. In particular, it yields a calibration curve that is *relatively* linear in both log-log and linear-linear representations, and relatively stable from assay to assay. It also yields valuable QC parameters, namely, Percent Bound ($\%B/B_{2000}$, or "%B/MB") values for the nonzero calibrators.

A still more informative graph, conveying a sense of within-assay reproducibility as a function of concentration, can be obtained by plotting the Percent Bound values of individual calibrator tubes, rather than first averaging the CPM of replicates. *It is good QC practice to construct the recommended log-log plot of the calibration curve,* even where the calculation of results is handled by computer.

Alternatives: Although Percent Bound can be calculated directly from Average CPM, correction for nonspecific binding usually produces a calibration curve that is more nearly linear throughout its range. A calibration curve can also be constructed by plotting CPM or Average CPM directly against Concentration on either log-log or linear-linear graph paper. (Semi-log graph paper should *not* be used.) This approach has the virtue of simplicity, but is less desirable from the standpoint of Quality Control.

Computerized Data Reduction: "Point to point" methods, including linear and cubic spline fits, are suitable for use with the IRMA-Count Ferritin system. However, since they provide little assistance in monitoring the integrity of an assay, it is important to prepare the recommended log-log plot of the calibration curve, either manually or by computer, as a Quality Control step.

Data reduction techniques based on the logistic model may also be applicable. Within this family, curve fitting routines based on the 4- or 5-parameter logistic are the most suitable candidates. Bear in mind, however, that some algorithms currently in use may not converge successfully, even when the logistic model is true to the data. If a logistic method is adopted, it is essential to verify its appropriateness for each day's assay by monitoring the backcalculation of the calibrators, and other parameters. In addition, a plot of the standard curve in a "logit-log" or log-log representation is highly recommended, as either of these is more informative than the conventional semi-log plot. (Suitable "logit-log" graph paper is available from DPC.)

Sample Handling: The instructions for handling and storing patient samples and components should be carefully observed. Dilute high patient samples with the kit's zero calibrator before assay. All samples, including the calibrators and controls, should be assayed at least in duplicate. It is good laboratory practice to use a *disposable-tip* micropipet, changing the tip between samples, in order to avoid carry-over contamination. (For the same reason, an automatic pipetter-diluter is not recommended.) Pairs of control tubes may be spaced throughout the assay to help verify the absence of significant drift. Inspect the results for agreement within tube pairs, and take care to avoid carry-over from sample to sample.

Gamma Counter: To minimize the possibility of spillover in multi-well gamma counters, the (optional) total counts tubes should be separated by one or more spaces from the other assay tubes. Alternatively, add only 25 µl of the Monoclonal [^{125}I] Anti-Ferritin Tracer to each of the total counts tubes at step 8, and multiply the observed counts per minute in these tubes by 4.

Appendix T
AM Blue 610

manual procedure insert

SERUM IRON/ UIBC

AM Blue 610® METHOD

U.S. Patent 4,154,929
Other patents and patents pending

INTENDED USE

The reagent system described is intended for use in the quantitative determination of iron and iron-binding capacity in serum.

METHODOLOGY

In the measurement of serum iron, ferric iron is dissociated from its carrier protein, transferrin, in an acid medium and simultaneously reduced to the ferrous form:

$$\text{transferrin} - 2Fe^{+3} \text{ complex} \xrightarrow{H^+} \text{transferrin} + 2Fe^{+3}$$
$$Fe^{+3} + e^- \xrightarrow{\text{ascorbate}} Fe^{+2}$$

Ferrous iron then is complexed with AM Blue 610®, American Monitor's brand of 9-(2-pyridyl)-acenaphtho-[1,2,*e*]-*as*-triazine, a very sensitive iron indicator:

$$Fe^{+2} + 3 \text{ AM Blue 610®} \longrightarrow Fe^{+2} - 3 \text{ AM Blue 610® complex (blue chromophore)}$$

The resulting blue chromophore exhibits maximum spectral absorbance at 610 nm, thereby minimizing interference from bilirubin, hemoglobin, and carotenoids. Routine use of a modified serum blank procedure eliminates problems commonly encountered with lipemic or turbid specimens.

To quantitate unsaturated iron-binding capacity, a known amount of ferrous iron is added, in excess, to the serum at an alkaline pH to enable saturation of the unoccupied iron-binding sites on the transferrin molecule:

$$\text{transferrin} + Fe^{+2} \xrightarrow{OH^-} \text{transferrin} + Fe \text{ complex} + \text{excess } Fe^{+2}$$

The iron that remains unbound to transferrin is then quantitated by complexing with AM Blue 610®:

$$\text{excess } Fe^{+2} + \text{AM Blue 610®} \longrightarrow Fe^{+2} - \text{AM Blue 610® complex (blue chromophore)}$$

By subtracting the amount of unbound iron thus measured from the total amount of iron originally added to the sample, the unsaturated iron-binding capacity may be calculated.

REAGENTS

For In Vitro Diagnostic Use Only

The Monitor AM Blue 610® Iron/UIBC assay system (Cat. No. 1610) is comprised of the following reagents of the indicated composition*:

① CHROMOGEN
Contains 9.7 mmol/l 9-(2-pyridyl)-acenaphtho-[1,2,*e*]-*as*-triazine and nonreactive preservatives.
Store at room temperature, 20° to 25°C.

② REDUCTANT
Each bottle contains 20 mmol ascorbic acid.
Store the unreconstituted reagent at room temperature, 20° to 25°C.

③ IRON BUFFER
Contains an acetate buffer, pH 4.5, with dimethylsulfoxide, thiourea, and surfactants.
CAUTION: Possible irritant. Avoid prolonged skin contact.
Store at room temperature, 20° to 25°C.

④ BINDING BUFFER
Contains a TRIS buffer, pH 8.3, with a nonreactive surfactant and antimicrobial agent added.
Store at room temperature, 20° to 25°C.

⑤ FERROUS REAGENT
Contains 3 mmol/l ferrous ammonium sulfate in citric acid.
Store at room temperature, 20° to 25°C.

IRON STANDARD, 200 µg/dl
Contains 200 µg/dl iron and a nonreactive preservative.
Store at room temperature, 20° to 25°C. Keep tightly capped.

IRON STANDARD, 600 µg/dl
Contains 600 µg/dl iron and a nonreactive preservative.
Store at room temperature, 20° to 25°C. Keep tightly capped.

When stored as directed, the above reagents will remain stable until the date indicated on the individual product labels.

*The composition of some of the above reagents may vary slightly from lot to lot within manufacturing tolerances, however, this will not affect or alter the results of the assay.

REAGENT PREPARATION

Reductant
Reconstitute the bottle of Iron Reductant with 15 ml of iron-free, deionized water and mix until dissolved. The prepared reagent will remain stable for at least one month when stored at 2° to 8°C and protected from exposure to light.

Buffered Ferrous Reagent
Add 1.0 ml of the Ferrous Reagent to the contents of the bottle of Binding Buffer. The prepared reagent will be stable for at least six months when stored at 20° to 25°C.

Working Reagent	Preparation	Storage	Stability
Chromogen	①as packaged	20° - 25°C	to exp. date
Reductant	②+ 15 ml water	2° - 8°C in brown bottle	one month
Iron Buffer	③as packaged	20° - 25°C	to exp. date
Buffered Ferrous Reagent	④+ 1.0 ml⑤	20° - 25°C	six months

SPECIMEN

Serum is the specimen of choice. Plasma derived from the use of anticoagulants should not be used. The serum should be centrifuged, removed from the clot, and then

Appendix T
AM Blue 610 (continued)

PROCEDURE OUTLINES

SERUM IRON

Step	Reagent Blank	Specimen, Control, or Standard
1. Add iron-free water. Add specimen, controls, and standard (if used)	0.5 ml – –	– – 0.5 ml
2. Add Iron Buffer Add Reductant. Mix.	2.0 ml 0.1 ml	2.0 ml 0.1 ml
3. Read A_1 at 610 nm vs. reagent blank.		
4. Add Chromogen. Mix. Wait 5 minutes.	0.1 ml	0.1 ml
5. Read A_2 at 610 nm vs. reagent blank.		
6. Calculate: $\Delta A = A_2 - A_1$		

SERUM UIBC

Step	Reference	Specimen or Control
1. Add iron-free water. Add specimen and controls.	0.5 ml – –	– – 0.5 ml
2. Add Buffered Ferrous Reagent. Add Reductant. Mix.	2.0 ml 0.1 ml	2.0 ml 0.1 ml
3. During 7-minute incubation, read A_1 at 610 nm vs. water.		
4. Add Chromogen. Mix and incubate 10 minutes at 37°C.	0.1 ml	0.1 ml
5. Read A_2 at 610 nm vs. water.		
6. Calculate: $\Delta A = A_2 - A_1$		
7. Calculate: $\Delta A_c = (\Delta A_1 - A_0) \times 1.2$		

Appendix U

Sigma Diagnostic Kits No. 352 and 352-3

Appendix U

Sigma Diagnostic Kits No. 352 and 352-3 (continued)

DIAGNOSTIC KITS and REAGENTS

Reagents for the Colorimetric, Enzymatic Determination of CHOLESTEROL in Serum or Plasma at 500 nm per Procedure No. 352 Discrete Analyzer Applications

CHOLESTEROL, TOTAL
No. 352 (Colorimetric)

Principle: Cholesterol Esters + H_2O $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + Fatty Acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + p-Hydroxybenzenesulfonate $\xrightarrow{\text{Peroxidase}}$ Quinoneimine Dye + $4H_2O$

The intensity of the color produced is directly proportional to the total cholesterol concentration in the sample.

KITS

Stock No.	Item	Assays	Quantity	US $
352-20	CHOLESTEROL 20	200	10 x 20 ml	123.00
352-50	CHOLESTEROL 50	500	10 x 50 ml	214.00
352-1000	CHOLESTEROL 1000	1000	1 liter	320.00
352-M	CHOLESTEROL 1000	4000	4 x 1 liter	1200.00

OTHER REQUIRED REAGENT

C 0284	CHOLESTEROL CALIBRATOR Cholesterol, 200 mg/dl, aqueous solution with stabilizer. Sodium azide added as preservative.	5 ml	9.00

OPTIONAL REAGENTS

C 0534	CHOLESTEROL CALIBRATOR SET Aqueous solution of cholesterol (bovine) with stabilizer. Sodium azide added as preservative. Set contains 2 x 5 ml each of calibrators with cholesterol concentrations of 100, 200 and 400 mg/dl.	6 x 5 ml	33.00
	ACCUTROL™ CHEMISTRY CONTROLS Enzymes and analytes in a human serum base. See Standards and Controls Section for details.		
A 2034	NORMAL	6 x 5 ml 20 x 5 ml	57.00 145.00
A 3034	ABNORMAL	6 x 5 ml 20 x 5 ml	57.00 145.00
	LIPID CONTROLS Assayed preparations containing triglycerides and cholesterol in a bovine serum base.		
L 1008	LIPID CONTROL-E, lyophilized Elevated levels	6 x 3 ml	32.00
L 2008	LIPID CONTROL-N, lyophilized Normal levels	6 x 3 ml	32.00

Reagents for the Determination of CHOLESTEROL using Technicon instrumentation.
See: Reagents for AutoAnalyzers Section.

CHOLESTEROL
(Automated)

Appendix U

Sigma Diagnostic Kits No. 352 and 352-3 (continued)

DIAGNOSTIC KITS and REAGENTS

CHOLESTEROL, HDL
No. 352-3
(Dextran Sulfate/Magnesium)
No. 352-4
(Phosphotungstic Acid/Magnesium)
No. 352-5
(Phosphotungstic Acid/Magnesium)

Reagents for the Isolation of HDL Cholesterol Fraction in Serum or Plasma per Sigma Procedure Nos. 352-3, 352-4 and 352-5. Discrete Analyzer Applications

Principle: HDL Cholesterol is measured after serum low density and very low density lipoproteins are selectively precipitated and removed by centrifugation. The supernatant contains the cholesterol associated with the soluble HDL fraction and is assayed for cholesterol by an enzymatic method such as Sigma Procedure No. 352. The amount of color produced is directly proportional to the concentration of HDL cholesterol in the sample.

Sigma Procedure No. 352-3 uses dextran sulfate with magnesium as the precipitating reagent.
Sigma Procedure No. 352-4 uses phosphotungstic acid with magnesium for precipitation.
Sigma Procedure No. 352-5 also uses phosphotungstic acid with magnesium for precipitation. The reagent is supplied in convenient pre-measured centrifuge tubes.

INDIVIDUAL REAGENTS

Stock No.	Item	Assays	Quantity	US $
352-3	HDL CHOLESTEROL REAGENT Dextran sulfate, 10 g/l, and Mg ions, 0.5 mol/l with buffer.	100 500	5 ml 5 x 5 ml	7.75 28.00
352-4	HDL CHOLESTEROL REAGENT (PTA/$MgCl_2$) Phosphotungstic Acid, 30.3 mmol/l, Magnesium Chloride, 100 mmol/l	100 600	10 ml 6 x 10 ml	10.00 41.00
352-5	HDL ISOSPIN™ Polypropylene centrifuge tubes containing 0.1 ml of HDL Cholesterol Reagent (PTA/$MgCl_2$)	100	100 ea.	75.00

OTHER REQUIRED REAGENTS

C 9908	CHOLESTEROL CALIBRATOR Cholesterol, 50 mg/dl, aqueous solution with stabilizer. Sodium azide added as preservative.	–	5 ml	6.50
352-20	CHOLESTEROL 20	200	10 x 20 ml	123.00
352-50	CHOLESTEROL 50	500	10 x 50 ml	214.00
352-1000	CHOLESTEROL 1000	1000	1 liter	320.00
352-M	CHOLESTEROL 1000	4000	4 x 1 liter	1200.00

(continued)

Appendix V
Individual Hematological Values

	a		b		c		d		e		f	
	Serum Ferritin		Serum Iron		Trans. Sat.		TIBC		Hct		Hgb	
EXERCISE GROUP:												
Subject	Week 0	11	0	11	0	11	0	11	0	11	0	11
1	86	84	111	117	33	35	331	326	44	44	14.7	15.3
2	48	53	87	146	26	45	333	324	**	**	15.8	15.9
* 3	379	**	78	83	27	28	288	289	42	42	14.3	15.3
4	148	155	87	179	30	60	289	295	46	44	15.4	15.7
5	<5	17	37	29	6	5	552	510	39	39	12.4	12.9
6	61	79	83	124	20	30	414	405	43	42	15.6	16.5
* 7	>1000	1677	109	111	48	46	227	240	49	48	18.4	18.3
8	46	69	121	102	36	30	334	332	39	42	13.4	14.5
9	61	56	98	84	42	34	230	247	38	39	13.5	13.8
10	118	170	109	96	**	27	**	348	45	45	15.6	15.8
NON-EXERCISE GROUP:												
1	86	129	113	75	27	26	409	281	50	44	17.7	16.4
2	67	80	99	81	36	29	274	279	46	45	15.8	15.5
3	58	62	133	97	38	30	342	319	46	45	16.4	15.6
4	68	106	168	162	58	64	287	253	45	45	16.1	15.9
5	44	70	84	74	24	19	343	384	48	46	16.5	15.9
6	173	206	106	174	37	71	285	243	47	45	17.6	16.1
7	91	66	98	65	30	20	324	324	47	43	16.4	17.3
* 8	91	51	86	78	25	23	336	332	42	45	16.4	16.0
9	206	267	85	89	29	27	290	320	43	43	14.9	16.1
10	111	100	114	91	33	25	338	357	44	44	16.1	15.1

a=serum ferritin, ng/dl
b=serum iron, mcg/dl
c=transferrin saturation, %
d=TIBC, mcg/dl
e=hematocrit, %
f=hemoglobin, mg/dl

*=subject omitted from study
**=value unavailable

Appendix W

Individual Values (minutes) to Reach 70% Max HR

Subject	Week 0 (minutes)	Week 11 (minutes)
A. Exercise Group		
1	4	8
2	2	6
3	4	5
4	4	10
5	4	6
6	7	12
7	6	8
8	3	9
B. Non-Exercise Group		
9	6	10
10	8	10
11	5	5
12	8	10
13	2	8
14	8	6
15	9	10
16	6	6

VITA

Violet Ryo-Hwa Woo was born May 30, 1964 in Plattsburg, New York. She obtained a Bachelor of Science in Sports Medicine at the University of Virginia, Charlottesville, Virginia in May 1987. In August 1988 she began her Master of Science at VPI&SU in the Department of Human Nutrition and Food and graduated July 1990. She had a poster exhibit at the 1990 FASEB Conference located in Washington, DC and at the Fifteenth Annual Database Conference located in Blacksburg, VA. In August 1990 she began her nine-month AP4 at Oklahoma State University, Stillwater, OK to become a registered dietician.

Violet Ryo Hwa Woo