

IRON STATUS AND BEHAVIORAL FACTORS RELATIVE TO  
DIETARY SOURCE OF PROTEIN INTAKE AMONG FEMALE  
ATHLETES AT VIRGINIA TECH

by

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LUCY S. GARMAN

(ABSTRACT)

Sixty-two female athletes at Virginia Tech participated in an investigation to compare iron status among those whose primary source of dietary protein was red meat (M), lacto-ovovegetarian (V), or poultry and fish (PF). Subjects' blood was collected three times over the six-month study period and analyzed for hemoglobin, hematocrit, serum iron, transferrin saturation, total iron-binding capacity, serum ferritin, free erythrocyte protoporphyrin, mean corpuscular volume, and red blood cell count. Means for all dietary groups were within normal limits for all blood variables. There were no significant differences among the dietary groups for any of the hematological parameters.

Six of the sixty-two subjects were found to be in stage 1 iron deficiency; two from Group M, one from Group V, and three from Group PF. None of the subjects was in stage 2 or 3 iron deficiency.

Subjects completed two 3-day dietary recalls which were analyzed for nutrient content. Group M consumed a significantly greater percent of kcals from protein than Group V and significantly more grams of protein than both Groups V and PF. There were no significant differences

among the groups for intake of iron; however, only Group M consumed the current RDA for iron for adult women.

Three subjects from each dietary group were interviewed to identify factors considered most important in the adoption of their dietary patterns. Primary factors identified were: Group M; habit, taste preference, and health; Group V; athletic performance and ethical treatment of animals, and Group PF; taste preference and health.

Iron status in this population does not appear to be affected by source of dietary protein.

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## CHAPTER I

### INTRODUCTION

Iron is an abundant element in the earth's crust and is of vital importance to all forms of life. The ease with which iron may participate in oxidation and reduction reactions is one reason why iron plays such an important role in many processes vital to the existence of plants, animals, and humans. Iron is necessary for essential metabolic and enzymatic functions in the human body. It is a primary component of hemoglobin, the protein which binds oxygen for transport from the lungs to the tissues, and myoglobin, which transports and stores oxygen for use during muscular contraction. Iron is also a major component of cytochromes responsible for oxidative metabolism for the production of adenosine triphosphate (ATP), the primary energy source for the body. In addition, it is a necessary component of several enzymes important for metabolic function, including NADH dehydrogenases, succinate dehydrogenase, aconitase, catalase and peroxidases.

The body closely regulates the amount of iron necessary for these functions through absorption from dietary sources and excretion via urine, feces, sweat, and gastrointestinal losses while maintaining storage sources of iron in the compounds ferritin and hemosiderin, found mainly in the liver, bone marrow, and reticuloendothelial cells (Dallman,

1990). Humans receive dietary iron from several sources providing the nutrient in either a heme or nonheme form. Sources of heme iron, which is the form most readily absorbed by the digestive tract, are mainly animal products, including meats. Nonheme sources are primarily plant products and are less well absorbed by humans.

Despite the close metabolic regulatory control of iron balance, many people worldwide, including those living in the United States, are considered to have impaired iron status or iron deficiency anemia. The World Health Organization has estimated the prevalence of iron deficiency anemia to be approximately 15% worldwide (DeMaeyer and Adiels-Tegman, 1985). In the United States women of child-bearing age are considered to be a high-risk group due to loss of iron through menstruation as well as insufficient dietary intake. Recent trends in America have emphasized thinness, especially among women, and this segment of the population often reduces its overall energy intake in order to lose weight. An intake of 1000 kilocalories (kcal) provides approximately six milligrams of available iron (Snyder et al., 1989); therefore, a reduction in overall energy intake reduces the amount of iron provided by the diet. Further, health researchers have recommended a reduction in consumption of red meat and fat, while encouraging the increased intake of complex carbohydrates,

chicken and fish, and protein from vegetarian sources to promote dietary habits believed to be more healthful. A reduction in the consumption of red meat, a source of highly bioavailable heme iron, as well as an increase in the consumption of complex carbohydrates and vegetarian sources of protein, which contain nonheme iron and substances which interfere with the absorption of iron, could result in less total bioavailable iron provided by such a diet.

An area of further concern for compromised iron status relates to athletes. There is some question, although controversial, that this group may experience what has come to be known as "sports anemia", so named by Yoshimura in 1959 in Japan and brought to attention in the United States in the 1970s (Yoshimura, 1970). This condition, as reflected in a decrease in hemoglobin and/or serum ferritin levels during exercise training, without the concurrent diagnostic characteristics of microcytic and hypochromic erythrocytes, has been observed by a number of researchers (Hunding et al., 1981; Plowman and McSwegin, 1981; Rowland et al., 1988; Manore et al., 1989; Clement et al., 1977; Selby and Eichner, 1986; Risser et al., 1988; Puhl et al., 1981; Parr et al., 1984; Nickerson et al., 1985; Nickerson and Tripp, 1983; Haymes et al., 1986; Frederickson et al., 1983; Ehn et al., 1980; and O'Toole et al., 1989). The condition is considered by many to be transient, and the

causes are still the subject of some debate. However, this phenomenon has been recognized among sufficient numbers of athletes to be of concern. Several researchers have found a significant relationship between hemoglobin concentrations and work performance in both humans and animals (Gardner et al., 1977; Wranne and Woodson, 1973). Thus, athletes whose iron status parameters are indicative of sports anemia could be susceptible to suboptimal performance. It would seem, then, that females who are also athletes may be at increased risk for developing poor iron status.

Certain dietary patterns of young female athletes in America today may be adopted for purposes of weight control and/or concerns about health. However, this observation is a presumption, as no studies have been conducted among this population to identify the specific reasons for the adoption of habitual dietary patterns.

The purpose of this study was to determine the effect of source of dietary protein intake on iron status among female athletes at Virginia Tech. Primary sources of dietary protein were either red meat, lacto-ovovegetarian, or poultry and fish. A secondary purpose of this study was to identify the primary factors affecting the athletes' choice of dietary pattern.

The following hypotheses were tested:

1. Iron status as indicated by the variables measured in

this study is not significantly different among female athletes whose primary source of dietary protein is red meat, lacto-ovovegetarian, or chicken and fish.

2. Dietary intake of iron is not significantly different among female athletes whose primary source of protein is red meat, lacto-ovovegetarian, or chicken and fish.
3. Dietary intake of protein (in total grams or percent of total kilocalories) is not significantly different among female athletes whose primary source of protein is red meat, lacto-ovovegetarian, or chicken and fish.

## CHAPTER II

### REVIEW OF LITERATURE

This review of literature is arranged with the following headings: iron in human metabolism, iron metabolism, iron deficiency, metabolic effects of the quantity and source of dietary protein, effects of exercise on iron status, indices of iron status, factors related to adoption of dietary pattern, and the personal interview.

### IRON IN HUMAN METABOLISM

This review of iron in human metabolism includes biological compounds and function.

#### Biological Compounds

Iron is found in the human body as a component of a number of different compounds involved in essential metabolic and enzymatic processes and storage and transport functions (Dallman, 1990). Most of the body's iron is found in the heme protein hemoglobin, which accounts for over 65% of the approximately 3.8 g and 2.3 g total iron in men and women, respectively (Dallman, 1990). The amount of total body iron varies according to weight, hemoglobin concentration, sex, and size of the storage compartment (Fairbanks and Beutler, 1988; Bernat, 1983). Myoglobin, another heme protein found in muscle, contains approximately 10% of total body iron. The intracellular heme enzymes

include cytochromes a, b, c, cytochrome P-450, cytochrome oxidase, catalase and peroxidase. The nonheme enzymes include nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinate dehydrogenase, and xanthine oxidase. The heme and nonheme enzymes together comprise approximately 3% of total body iron (Dallman, 1990). Other enzymes require iron for proper function and include aconitase, phosphoenolpyruvate (PEP) carboxykinase, and ribonucleotide reductase. The major storage compounds are ferritin and hemosiderin, found mainly in liver, bone marrow, and reticuloendothelial cells. The amount of storage iron varies widely and averages approximately 12% in women and 25% in men of total body iron (Dallman, 1990).

The heme proteins contain an iron-porphyrin prosthetic group which stabilizes the iron (Prasad, 1978). They serve mainly in oxidative energy metabolism processes (Dallman, 1990). The nonheme enzymes are iron-flavoprotein complexes or those requiring iron as a cofactor (Prasad, 1978). These are also involved in the production of energy, as well as detoxification processes, and DNA synthesis (Dallman, 1990). Transferrin is the protein responsible for transport of iron through the blood and accounts for approximately 0.1% of the total body iron (Fairbanks and Beutler, 1988).

## Function

Hemoglobin is the major protein associated with the erythrocyte and serves to reversibly bind oxygen for delivery from the lungs to the tissues for cellular respiration (Wrigglesworth and Baum, 1980; Dallman, 1990). It also returns carbon dioxide to the lungs. The structure of hemoglobin is that of four globin chains, each with a heme group in a porphyrin ring structure containing one iron atom (Dallman, 1990). Thus, each hemoglobin molecule can bind four atoms of oxygen. The protective globin structure makes both hemoglobin and myoglobin resistant to autoxidation (Wrigglesworth and Baum, 1980). The life-span of an erythrocyte is approximately 120 days, after which it undergoes phagocytosis by macrophages in the reticuloendothelial cells of the liver, spleen, and bone marrow which releases the iron to the cytoplasm (Lynch et al., 1974). This process liberates approximately 20 mg of endogenous iron daily (Prasad, 1978). The iron then enters a pool where it may be transported out of the cell into the plasma by transferrin or stored as ferritin or hemosiderin (Lynch et al., 1974). The iron is now available for resynthesis of hemoglobin or other compounds as needed.

Myoglobin is similar in structure to hemoglobin but contains only one globin moiety in an iron-porphyrin complex (Dallman, 1990). It is found in muscle in a concentration

of approximately 5 mg myoglobin/g of tissue (Dallman, 1990). Its high affinity for binding oxygen allows for the release of oxygen from hemoglobin to myoglobin where it is then transported across the muscle cell and stored for use during muscular contraction (Williams, 1974; Dallman, 1982).

Myoglobin provides cellular respiration to the tissue for the inflow of oxygen and nutrients into cells and the elimination of end products of metabolism (Bernat, 1983). Cellular respiration is also partly responsible for the liberation, storage, and utilization of energy by the cell (Bernat, 1983). The iron in myoglobin has a life span of 150 days, slightly longer than that in hemoglobin (Hughes, 1978).

All living cells are subject to the potentially toxic effects of one by-product of the combination of oxygen and water, hydrogen peroxide. The enzymes peroxidase and catalase, comprised of one and four heme groups, respectively, convert hydrogen peroxide to innocuous products. Both enzymes are widely distributed in the body, but catalase is most prevalent in the erythrocyte and liver (Dallman, 1974).

Cytochromes a, b, and c are heme proteins of the electron transport chain (ETC) located within the cristae of mitochondria in all aerobic cells (Dallman, 1990). They serve as electron acceptor-donors for the oxidative

production of ATP (Prasad, 1978). The structure of cytochrome c is better known than are the structures of other cytochrome molecules. It is similar to myoglobin with one globin chain and one heme group; however, the heme moiety in cytochrome c is linked with the protein by thioether bonds (Prasad, 1978). Cytochrome P-450 is located within the microsomal membranes of the liver and serves to detoxify drugs and endogenous substrates by oxidative degradation (Dallman, 1990). Cytochrome  $b_5$  is a component of many membranes and is also found in the matrix of erythrocytes (Dallman, 1990). In the endoplasmic reticulum it is thought to provide energy for protein synthesis, whereas in the erythrocyte it is believed to catalyze the reduction of methemoglobin, formed in very small amounts from the slow oxidation of hemoglobin (Dallman, 1974; Dallman, 1990). Cytochrome oxidase, a complex of cytochromes a and  $a_3$ , is the terminal member of the ETC and, thus, is responsible for the reduction of molecular oxygen to water (Wrigglesworth and Baum, 1980). Cytochromes a and  $a_3$  differ from the other heme enzymes in the structure of their iron-porphyrin prosthetic group, which has a formyl group in place of one of the methyl groups and a hydrocarbon chain in place of one of the vinyl groups (Stryer, 1981).

The nonheme proteins include a group of iron-sulfur proteins which are proteins containing either acid-labile

sulfide or cysteinyl sulfur (Prasad, 1978). They include succinate dehydrogenase, NADH dehydrogenase, and xanthine oxidase. Succinate dehydrogenase, found in the mitochondria of cells, contains four iron atoms per molecule (Bernat, 1983). Succinate dehydrogenase is an enzyme in the tricarboxylic acid (TCA) cycle important for the production of ATP (Stryer, 1981). NADH dehydrogenase serves as an electron carrier in the ETC (Stryer, 1981). Xanthine oxidase contains a flavin molecule as well as molybdenum and can oxidize a wide variety of substrates (Wrigglesworth and Baum, 1980). It is thought to play an important role in the release of iron from storage sites and in iron absorption from the intestine (Dallman, 1974).

Those enzymes requiring iron for proper function include aconitase, phosphoenolpyruvate (PEP) carboxykinase, and ribonucleotide reductase (Dallman, 1990). Aconitase functions in the TCA cycle; PEP carboxykinase is a rate-limiting enzyme in the gluconeogenic pathway, and ribonucleotide reductase catalyzes the reduction of the four common ribonucleotides to their corresponding deoxyribonucleotides, an essential step in DNA synthesis (Dallman, 1990; Wrigglesworth and Baum, 1980).

The major storage compounds for iron, ferritin and hemosiderin, are found mainly in the reticuloendothelial system of the liver, spleen, and bone marrow, and may be

nearly depleted of their iron content or hold almost 20 times the normal amount before serious metabolic consequences are seen (Dallman, 1990). Each compound usually contains half the total storage iron; however, as liver iron stores increase, hemosiderin holds a greater proportion of total storage iron (Dallman, 1990).

Hemosiderin is thought to be aggregated ferritin partially degraded of its protein component, apoferritin (Fairbanks and Beutler, 1988). Both forms may be easily mobilized when iron is needed for hemoglobin synthesis, but the exact mechanism by which ferritin iron is released to the plasma is not known (Fairbanks and Beutler, 1988).

Transferrin, the iron transport protein, is synthesized in the liver and has a half-life of 8-10.5 days (Fairbanks and Beutler, 1988). Transferrin binds iron from the intestinal tract, storage sites, or the catabolism of hemoglobin and delivers the iron to locations where needed for synthesis of hemoglobin or enzymes, or return to storage (Fairbanks and Beutler, 1988). The cells, which receive the iron, contain receptors specific for transferrin in their membranes which bind the transferrin-iron complex and transport it into the cell where the iron is released (Dallman, 1990). The number of receptors is closely controlled so that there are fewer receptors where iron is plentiful and more receptors where iron is less plentiful

(Dallman, 1990).

### IRON METABOLISM

This review of iron metabolism includes absorption, the effects of protein on iron metabolism, and excretion.

#### Absorption

Iron balance is closely regulated metabolically and is maintained by reutilizing iron from the catabolism of cells, storing iron in ferritin, and most importantly through control of absorption of dietary iron intake (Hallberg, 1982). Absorption is increased when iron is deficient and decreased when the body has excess iron (Hallberg, 1982). The absorption of iron is also influenced by the amounts and chemical form of dietary iron and the presence of enhancing or inhibiting factors which can cause the bioavailability of iron to vary from <1% to >50% (Hallberg, 1982, Dallman, 1990).

Dietary iron exists in either heme or nonheme form, the former coming mostly from animal products and the latter found mainly in plant products (Dallman, 1990). Most dietary iron is from nonheme sources which exist as ferric iron salts and must be reduced to the ferrous form for better solubility and chelation at neutral pH for intestinal absorption (Fairbanks and Beutler, 1988). Substances which enhance these processes include ascorbic acid, meat, fish,

and poultry (Cook, 1990). Inhibitors of nonheme iron absorption bind the iron or make it insoluble and include tannic acid in tea and vegetables, ethylenediaminetetraacetic acid (EDTA), calcium phosphate, phosvitin in egg yolk, phytates, soy products, and antacids (Monsen et al., 1978; Raper et al., 1984).

Heme iron comes mainly from the hemoglobin and myoglobin in meat, poultry, and fish, and although it comprises only 10-15% of dietary iron among those who consume meat, it may provide nearly one-third of absorbed iron due to its high bioavailability (Cook, 1990). It is absorbed as an intact porphyrin complex and, thus, does not have contact with substances which might hinder absorption (Cook, 1990). It is broken down by heme oxygenase, after which the released iron enters a common intracellular iron pool where it is available for transport (Cook, 1990). The proportion of heme iron in animal tissues has been estimated to average 40% for meat, liver, poultry, and fish (NRC, 1989).

Diets which derive most iron from nonheme sources have been estimated to have an absorption of 2.5-5%, while those diets containing meat and ascorbic acid may have an absorption of from 15-25% of total iron intake (Cook, 1990; Dallman, 1990). The amount of bioavailable iron from a typical mixed Western diet has been estimated to be

approximately 6mg/1000 kcal (Snyder et al., 1989). Models have been developed to estimate dietary iron based upon total intake, proportion of heme and nonheme iron, and presence of enhancers to absorption (Cook, 1990). However, these models do not take into account inhibiting factors to absorption; knowledge of the effect of these factors would produce more reliable estimates of bioavailable iron.

#### The Effects of Protein on Iron Metabolism

One of the factors affecting the bioavailability of nonheme iron is the presence of proteins ingested along with the source of nonheme iron. The use of extrinsic radioactive iron labeling techniques has elucidated much that is known regarding the absorption of iron. Most proteins inhibit the absorption of nonheme iron; however, cellular animal proteins enhance absorption. The proposed mechanism by which proteins either inhibit or enhance absorption of nonheme iron is the formation of chelates between nonheme iron and protein or protein degradation products (Monsen, 1988). Noncellular proteins which inhibit the absorption of nonheme iron include egg albumin, dried whole egg, milk, cheese, and soy protein (Monsen, 1988).

By contrast, cellular animal proteins may enhance the absorption of nonheme iron by as much as three times when substituted for a substance such as egg albumin in a meal

(Monsen, 1988). A similar level of enhanced absorption is seen in meals containing beef, lamb, or pork; however, when compared to those meats, substitution in the same meal with chicken or fish resulted in a 17% and 28% reduction in absorption of nonheme iron, respectively (Monsen, 1988). When noncellular animal protein such as egg albumin was substituted for beef in the same meal, the absorption of nonheme iron was reduced by 66% (Monsen, 1988). Further, the enhancement of nonheme iron absorption by cellular animal protein was shown to be dose-related by substituting increasing amounts of beef for egg albumin in a meal, resulting in a steady rate of increased absorption of nonheme iron (Monsen, 1988). Both raw and cooked meat increase nonheme iron absorption, and specific amino acids such as cysteine have been shown to have enhancing effects, apparently through an effect by free sulfhydryl groups (Monsen, 1988).

### Excretion

Iron losses in a normal adult male from the gastrointestinal tract via the feces are comprised of bile, desquamated mucosal cells, minute losses of blood, and amount to approximately 0.6 mg/d (Dallman, 1990). Other losses occur through sweat, amounting to 0.2-0.3 mg/d, and urinary losses of approximately 0.1 mg/d (Dallman, 1990).

These losses amount to 12-14 mcg/kg/d and are assumed to be similar among females and all age groups when expressed by body weight (Cook, 1990). In addition, premenopausal women experience another 25-30 ml/mo loss through menstruation, or 0.5 mg iron/d averaged over the entire menstrual cycle (Cook, 1990). However, this amount varies considerably among women and can amount to three times normal in approximately 5% of women (Cook, 1990). Further, women taking oral contraceptives will experience smaller losses and those using intrauterine devices for birth control will have heavier losses (Soustre et al., 1986).

### IRON DEFICIENCY

This review of iron deficiency includes prevalence, stages, metabolic consequences, recommended dietary intake, dietary iron intake in the general population, and iron intake among female athletes.

#### Prevalence

Estimates of the prevalence of iron deficiency throughout the world vary considerably due to differences in parameters measured, reference values and sampling methods. There is agreement, however, that iron deficiency is the most common single nutritional deficiency in both the developing and developed countries (ESWG, 1985).

Based on the data from the second National Health and Nutrition Examination Survey from 1976-1980 (NHANES II), the prevalence of iron deficiency in the United States was estimated by the Expert Scientific Working Group (ESWG) of the Life Sciences Research Office (LSRO) using three different models: ferritin model, mean corpuscular volume (MCV) model, and hemoglobin percentile shift model (ESWG, 1985). The range of prevalence estimates was highest for children aged 1-2 years (9.2-9.4%), males aged 11-14 years (3.5-12.1%), and females aged 15-44 years (2.5-14.2%), depending on the model used (ESWG, 1985). Dallman et al. (1984), using the same data, found the prevalence of the third stage of iron deficiency, anemia, to be highest in infants (5.7%), teenage girls (5.9%), young women (5.8%), and elderly men (4.4%). However, excluded from the analysis of Dallman et al. were those individuals from the reference population who had laboratory values indicative of iron deficiency. This was done in order to get a 95% reference range for age and sex of hemoglobin values for determination of iron deficiency anemia. The percent of women, aged 25-44, in the NHANES II population who were excluded by Dallman et al. and, thus, were iron deficient was 21.7 (Dallman et al., 1984).

### Stages

Iron deficiency develops across three stages of impaired iron status. With an increased need or decreased intake, iron must be mobilized from stores to meet needs for hemoglobin synthesis and other functions. Thus, there is a decrease in ferritin, the major storage source of iron, which is reflected by the measurement of serum ferritin. Transferrin is the protein which transports iron in the blood to where it is needed. When stores of iron are depleted the amount of transferrin which is saturated with iron is reduced, thus limiting the iron supply for the development of the red blood cell, resulting in signs of the second stage of deficiency, iron-deficiency erythropoiesis, manifested by increased free erythrocyte protoporphyrin and decreased percent transferrin saturation. Hemoglobin concentration may decline slightly at this stage, but is not definitive. However, as the deficiency progresses to the third stage, insufficient iron for hemoglobin synthesis results in subnormal hemoglobin concentration and an increase in numbers of microcytic (abnormally small) erythrocytes, producing a decrease in mean corpuscular volume (MCV) characteristic of iron deficiency anemia (ESWG, 1985).

### Metabolic Consequences

Symptoms of iron deficiency are usually vague and without serious manifestations until the condition becomes severe. Several studies in rats, as well as humans, have found a decrease in work performance relative to hemoglobin concentration. The change in hemoglobin concentration need not be dramatic to see a significant impairment of work capacity (Gardner et al., 1977). Ohira et al. (1983) reported their previous findings of a correlation between the severity of anemia and the decrement in work capacity in both rats and humans. Gardner et al. (1977) studied female Sri Lankan tea estate laborers and found that subjects with hemoglobin concentrations between 11.0 and 11.9 g/dl exhibited a 20% decrease in work performance on a treadmill compared to subjects whose hemoglobin concentration was above 13 g/dl. In those studies showing a correlation between hemoglobin concentration and work performance, deficient subjects who were supplemented with iron improved their work performance, with the greatest improvement seen in those with the lowest initial hemoglobin concentration (Dallman, 1990).

Since hemoglobin mediates the oxygen carrying capacity of the blood, it was considered to be responsible for the decrement in work performance observed in those subjects with lower hemoglobin concentrations. However, inadequate

tissue iron may contribute to submaximal work performance through the reduced participation of iron-containing enzymes in cellular respiration and energy production. Finch et al. (1979) normalized hemoglobin levels in iron-deficient rats with blood transfusions which corrected the anemia without affecting tissue iron levels; however, decreased work performance was still observed. A reduction in activity of the TCA cycle could occur with diminished levels of aconitase, a rate limiting enzyme, as well as succinate dehydrogenase. A decrease in energy production through the TCA cycle would promote anaerobic metabolism, resulting in increased lactate production (McDonald and Keen, 1988). This has been demonstrated in several studies (Ekblom et al., 1972; Schoene et al., 1983). However, the extent to which these responses affect diminished work performance remains to be elucidated.

Studies evaluating cognitive performance and behavior in infants between the ages of six months and two years of age have found a significant decrease in responsiveness and activity and increased body tension, fearfulness, and fatigue among those who were only mildly iron deficient (Dallman, 1990). These abnormalities appeared to be related to the duration of iron deficiency and were not fully corrected after 1-3 months supplementation with iron (Dallman, 1990).

Recent studies have implicated iron deficiency in an impairment of thermoregulation, or the body's ability to regulate its temperature. The maintenance of normal body temperature in cold conditions depends on heat production generated through both shivering and nonshivering thermogenesis (Beard and Borel, 1988). Muscular contraction provides shivering thermogenesis, while nonshivering thermogenesis is provided by heat via the stimulation of metabolic processes also known as facultative thermogenesis, which can be maintained for longer periods of time than can shivering thermogenesis (Beard and Borel, 1988). Facultative thermogenesis is mediated by the sympathetic nervous system (SNS) through the action of norepinephrine (NE), and by the thyroid hormone system through the action of triiodothyronine ( $T_3$ ), formed from thyroxine ( $T_4$ ) (Beard and Borel, 1988). Beard and Borel (1988) reported the findings of Beard et al. (1988) who exposed iron-deficient women and normal control women of similar body fatness to a cool water bath for 100 minutes for two to three trials. The iron-deficient subjects were then given 12 weeks of supplementation after which both groups were retested. Before supplementation the iron-deficient women showed an impaired thermoregulatory capacity compared to control women as indicated by a significantly greater decline in mean body core temperature, as well as significantly lower levels of

mean plasma  $T_3$  and  $T_4$  concentrations. After iron supplementation the anemia was corrected and a significant improvement in the ability to maintain body core temperature was found along with an increase in thyroid hormone concentrations (Beard and Borel, 1988).

A decreased resistance to infection has been demonstrated in both humans and animals who are iron-deficient, although a sufficient cause and effect relationship has not been established (Dallman, 1990). Conversely, an excess of iron may contribute to an increased risk of infection which may be related to the requirement by bacteria for iron for growth (Dallman, 1990).

#### Recommended Dietary Intake

The Food and Nutrition Board has been responsible for establishing the Recommended Dietary Allowances (RDA) for nutrients since 1941 (NRC, 1989). RDAs are defined as "the levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy persons" (NRC, 1989). In setting the requirement for iron, the subcommittee of the Board decided to assign a level of intake which would provide iron stores of 300 mg, which they deemed necessary as a reserve to meet the needs for several months of someone

whose diet was devoid of iron. This was based upon data estimating average stores to be 300 mg in women and 1000 mg in men (NRC, 1989). Daily iron losses were estimated to be approximately 1 mg/d for a healthy adult male; to this was added an average loss of 0.5 mg/d for women through menstruation. Using data from several U.S. population surveys, the mean consumption of iron was found to be approximately 10 mg/d among a population in which evidence of iron deficiency was identified in 2.5-4%, suggesting this level of intake was associated with adequate iron status in at least 86% of the population of women 15-44 years of age (NRC, 1989). The subcommittee accepted an absorption level of 10-15% of total dietary iron intake; they, therefore, concluded that an intake of 15 mg/d would be sufficient to meet the needs of most women. This was a reduction from the previous RDA for iron for women of 18 mg/d.

The breast-fed infant has stores of iron which will provide adequate iron status for the first three months of life. The RDA for children aged 6 months to 3 years is currently set at 10 mg/d.

Children and adolescents require iron for normal stores as well as for increased iron mass related to growth. The RDA for this population has been set at 10 mg/d with an additional 2 mg/d recommended for males during puberty to provide for a normal spurt in growth. Females would need an

extra 5 mg/d upon reaching puberty, as determined for the requirement of adult women.

In setting the RDAs, the subcommittee assumed a daily dietary intake of 30 to 90 g of meat, poultry or fish, or foods containing 25-75 mg of ascorbic acid after preparation. Those people not consuming such diets are considered to have a higher requirement for iron than that established by the RDAs (NRC, 1989).

#### Dietary Iron Intake in the General Population

Raper et al. (1984) evaluated data from the United States Department of Agriculture's (USDA) 1977-78 Nationwide Food Consumption Survey for levels of total and available iron. The source was a 1-day dietary recall and covered 9,547 individuals one year and older. The model of Monsen and Balintfy (1982) was used to estimate available iron from total iron intake. Mean total and available iron were found to be 9.2 and 0.65 mg, respectively for children aged 1-8 years. The percent of total iron that was available was 7. For all males aged 9 and older the mean total and available iron intakes were 14.3 and 1.18 mg, respectively, resulting in a mean 8.3% available iron. For females aged 9 and older the mean total and available iron intakes were 10.4 and 0.82, respectively, resulting in a mean 7.9% available iron (Raper et al., 1984). Comparing these values to the current

RDA, it is seen that only the males aged 9 and older satisfied their daily requirement for iron. In no group did the percent available iron meet the 10% assumed by the current RDA. The 337 women in the group aged 19-22 years consumed 1.0 mg of heme iron/d from a total intake of 10.7 mg iron/d. This, considered with the nonheme iron portion of their diet and application of the model gave an estimate of 0.83 mg available iron, or 8.2% of total intake (Raper et al., 1984).

Murphy and Calloway (1986) used data from the NHANES II to estimate nutrient intakes of 1,066 women aged 18-24 years by means of a shortened nutrient data base. Their finding of a mean total daily iron intake of 10.7 mg was very close to the NHANES II figure of 10.4 mg. This represents 71% of the current RDA for iron for premenopausal women. The heme iron content of this intake was estimated to be 1.3 mg.

#### Iron Intake Among Female Athletes

Assessment of nutrient intake among the 19 members of the Canadian Women's Olympic Field Hockey Team of 1984 by means of a 3-day dietary record found a mean daily iron intake of 11 mg (Ready, 1987). Two studies by Lukaski et al. (1988, 1989) of 12 and 21 female college swim team members found nearly identical intakes of daily iron, based upon 7-day dietary records, of 13.2 mg at the beginning of

the competitive season in both studies and 12.0 and 12.1 mg at the end of the season, respectively. Manore et al. (1989) used 3-day dietary recall records to assess iron intake in 10 female runners. The results of two records taken three weeks apart indicated a daily total iron intake of 12.7 mg and heme iron intake of 0.7 mg (Manore et al., 1989). Deuster et al. (1986) also evaluated 3-day dietary records in 51 highly trained female runners for daily iron intake. Nearly half of the women were taking supplements which included iron and the respective amounts of iron from food and supplements were not given. The authors stated, however, that the amount of iron from both sources was similar. The mean daily intake for iron from both sources was 41.9 mg (Deuster et al., 1986). Despite this relatively high intake, 30% of the subjects consumed less than the current RDA of 15 mg/d of iron (Deuster et al., 1986). Another study of 17 female endurance runners found a mean daily iron intake of 12.5 mg by means of 7-day dietary records (Clement and Asmundson, 1982). Ninety percent of the subjects consumed less than the Recommended Daily Intake (RDI) for Canada, which was 14 mg; thus, at least the same percent would consume less than the current USRDA. The mean daily intake of iron among eight highly trained female cyclists was determined to be 10.6 mg (Keith et al., 1989). A study of 31 female high school athletes who completed 24-

hour diet recalls found a mean daily iron intake of 11 mg (Perron and Endres, 1985).

Although these studies have varied in the number of subjects, athletic activity, and means of assessment, only the study by Deuster et al. (1986), found a dietary intake meeting the current USRDA, and over half of those subjects received part of their iron intake through supplementation.

#### METABOLIC EFFECTS OF THE QUANTITY AND SOURCE OF DIETARY PROTEIN

Absorption of iron is reduced in rats with protein-calorie malnutrition and is corrected when adequate protein is provided (Enwonwu et al., 1972). Turnbull (1974) reported the findings of Conrad et al. (1967) of weight loss and reduced plasma iron turnover and erythrocyte utilization in rats deprived of protein.

Assessment of iron status based upon habitual source of dietary protein has been evaluated by a number of researchers with particular emphasis applied to the nutritive quality of vegetarian diets. Because many vegetarian diets contain little or no sources of heme iron, high levels of nonheme iron with low bioavailability, along with the presence of inhibitors to the absorption of iron, concern has been generated as to the adequacy of these diets to meet the body's need for iron.

Harland and Peterson (1978) studied the diets of 16 lacto-ovovegetarian Trappist monks for one month. Total mean daily iron intake ranged from 7-18 mg; this was accompanied by relatively high intakes of phytate and crude fiber; however, high levels of ascorbic acid were also consumed (Harland and Peterson, 1978). Nine of the sixteen consumed less than the RDA for protein. One of the monks had a hematocrit below normal; two had both hematocrit and hemoglobin below normal (Harland and Peterson, 1978).

Iron status in a group of 59 male and 55 adult female East Indian immigrants to Canada who were lacto-ovovegetarians was assessed by means of 3-day dietary record and biochemical indices (Bindra and Gibson, 1986). Mean daily total iron intake was 18.7 and 14.4 mg for males and females, respectively. Available iron was calculated to be 1.27 and 1.08 mg for males and females, representing only 6.8% and 7.5% of total iron intake, respectively (Bindra and Gibson, 1986). The prevalence of impaired iron status, depending on which of three models was applied, ranged from 3-5.1% for the males and from 12-33.3% for the females (Bindra and Gibson, 1986). The authors attributed the relatively high prevalence of impaired iron status in the women to the inadequate amounts of readily available dietary iron in their lacto-ovovegetarian diets (Bindra and Gibson, 1986).

The iron status of a group of 56 long-term vegetarian women was studied by Anderson et al. (1981). Nine were vegans with the remaining subjects being lacto-ovovegetarians. The mean daily intake of total iron was 12.5 mg; the calculated available iron was 0.62 mg for premenopausal women and 0.73 mg for postmenopausal women, both less than that considered adequate based on the RDA (Anderson et al., 1981). Only seven had a hemoglobin concentration below 12.0 g/dl, considered indicative of iron deficiency anemia (Anderson et al., 1981). The authors concluded that the relatively low prevalence of impaired iron status seen in this population indicated that any adverse effects possible from a vegetarian diet were insufficient to produce significant negative effects; they further postulated that the women may have adapted to their vegetarian diets by increased absorption of dietary iron (Anderson et al., 1981).

Bergan and Brown (1980) studied the nutritional status of 76 "new" vegetarians, a term denoting mostly young people who have recently adopted vegetarian diets in response to popular trends, and who have been following a mostly vegetarian diet for at least one year. The dietary intakes of iron were not reported except to note that the men consumed sufficient intakes and 50% of the women consumed less than 60% of the RDA for iron, which was 18 mg/d at the

time (Bergan and Brown, 1980). Based on the guidelines used in the Ten-State Nutrition Survey for the interpretation of blood data, 14% of the vegetarians had values for all four indicators of iron status which were unacceptable (Bergan and Brown, 1980). Of those vegetarians whose indicators of iron status were in the acceptable range, there was a high prevalence of values in the low range (Bergan and Brown, 1980).

Several studies have compared the nutrient intake and/or iron status of vegetarians and omnivores. Mason et al. (1978) analyzed data obtained from 78 women; 42 vegetarians, all but one lacto-ovovegetarians, and 36 nonvegetarians who consumed seven or more servings of meat per week. Dietary intakes were determined based upon 7-day dietary records. The mean daily intake of total iron was slightly higher among the vegetarians, 11.2 mg, compared to the nonvegetarians, 10.8 mg (Mason et al., 1978). However, the average age of the subjects was 57.1 years, and the authors did not report dietary intakes relative to menopausal status, thus making it difficult to determine adequacy of dietary iron intake.

Another study which compared iron status between those considered "new" vegetarians and omnivores was conducted by Helman and Darnton-Hill (1987). Vegetarians in this study were defined as those who ate no meat, poultry, or fish.

Although the vegetarians were considered "new" in the sense that they were mainly a younger group who had adopted their dietary pattern mostly in response to recent trends; the mean length of time they had been following a vegetarian diet was 7.3 years (Helman and Darnton-Hill, 1987). The only biochemical indicator used to assess iron status was serum ferritin. Low ferritin levels were found in 5% of the male and 27% of the female vegetarians, while among the omnivores 8% of the males but only 12% of the females were found to have values in the low range (Helman and Darnton-Hill, 1987).

Premenopausal women who were either vegetarians or omnivores were studied for comparison of nutritional status by Reddy and Sanders (1990). Dietary intakes were evaluated based upon 7-day dietary records. Omnivores consumed a mean of 12.1 mg iron/d, while vegetarians who were Indian consumed 12.7 mg iron/d and Caucasian vegetarians consumed 13.8 mg iron/d (Reddy and Sanders, 1990). Serum ferritin concentrations in both groups of vegetarians were approximately half that of omnivores and were below normal in the majority of vegetarian subjects not taking supplements (Reddy and Sanders, 1990). A significant correlation was found between intake of heme iron and serum ferritin concentration in the omnivores (Reddy and Sanders, 1990). Hemoglobin concentrations were significantly lower

in the Indian vegetarians than in either the Caucasian vegetarians or omnivores, as were mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) (Reddy and Sanders, 1990). The authors concluded that both Indian and Caucasian vegetarians had an increased risk of developing iron-deficiency anemia.

Another comparison between omnivores and lacto-ovovegetarians was made by McEndree et al. (1983). Groups were divided according to sex, dietary pattern and status in the community (student or adult). Male omnivores who were students consumed a mean daily iron intake of 13.2 mg; those who were adults consumed 11.3 mg. Male vegetarians consumed 18.4 mg iron/d. All males had blood parameters indicative of normal iron status. Female omnivores who were students consumed a mean daily intake of iron of 15.2 mg; those who were adults consumed 11.9 mg. Female vegetarians who were students consumed a mean daily iron intake of 12.1 mg; those who were adults consumed 11.6 mg. Data for indicators of iron status were within normal limits for all groups and not statistically different from each other; however, there was a trend toward higher serum ferritin and serum iron values among the omnivores compared to the vegetarians (McEndree et al., 1983). The authors concluded that although iron intakes were low for groups with the exception of vegetarian males, this was not reflected in biochemical parameters of

iron status.

One study of premenopausal women separated groups by habitual intake of dietary protein into those who consumed either red meat, poultry and fish, or lacto-ovovegetarian sources of protein (Worthington-Roberts et al., 1988). Two 3-day dietary recall records were obtained to confirm dietary pattern and for nutrient analysis. No significant differences were seen among the groups in daily intake of kilocalories, iron, or fat; however, lacto-ovovegetarians consumed significantly less protein and more carbohydrate than the other two groups (Worthington-Roberts et al., 1988). Mean daily intakes of iron were less than the current RDA for all groups. Mean hemoglobin and hematocrit values were normal for all groups; however, those who consumed red meat had significantly higher values for both variables than those in the other two groups (Worthington-Roberts et al., 1988). An even greater difference was seen in the higher serum ferritin values for the red meat users compared to the other two groups. An unexpected finding was that the lowest mean serum ferritin values occurred among the consumers of chicken and fish (Worthington-Roberts et al., 1988). The authors concluded that superior iron status was associated with regular consumption of red meat.

### THE EFFECTS OF EXERCISE ON IRON STATUS

This review of the effects of exercise on iron status includes an historical perspective, proposed mechanisms for development of sports anemia (including erythrocyte destruction and hemodilution effects, iron losses in sweat, gastrointestinal blood loss, decreased iron absorption, and decreased iron intake), and the effects of exercise and vegetarian diet on iron status.

#### Historical Perspective

The development of anemia during strenuous muscular exercise accompanied by erythrocyte destruction was noted by Broun in 1922 based on experiments with dogs. Broun proposed the mechanism of increased erythrocyte destruction to be a reduction in resistance of the erythrocyte membranes caused by the damage from increased circulation through the capillaries (Yoshimura, 1970). Davis in 1935 further speculated that this decreased resistance to erythrocyte destruction was due to the increased body temperature induced by heavy muscular work (Yoshimura, 1970). Yamaji, in studying human subjects during sports training, also observed the development of a significant anemia and hypoproteinemia which returned to normal upon the cessation of training (Yoshimura, 1970). Yamada measured the osmotic resistance of erythrocytes in athletes during training and

found anemia and increased erythrocyte fragility which were corrected after two weeks' rest (Yoshimura, 1970). He also demonstrated the increased deposition of hemosiderin in rats' spleens after they were subjected to one week's hard muscular exercise (Yoshimura, 1970). Yoshimura in 1959 summarized these findings and described the phenomenon as "sports anemia". Through experiments which measured nitrogen balance and blood parameters indicative of iron status on exercising subjects who consumed protein at various levels, he concluded that the ingestion of 2 g protein/kg body weight/day could prevent an athlete in training from developing sports anemia (Yoshimura, 1970).

In an attempt to identify the mechanism responsible for the increased destruction of erythrocytes, Shiraki proposed that there is some hemolyzing factor which is liberated from the spleen during strenuous muscular exercise (Yoshimura, 1970). Yoshimura in 1966 proposed this to be an adaptive mechanism by which the body retrieves hemoglobin released from erythrocyte destruction and reutilizes it for the increased production of myoglobin necessary for hypertrophy of muscle during physical training (Yoshimura, 1970).

Since these early works, iron deficiency or impaired iron status, with or without iron deficiency anemia have been identified by a number of researchers among athletes (Clement et al., 1977; Clement and Asmundson, 1982; Hunding

et al., 1981; Selby and Eichner, 1986; Puhl et al., 1981; Puhl and Runyan, 1980; Nickerson et al., 1985; Nickerson and Tripp, 1983; Frederickson et al., 1983; Ehn et al., 1980; Parr et al., 1984). Other researchers have not found this effect (Haymes et al., 1986; Durstine et al., 1987; Lukaski et al., 1989; Lukaski et al., 1990; Risser et al., 1988; Rowland and Kelleher, 1989; Wirth et al., 1978).

Differences among studies regarding subjects, methodology, and parameters used as indicators of iron status provide justification for continued debate over this area of controversy.

#### Proposed Mechanisms for Development of Sports Anemia

Several theories have been proposed to explain the incidence of impaired iron status among athletes. These include erythrocyte destruction, increased plasma volume producing a hemodilution effect on hemoglobin concentration, increased iron losses through sweat, gastrointestinal bleeding, and hematuria, and insufficient intake of dietary iron.

Erythrocyte Destruction and Hemodilution Effects. The possibility has been suggested that reduced levels of hemoglobin concentrations observed in some athletes could be due to a hemodilution effect resulting from an increase in plasma volume. McDonald and Keen (1988) cited Brotherhood

et al. (1975) who reported that blood volume can increase as much as 20% during training. This, however, would not alter actual oxygen-carrying capacity of blood.

Puhl and Runyan (1980) studied 19 untrained college females engaged in a 9-week aerobic fitness training program and found progressive decreases in mean hemoglobin, hematocrit, and red blood cell (RBC) count through the first seven weeks of the program, which returned to near pre-training values by the ninth week of the study. The researchers also observed an increase in mean cell volume (MCV) which could be indicative of erythrocyte destruction as was pointed out by Harris and Kellermeyer who suggested that older, smaller red blood cells hemolyze at a lower osmotic stress compared to younger cells which are more resistant to osmotic effects (Puhl and Runyan, 1980). A loss of older, smaller cells during training could leave a greater proportion of younger, larger cells, which would be reflected by an increase in MCV (Puhl and Runyan, 1980). Puhl and Runyan concluded that the changes they found in their study were more indicative of erythrocyte destruction than a hemodilution effect, although they did not measure change in blood volume. Puhl et al. (1981) further explored this possibility in a study performed on younger women involved in more intensive physical training, eight high-school cross-country runners, and a comparison group of non-

runners. During the competitive season the runners experienced significant changes in blood variables which were most marked during the first week: decreases in hemoglobin (8.0%), hematocrit (7.7%), and red blood cell (RBC) count (6.8%); and increases in RBC volume (1.8%) and MCV (data not given). By contrast, the only significant change in the comparison group was an initial decrease in RBC volume of 1.9%. Again, these effects proved transitory, with the return of preseason values by the eighth week of the study, with the exception of RBC volume. Although the researchers did not monitor blood volume and so could not exclude the possibility of a hemodilution effect, they concluded that the results supported an increased destruction of erythrocytes as the cause of the changes in blood variables, citing the increase in MCV as supportive evidence (Puhl et al., 1981). A similar study by Frederickson et al. (1983) confirmed the results of Puhl et al. (1981) and provided further argument that the changes in blood variables occurred as a result of increased erythrocyte destruction rather than a hemodilution effect, by calculating that the degree of change in hemoglobin concentration was greater than that accountable to increased plasma volume. Further evidence of increased erythrocyte destruction was compiled by Magnusson et al. (1984) who studied 43 male runners whose blood values were compared

with two groups of control subjects. The runners had significantly lower hematocrit, serum iron, transferrin saturation and serum ferritin values than the 100 healthy non-athlete controls (Magnusson et al., 1984). Moreover, the low serum haptoglobin observed in most of the runners was considered indicative of an increased intravascular hemolysis, since haptoglobin forms a complex with hemoglobin released by erythrocytes (Magnusson et al., 1984). This complex is taken up by hepatocytes resulting in a reduced return of catabolized cells to the reticuloendothelial system (Magnusson et al., 1984). This was reflected in the lower levels of ferritin and hemosiderin found in the athletes. Most researchers have considered the susceptibility to increased erythrocyte destruction to be greater among runners who may experience greater mechanical trauma. However, a study by Selby and Eichner (1986) found evidence of this phenomenon among a group of collegiate swimmers. There was a steady decline in hemoglobin values throughout the competitive season, with a return toward preseason levels two weeks following the end of the season (Selby and Eichner, 1986). The researchers were able to correlate the degree of decline in hemoglobin to increase in swimming distance (Selby and Eichner, 1986). Below normal serum haptoglobin levels were found in 25.9% of the swimmers at preseason (Selby and Eichner, 1986). During competition

this value decreased further, with only 3% of the swimmers not showing a decrease in their haptoglobin levels (Selby and Eichner, 1986).

Iron Losses in Sweat. Iron losses through sweat for the average person have been estimated to be rather low, approximately 0.2-0.3 mg/d (Dallman, 1990). However, the increased sweating that may occur from prolonged intense physical activity may increase this amount significantly. Newhouse and Clement (1988) estimated a potential loss of sweat from one day's endurance running to be 1-3 liters and extrapolated iron losses equivalent to an additional 0.4-1.0 mg/d over normal, based upon Vellar's finding of 0.4 mg iron/l sweat (Newhouse and Clement, 1988). Paulev et al. (1983) used two different procedures to measure iron concentration of sweat in two groups of distance runners while they cycled on an ergometer. One procedure included the first collection of cell-rich sweat, while the second procedure collected sweat after the skin had been wiped clean of desquamated cells. The mean iron concentration was 0.29 mg/l sweat (Paulev et al., 1983). The mean iron concentration for the cell-free sweat was significantly less, as expected, which when added to that found in the cell-rich sweat, would result in total iron losses of 1.17 mg for a training session with a sweat loss of six liters (Paulev et al., 1983). Brune et al. (1986) found an iron

concentration of only 0.02 mg/l sweat; however, the researchers felt the amount of iron in the first sweat collection should not be included in the calculations due to its high content of desquamated cells. They wanted to quantify the amount of iron lost over steady state conditions which might occur with prolonged sweating. Variability in testing protocol makes it difficult to draw conclusions about the additional loss of iron an athlete exercising under conditions conducive to heavy sweating might endure.

Gastrointestinal Blood Loss. McMahon et al. (1984) used the hemocult guaiac card method to evaluate stool samples from runners participating in the 1983 Boston Marathon. They found 22% to have guaiac-positive stools and these runners had significantly faster race times than those whose stools were negative, which would imply an exertion effect (McMahon et al., 1984). They speculated that the causes of gastrointestinal blood loss included intestinal ischemia, stress gastritis, drug-induced lesions, and blood loss from preexisting lesions. Although the subjects reported a high prevalence of aspirin and nonsteroidal drug use, the prevalence did not differ significantly from those whose stools were negative.

Stewart et al. (1984) used a more sensitive test for the detection of fecal hemoglobin, the HemoQuant assay.

This test was used to compare gastrointestinal blood loss in a group of long-distance runners with a group of matched nonrunning controls. Fecal hemoglobin levels were comparable between the two groups before the runners participated in a race; after the race mean fecal hemoglobin levels increased in 21 of the 24 runners, with a mean peak of 3.96 mg/g stool compared to 0.99 mg/g stool in controls (Stewart et al., 1984). The researchers controlled for possible interfering variables and speculated that the running-induced bleeding might be due to transient gut ischemia (Stewart et al., 1984).

Decreased Iron Absorption. Iron absorption is usually enhanced in cases of inadequate iron stores. This has not always proved true among athletes. Using radioactive iron labeling, Ehn et al. (1980) compared iron absorption in athletes and controls who were both iron deficient. They found an iron absorption of only 16.4% in the runners compared to 30% for the controls (Ehn et al., 1980). These results were supported in an unpublished study by Clement et al. who found an average iron absorption in a group of iron-deficient female athletes of 29%, which was compared to previously reported iron absorption values of over 70% in iron-deficient, nonexercising females (Clement and Sawchuk, 1984). The mechanism by which this malabsorption occurs is unknown, but some researchers have postulated that an

elevation in plasma transferrin saturation could inhibit the release of iron from intestinal mucosal cells (Newhouse and Clement, 1988).

Studies with rats have produced conflicting results. Ruckman and Sherman (1981) demonstrated decreased apparent iron absorption in exercised male rats compared to sedentary controls; however, this effect was not seen among the female rats. Strause et al. (1983) found an increase in iron absorption in exercised rats compared to sedentary controls. They concluded this to be one of several adaptive mechanisms to provide iron available for increased muscular requirements due to exercise (Strause et al., 1983).

Decreased Iron Intake. Because of iron's essential role in oxidative energy metabolism and the potential for athletes to experience greater iron losses than the average person, an adequate dietary intake of iron assumes an important role in an athlete's diet. The average Western diet supplies approximately 5-6 mg iron per 1000 kilocalories (kcal), so a caloric intake under 2000 kcal can provide less than the RDA (Clement and Sawchuk, 1984). Also, athletes involved in sports which require a relatively low percent body fat would be inclined to consume less kilocalories. In addition, the general emphasis in the U.S. for several decades has been toward thinness, especially among women. Thus, a female who is also an athlete may be

inclined to follow a diet deficient in iron.

Clement and Asmundson (1982) reported dietary intakes of iron among college athletes and found the mean daily intake among males to be 18.5 mg/d; however, among the females it was only 12.5 mg/d, less than the RDA. Although the average hemoglobin values were within normal range, 29% of the men and 82% of the women were at risk for iron deficiency, based on plasma ferritin concentrations (Clement and Asmundson, 1982). Ready (1987) reported the nutrient intake of the Canadian Women's Olympic Field Hockey Team to be 11.0 mg/d for iron. Lukaski et al. (1989) compared dietary intakes among female college swimmers at the beginning and end of the competitive season. The mean daily intake for iron was 13.2 mg at the beginning and 12.1 mg at the end of the season (Lukaski et al., 1989). All blood variables had means within the normal ranges; however, hemoglobin and hematocrit showed a slight decrease and TIBC was slightly increased at the end of the season (Lukaski et al., 1989). Manore et al. (1989) studied female runners over a nine week period for nutrient intake and iron status. Mean daily iron intake was calculated at three instances and was found to steadily decline from 13.7 to 11.6 to 10.5 mg/d (Manore et al., 1989). Indices assessing iron status were within normal limits; however, the runners' hemoglobin, hematocrit, erythrocyte count, and ferritin levels showed

steady declines, and TIBC rose over the study period (Manore et al., 1989).

Deuster et al. (1986) evaluated nutrient intake and iron status in a group of women who competed in the First Women's Olympic Marathon Trials in 1984. Approximately half of the women took nutritional supplements and the data were not distinguished accordingly. However, the researchers stated that 43.1% of the women were consuming less than the RDA for iron, and that supplemental iron contributed more to total intake than did food iron (Deuster et al., 1986). In 35% of the women serum ferritin concentrations were <12 ng/ml, indicative of inadequate iron stores (Deuster et al., 1986). Keith et al. (1989) evaluated dietary status in female cyclists and found a mean daily iron intake of 10.6 mg. Although mean values for hemoglobin and hematocrit were normal, most hemoglobin values were in the lower 50% of normal range (Keith et al., 1989). Perron and Endres (1985) studied female high school athletes and found their mean daily intake of iron to be 11 mg. Although their scores on a nutritional knowledge test were high, analysis of the data found that nutrition knowledge explained little of the variation in any of the nutrient values, so knowledge was not always applied to dietary practices (Perron and Endres, 1985). The mean daily caloric intake was 1799 kcals, below the RDA for that age group (Perron and Endres, 1985).

Further, 81% of the subjects were unhappy with their present weight and 73% wanted to lose weight (Perron and Endres, 1985). One study by O'Toole et al. (1989) of ultraendurance triathletes did find dietary intakes of 35.2 mg and 18.5 mg for men and women, respectively, which are both above the RDA. However, 30% had at least one suboptimal index of iron status (O'Toole et al., 1989).

#### EFFECTS OF EXERCISE AND VEGETARIAN DIET ON IRON STATUS

In a study conducted by Liebman et al. (1987) female runners who ran between 10-25 miles per week were divided into two groups. Both groups adopted a lacto-ovovegetarian diet for six months, but the experimental group increased the duration and intensity of their training program while the control group maintained their pre-study exercise levels. Thus, an attempt was made by the researchers to separate the effects of the increased exercise from those of the vegetarian diet.

There were four test periods through the length of the study which consisted of biochemical analyses and collection of 7-day food records. Maximum oxygen consumption was determined in the first and fourth testing period of the study.

The results found no significant differences in mean hemoglobin, hematocrit, plasma iron, total iron binding

capacity, or transferrin saturation between the two groups at any of the four test periods nor across time (Liebman et al., 1987). However, a significant decrease in plasma ferritin occurred in both groups over the course of the study (Liebman et al., 1987). This decrease was greater in the experimental group compared to the control group (55 vs. 32% decrease) (Liebman et al., 1987).

The researchers concluded that the significant decrease in ferritin levels in both groups suggested that this change was due to the institution of the lacto-ovovegetarian diet, since the changes were seen in the control group which had not altered its exercise levels (Liebman et al., 1987). However, the authors also speculated that since the magnitude of the changes in indicators of iron status was greater among the experimental group for all parameters measured, an exercise-induced effect might have been demonstrated with a larger group of subjects and/or a study of longer duration (Liebman et al., 1987).

#### INDICES OF IRON STATUS

Currently, no single biochemical indicator is available which is consistently diagnostic of iron deficiency; therefore, the use of several indicators of iron status is necessary to produce the best assessment.

A number of laboratory tests are available to assess

blood variables associated with iron status. The accuracy and reliability of the tests depend upon a number of factors, including operator technique, instrument calibration, testing protocol, and interfering conditions which may be present in subjects. A sample of venous blood from a fasted individual is necessary for accurate determination of all blood indices of iron status. This section includes a discussion of the following indices: hemoglobin, hematocrit, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, serum ferritin, serum iron, iron binding capacity and transferrin saturation, and free erythrocyte protoporphyrin.

### Hemoglobin

Iron deficiency progresses across a spectrum with several variables considered indicative of stages which may be vague at times. However, a drop in hemoglobin is considered diagnostic of severe iron deficiency indicative of chronic negative iron balance (Blum et al., 1986).

Hemoglobin is intensely colored and this characteristic is used in several methods to estimate its concentration in blood (Williams et al., 1990). Whole blood treated with an anticoagulant such as disodium or tripotassium EDTA or heparin is used for this determination and concentration is

given in grams of hemoglobin per deciliter of blood. Sources of error for the determination of hemoglobin include turbidity from improperly lysed red blood cells or from high concentrations of lipids or nucleated cells (Williams et al., 1990). For a normal population, hemoglobin concentration ranges from 14.0-17.5 in adult males with a mean of 15.7 g/dl and from 12.3-15.3 with a mean of 13.8 g/dl in adult females (Williams et al., 1990). Normal values for hemoglobin for females at Montgomery Regional Hospital (MRH) Laboratory are 12-16 g/dl.

### Hematocrit

The hematocrit refers to the portion of the blood occupied by erythrocytes and is determined by centrifuging whole blood with sufficient force to pack the cells in as small a volume as possible (Williams et al., 1990). A sample of anticoagulated blood is also used for this determination. If an electronic instrument is used the hematocrit is calculated from measurements of the erythrocyte count and the mean corpuscular volume (MCV) (Williams et al., 1990). Hematocrit is expressed as a percentage or ratio. Normal values for hematocrit range from 0.42-0.50 with a mean of 0.46 for adult males, and from 0.36-0.45 with a mean of 0.40 for adult females (Williams et al., 1990). Errors in this procedure may occur from

hemoconcentration due to prolonged tourniquet application on collection, excessive EDTA anticoagulant, inadequate mixing of the sample, or operator error (Williams et al., 1990). Normal values for females for hematocrit at MRH Laboratory are 37-47%.

### Red Blood Cell Count

The red blood cell count is the number of red blood cells in a liter of venous or capillary blood. Electronic cell counting, performed on an instrument such as a Coulter Counter, provides an accurate count of red blood cells and is widely used. Red cells are counted in diluted blood samples which also contain leukocytes and platelets as well as red blood cells. Platelets are quite small and, thus, do not usually cause errors in the red cell count (Williams et al., 1990). The number of red blood cells is normally greater than leukocytes by a factor of 500, therefore, the error in RBC count due to their presence is usually negligible (Williams et al., 1990). However, RBC count can be significantly altered when there is an abnormally high leukocyte count. High altitude causes a moderate elevation in RBC count (Tilkian et al., 1987). RBC count declines as iron deficiency anemia progresses (Williams et al., 1990). The reference range for RBC count at Montgomery Regional Hospital is  $4.7-6.1 \times 10^6/\text{ml}$  for males and  $4.2-5.4 \times 10^6/\text{ml}$

for females.

### Mean Corpuscular Volume

The mean corpuscular volume (MCV) is the average volume of the erythrocytes and may be calculated from the erythrocyte count, which provides the number of cells per microliter of blood, and the packed cell volume, or hematocrit (Williams et al., 1990). It also is performed on anticoagulated whole blood. MCV may be calculated using the formula

$$\text{MCV} = \frac{\text{hematocrit (\%)}}{\text{RBC count ( x } 10^6/\text{mcl)}}$$

MCV is measured in cubic micrometers or femtoliters (fl). MCV may be measured by electronic cell counters, such as the Coulter counter which gives excellent linearity of RBC counts and a stable MCV regardless of RBC counts (Williams et al., 1990). Normal range for MCV for both sexes is 80.0-96.1 with a mean of 88.0 fl/red cell (Williams et al., 1990). Normal values for MCV for females at MRH Laboratory are 81-99 fl. A low MCV and/or MCH assist in the diagnosis of iron deficiency (Dallman, 1990). The MCV is much more sensitive than is the mean corpuscular hemoglobin concentration (MCHC) in detecting changes of iron deficiency

due to variability in plasma trapping in the packed column of erythrocytes which may occur from a centrifuged hematocrit (Beutler and Fairbanks, 1980).

### Mean Corpuscular Hemoglobin

Mean corpuscular hemoglobin (MCH) is determined on anticoagulated whole blood and calculated by dividing the amount of hemoglobin in one liter of blood by the number of erythrocytes per liter of blood, as follows:

$$\text{MCH} = \frac{\text{Hb (g/dl)}}{\text{RBC ( x } 10^6/\text{mcl)}} \times 10$$

MCH is expressed as picograms (pg;  $\text{g} \times 10^{-12}$ ) of hemoglobin per cell. Normal values for both sexes range from 27.5-33.2 with a mean of 30.4 pg/red cell (Williams et al., 1990). Normal values for MCH for females at MRH Laboratory are 27-31 pg. A low MCH provides supportive evidence with hemoglobin concentration for diagnosis of iron deficiency (Dallman, 1990). MCH is an accurate aid in this determination since both the red cell and the hemoglobin are directly measured when performed on an electronic counter (Bothwell et al., 1979). MCH can be affected by conditions, such as hyperlipidemia, which falsely elevate the hemoglobin level (Williams et al., 1990). MCH was not one of the blood

variables used to establish iron status in this study.

#### Mean Corpuscular Hemoglobin Concentration

The concentration of hemoglobin in the erythrocytes or mean corpuscular hemoglobin concentration (MCHC) is expressed as a percentage and determined by dividing the amount of hemoglobin per deciliter of blood by the packed cell volume expressed as a percentage, given by

$$\text{MCHC} = \frac{\text{hemoglobin, in g/dl} \times 100}{\text{packed cell volume, percent}}$$

The determination of MCHC is made on anticoagulated whole blood and normal values for both sexes range from 33.4-35.5 with a mean of 34.4 g/dl RBC (Williams et al., 1990). Normal values for MCHC for females at MRH Laboratory are 32-36 g/dl. MCHC was not one of the blood variables used to establish iron status in this study.

#### Serum Ferritin

Measurements of serum ferritin are believed to give an accurate representation of iron storage as reported by Newhouse and Clement (1988) from high correlations determined by phlebotomy and original serum ferritin concentrations. Further confirmation of the high

correlation between serum ferritin and storage iron was determined by  $^{59}\text{Fe}^{++}$  absorption, a sensitive indicator of iron storage, and serum ferritin concentration (Newhouse and Clement, 1988). These and other studies provided an estimation that 1 mcg/l of ferritin is equivalent to 8 mg of storage iron (Newhouse and Clement, 1988). However, some researchers have reported falsely elevated serum ferritin values for athletes for several days following strenuous exercise (Newhouse and Clement, 1988). Also, serum ferritin will be falsely elevated in conditions such as infection, inflammation, or malignancy, while it is reported that iron deficiency is the only condition in which serum ferritin concentration will be decreased (Newhouse and Clement, 1988). Serum ferritin may be determined by radioimmunoassay or enzyme-linked immunoassay (Tilkian et al., 1987). Normal value for both sexes is 60 ng/ml (Cook and Finch, 1979); first stage iron deficiency or iron depletion is indicated by <12 ng/ml (Blum et al., 1986). The range of normal values for Coat-A-Count Ferritin IRMA procedure is 12-130 ng/ml.

### Serum Iron

The measurement of serum iron concentration is subject to many sources of error, including diurnal variations in serum iron levels, which are lowest in late afternoon and

evening, reaching a maximum between 7 and 10 a.m. (Williams et al., 1990). Fluctuations also occur around the menstrual cycle, with low levels at the time of menstrual bleeding, reaching a maximum at the mid-point of the menstrual cycle (Zilva and Patston, 1966). Individuals may experience a day-to-day variability in serum iron of 20-25% (Tilkian et al., 1987). Serum iron is also reduced in cases of inflammation or malignancy, while it is elevated during chemotherapy (Williams et al., 1990). Because of the potential variation in serum iron levels, it is considered a less reliable indicator of iron status than other tests.

There are many different methods of assessing serum iron, but those generally considered more accurate are based on deproteinization (Bothwell et al., 1979). The basic steps include acidifying the serum to dissociate the iron-transferrin complex, precipitation of serum proteins with trichloroacetic acid or heating and removal by centrifugation, and the addition of a sensitive iron chromogen to the supernatant to determine the iron concentration colorimetrically (Bothwell et al., 1979). Normal values vary depending on the specific procedure and instrumentation. The reference range for serum iron from analysis by Olympus AU5000, Roche Biomedical Laboratories, Inc. is 40-180 mcg/dl.

### Iron Binding Capacity and Transferrin Saturation

The iron-binding capacity measures the amount of transferrin, the transport protein for iron, in circulating blood. The degree to which circulating transferrin is saturated with iron is believed to be a more reliable indicator of iron supply to the developing red cell than the serum iron concentration (Bothwell et al., 1979). The addition of the serum iron concentration to the unsaturated iron-binding capacity (UIBC), which is the concentration of iron that can be taken up by the unbound transferrin in the plasma gives the total iron-binding capacity (TIBC).

Indirect colorimetric methods for determining iron-binding capacity involve the addition of sufficient iron to the serum to fully saturate any unbound transferrin. An iron chromogen is added to react with the unbound excess iron which is quantified and subtracted from the amount of excess iron originally added to the serum to give UIBC (Bothwell et al., 1979). The reference range for TIBC from KDA Micro Analyzer utilized by Roche Biomedical Laboratories, Inc. is 250-450 mcg/dl. With less iron available to bind transferrin, a decreased transferrin saturation results. Transferrin saturation (TS) is given by the ratio of serum iron concentration to total iron-binding capacity multiplied by 100. The reference range for TS from Roche Biomedical Laboratories, Inc. is 15-55%.

### Free Erythrocyte Protoporphyrin

When transferrin saturation falls below 16%, there is insufficient iron for the production of normal red blood cells (Blum et al., 1986). This is characteristic of the second stage of impaired iron status, iron deficiency erythropoiesis. Without iron to attach to the porphyrin molecule, there are elevated levels of free erythrocyte protoporphyrin (FEP) in the blood.

FEP's do not fluctuate as much as serum iron and transferrin saturation in response to dietary intake (Nathan and Oski, 1974).

Erythrocyte porphyrins are extracted in a solvent mixture, followed by acid extraction. The porphyrins can then be quantified fluorometrically by comparison with a standard solution of protoporphyrin. FEP concentration is a stable indicator of the balance between storage iron requirement and available supply; however, it is also elevated in lead poisoning and anemias accompanying inflammation (Bothwell et al., 1979; Williams et al., 1990). Normal values for FEP are <60 mcg/dl of blood; moderately elevated values of 60-189 mcg/dl are seen in lead intoxication and iron deficiency anemia (Piomelli, 1977).

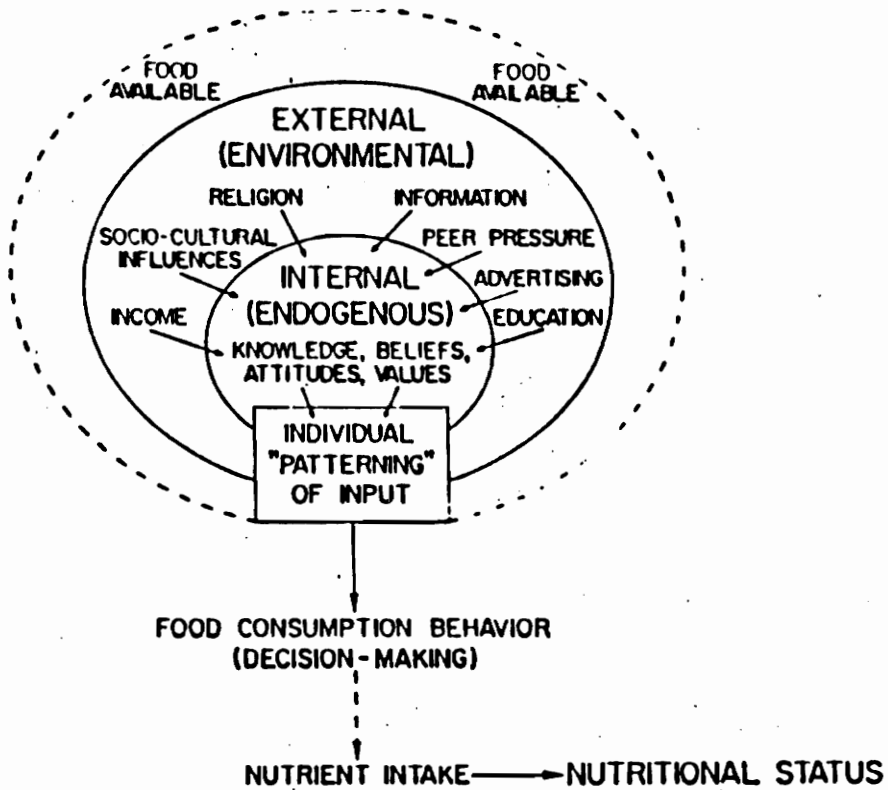
### FACTORS RELATED TO ADOPTION OF DIETARY PATTERN

Individuals are subject to many forces which influence

them, thus affecting their attitudes, beliefs, values, and ultimately, their behavior. Urie Bronfenbrenner (Lefrancois, 1992) has developed an ecological systems theory in which ways an individual interacts with his or her environment may be defined. Bronfenbrenner observes that psychological, biological, and social systems are open systems, in that their existence depends on interaction and they are subject to constant change relative to interaction (Lefrancois, 1992). A person is influenced by his or her biological makeup, the immediate environment, a social and economic context, and a cultural context (Sroufe et al., 1992).

The adoption of a particular dietary pattern is a behavior which evolves from the influences of these different interactions. Sims (1978) adapted a model to illustrate the influences of various factors on food consumption behavior (Figure 1). A person's food consumption depends on internal factors such as his or her knowledge, beliefs, attitudes, and values, which in turn are influenced by factors in the external environment such as income, socio-cultural influences, religion, information, peer pressure, advertising, and education (Sims, 1978).

This model was used by Sims to evaluate the food-related values, attitudes, and beliefs of vegetarians and non-vegetarians. The values of the vegetarian group were



**FIGURE 1** Conceptual framework: Influences on food consumption behavior.

(Sims, 1978)

more strongly oriented toward ethics, religion, and health than the non-vegetarians (Sims, 1978). Health concerns were cited as the most important reason for adopting a vegetarian diet (Sims, 1978). Beliefs between the two groups differed also in that the vegetarian group believed in consuming "health" foods and distrusted food processing and additives (Sims, 1978). However, non-vegetarians showed a stronger positive attitude to the concept that nutrition is important than the vegetarians (Sims, 1978).

In a study of vegetarians by Cooper et al. (1985), health concerns were the predominant reason given for their dietary preferences. Other reasons cited included a desire to avoid cruelty to animals, the dislike of eating animal flesh, fear of world food shortage, and the influence of a spouse (Cooper et al., 1985).

#### The personal interview

Qualitative data consist of detailed descriptions of situations, people, interactions, behaviors, and direct quotations from people about their experiences, attitudes, beliefs, and thoughts (Patton, 1980). One of the methods used to collect such data is the personal interview. With a personal interview, the researcher can gain access to the subject's perspective on an issue.

An interview comprised of mostly open-ended questions

will afford the subject the opportunity to provide responses with minimal bias on the part of the researcher (Patton, 1980). One basic approach to open-ended interviewing is to have a general interview guide (Patton, 1980). This involves identifying a set of issues to be explored prior to the interview by the researcher. This serves as a basic checklist during the interview to assure that all relevant topics are covered in each interview. This guide presumes that there is information to be obtained common to all subjects, but does not provide a standardized set of questions prepared in advance, nor does it require a specific order for the questioning, thus allowing a natural evolution of thoughts to occur with each unique interview.

The personal interview, while capable of obtaining an in-depth perspective on an individual, is a subjective procedure and thus is subject to some bias. Interview data may be subject to biases incurred by the emotional state of the interviewee at the time of the interview, recall error, reactivity of the interviewee to the interviewer, and self-serving responses (Patton, 1980).

## CHAPTER III

### MATERIALS AND METHODS

The objective of this study was to compare dietary intakes of protein and iron among female athletes who were regular consumers of meat, poultry and fish, or lacto-ovovegetarian sources of protein, and to compare hematological indicators of iron status among these groups. A further objective was to identify those factors most influential in the adoption of habitual dietary patterns among these groups.

This chapter on the materials and methods of the research study includes sections on selection of subjects, data collection, the personal interview, and statistical analysis. The experimental protocol for this study was accepted by the Institutional Review Board for Research Involving Human Subjects (See documentation in Appendix A).

#### Selection of Subjects

Subjects were those females at Virginia Tech who identified themselves as:

- a) athletes, and
- b) following one of three dietary patterns based upon habitual intake of protein and who were assigned membership in groups as:

Group 1 - Meat (M): Those who consume meat, to include beef, pork, lamb, and veal at least four times per week,

Group 2 - Lacto-ovovegetarian (V): Those who consume protein primarily from vegetarian sources with no more than one serving of poultry or fish per week and no consumption of meat, or

Group 3 - Poultry/Fish (PF): Those who consume protein primarily from poultry or fish, with no more than one serving of meat per week.

Recruitment of subjects was through flyers distributed around campus and visits to practice sessions of teams, including basketball, volleyball, track and field, field hockey, swimming, gymnastics, dance, High Techs, lacrosse, and tennis. Over 150 athletes volunteered to participate in the study. The study was designed to have 20 participants in each dietary group for a total of 60 subjects; therefore, volunteers were eliminated based upon determination of:

- 1) nonconformity to the parameters defined for each dietary group,
- 2) use of cigarettes, iron supplements, or oral contraceptive agents.

Sixty-five volunteers were accepted into the study initially in anticipation of potential drop-outs. There were 25 members in group 1 (M), 18 in group 2 (V), and 22 in

group 3 (PF) at the beginning of the study in October, 1991. Written permission was secured from each of the subjects prior to participation in the study (Appendix B).

### Data collection

Subjects completed a health history providing information on demographic characteristics, exercise activity, and dieting practices; additional questions deemed necessary to establish adequate psychological and physical health were also included (Appendix C).

Subjects were given a sub-maximal Physical Work Capacity (PWC) test (Astrand and Rhyning, 1954) to determine aerobic fitness. This was conducted in the Human Performance Laboratory by a graduate student from the Department of Human Nutrition and Foods experienced in the procedure. Measurements of weight, height, body fat, and resting heart rate were obtained prior to testing. Weight in kilograms and height in centimeters were measured on subjects wearing light clothes and no shoes on a Detecto-Medic scale (Detecto Scales, Inc., Brooklyn, N.Y.). Skinfold thickness was measured at seven sites in triplicate using Harpenden calipers (Quinton Equipment, Seattle, WA). Three of the seven sites measured were used in an equation for determination of percent body fat (Jackson et al., 1980).

A Body Guard 660 ergometer was used for the PWC test and was calibrated several times daily throughout the testing procedure.

Subjects completed two 3-day dietary intake records covering two week days and one weekend day which were analyzed for nutrient content and confirmation of dietary group membership (Appendix D). One record was taken at the beginning and another near the end of the six-month study. An instructional session which included food models was held to inform subjects about proper completion of these records. Diet records were coded and analyzed using the computer program Nutritionist III (N-Squared Computing, Salem, Oregon) for total kilocalories consumed, percent of calories from protein, carbohydrate, and fat, grams of protein and fiber, and milligrams of iron and vitamin C.

Blood samples were obtained by a qualified medical technician three times during the course of the six-month study; at the beginning, from mid-October to mid-November (time 1), middle, from mid-January to mid-February (time 2), and end, from mid-March to mid-April (time 3). Blood sampling was timed to occur 14 days  $\pm$  3 days from the start of the subjects' menstrual cycles, which has been shown to be time at which serum iron is at its peak among women (Zilva and Patston, 1966). A sample of blood was drawn from an antecubital vein between 7-9 a.m. after the

subjects had maintained a 10-12 hour fast. One 5-ml vacutainer treated with EDTA (Becton Dickinson Vacutainer Systems, Rutherford, NJ), and two 7-ml mineral-free vacutainers (Becton Dickinson Vacutainer Systems, Rutherford, NJ) were filled per subject. Complete blood counts were performed at Montgomery Regional Hospital Laboratory (Coulter S+4 Counter, Coulter Electronics, Inc, Hialeah, Fl), on the EDTA-treated blood. An aliquot of whole blood was removed and refrigerated until time of analysis for determination of free erythrocyte protoporphyrin (FEP) by the method of Piomelli (1977) (Porphyrin Products, Logan, Utah). Remaining tubes were centrifuged on a Fisher Centrifuge Centrifuge, Model 225, for 10 minutes at 2,000 x g. After centrifugation, serum was removed by serum separator tubes (Fisher Ulti-Sep Serum Separators, Fisher Scientific Co., Pittsburgh, Pa) and analyzed for serum iron (Olympus AU5000, Roche Biomedical Laboratories, Inc., Burlington, NC), and total iron binding capacity (TIBC) (KDA Micro Analyzer, Roche Biomedical Laboratories, Inc., Burlington, NC). A sample of serum was frozen until analysis could be performed for serum ferritin (Coat-A-Count Ferritin IRMA, Diagnostic Products Corp, Los Angeles, CA).

After calculating means for all blood variables, results were analyzed for prevalence of subjects who were in

any stage of iron deficiency. The model of Cook and Finch (1979) was used to categorize deficient subjects into one of three stages as indicated by:

Stage 1 - Iron depletion: Serum ferritin < 12 ng/ml

Stage 2 - Iron deficiency erythropoiesis: Transferrin saturation < 16%; Free erythrocyte protoporphyrin > 100 mcg/dl

Stage 3 - Iron deficiency anemia: Hemoglobin < 12 g/dl.

### The Personal Interview

A subset of three subjects from each group was requested to voluntarily participate in a personal interview with the researcher. Those asked to participate were assessed by the researcher to be serious athletes who had followed their current dietary pattern for at least one year. The purpose of the interview was to explore those factors considered most important by the subjects in their choice of dietary pattern. Interviews were audiotaped and transcribed. The interview consisted of primarily open-ended questions of a broad nature posed to the subjects in order to elicit spontaneous unbiased responses. A general outline of the questions posed to the subjects appears in Appendix E. A pilot interview with a female athlete not participating in the study was conducted to identify potential areas of difficulty and to familiarize the

researcher with the process.

### Statistical Analysis

Analysis of variance was used to statistically evaluate differences in dietary data and variables assessing iron status between groups. Tukey's test was applied to identify group differences for variables where significant F values were obtained. Percent protein as determined from dietary records followed a skewed distribution that was normalized by arcsine square root transformation before application of analysis of variance. Multiple analysis of variance was used to test for differences between groups over time. Regression analysis was used to test for correlations between serum ferritin and hours of exercise, grams of protein, and milligrams of iron ingested. The computer software program SAS (SAS Institute Inc., Cary, NC) was used for all statistical analyses. A level of  $p < 0.05$  was used to determine significance.

Upon completion of statistical analyses, subjects were provided with the results of their own individual as well as group means for the hematological and dietary data (Appendix F).

## CHAPTER IV

### RESULTS AND DISCUSSION

This chapter reports and discusses the findings of the research. It is arranged as follows: subject description, hematological parameters, iron status, dietary analysis, and behavioral factors relative to dietary protein intake.

#### Subject Description

Sixty-five subjects were accepted into the study which was conducted from October 1991 to April 1992. Group membership was comprised of 25 in Group 1 (M), 18 in Group 2 (V), and 22 in Group 3 (PF). Three subjects were dropped from the study; one from Group V withdrew from school and one each from Groups V and PF were dropped for continued use of iron supplements, resulting in final group memberships of 25 in Group M, 16 in Group V, and 21 in Group PF. The final number of participants was 62, for a percent retention of 95, and data are reported on those subjects.

Descriptive characteristics of subjects are shown in Table 1. Subjects ranged in age from 17 to 25 with a mean of  $19.5 \pm 1.4$  years. Group PF was significantly older than Group M ( $p < .02$ ). The mean height of the subjects was  $166.6 \pm 7.6$  cm with a range of 152-185 cm. The mean weight of the subjects was  $61.2 \pm 8.3$  kg with a range of 44.1-83.2 kg. The mean number of hours spent exercising per week was 10.1

Table 1  
Descriptive characteristics of subjects<sup>a</sup>

Variable	All Subjects (N=62)	Meat (N=25)	Lacto-Ovo- vegetarian (N=16)	Poultry/Fish (N=21)
Age (yrs) <sup>b</sup>	19.5 ± 1.4 (17-25)	19.0 ± 1.3 (17-21)	19.3 ± 1.8 (18-25)	20.1 ± 1.1 (18-22)
Height (cm)	166.6 ± 7.6 (152-185)	168.8 ± 7.6 (154.5-185.0)	163.8 ± 6.3 (154-176)	166.2 ± 8.0 (152-182.5)
Weight (kg)	61.2 ± 8.3 (44.1-83.2)	62.6 ± 8.4 (44.1-83.2)	59.2 ± 7.5 (45.7-72.5)	61.2 ± 8.8 (47.5-79.7)
% Body Fat	20.4 ± 3.8 (12.2-31.8)	21.0 ± 3.9 (13.7-27.1)	20.6 ± 4.3 (13.9-31.8)	19.4 ± 3.2 (12.2-25.6)
PWC Test <sup>c</sup> (VO <sub>2</sub> max, ml/min/kg)	45.5 ± 9.2 (30.3-73.2)	44.3 ± 7.4 (30.3-60.0)	49.3 ± 11.3 (34.9-72.3)	44.0 ± 8.9 (32.2-73.2)
Exercise (hrs/wk)	10.1 ± 8.6 (1.5-60.0)	10.2 ± 6.2 (1.5-27.0)	11.3 ± 13.3 (4.0-60.0)	9.0 ± 6.8 (2.5-33.0)

<sup>a</sup>Values are Mean ± SD and (range).

<sup>b</sup>poultry/Fish group differs from Meat and Lacto-Ovovegetarian groups, p<0.02; all other differences are not significant, p<0.05.

<sup>c</sup>Physical Work Capacity Test.

$\pm 8.7$ . Percent body fat for all subjects had a mean of  $20.4 \pm 3.8$  with a range of 12.2-31.8%. The mean value for the Physical Work Capacity (PWC) test was  $45.5 \pm 9.2$  with a range of 30.3-73.2 ( $VO_2$ max, ml/min/kg). Ranges for women aged 20-29 are classified as  $<24$  = low, 24-30 = fair, 31-37 = average, 38-48 = good, and  $\geq 49$  = high; therefore, the mean PWC value for athletes in this study was good (Baumgartner and Jackson, 1987). There were no significant differences among the groups for height, weight, percent body fat, hours of exercise, or PWC test.

#### Hematological Parameters

Results for the hematological parameters for the three dietary groups are presented in Table 2. Means for individual subjects can be found in Appendix G. Acceptable ranges are included as a point of reference. Multiple analysis of variance (MANOVA) found four of the nine hematological parameters to show no significant changes over time; thus, values were averaged for the three blood collections to determine means for each subject. Grand means for all three groups were within normal ranges and there were no significant differences among the groups for those four blood variables used to assess iron status. The number of subjects whose means fell outside normal values for all nine parameters is presented in Table 3.

Table 2  
Hematological Parameters of Subjects<sup>a</sup>

Parameter	All Subjects (N=62)	Meat (N=25)	Lacto-Ovovegetarian (N=16)	Poultry/Fish (N=21)
Hb (g/dl) (12-16) <sup>b</sup>	13.7 ± 0.9	13.9 ± 0.8	13.7 ± 1.1	13.5 ± 0.8
TS (%) (16-55)	27.1 ± 8.6	29.1 ± 11.0	25.4 ± 6.9	26.0 ± 6.2
SI (mcg/dl) (40-180)	93.9 ± 24.5	99.0 ± 26.2	90.5 ± 28.5	90.5 ± 18.4
TIBC (mcg/dl) (250-450)	349.7 ± 41.3	350.8 ± 48.5	348.7 ± 37.1	350.0 ± 36.5

<sup>a</sup>Values are Mean ± SD, all variables not significantly different, p<0.05. Abbreviations used are as follows: Hb, Hemoglobin; TS, Transferrin Saturation; SI, Serum Iron; TIBC, Total Iron-Binding Capacity.

<sup>b</sup>(normal values).

Table 3  
 Percent and number of subjects in each dietary group whose means for indices of iron status are outside normal values<sup>a</sup>

Parameter	M (N=25)		V (N=16)		PF (N=21)	
	%	#	%	#	%	#
HB	0	0	12.5	2	14.3	3
Hct	0	0	6.3	1	14.3	3
SF	12	3	12.5	2	23.8	5
TS	12	3 <sup>b</sup>	12.5	2	9.5	2
FEP	0	0	0	0	0	0
SI	0	0	6.3	1	0	0
TIBC	4	1	0	0	0	0
RBC	12	3 <sup>b</sup>	18.8	3	9.5	2
MCV	8	2 <sup>c</sup>	0	0	4.8	1

<sup>a</sup>Comparisons are among meat (M), lacto-ovovegetarian (V), and poultry/fish (PF) dietary groups. Abbreviations used are as follows: Hb, Hemoglobin; Hct, Hematocrit; SF, Serum Ferritin; TS, Transferrin Saturation; FEP, Free Erythrocyte Protoporphyrin; SI, Serum Iron; TIBC, Total Iron-Binding Capacity; RBC, Red Blood Cell; MCV, Mean Corpuscular Volume.

<sup>b</sup>Two subjects are below normal; one is above normal.

<sup>c</sup>One subject is below normal; one is above normal.

Hemoglobin (Hb). The mean value for hemoglobin for all subjects was  $13.7 \pm 0.9$  g/dl. Means for groups M, V, and PF were  $13.9 \pm 0.8$ ,  $13.7 \pm 1.1$ , and  $13.5 \pm 0.8$  g/dl, respectively. The number of subjects whose mean was below the normal range of 12-16 g/dl was none in Group M, two in Group V, and three in Group PF for a total of five.

Transferrin Saturation (TS). The mean value for transferrin saturation for all subjects was  $27.1 \pm 8.6\%$ . Means for groups M, V, and PF were  $29.1 \pm 11.0$ ,  $25.4 \pm 6.9$ , and  $26.0 \pm 6.2\%$ , respectively. The number of subjects whose mean was below the normal range of 16-55% was two in each group for a total of six. There was one subject in Group M whose TS was above normal.

Serum Iron. The mean value for serum iron for all subjects was  $93.9 \pm 24.5$  mcg/dl. Means for groups M, V, and PF were  $99.0 \pm 26.2$ ,  $90.5 \pm 28.5$ , and  $90.5 \pm 18.4$  mcg/dl, respectively. There was only one subject in Group V whose mean was below the normal range of 40-180 mcg/dl.

Total Iron-Binding Capacity (TIBC). The mean value for TIBC for all subjects was  $349.7 \pm 41.3$  mcg/dl. Means for groups M, V, and PF were  $350.8 \pm 48.5$ ,  $348.7 \pm 37.1$ , and  $350.0 \pm 36.5$  mcg/dl, respectively. There was only one subject in Group M whose mean was above the normal range of 250-450 mcg/dl.

### Hematological Parameters Over Time

Multiple analysis of variance (MANOVA) was used to test for significant differences among groups over time for all blood variables. Results are shown in Table 4. Of the nine blood variables, five showed a significant effect over time: SF, Hct, MCV, RBC, and FEP. Representations of these changes are depicted in Figures 2-6. Four of these five variables exhibited the same pattern over time among all the groups: for SF, Hct, and RBC, means for all groups increased over time; for MCV, the means for all groups decreased over time. Means for the fifth variable, FEP, also decreased over time, but also exhibited a time by group effect. FEP for groups M and PF decreased from time 1 to time 2 and again to time 3, while means for group V decreased from time 1 to time 2 but increased slightly from time 2 to time 3. Tukey's test found FEP for group M to be significantly greater than group PF at time 1 (28.4 vs. 22.5 mcg/dl, respectively). No other groups were found to differ significantly from each other for any of the other blood variables at any of the three times of collection. Means for all groups at all three times were within normal limits for each blood variable.

The progressive increase over time in SF and Hct, and decrease in FEP for all groups indicate an improvement in iron status, while the progressive increase in RBC and

Table 4  
Hematological Parameters Within Groups Over Time<sup>a</sup>

Parameter	Time 1			Time 2			Time 3			Manova Time * Group Effect		
	Group M	Group V	Group PF	Group M	Group V	Group PF	Group M	Group V	Group PF			
Hct (%) (37-47) <sup>b</sup>	40.3± 2.4	39.8± 3.6	40.2± 3.8	41.6± 2.3	41.4± 3.4	40.0± 2.4	41.3± 2.2	40.5± 3.2	39.7± 2.3	NS	0.01	NS
SF (ng/ml) (12-130)	21.3± 28.5	27.0± 21.6	18.8± 15.3	37.0± 28.9	30.9± 29.6	24.0± 16.6	36.8± 24.4	28.9± 19.0	32.8± 21.7	NS	0.004	NS
FEP (mcg/dl) (<60)	28.4± 8.2	25.5± 6.0	22.5± 5.2	17.5± 5.0	14.9± 3.2	17.2± 5.5	17.5± 4.4	15.2± 4.2	16.0± 5.6	NS	0.0001	0.01
RBC (x10 <sup>6</sup> ) (4.2-5.4)	4.53± 0.33	4.41± 0.40	4.46± 0.39	4.68± 0.45	4.62± 0.34	4.45± 0.29	4.70± 0.42	4.60± 0.36	4.48± 0.24	NS	0.002	NS
MCV (fl) (81-99)	89.4± 6.0	90.3± 3.4	90.0± 4.2	89.4± 5.8	89.8± 4.2	90.1± 4.5	88.0± 5.7	88.0± 4.0	88.7± 3.8	NS	0.0001	NS

<sup>a</sup>Values are Mean ± SD; Time 1 = October-November 1991, Time 2 = January-February 1992, Time 3 = March-April 1992. Abbreviations used are as follows: Hct, Hematocrit; SF, Serum Ferritin; FEP, Free Erythrocyte Protoporphyrin; RBC, Red Blood Cell Count; MCV, Mean Corpuscular Volume.

<sup>b</sup>(normal values)

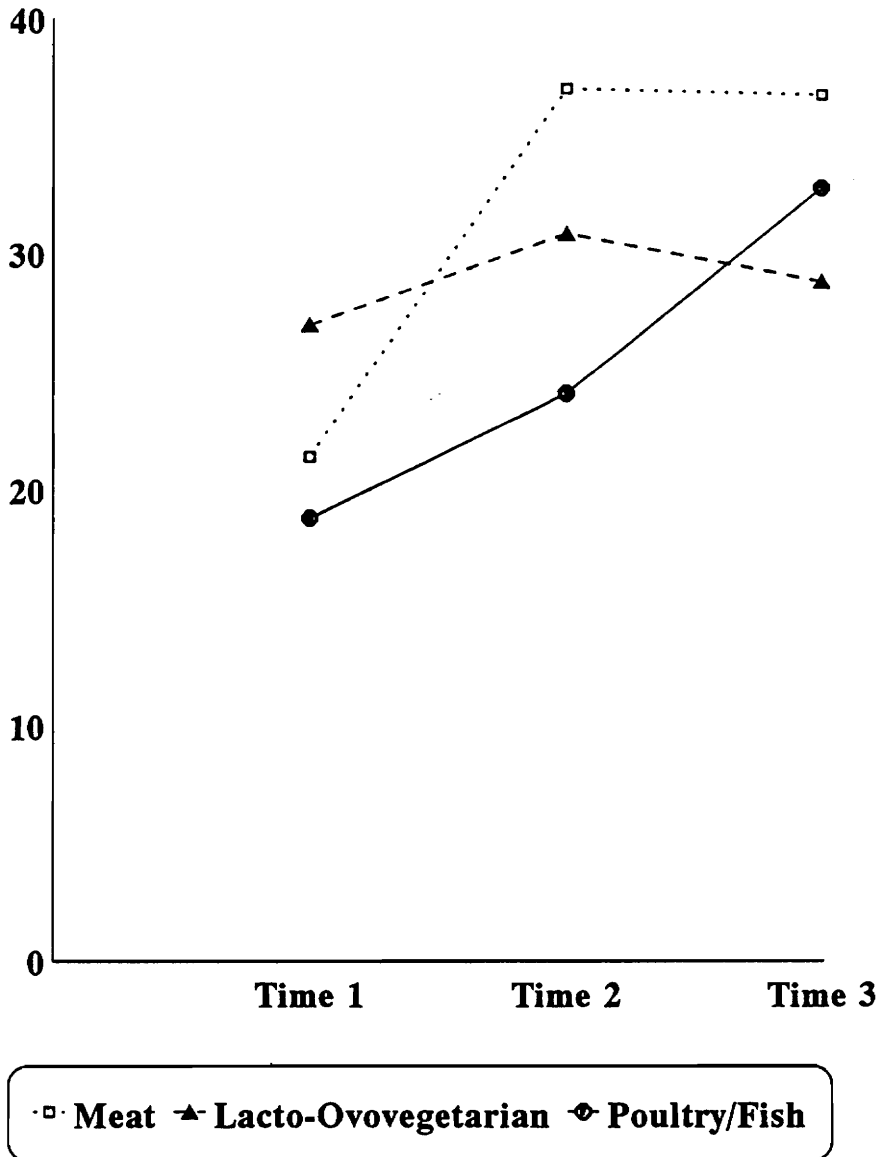


FIGURE 2: Serum Ferritin (ng/ml) by Group Over Time<sup>a</sup>

<sup>a</sup>Values are means at Time 1 (October-November 1992), Time 2 (January-February 1992), and Time 3 (March-April 1992).

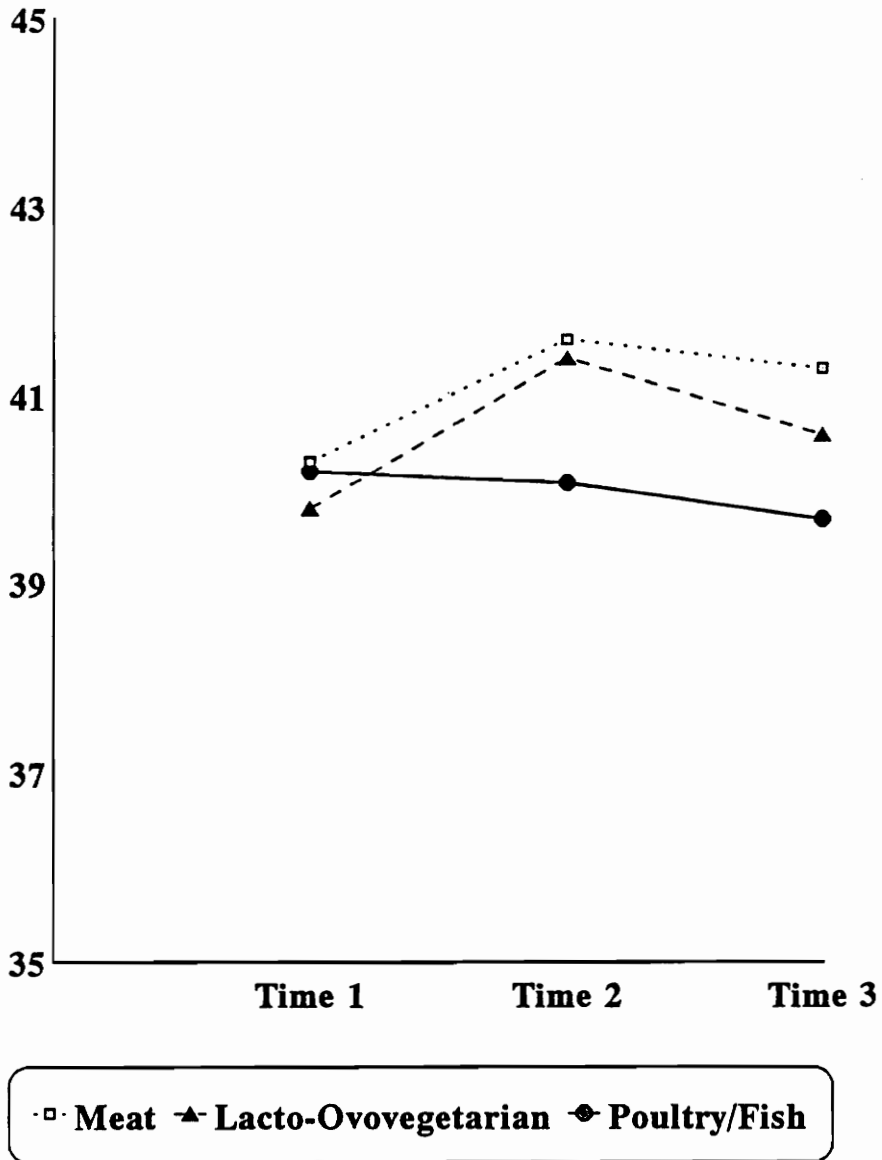


FIGURE 3: Hematocrit (%) by Group Over Time<sup>a</sup>

<sup>a</sup>Values are means at Time 1 (October-November 1992), Time 2 (January-February 1992), and Time 3 (March-April 1992).

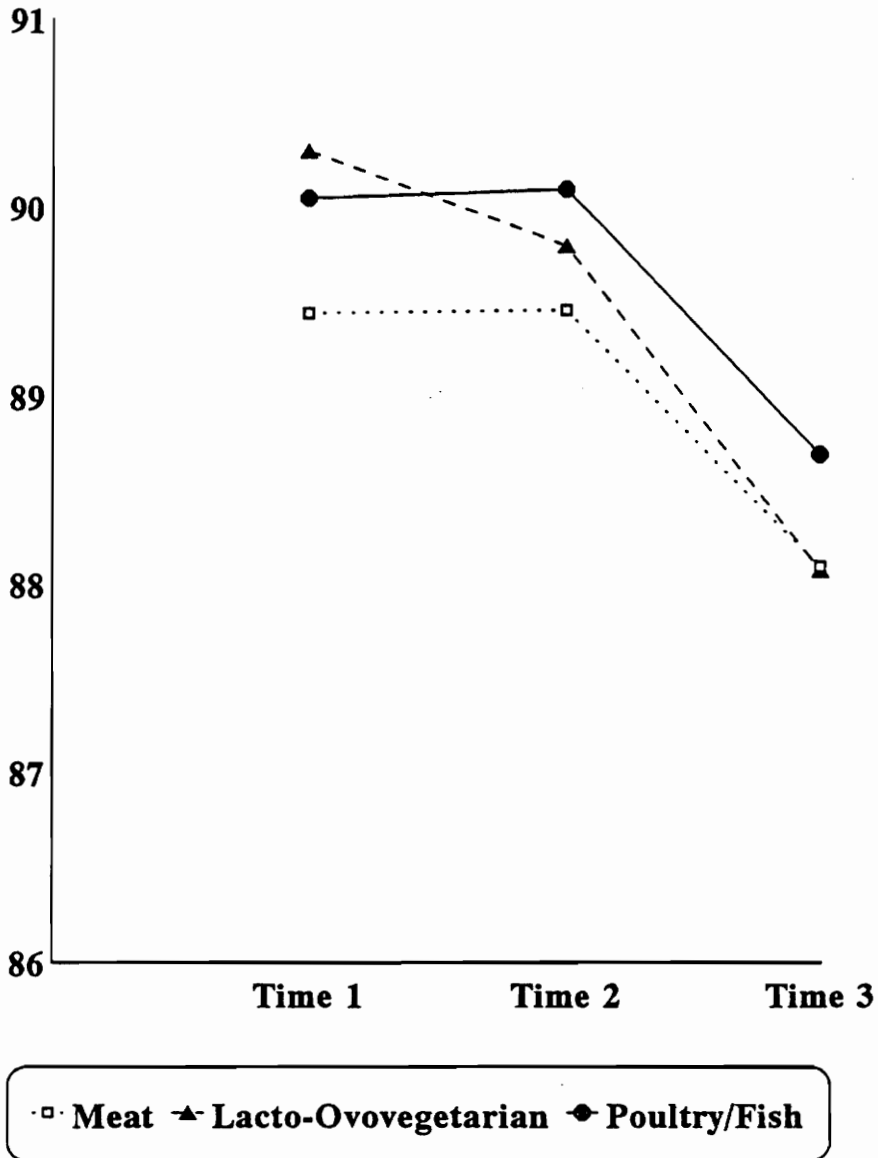


FIGURE 4: Mean Corpuscular Volume (fl) by Group Over Time<sup>a</sup>

<sup>a</sup>Values are means at Time 1 (October-November 1992), Time 2 (January-February 1992), and Time 3 (March-April 1992).

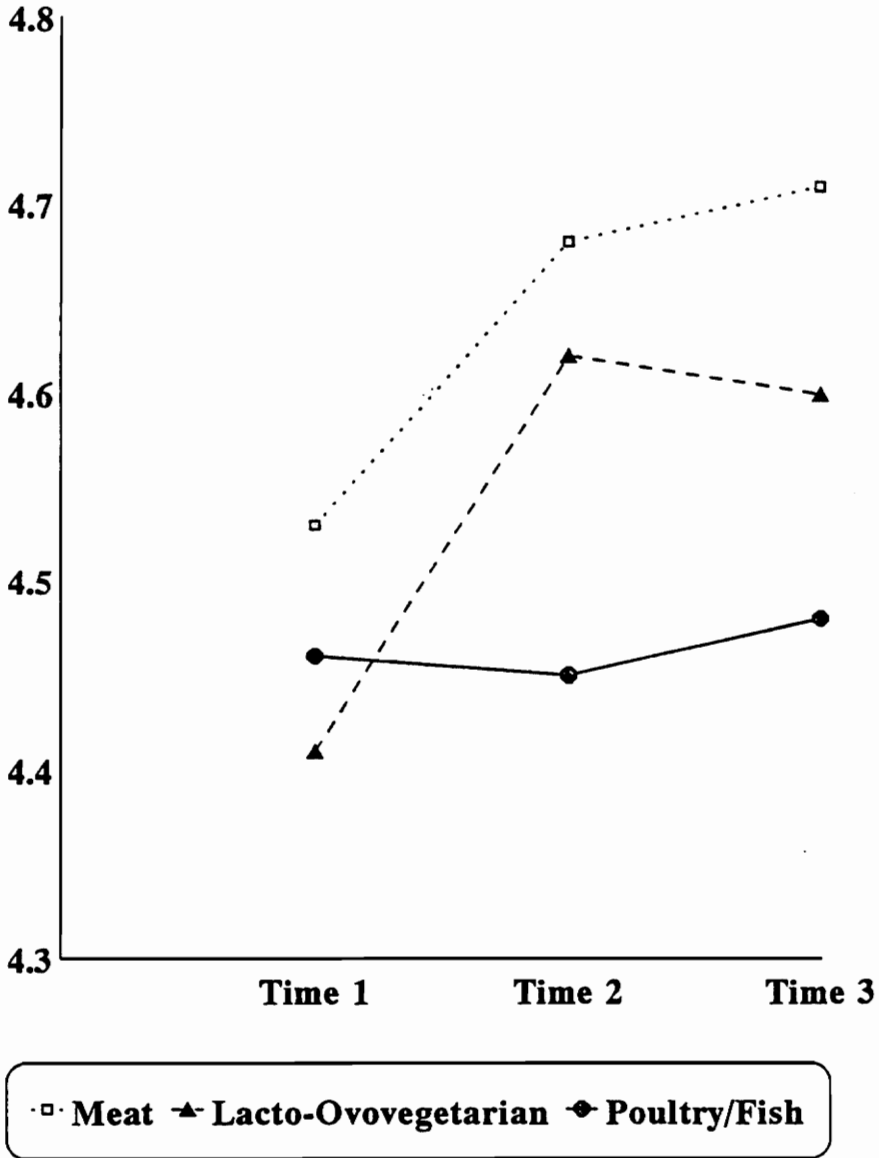


FIGURE 5: Red Blood Cell Count ( $\times 10^6$ ) by Group Over Time<sup>a</sup>

<sup>a</sup>Values are means at Time 1 (October-November 1992), Time 2 (January-February 1992), and Time 3 (March-April 1992).

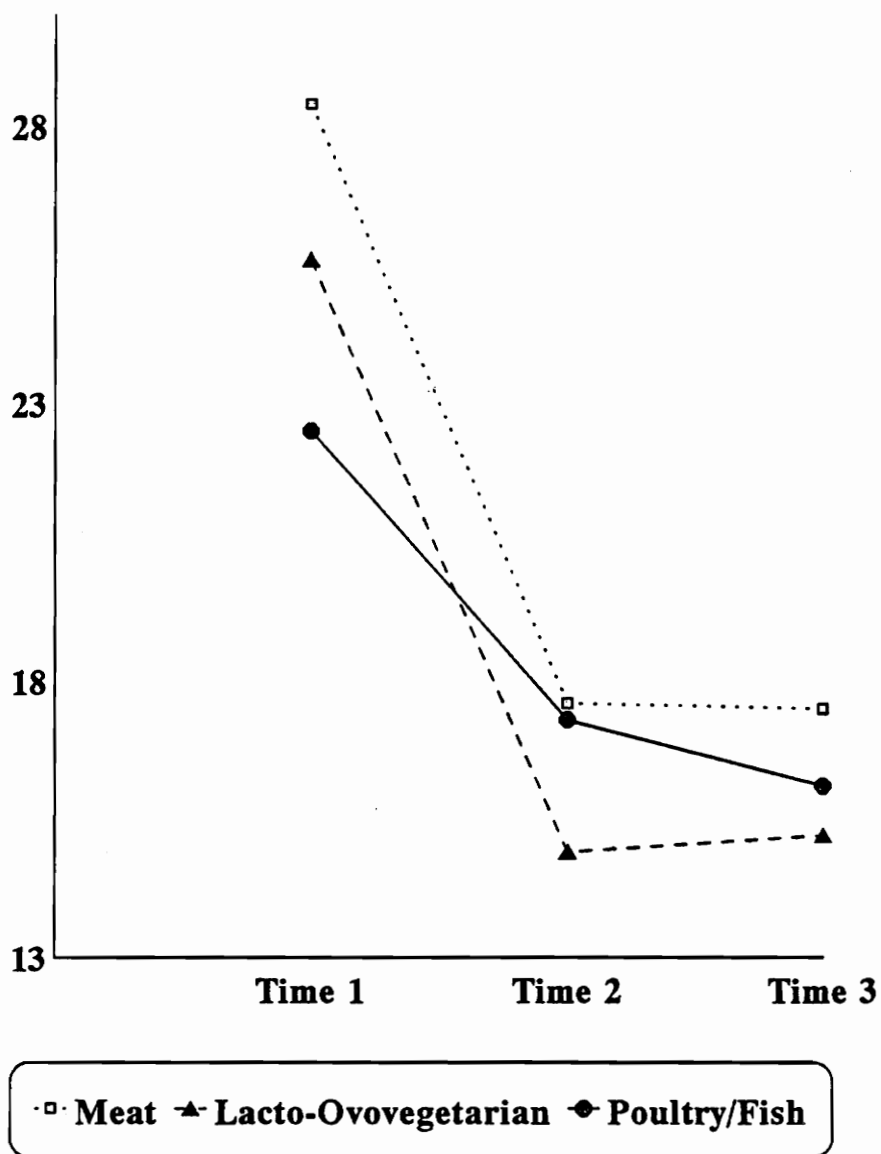


FIGURE 6: Free Erythrocyte Protoporphyrin (mcg/dl) by Group Over Time<sup>a</sup>

<sup>a</sup>Values are means at Time 1 (October-November 1992), Time 2 (January-February 1992), and Time 3 (March-April 1992).

decrease in MCV for all groups indicate a deterioration in iron status; thus, no clear trends can be seen to implicate a significant change in iron status for the three groups. Also, the fact that all groups were within normal limits for all nine blood variables would suggest no biological change in iron status can be demonstrated for any group for the six month period.

### Iron Status

Hematological parameters for all subjects were analyzed for presence of any of the three stages of iron deficiency. The three stages were identified by:

Stage 1 - Serum ferritin <12 ng/ml; TS>16%; FEP<100 mcg/dl; Hb>12 g/dl.

Stage 2 - Serum ferritin <12 ng/ml; TS<16%; FEP>100 mcg/dl; Hb>12 g/dl.

Stage 3 - Serum ferritin <12 ng/ml; TS<16%; FEP>100 mcg/dl; Hb<12 g/dl.

Results are shown in Table 5. A total of six of the sixty-two subjects (9.7%) were identified as being in stage 1 iron deficiency; two from Group M (8%), one from Group V (6.3%), and three from Group PF (14.3%). None of the subjects was in stages 2 or 3 iron deficiency. Although there were several subjects whose serum ferritin and TS were abnormal, in none of those was the FEP abnormal as well,

Table 5  
Incidence of stage 1 iron deficiency in female athletes<sup>a</sup>

Group	Number	Percent
All subjects (N=62)	6	9.7
Meat (N=25)	2	8.0
Lacto-ovovegetarian (N=16)	1	6.3
Poultry/fish (N=21)	3	14.3

<sup>a</sup>Stage 1 iron deficiency determined by serum ferritin <12 ng/dl; transferrin saturation >16%; free erythrocyte protoporphyrin <100 mcg/dl; hemoglobin >12 g/dl.

thus preventing any subjects from being classified as stage 2 iron deficient.

The data of the six subjects who were classified as being in stage 1 iron deficiency were deleted from statistical analysis in order to see if this had any significant effect on any other variables tested. The only change which was generated by this deletion was the absence of a significant difference in age among the three groups (previously, Group PF was significantly older than Group M). None of the hematological or dietary parameters was affected.

In evaluating the results of the hematological parameters, comparisons to other studies is difficult due to differences in subjects, methodology, and criteria used in classifying parameters as abnormal. Nickerson and Tripp (1983) found 2 of 18 (11%) adolescent female runners to have hemoglobin levels of <12 g/dl, compared to 5 of 62, or 8% of subjects in this study. Of the 18 adolescent runners, 8 had serum ferritin levels <12 ng/ml for 44%, versus 10 of 62 for 16% in this study. O'Toole et al. (1989) found 1 of 19 female triathletes to have hemoglobin levels <12 g/dl for 5%; 2 of the 19 had serum ferritin values <12 ng/ml for 10.5%; and 5 of the 19 or 26% had transferrin saturation of <16%. In this study, 6 of 62 or 9.7% had TS<16%.

Frederickson et al. (1983) evaluated 8 young female

cross-country runners for changes in iron status over the competitive season. At the beginning of the season, all hematological parameters were within normal ranges for all subjects. Over the course of the season, however, Hb, SI and TS values fell, while TIBC and FEP values rose. The current study did not address any possible training effects on iron status.

Risser et al. (1988) compared 100 female college athletes to 66 female controls for iron status and found 13% of the athletes to be in stage 1 iron deficiency using SF and TS criteria used in this study. This compares to 9.7% of the 62 subjects in this study who were found to be in stage 1 iron deficiency. Comparisons of stages 2 and 3 between the studies is not possible as the Risser study did not use FEP as a parameter in the evaluations.

Newhouse and Clement (1988) surveyed the results from a number of studies which assessed iron status in exercising individuals. Of the 14 studies surveyed for SF and Hb levels which involved females, only one used 12 ng/ml as the lower limit for deficiency and found 9% of the women to be classified as such. Three of the 14 studies found from 2-8.5% of the females to be anemic with a Hb value of <12 g/dl.

The study which separated dietary groups in a similar manner to this study was that of Worthington-Roberts et al.

(1988); however, the subjects in that study were premenopausal women who were not athletes. The group members in the Worthington-Roberts et al. study also were older than those in the current study, with a mean age of 31.0, 27.9, and 28.9 years for the red meat, poultry/fish, and lacto-ovovegetarian groups, respectively. The researchers found that although Hb and Hct values were within normal limits for all dietary groups, the red-meat group had significantly higher values for both parameters. In addition, TIBC was significantly lower in the meat group than in the poultry and fish or lacto-ovovegetarian groups. The greatest difference among the groups was seen with serum ferritin, which was significantly higher in the meat group, with the poultry and fish group showing the lowest mean SF of all. Worthington-Roberts et al. concluded that premenopausal females who were red-meat eaters exhibited superior iron status to women who consumed mainly poultry and fish or lacto-ovovegetarian sources of protein. These findings are in contrast to the present study which found no significant differences among the dietary groups in any of the measures selected to determine iron status.

In attempting to explain the lack of significant differences among the three dietary groups for all parameters measuring iron status, a number of possibilities can be hypothesized. First, one can conclude that there are

no significant differences in iron status among female athletes at Virginia Tech relative to source of dietary protein. Another explanation could be that because the subjects included in the study were volunteers, only those athletes interested in nutrition and/or iron status volunteered to participate; thus, the sample could have been unintentionally biased toward those athletes already practicing sound nutritional habits. Or, one could postulate that athletes in general have a greater awareness of, and interest in, adequate dietary practices than a similar group of non-athletes. A further possibility for the lack of statistical significance in a number of the findings could have been the large standard deviations for some of the variables. Also, the design of the study itself could have precluded the determination of significant differences in iron status which might actually have been present. The exclusion from this study of athletes using iron supplements could have eliminated a part of the female athletic population already known to be anemic. Also, the present study was not designed to measure the amount of dietary iron which was absorbed by the subjects, taking into account factors such as intake of both heme and nonheme iron, or enhancers of, and inhibitors to, the absorption of dietary iron. Nor was the present study able to measure iron losses or make laboratory determinations of the

contents of the subjects' diets, thus relying solely on accurate self-reporting by the athletes. Also, no attempt was made to correlate the subjects' physical activity relative to the seasonality of the different sports to their iron status. It is conceivable that some athletes did experience a transient anemia during periods of most intense activity which was not detected. A final possibility regarding the failure to detect any subjects with stage 2 or 3 iron deficiency was the test for free erythrocyte protoporphyrin. The inclusion of this variable in the criteria for stage 2 or 3 iron deficiency precluded some subjects, who had low serum ferritin, transferrin saturation, and hemoglobin, from being identified as being in these stages. Although no subjects' FEP values came near to being abnormal, an investigation into the reliability of the methodology could be warranted. If FEP had not been used as an indicator for stages 2 and 3 iron deficiency, five subjects would have been classified as being in stage 2 iron deficiency; two from Group M, one from Group V, and two from Group PF, which follows a similar pattern to that seen with stage 1 iron deficient subjects. Only two subjects would have been classified as stage 3 iron-deficient without using FEP values; one each from Group V and PF.

### Dietary Analysis

Mean daily nutrient intakes of the three dietary groups are presented in Table 6. Mean values for individual subjects can be found in Appendix G. No significant differences were found for the dietary data from baseline to the final dietary record; thus, results from the two 3-day diet recalls were averaged to determine means for daily intake of total kilocalories (kcal), total grams (g) of protein and fiber, milligrams (mg) of iron and vitamin C, and percent of total kcals from protein, carbohydrate, and fat. Percent protein followed a skewed distribution; therefore, data were normalized using arcsine square root transformation before being tested for statistical significance (SAS Institute Inc., 1990).

Total Kilocalories. The mean value for all subjects for total kcals consumed was  $1951 \pm 898$ . The means for groups M, V, and PF were  $2229 \pm 1105$ ,  $1758 \pm 833$ , and  $1768 \pm 558$  kcals, respectively. Although Group M consumed more kcals than the other groups, there were no significant differences among the groups for total kcals, which can most likely be attributed to the large standard deviations.

Percent Protein. The percent of total kcals which was attributed to protein for all subjects was  $14.8 \pm 3.2$ . Mean values for groups M, V, and PF were  $15.8 \pm 3.0$ ,  $12.8 \pm 2.2$ , and  $15.1 \pm 3.6\%$ , respectively. Tukey's Studentized Range

Table 6  
 Mean daily dietary intake of selected nutrients of female athletes<sup>a</sup>

Nutrient	All Subjects (N=62)	M (N=25)	V (N=16)	PF (N=21)	P
Kcals	1951±898	2229±1105	1758±833	1768±558	NS
% kcal protein	14.8±3.2	15.8±3.0 <sup>b</sup>	12.8±2.2 <sup>c</sup>	15.1±3.6 <sup>b</sup>	0.0102
% kcal carbohydrate	57.4±8.3	52.2±6.2 <sup>b</sup>	64.9±7.7 <sup>c</sup>	58.0±6.4 <sup>d</sup>	0.0001
% kcal fat	27.1±7.6	31.1±5.5 <sup>b</sup>	22.0±6.9 <sup>c</sup>	26.3±8.0 <sup>c</sup>	0.0004
Protein (g)	72.1±30.5	87.9±34.8 <sup>b</sup>	56.5±27.0 <sup>c</sup>	65.3±6.8 <sup>c</sup>	0.0016
Iron (mg)	14.2±7.9	15.7±9.3	14.9±9.1	12.0±4.3	NS
Vitamin C (mg)	135.1±88.6	119.7±1.9	135.3±8.5	153.3±10.9	NS
Fiber (g)	12.7±7.0	12.4±0.5	15.8±0.5	10.7±1.0	NS

<sup>a</sup>Statistical comparison for meat (M), lacto-ovo-vegetarian (V), and poultry/fish (PF) groups are by analysis of variance; values are means ± SD. Means not sharing a common superscript letter are significantly different by post hoc Tukey's test (p<0.05).

Test identified Group M as consuming a significantly greater percent of kcals from protein than Group V.

Percent Carbohydrate. The percent of total kcals which was attributed to carbohydrate for all subjects was  $57.4 \pm 8.3$ . Mean values for groups M, V, and PF were  $52.2 \pm 6.2$ ,  $64.9 \pm 7.7$ , and  $58.0 \pm 6.4\%$ , respectively. Tukey's Test identified all groups as being significantly different from each other.

Percent Fat. The percent of total kcals which was attributed to fat for all subjects was  $27.1 \pm 7.6$ . Mean values for groups M, V, and PF were  $31.1 \pm 5.5$ ,  $22.0 \pm 6.9$ , and  $26.3 \pm 8.0\%$ , respectively. Tukey's Test identified Group M as consuming a significantly higher percent fat than Groups V and PF.

Protein (g). The mean total number of grams of protein consumed by all subjects was  $72.1 \pm 30.5$ . Mean values for groups M, V, and PF were  $87.9 \pm 34.8$ ,  $56.5 \pm 27.0$ , and  $65.3 \pm 16.8$  g, respectively. Tukey's Test identified Group M as consuming significantly more grams of protein than Groups V and PF.

Fiber (g). The mean total number of grams of fiber consumed by all subjects was  $12.7 \pm 7.0$ . Mean values for groups M, V, and PF were  $12.4 \pm 0.5$ ,  $15.8 \pm 0.5$ , and  $10.7 \pm 1.0$  g, respectively. No significant differences were found among the groups for amount of fiber consumed.

Vitamin C (mg). The mean total number of milligrams of vitamin C consumed by all subjects was  $135.1 \pm 88.6$ . Mean values for groups M, V, and PF were  $119.7 \pm 1.9$ ,  $135.3 \pm 8.5$ , and  $153.3 \pm 10.9$  mg, respectively. No significant differences were found among the groups for amount of vitamin C consumed.

Iron (mg). The mean total number of milligrams of iron consumed by all subjects was  $14.2 \pm 7.9$ . This is below the current RDA for all females aged 11-50 of 15 mg/d. Mean values for groups M, V, and PF were  $15.7 \pm 9.3$ ,  $14.9 \pm 9.1$ , and  $12.0 \pm 4.3$  mg, respectively. There was no significant difference among the groups for intake of iron. Table 7 shows the number of subjects in each dietary group whose iron intake was below the current RDA.

Comparisons between this study and other studies which have assessed dietary iron intake are difficult due to various differences in study design. Manore et al. (1989) found the mean daily iron intake in 10 premenopausal women runners to be 11.9 mg. Anderson et al. (1981) studied the iron and zinc status of 56 long-term vegetarian women and found the mean daily iron intake was  $12.5 \pm 3.0$  mg. Bindra and Gibson (1986) found a mean daily iron intake of  $14.4 \pm 4.2$  mg for 44 adult females who ate meat, poultry, or fish no more than twice a week. Blum et al. (1986) compared iron status in women who exercised versus that of sedentary

Table 7  
Subjects with mean daily dietary iron intake below  
Recommended Daily Allowance

Group	Number	Percent
All subjects (N=62)	40	64.5
Meat (N=25)	16	64.0
Lacto-ovovegetarian (N=16)	9	56.3
Poultry/fish (N=21)	15	71.4

women. Prior to the exercise protocol, the groups were consuming a mean daily intake of  $12.0 \pm 0.8$  and  $11.4 \pm 1.3$  mg iron, respectively. Clement and Asmundson (1982) found a mean daily iron intake of  $12.5 \pm 3.6$  mg for 17 female endurance runners. Data from NHANES II on 1066 women aged 18-24 found a mean iron intake of  $10.4 \pm 5.6$  mg from 24-hour dietary recalls (Murphy and Calloway, 1986). Perron and Endres (1985) found a mean iron intake of  $11.0 \pm 6.0$  mg from 24-hour dietary recalls in 26 female high school athletes. In the 1977-78 Nationwide Food Consumption Survey, women aged 19-22 had a mean iron intake of 10.7 mg from 24-hour dietary recalls. Snyder et al. (1989) compared female runners who included red meat in their diets to a similar group which excluded red meat from their diets and found a mean daily iron intake of  $14.0 \pm 2.2$  and  $14.7 \pm 2.0$  mg in the meat and meatless groups, respectively. Finally, Worthington-Roberts et al. (1988) found a mean daily iron intake of  $12.7 \pm 1.1$  mg in the red meat group,  $11.9 \pm 1.2$  mg in the fish and poultry group, and  $11.8 \pm 0.8$  mg in the lacto-ovovegetarian group. In none of these studies did the mean daily iron intake reach the current RDA for women of 15 mg/d. However, in the current study the meat group exceeded the RDA with a mean of  $15.7 \pm 9.3$ , and the lacto-ovovegetarian group came close at  $14.9 \pm 9.1$  mg/d. Although the poultry and fish group consumed the least dietary iron

with a mean of  $12.0 \pm 4.3$  mg/d, the differences among the groups were not significant.

The mean amount of protein consumed by Group M exceeded both that recommended by the current RDA for adult women for protein of 0.75g/kg body weight, and the upper end of the suggested range (1.0 g/kg body weight) for protein in consideration of possible needs for athletic performance. Group V consumed slightly less, and Group PF consumed slightly more than the RDA for protein. Although Group M consumed a greater amount of protein and iron than Groups V and PF, this did not improve their iron status relative to the other two groups, demonstrated by the lack of any statistically significant differences among the groups in the values of the blood variables measured in this study. This could also imply that although Group V consumed less protein, they chose protein sources carefully, and consumed foods which enhance absorption of nonheme iron while limiting foods which might inhibit absorption of nonheme iron. In fact, all three dietary groups consumed much more vitamin C than the current RDA of 60 mg, and although group V consumed more fiber than groups M and PF (15.8 vs. 12.4 and 10.7 g, respectively), the amount is not considered high enough to inhibit absorption of non-heme iron (Mason et al., 1990).

Considering the percent of total kcals consumed from

protein, carbohydrate, and fat, all three groups fell within the current dietary guidelines for what is considered a healthy diet for all adults, with the exception of a slightly greater percent consumption of fat by Group M than the current recommendation of a maximum of 30%. Perhaps making the decision to either become a vegetarian or eliminate red meat from the diet and replace it with poultry and fish indicates a greater interest in nutrition among those individuals than would be found in the meat group, members of which seem to be maintaining dietary habits from childhood. This could suggest a need for more nutrition education for those individuals who follow a dietary pattern based upon habit rather than specific nutrition knowledge.

#### Regression Analysis

Regression analysis was performed to assess possible correlations between serum ferritin, thought to be the most sensitive indicator of iron status, and amount of exercise (hours/week), grams of protein and milligrams of iron ingested. Results are shown in Table 8. None of the correlations were significant, either at baseline or the end of the study. There were no significant differences between the groups for hours of exercise or consumption of iron; however, Group M consumed significantly more grams of protein than both groups V and PF. Yet this greater ingestion of protein did not affect iron status as reflected

Table 8  
Correlations between serum ferritin and selected variables

Variable	Correlation Coefficient	P
Exercise (hrs/wk)		
Baseline	0.02	NS
6 months	-0.03	NS
Iron intake (mg)		
Baseline	0.18	NS
6 months	-0.22	NS
Protein intake (g)		
Baseline	0.04	NS
6 months	-0.10	NS

in serum ferritin, as seen in the very low correlation coefficient obtained in the regression analysis. This would suggest that since all groups consumed above the RDA for protein for women in this age group of 0.75 g/kg body weight/day, this was sufficient to maintain adequate iron status, and no further improvement in iron status was obtained from an increase in protein intake.

#### Behavioral Factors Relative To Dietary Protein Intake

Three subjects from each dietary group volunteered to participate in a personal interview. The interviews were of 30-45 minutes' duration.

From the lacto-ovovegetarian group, one was a triathlete who had been a lacto-ovovegetarian for seven years, one was on the crew team and had been a lacto-ovovegetarian for 5 1/2 years (the last year she became a vegan), and the third was on the volleyball team and had been a lacto-ovovegetarian for 11 years. From the meat group, one was on the basketball team, one was a cyclist, and one was a heptathlete; all had been meat-eaters their entire lives.

From the poultry/fish group, one was on the basketball team and had followed her dietary pattern for approximately 10 years, one ran track and had eaten primarily poultry and fish for three years, and the third was on the volleyball

team and had been a poultry and fish eater all her life, but with special emphasis for the last three years.

Six of the nine athletes lived off-campus and were responsible for preparing their own meals. The three living on-campus ate primarily in the dining halls.

#### Factors for Adoption of Dietary Pattern

Table 9 lists the factors identified by the subjects as being of primary and secondary importance in the adoption of their current dietary pattern.

In the meat group, while some subjects identified taste preference, habit, and health reasons as factors of primary importance in the adoption of current dietary pattern, habit and taste preference were cited as the factors of secondary importance by some subjects in their decision for adoption of current dietary pattern.

Among the vegetarians, athletic performance and ethical treatment of animals were cited as being the most important reasons for adopting their dietary pattern. Of secondary importance were long-term health, ecological concerns, and the development of an alternative lifestyle.

In the poultry and fish group, the factors given primary importance to the adoption of their dietary patterns by some subjects included taste preference and health concerns. Of secondary importance to some subjects were

Table 9  
 Primary and secondary factors for adoption of dietary pattern<sup>a</sup>

	Meat (N=3)	Lacto- Ovovegetarian (N=3)	Poultry/Fish (N=3)
Habit	1,2	--	--
Taste Preference	1,2	--	1,1,2
Health	1	2	1,2,2
Athletic Performance	2	1	--
Ethical Treatment of Animals	--	1,1	--
Ecological Concerns	--	2	--
Alternative Lifestyle	--	2	--

<sup>a</sup>1 denotes primary factor; 2 denotes secondary factor.

also health concerns and taste preference, as well as habit.

#### Primary Source of Influence on Dietary Pattern

Table 10 lists the sources cited by the subjects as being the most important influence on their decision to adopt and/or maintain their current dietary pattern.

Among the meat group, all three athletes named habitual family dietary pattern as being the most important influence. Among the vegetarian group, family, especially the influence of an older sibling, and subjects' own decision were the factors cited as motivating a change in dietary pattern. All vegetarians had been raised in families which consumed meat. Among the poultry and fish eaters, family, nutrition and health information, and subjects' own decision were the primary influences on the decision to adopt current dietary patterns. The two in the poultry and fish group which did not cite family influence were raised in families which consumed meat.

#### Attitudes Regarding Dietary Pattern

All nine athletes interviewed felt that diet was important in their lives. When asked if they could foresee ever changing their current dietary pattern all vegetarians stated they could never see themselves eating meat again. Among the meat group, all intend to continue to include meat

Table 10  
Main influence on adoption of dietary pattern

	Meat (N=3)	Lacto- Ovovegetarian (N=3)	Poultry/Fish (N=3)
Family	3	1	1
Self	--	2	1
Health Information	--	--	1

in their diets; two stated a desire to include more vegetables in their diets and one would prefer more variety in her meals. Among the poultry and fish eaters, one said she would resume eating meat if she became pregnant and was interested in increasing her current intake of complex carbohydrates, one wished to consume more fresh vegetables and fruit, and one said she would make no changes in her diet.

When asked to rate their overall satisfaction with their diets, answers ranged from pretty satisfied to very satisfied, except for one person in the meat group who was unhappy with the quality of the food in the dining halls and wished more healthful choices were available.

## CHAPTER V

### SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Sixty-two female athletes volunteered to participate in a study to assess iron status relative to habitual source of dietary protein. The subjects were divided into three dietary groups designated as Meat (M), Lacto-ovovegetarian (V), and Poultry/Fish (PF). Group membership was comprised of 25 in Group M, 16 in Group V, and 21 in Group PF. The study was of six months' duration, beginning in October, 1991 and ending in April, 1992.

Determinations were made of the subjects' age, height, weight, percent body fat, and number of hours spent exercising per week; a Physical Work Capacity Test (PWC) was conducted to assess aerobic capacity. The mean age of the subjects was 19.5 years, the mean height was 166.6 centimeters, the mean weight was 61.2 kilograms, the mean percent body fat was 20.4, and the mean value for the PWC test was 45.5  $\text{VO}_2$  Max (ml/min/kg). Group PF was found to be significantly older than Group M (20.1 vs. 19.0 years, respectively). No significant differences were found among the dietary groups for height, weight, percent body fat, hours of exercise or PWC test.

Three blood collections were obtained from each subject at the beginning, middle, and end of the study and were timed to coincide with the mid-point of the menstrual cycle,

+/- 3 days. Blood samples were evaluated for the following indicators of iron status: hemoglobin, hematocrit, red blood cell count, mean corpuscular volume, serum iron, total iron-binding capacity, transferrin saturation, serum ferritin, and free erythrocyte protoporphyrin.

The first hypothesis of this investigation was as follows: Iron status as indicated by the variables measured in this study is not significantly different among female athletes whose primary source of dietary protein is red meat, lacto-ovovegetarian, or chicken and fish. Grand means for subjects in each dietary group were within normal limits for all blood variables measured. No significant differences were found among the dietary groups for any of the blood variables measured.

The mean value for hemoglobin for all subjects was  $13.7 \pm 0.9$  g/dl. Means for groups M, V, and PF were  $13.9 \pm 0.8$ ,  $13.7 \pm 1.1$ , and  $13.5 \pm 0.8$  g/dl, respectively. The mean value for hematocrit for all subjects was  $40.6 \pm 2.6\%$ . Means for groups M, V, and PF were  $41.1 \pm 2.1$ ,  $40.6 \pm 3.2$ , and  $40.0 \pm 2.5\%$ , respectively. The mean value for serum ferritin for all subjects was  $28.9 \pm 18.7$  ng/ml. Means for groups M, V, and PF were  $31.8 \pm 19.2$ ,  $29.0 \pm 21.2$ , and  $25.3 \pm 16.1$  ng/ml, respectively. The mean value for transferrin saturation for all subjects was  $27.1 \pm 8.6\%$ . Means for

groups M, V, and PF were  $29.1 \pm 11.0$ ,  $25.4 \pm 6.9$ , and  $26.0 \pm 6.2\%$ , respectively. The mean value for FEP for all subjects was  $19.6 \pm 4.7$  mcg/dl. Means for groups M, V, and PF were  $21.2 \pm 5.3$ ,  $18.6 \pm 3.6$ , and  $18.6 \pm 4.4$  mcg/dl, respectively. The mean value for serum iron for all subjects was  $93.9 \pm 24.5$  mcg/dl. Means for groups M, V, and PF were  $99.0 \pm 26.2$ ,  $90.5 \pm 28.5$ , and  $90.5 \pm 18.4$  mcg/dl, respectively. The mean value for TIBC for all subjects was  $349.7 \pm 41.3$  mcg/dl. Means for groups M, V, and PF were  $350.8 \pm 48.5$ ,  $348.7 \pm 37.1$ , and  $350.0 \pm 36.5$  mcg/dl, respectively. The mean value for RBC for all subjects was  $4.56 \pm 0.34 \times 10^6$ . Means for groups M, V, and PF were  $4.64 \pm 0.39$ ,  $4.55 \pm 0.35$ , and  $4.47 \pm 0.26 \times 10^6$ , respectively. The mean value for MCV for all subjects was  $89.3 \pm 4.8$  fl. Means for groups M, V, and PF were  $89.0 \pm 5.8$ ,  $89.4 \pm 3.9$ , and  $89.6 \pm 4.2$  fl, respectively.

Hematological parameters of the subjects were evaluated for classification into any of the three stages of iron deficiency. Six subjects were classified as being in stage 1 iron deficiency; 2 from Group M, 1 from Group V, and 3 from Group PF. No subjects were classified as being in stage 2 or 3 iron deficiency.

Multiple analysis of variance (MANOVA) was used to test for differences among groups over time for all blood variables. Means for all groups for SF, Hct, and RBC

increased significantly over the six months time period. Means for all groups for MCV decreased significantly over time. Means for all groups for FEP also decreased over time; in addition, means for groups M and PF decreased progressively from baseline to the end of the study, while means for group V decreased from time 1 to time 2, then increased slightly from time 2 to time 3.

Subjects also completed two 3-day dietary recalls to confirm appropriate group membership and for nutrient analysis which was evaluated by the computer program Nutritionist III. The analysis included determinations of total kilocalories consumed, percent of kilocalories from protein, fat, and carbohydrate, grams of protein and fiber, and milligrams of iron and vitamin C. No significant differences were found for any of the dietary variables between the baseline and final dietary recalls; thus, results of the two dietary recalls were averaged and means compared for statistical analysis.

The second hypothesis of this investigation was as follows:

Dietary intake of iron is not significantly different among female athletes whose primary source of protein is red meat, lacto-ovovegetarian, or chicken and fish. While there were no significant differences among the dietary groups for milligrams of iron consumed, only Group M consumed the

current RDA of 15 mg/d for women in this age group; they consumed  $15.7 \pm 9.3$  mg/d. Group V consumed  $14.9 \pm 9.1$  mg while Group PF consumed  $12.0 \pm 4.3$  mg/d.

The third hypothesis of this investigation was as follows:

Dietary intake of protein (in total grams or percent of total kilocalories) is not significantly different among female athletes whose primary source of protein is red meat, lacto-ovovegetarian, or chicken and fish. Group M consumed significantly more protein as a percent of total kcals than Group V ( $15.8 \pm 3.0$  vs.  $12.8 \pm 2.2\%$ , respectively). Group PF consumed  $15.1 \pm 3.6\%$  protein. Group M also consumed significantly more grams of protein than both Groups V and PF ( $87.9 \pm 34.8$  vs.  $56.5 \pm 27.0$  and  $65.3 \pm 16.8$  g, respectively).

The results of the other dietary factors analyzed indicated other differences among the groups. Each group differed significantly from the other two with respect to percent of kcals from carbohydrate. Values were  $52.2 \pm 6.2\%$  for Group M,  $64.9 \pm 7.7\%$  for Group V, and  $58.0 \pm 6.4\%$  for Group PF. Group M consumed significantly more fat as a percent of total kcals than Groups V and PF ( $31.1 \pm 5.5$  vs.  $22.0 \pm 6.9$  and  $26.3 \pm 8.0\%$ , respectively).

There were no significant differences found among the dietary groups with respect to total kilocalories, grams of

fiber, or milligrams of vitamin C consumed. Group M consumed  $2229 \pm 1105$ , Group V consumed  $1758 \pm 833$ , and Group PF consumed  $1768 \pm 558$  kcals. Mean values for grams of fiber were  $12.4 \pm 0.5$ ,  $15.8 \pm 0.5$ , and  $10.7 \pm 1.0$  for groups M, V, and PF, respectively. Mean values for milligrams of vitamin C were  $119.7 \pm 1.9$ ,  $135.3 \pm 8.5$ , and  $153.3 \pm 10.9$ , for groups M, V, and PF, respectively.

Regression analysis found no significant correlations between serum ferritin and hours of exercise, amount of iron consumed, or amount of protein consumed.

A subset of three subjects from each of the dietary groups was interviewed in order to identify behavioral factors associated with their choice of dietary pattern. The factors identified as being most important in the adoption of dietary pattern in Group M were habit, health, taste preference, and athletic performance. The factors cited as being the most important in Group V were health, ethical treatment of animals, ecological concerns, athletic performance, and alternative lifestyle. The factors cited by Group PF as being most important were health, and taste preference.

The main influences on the subjects' adoption of dietary pattern were family for Group M, family and self for Group V, and family, self, and health information for Group PF.

It is concluded that there are no significant differences in iron status among female athletes at Virginia Tech whose main sources of dietary protein are red meat, lacto-ovovegetarian, or poultry and fish. Although only Group M consumed more than the RDA for iron, iron deficiency does not appear to be a problem among female athletes at Virginia Tech.

Female athletes from three dietary groups at Virginia Tech differ in nutrient analysis of percent of kcals from protein, fat, and carbohydrate and total grams of protein consumed.

Future studies evaluating iron status in female athletes could include aspects not addressed in the current study in an attempt to further investigate this question. A study which controlled for seasonality of individual sports might elucidate possible training effects. A metabolic balance study, although rather complicated and expensive, could evaluate iron intakes as well as losses and might give a more precise determination of true iron status. An attempt to evaluate bioavailability of dietary iron, taking into consideration both heme and nonheme sources of iron, as well as enhancers and inhibitors to absorption of iron, could provide a better assessment of dietary iron than just total milligrams of iron consumed. Finally, if free erythrocyte protoporphyrin is to be included as a diagnostic

indicator of iron deficiency, an investigation of alternative methodologies for this parameter should be made.

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## APPENDIX A

### REQUEST FOR APPROVAL OF INVESTIGATION INVOLVING HUMAN SUBJECTS

Principal Investigator(s) Dr. Charlotte Pratt and Dr. Janette Taper Department Humns Nutrition & Foods

Project Title: Comparison of iron status among female athletes who are regular users of beef, lacto-ovo vegetarians, and regular users of fish or poultry.

Source of Support: Departmental Research  Sponsored Research  Proposal No. \_\_\_\_\_

1. The criteria for "expedited review" by the Institutional Review Board for a project involving the use of human subjects and with minimal risk\* is one or more of the following. Please initial all applicable conditions and provide a substantiating statement of protocol.

- a. Collection of:
  - 1) hair or nail clipping in a non-disfiguring manner;
  - 2) deciduous teeth;
  - 3) permanent teeth if patient care indicates need of extraction.
- b. Collection of excreta and external secretions: sweat, uncanalated saliva, placenta removed at delivery, amniotic fluid obtained at time of rupture of the membrane.
- c. Recording of data from subjects 18 years or older, using noninvasive procedures routinely employed in clinical practice. Exemption does not include exposure to electromagnetic radiation outside the visible range.
- d. Collection of blood samples by venipuncture (not exceeding 150 ml/8 week period, and no more than twice a week) from subjects 18 years or older, in good health and not pregnant.
- e. Collection of supra- and subgingival dental plaque and calculus, provided the procedure is no more invasive than routine scaling of the teeth.
- f. Voice readings.
- g. Moderate exercise by healthy volunteers.
- h. Study of existing data, documents, records, pathological specimens or diagnostic specimens.
- i. Research on drugs or devices for which an investigational exemption is not required.

2. If the project involves human subjects who are exposed to "more than minimal risk" and are not covered by the criteria above (1 to 9), the IRB review must involve the full IRB board. Please check if the research involves more than minimal risk\*\*  and provide a substantiating statement of protocol.

3. Human subjects would be involved in the proposed activity as either: Minors and/or Children\* , Fetuses , Abortuses , Pregnant Women , Prisoners , Mentally Retarded , Mentally Disabled .

Note that if children are involved in the research as human subjects, they may have to provide consent as well as their parents.

Whether or not the project may undergo "expedited review" or must be reviewed by the full Institutional Review Board, it is necessary that the required informed consent forms also be reviewed. These should be submitted with the proposal. However, if there is insufficient time to meet the sponsor's deadline, submittal can be delayed up to thirty days after submittal of the proposal without jeopardizing the IRB certification to the prospective sponsor.

\*Minimal risk means that the risks of harm anticipated in the proposed research are not greater, considering the probability and magnitude, than those encountered in daily life or during performance of routine physical or psychological examinations or tests.

\*\*Subject at risk is an individual who may be exposed to the possibility of injury as a consequence or participation as a subject in any research, development or related activity which departs from the application of those established and accepted methods necessary to meet his needs, or which increases the ordinary risks of daily life, including the recognized risks inherent in a chosen occupation or field of science.

This is to certify that the project identified above will be carried out as approved by the Human Subject Review Board, and will neither be modified nor carried out beyond the period approved below without express review and approval by the Board.

Charlotte Pratt 5/8/91  
Principal Investigator/Date

Michael J. Blumenthal 5/10/91  
Departmental Reviewer/Date

The Human Subjects Review Board has reviewed the protocol identified above, as it involves human subjects, and hereby approves the conduct of the project for 12 months, at which time the protocol must be resubmitted for approval to continue.

[Signature] 5/22/91  
Board Chairman/Authorized Reviewer Date

APPENDIX B

Authorization for Participation in Study  
(Written Consent Form)

I have received an oral and written explanation of the study. I understand the following:

The purpose of this study is to compare iron status among female athletes who are regular users of beef, lacto-ovo vegetarians and regular users of poultry or fish.

I will be asked to provide the following information: my smoking status, use of oral contraceptive agents, medications, or vitamin/mineral supplements, age, exercise level, weight, height, two 3-day food intake records, health status, information on frequency of food use and information concerning dietary intake patterns.

I will be given a Physical Work Capacity test.

I will be assigned to one of three groups: Group 1, regular beef users (use of beef at least four times per week), Group 2, lacto-ovo vegetarians (no beef, poultry or fish) or Group 3, regular poultry or fish users. Blood samples (45 ml) will be taken from me each month for 6 months. The samples will be analyzed to determine my iron status. The study duration is 6 months.

An honorarium of \$50 will be provided to me at the completion of the study. In addition, the average values from the blood analyses will be provided to me.

All information obtained from me will be held strictly confidential. I understand that participation is voluntary and I may discontinue the study if, at any time, I or the investigators feel it is in my best interest.

The following people may be contacted if I have questions about the study.

Charlotte A. Pratt  
Dept. of Human Nutrition and Foods  
VPI & SU  
Blacksburg, VA 24061-0430  
(703) 231-5549

L. Janette Taper  
Dept. of Human Nutrition and Foods  
VPI & SU  
Blacksburg, VA 24060-0430  
(703) 231-5549

I understand the above and agree to participate in the study from October, 1991 to April, 1992.

Name \_\_\_\_\_ Date \_\_\_\_\_

Address \_\_\_\_\_ Phone # \_\_\_\_\_

Group 1 \_\_\_\_\_  
2 \_\_\_\_\_  
3 \_\_\_\_\_

# Health and Fitness Appraisal

## MEDICAL HISTORY QUESTIONNAIRE

---

Last Name                                      First Name                                      Middle Initial

---

Date of Birth                                      Sex                                      School Phone

---

School Address                                      City, State                                      Zip

---

Social Security Number                                      Family Physician

---

Home Address                                      Home Phone

In order to assess cardiovascular function, body composition, and other physical fitness components, the undersigned hereby voluntarily consents to engage in one or more of the following tests (check the appropriate boxes):

- Graded exercise stress test
- Underwater weighing
- Muscle fitness tests
- Flexibility tests

#### EXPLANATION OF THE TESTS

The graded exercise stress test is performed on a bicycle ergometer or motor-driven treadmill. The work load is increased every few minutes until exhaustion or until other symptoms dictate termination of the test. We may stop the test at any time because of fatigue or discomfort.

The underwater weighing procedure involves being completely submerged in a tank or tub while breathing through respiratory equipment. This test provides an accurate assessment of your body composition.

For muscle fitness testing, you lift weights for a number of repetitions using barbells or exercise machines. These tests assess the strength and endurance of the major muscle groups in the body.

For evaluation of flexibility, you perform a number of calisthenic-type exercises. During these exercises, we measure the range of motion in your joints.

#### RISKS AND DISCOMFORTS

During the graded exercise stress test, certain changes may occur. These changes include abnormal blood pressure responses, fainting, irregularities in heartbeat, and heart attack. Every effort is made to minimize these occurrences. Emergency equipment and trained personnel are available to deal with these situations if they occur.

You may experience some discomfort during the underwater weighing, especially if you have a fear of being submerged. Breathing through respiratory equipment while underwater should minimize this discomfort. If necessary, alternative procedures (e.g., skinfold techniques) are used to estimate body composition.

There is a slight possibility of pulling a muscle or spraining a ligament during the muscle fitness and flexibility testing. In addition, you may experience muscle soreness 24-48 hr after testing. These risks can be minimized by performing warm-up exercises prior to taking the tests. If muscle soreness occurs, appropriate stretching exercises to relieve this soreness will be demonstrated.

#### EXPECTED BENEFITS FROM TESTING

These tests allow us to assess your physical working capacity scientifically and to appraise your physical fitness status clinically. The results are used to prescribe a safe, sound exercise program for you. Records are kept strictly confidential unless you consent to release this information.

***INQUIRIES***

Questions about the procedures used in the physical fitness tests are encouraged. If you have any questions or need additional information, please ask us to explain further.

**FREEDOM OF CONSENT**

Your permission to perform these physical fitness tests is strictly voluntary. You are free to deny consent if you so desire.

\_\_\_\_\_

I have read this form carefully and I fully understand the test procedures. I consent to participate in these tests.

\_\_\_\_\_  
Signature of patient

\_\_\_\_\_  
Date

\_\_\_\_\_  
Witness

QUESTIONS: \_\_\_\_\_

\_\_\_\_\_  
RESPONSE: \_\_\_\_\_

4. Give the following information pertaining to the last three times you have been hospitalized. (Women: do not list normal pregnancies.)

	Hospitalization Number 1	Hospitalization Number 2	Hospitalization Number 3
Type of operation			
Month and year hospitalized			
Name of hospital			
City and state			

During the past twelve months...

1. Has a physician prescribed any form of medication for you? Yes  No
2. Has your weight fluctuated more than a few pounds? Yes  No
3. Did you attempt to bring about this weight change through diet and/or exercise? Yes  No
4. Have you experienced any faintness, lightheadedness, blackouts? Yes  No
5. Have you occasionally had trouble sleeping? Yes  No
6. Have you experienced any blurred vision? Yes  No
7. Have you had any severe headaches? Yes  No
8. Have you experienced chronic morning cough? Yes  No
9. Have you experienced any temporary change in your speech pattern such as slurring or loss of speech? Yes  No
10. Have you felt unusually nervous or anxious for no apparent reason? Yes  No
11. Have you experienced unusual heartbeats such as skipped beats or palpitations? Yes  No
12. Have you experienced periods in which your heart felt as though it were racing for no apparent reason? Yes  No

At present...

1. Do you experience shortness of breath or loss of breath while walking with others your own age? Yes  No
2. Do you experience sudden tingling, numbness, or loss of feeling in your arms, hands, legs, feet, or face? Yes  No
3. Have you ever noticed that your hands or feet sometimes feel cooler than other parts of your body? Yes  No
4. Do you experience swelling of your feet and ankles? Yes  No
5. Do you get pains or cramps in your legs? Yes  No
6. Do you experience any pain or discomfort in your chest? Yes  No
7. Do you experience any pressure or heaviness in your chest? Yes  No

8. Have you ever been told that your blood pressure was abnormal? Yes  No
9. Have you ever been told that your serum cholesterol or triglyceride level was high? Yes  No
10. Do you have diabetes? Yes  No   
If yes, how is it controlled?  
 Dietary means     Insulin injection  
 Oral medication     Uncontrolled
11. How often would you characterize your stress level as being high?  
 Occasionally     Frequently     Constantly
12. Have you ever been told that you have any of the following illnesses?  
 Myocardial infarction     Arteriosclerosis     Heart disease     Heart block  
 Coronary thrombosis     Rheumatic heart     Heart attack     Aneurysm  
 Coronary occlusion     Heart failure     Heart murmur     Angina
- 

Has any member of your immediate family been treated for or suspected to have had any of these conditions? Please identify their relationship to you (father, mother, sister, brother, etc.).

- A. Diabetes  
B. Heart disease  
C. Stroke  
D. High blood pressure
-

---

**SMOKING HABITS**

1. Have you ever smoked cigarettes, cigars, or a pipe? Yes  No
2. Do you smoke presently? Yes  No

Cigarettes \_\_\_\_\_ per day

Cigars \_\_\_\_\_ per day

Pipefuls \_\_\_\_\_ per day

3. At what age did you start smoking? \_\_\_\_\_ years
  4. If you have quit smoking, when did you quit? \_\_\_\_\_
- 

**DRINKING HABITS**

1. During the past month, how many days did you drink alcoholic beverages?  
\_\_\_\_\_ days
2. During the past month, how many times did you have five or more drinks per occasion?  
\_\_\_\_\_ times
3. On the average, how many glasses of beer, wine, or highballs do you consume per week?

Beer \_\_\_\_\_ glasses or cans

Wine \_\_\_\_\_ glasses

Highballs \_\_\_\_\_ glasses

Other \_\_\_\_\_ glasses

---

**EXERCISE HABITS**

1. Do you exercise vigorously on a regular basis? Yes  No
2. What activities do you engage in on a regular basis?

3. If you walk, run, or jog, what is the average number of miles you cover per workout?  
\_\_\_\_\_ miles
4. How many minutes on the average is each of your exercise workouts?  
\_\_\_\_\_ minutes
5. How many workouts per week do you participate in on the average?  
\_\_\_\_\_ workouts
6. Is your occupation:  
\_\_\_\_\_ Inactive (e.g., desk job)  
\_\_\_\_\_ Light work (e.g., housework, light carpentry)  
\_\_\_\_\_ Heavy work (e.g., heavy carpentry, lifting)
7. Check those activities that you would prefer in a regular exercise program for yourself:
- |                               |                                  |
|-------------------------------|----------------------------------|
| _____ Walking/running/jogging | _____ Handball/raquetball/squash |
| _____ Stationary running      | _____ Basketball                 |
| _____ Jumping rope            | _____ Swimming                   |
| _____ Bicycling               | _____ Tennis                     |
| _____ Stationary cycling      | _____ Aerobic dance              |
|                               | _____ Others (specify)           |
- 

#### DIETARY HABITS

1. What is your current weight? \_\_\_\_\_lb height? \_\_\_\_\_in.
2. What would you like to weigh? \_\_\_\_\_lb
3. What is the most you ever weighed as an adult? \_\_\_\_\_lb
4. What is the least you ever weighed as an adult? \_\_\_\_\_lb

5. What weight loss methods have you tried?
6. Which do you eat regularly?  
 Breakfast  Mid-afternoon snack  
 Mid-morning snack  Dinner  
 Lunch  After-dinner snack
7. How often do you eat out per week? \_\_\_\_\_times
8. What size portions do you normally have?  
 Small  Moderate  Large  Extra large  
 Uncertain
9. How often do you eat more than one serving?  
 Always  Usually  Sometimes  Never
10. How long does it usually take you to eat a meal? \_\_\_\_\_minutes
11. Do you eat while doing other activities (e.g., watching TV, reading, working)?
12. When you snack, how many times per week do you eat the following?  
 Cookies, cake, pie \_\_\_\_\_ Candy \_\_\_\_\_ Diet soda \_\_\_\_\_  
 Soft drinks \_\_\_\_\_ Doughnuts \_\_\_\_\_ Fruit \_\_\_\_\_  
 Milk or milk beverage \_\_\_\_\_ Potato chips, pretzels, etc. \_\_\_\_\_  
 Peanuts or other nuts \_\_\_\_\_ Ice cream \_\_\_\_\_  
 Cheese and crackers \_\_\_\_\_ Other \_\_\_\_\_
13. How often do you eat dessert? \_\_\_\_\_times per day \_\_\_\_\_times per week
14. What dessert do you eat most often? \_\_\_\_\_
15. How often do you eat fried foods? \_\_\_\_\_per week
16. Do you salt your food at the table? Yes  No   
 Before tasting it  After tasting it

Respond to the following questions with yes or no answers:

Yes    No

- |     |     |    |  |
|-----|-----|----|--|
| ___ | ___ | 1. | I have an intense sustained drive to get ahead.                        |
| ___ | ___ | 2. | I'm anxious to reach my goals, but I'm uncertain what those goals are. |
| ___ | ___ | 3. | I feel a need to compete and win.                                      |
| ___ | ___ | 4. | I have a persistent desire for recognition.                            |
| ___ | ___ | 5. | I always seem to be involved in too many things at once.               |
| ___ | ___ | 6. | I'm always racing the clock, constantly on edge, have deadlines.       |
| ___ | ___ | 7. | I have a need to speed things up, get things done faster.              |
| ___ | ___ | 8. | I'm extraordinarily alert mentally and physically.                     |

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From Falls, H. B., Baylor, A. M., and Dishman, R. K. *Essentials of Fitness*. Philadelphia: Saunders College Publishing, 1980. Reprinted with permission of the publisher.

Answer yes or no to each of the following questions:

Yes    No

- |     |     |   |
|-----|-----|---|
| ___ | ___ | 1. Do you often experience headaches or backaches?  |
| ___ | ___ | 2. When sitting in a chair and talking to someone, do you continually move in the chair to seek a comfortable position? |
| ___ | ___ | 3. When retiring for the night, are you unable to fall asleep immediately?  |
| ___ | ___ | 4. Do you often grind your teeth when you are confronted with an unpleasant experience?                                 |
| ___ | ___ | 5. Do you easily become angry or frustrated when you are faced with a problem for which there is no immediate solution? |
| ___ | ___ | 6. Do you often complain of being tired?  |
| ___ | ___ | 7. Does your face often hold expressions of intense concentration?  |
| ___ | ___ | 8. Do you often drum your fingers aimlessly or forcibly to express irritation?  |
| ___ | ___ | 9. Does your posture appear stiff when you sit or walk?   |
| ___ | ___ | 10. Are you unable to concentrate on one problem at a time?   |
| ___ | ___ | 11. Are you unable to relax voluntarily?  |
| ___ | ___ | 12. Do you often experience nervousness and uneasy feelings?  |
| ___ | ___ | 13. Do you become upset when your plans are interrupted or must be changed?   |
| ___ | ___ | 14. Are you highly competitive in sports, in your test grades, in your daily responsibilities?                          |
| ___ | ___ | 15. Are you time-conscious?   |
| ___ | ___ | 16. Do you experience extreme dissatisfaction and anxiety when you fail to achieve success in your endeavors?           |
| ___ | ___ | 17. Are you an aggressive person?   |
| ___ | ___ | 18. Are you often too busy to allow time for physical activity?   |
| ___ | ___ | 19. Do you plan your day's activities and often budget your time?   |
| ___ | ___ | 20. Are you critical of yourself when you make a mistake?   |

- \_\_\_ \_\_\_ 21. Do you feel "uptight" at the end of the day?
- \_\_\_ \_\_\_ 22. Are you impatient when others are late for an appointment with you?
- \_\_\_ \_\_\_ 23. Do you often set high goals or levels of achievement for yourself?
- \_\_\_ \_\_\_ 24. Do you experience bad moods often?
- \_\_\_ \_\_\_ 25. Are you unyielding when others disagree with your beliefs or convictions?

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From Müller, D. K., and Allen, T. E. *Fitness: A Lifetime Commitment* (ed. 2). Minneapolis, MN: Burgess Publishing Co., 1982. Reprinted with permission of the publisher.





## APPENDIX E

### Guideline for Personal Interview

1. Tell me something about yourself; your background, family, where you come from, how you came to be at Virginia Tech, etc.
2. Tell me about your life at Virginia Tech; describe your living situation, schoolwork, activities, friends.
3. Describe what your diet is like.
4. How long have you followed the type of diet that you do?
5. How is it you have come to follow the diet you do?
6. What factors would you give greatest importance to in the adoption of your dietary pattern?
7. How would you describe your level of satisfaction with your diet?
8. What changes, if any, would you make in your diet?
9. How important do you consider your diet in your way of life?
10. Do you find it difficult to follow your diet when you eat out, visit people, or try to find ingredients?

## APPENDIX F

### TO ALL PARTICIPANTS IN THE IRON STUDY WITH FEMALE ATHLETES:

You will find enclosed a report giving both your individual data on all variables measured and means for these variables for each dietary group. Please remember that group 1 was the meat group, group 2 was the vegetarian group, and group 3 was the poultry and fish group. We hope you still have your information sheets we provided containing the explanations of the meaning of the blood variables measured. Normal values are given in parentheses under each blood variable. The dietary data is an average of the two diet records and indicate your average daily intake of total calories, the percent of total calories which may be attributed to protein, carbohydrate, and fat, and your total protein and iron intakes in grams and milligrams, respectively.

If you have any immediate questions regarding this information, you may call us at school at (703) 231-8768. If you wish to wait until returning for the school year in August, Susie will be gone to South Carolina where she got a job teaching at Winthrop College (hooray!); however, Lucy should be able to be contacted through the Department of Human Nutrition and Foods. Additionally, if you feel any interest in seeking nutritional counseling concerning these results, or for any other reason, Mary Pat Ward, who is an instructor in our department, does nutritional counseling through the Student Health Center. Please feel free to contact her. She is very personable, and has lots of experience dealing with athletes and their special needs.

We want to thank you all for your interest and participation in this study. Through the kind cooperation from volunteers such as yourselves, much research is able to be accomplished. We enjoyed working with you and sincerely thank you for all your efforts. We hope this has been a positive experience for all of you and hope if the occasion arises in the future to participate in scientific research, you may feel motivated to do so.

NAME OF SUBJECT:	DIETARY GROUP:			
PARAMETER	MEAN	MEAN, GRP 1'	MEAN, GRP2'	MEAN, GRP3'
% Body fat		21.1 +/- 3.9	20.6 +/- 4.4	19.4 +/- 3.2
PWC test VO max. (ml/min/kg)		44.3 +/- 7.4	48.3 +/- 11.3	44 +/- 8.8
Hemoglobin (12-16 g/dl)		13.9 +/- 0.8	13.3 +/- 1.2	13.5 +/- 0.8
Hematocrit (37-47%)		41.1 +/- 2.1	40.6 +/- 3.3	40 +/- 2.5
MCV (81-99 fl)		89 +/- 5.3	89.4 +/- 3.9	90 +/- 4.2
Ferritin (12-130 ng dl)		31.2 +/- 19.1	29 +/- 21.2	25.3 +/- 16.1
Serum iron (40-180 mcg/dl)		89 +/- 28.2	80.5 +/- 28.5	90.5 +/- 18.4
TS (16-55)		28.1 +/- 11	25.4 +/- 6.9	26 +/- 6.2
TIBC (250-450 mcg/dl)		351 +/- 48.5	348 +/- 37.1	350 +/- 37
FEP (460 mcg dl)		11.2 +/- 5.2	13.6 +/- 3.6	18.6 +/- 4.4
RBC x 10 <sup>6</sup> (4.2 - 5.4)		4.64 +/- 0.4	4.55 +/- 0.4	4.47 +/- 0.3
Total kcal		2029 +/- 1105	1758 +/- 862	1768 +/- 558
% Protein		15.2 +/- 3	12.8 +/- 2.7	15.1 +/- 3.6
% CHO		52.2 +/- 6.2	64.9 +/- 8.1	58 +/- 6.4
% Fat		31.1 +/- 5.5	22.0 +/- 7.3	26.3 +/- 8
Iron (mg) (RDA=15 mg/day)		15.7 +/- 9.3	14.9 +/- 10.2	12 +/- 4.3
Protein (g)		87.9 +/- 15	58.5 +/- 28.2	65.3 +/- 17

\* Mean +/- Standard deviation

PWC = Physical Work Capacity

MCV = Mean Corpuscular Volume

TS = Transferrin Saturation

TIBC= Total Iron Binding Capacity

FEP = Free Erythrocyte Protoporphyrin

RBC = Red Blood Cell

Kcal= Kilocalories

CHO = Carbohydrate

## APPENDIX G

### Individual Values for All Parameters

Subj = Subject number  
 Group = Group number  
 PCBF = Percent body fat  
 PWC = Physical Work Capacity (VO<sub>2</sub> Max, ml/min/kg)  
 HGB = Hemoglobin (g/dl)  
 TBC = Total iron-binding capacity (mcg/dl)  
 FEP = Free erythrocyte protoporphyrin (mcg/dl)  
 FER = Serum ferritin (ng/ml)  
 TST = Transferrin saturation (%)  
 HCT = Hematocrit (%)  
 CAL = Kilocalories  
 PPR = % Protein  
 PPRT = % Protein (Arcsine Sqrt)  
 CHO = % Carbohydrate  
 FAT = % Fat  
 IRON = Iron (mg)  
 GPR = Protein (g)  
 MCV = Mean Corpuscular Volume (fl)  
 RBC = Red Blood Cell (x 10<sup>6</sup>/ml)  
 SFE = Serum Iron (mcg/dl)  
 Fi = Fiber (g)  
 C = Vitamin C (mg)  
 Ex = Exercise (hrs/wk)

OBS	SUBJ	GROUP	HGB	TBC	FEP	FER	TST	HCT	CAL	PPR	PPRT
1	1	1	13.8000	330.000	28.0667	37.5000	30.6667	40.2333	1782.0	14.0	0.38350
2	2	1	14.2333	450.000	25.6667	13.6667	17.0000	42.5667	1648.5	14.5	0.39065
3	3	1	14.4667	309.667	19.4333	45.1667	35.0000	42.1000	1792.5	18.0	0.43815
4	4	1	12.9000	470.000	22.6333	30.6333	28.0000	40.8667	1321.0	14.5	0.39065
5	5	1	14.9333	291.333	15.5667	36.1667	40.0000	44.4000	1868.5	21.5	0.48215
6	6	1	13.5333	330.000	13.9333	43.6667	31.3333	39.5667	1895.0	19.5	0.45737
7	7	1	13.4333	377.000	22.7000	23.1667	20.3333	39.1667	1681.5	14.5	0.39065
8	8	1	14.2000	335.667	19.7000	15.0000	20.6667	42.2333	2066.5	17.0	0.42499
9	9	1	13.0333	333.333	18.0000	52.4000	31.6667	39.0667	1505.5	14.0	0.38350
10	10	1	16.0333	358.333	24.0667	11.5333	22.3333	47.0333	2363.0	12.5	0.36137
11	11	1	13.4333	377.333	17.3333	36.5333	31.0000	39.3667	2241.0	16.5	0.41829
12	12	1	14.2000	358.000	15.8667	23.2667	29.3333	41.7333	1825.5	15.0	0.39770
13	13	1	12.9333	354.500	19.7333	14.1667	30.5000	38.4000	2051.5	16.0	0.41152
14	14	1	14.7000	271.333	34.6333	30.6667	42.6667	43.1000	1893.5	23.5	0.50610
15	15	1	13.7667	301.667	18.7667	33.6333	44.3333	40.3667	2421.5	17.0	0.42499
16	16	1	14.0333	350.667	20.0333	16.6333	33.0000	41.6333	2199.5	16.5	0.41829
17	17	1	13.6000	297.667	14.2000	43.2000	33.3333	39.7667	1823.5	15.5	0.40485
18	18	1	15.1000	371.667	20.0333	75.7667	33.0000	45.1333	2553.5	17.0	0.42499
19	19	1	12.8333	347.333	24.9333	20.3333	20.3333	36.4667	2428.5	16.5	0.41829
20	20	1	14.1667	348.333	21.6333	75.3667	28.0000	41.3667	2449.0	9.5	0.31332
21	21	1	13.8000	375.667	16.4667	45.7667	14.3333	40.9667	1503.0	12.0	0.35374
22	22	1	13.8000	268.000	15.2667	46.7333	60.6667	40.1000	3032.0	17.0	0.42499
23	23	1	13.2667	374.667	23.7000	7.1333	10.6667	39.6667	7202.0	11.5	0.34598
24	24	1	13.8000	377.000	26.3000	11.4333	26.6667	40.9000	2085.5	14.0	0.38350
25	25	1	13.4000	409.667	31.0000	4.3000	13.6667	39.5000	2122.5	17.5	0.43161
26	26	2	14.1667	353.000	20.5000	29.4667	28.0000	41.2667	1919.5	11.5	0.34598

OBS	CHO	FAT	IRON	GPR	MCV	RBC	SFE	CC	FI	EX
1	56.5	29.5	4.85	63.35	89.5667	4.49333	102.667	19.15	6.40	14.0
2	55.5	30.0	11.00	61.30	87.8667	4.84000	80.667	132.00	12.25	18.0
3	51.0	31.0	18.35	86.15	86.7333	4.74333	109.000	174.00	16.40	27.0
4	67.5	18.0	12.05	48.20	69.3667	5.89000	121.667	127.45	9.80	3.0
5	50.0	28.5	14.40	104.00	89.7333	4.94333	117.333	74.85	16.00	7.5
6	44.0	36.5	11.05	94.10	90.3667	4.38000	105.000	146.00	10.70	1.5
7	52.5	30.0	20.15	59.55	91.7333	4.27000	77.000	82.05	12.60	5.0
8	42.5	40.5	7.55	89.50	88.8667	4.89000	71.333	78.15	6.30	3.0
9	56.0	30.0	11.75	54.50	95.8667	4.07333	106.000	134.00	6.05	6.5
10	58.0	30.0	14.55	78.50	90.5667	5.19667	81.000	191.00	7.75	12.5
11	52.5	22.5	14.25	93.25	91.6333	4.29667	117.667	140.90	15.60	18.0
12	53.5	31.5	7.35	68.65	92.4333	4.58333	106.667	66.80	8.05	9.0
13	51.0	28.5	16.75	81.90	89.0000	4.31333	109.500	68.20	11.15	2.5
14	48.5	28.5	16.75	112.00	93.3000	4.62000	109.000	54.35	17.45	6.5
15	50.5	32.5	15.20	105.65	93.0333	4.38667	136.000	186.75	13.55	10.0
16	43.0	40.5	12.80	92.05	84.9333	4.90333	118.333	66.70	5.85	12.0
17	50.5	35.0	17.75	68.25	89.6000	4.43000	88.000	46.70	9.90	3.0
18	62.0	21.0	32.60	110.00	95.7667	4.90333	107.667	197.50	25.55	10.0
19	51.0	30.0	49.60	104.75	85.5667	4.50000	80.000	101.35	22.30	5.5
20	64.0	26.0	13.10	60.60	93.5333	4.46000	104.333	213.50	11.00	15.0
21	53.0	35.0	7.15	45.95	84.7000	4.60667	59.333	79.90	6.20	8.0
22	46.5	36.5	13.65	131.00	99.3000	4.04333	163.333	209.00	12.80	18.0
23	51.0	37.0	27.15	217.50	84.6000	4.69000	42.333	169.50	24.20	12.5
24	49.5	36.5	11.35	74.30	85.4667	4.78333	102.667	148.50	11.90	15.0
25	45.5	33.0	10.75	93.85	81.2333	4.86333	58.000	86.45	11.55	10.0
26	67.5	21.0	24.35	55.95	89.4667	4.61000	99.333	81.50	14.80	5.0

## Susie &amp; Lucy Blood Values for Anova 5/92 (ALL) 10:57 Friday, December 4, 1992 2

OBS	SUBJ	GROUP	HGB	TBC	FEP	FER	TST	HCT	CAL	PPR	PPRT
27	27	2	13.8000	332.000	15.1000	27.9000	24.5000	41.3333	1722.0	11.5	0.34598
28	28	2	14.1000	349.500	25.2000	23.4667	23.5000	41.9000	4245.0	8.5	0.29584
29	29	2	11.9667	343.000	19.4667	26.5333	15.3333	35.5333	3110.0	17.0	0.42499
30	30	2	14.0000	384.667	13.8000	21.6000	20.0000	41.1000	1786.0	12.5	0.36137
31	31	2	12.7333	332.000	19.3667	12.9333	29.6667	37.6333	1814.5	13.0	0.36886
32	32	2	10.6333	287.667	18.9667	4.9000	13.3333	32.1000	1101.5	12.0	0.35374
33	33	2	13.8333	367.333	18.2000	34.0000	29.0000	40.5667	1521.0	12.0	0.35374
34	34	2	13.8000	355.333	13.7667	43.2000	35.0000	40.7000	1331.5	10.5	0.32999
35	35	2	13.6000	320.667	20.3000	23.9667	16.0000	40.5667	1270.5	14.0	0.38350
36	36	2	14.7333	361.000	15.0667	26.6667	19.6667	43.2333	1309.0	14.5	0.39065
37	37	2	15.0667	286.000	17.5667	63.2333	33.0000	44.9000	991.0	11.0	0.33807
38	38	2	14.8000	342.333	19.5000	88.7333	33.6667	43.9000	1174.0	14.0	0.38350
39	39	2	14.3667	354.667	15.2667	9.6667	25.0000	42.0667	1202.0	12.5	0.36137
40	40	2	14.8333	347.000	19.1667	14.0000	27.6667	43.9333	1758.5	14.5	0.39065
41	41	2	13.5000	447.000	25.9000	13.9000	32.6667	39.5667	1871.0	16.5	0.41829
42	42	3	13.6333	344.333	18.9000	22.1333	28.0000	40.9000	1408.5	14.5	0.39065
43	43	3	13.3667	413.000	17.3667	11.9333	15.3333	40.0667	1532.0	12.5	0.36137
44	44	3	14.0667	381.000	16.3333	4.9667	26.0000	41.8333	2196.0	10.5	0.32999
45	45	3	14.3000	344.500	14.5333	5.8333	32.5000	43.0667	1489.5	13.0	0.36886
46	46	3	14.9333	298.000	15.5000	23.9333	35.0000	44.0667	1784.0	14.5	0.39065
47	47	3	13.9333	380.500	18.4000	40.4333	21.0000	41.5667	1323.0	18.0	0.43815
48	48	3	13.0667	308.000	14.8667	57.3333	19.6667	38.7667	2836.5	14.0	0.38350
49	49	3	13.2333	297.667	12.0000	31.4333	26.6667	38.6000	1143.0	20.5	0.46987
50	50	3	13.5000	88.333	11.1000	17.4000	27.5000	40.1667	1879.5	17.0	0.42499
51	51	3	14.2333	361.000	21.6333	9.9667	26.0000	42.2667	1796.5	12.5	0.36137
52	52	3	11.5000	375.000	29.9667	4.7333	11.3333	34.9000	2674.0	11.0	0.33807

OBS	CHO	FAT	IRON	GPR	MCV	RBC	SFE	CC	FI	EX
27	51.5	34.0	7.50	48.75	94.0000	4.40000	78.500	140.00	12.40	4.0
28	77.5	14.0	33.70	95.30	91.6333	4.57333	85.500	280.50	27.00	60.0
29	55.5	26.0	32.70	135.50	88.1000	4.03000	54.000	216.00	49.30	5.0
30	61.5	26.0	18.25	54.70	86.1000	4.77333	78.000	68.50	13.35	10.0
31	60.0	27.5	8.55	58.55	89.5333	4.20667	100.000	114.15	16.15	10.5
32	64.0	24.0	9.35	34.00	83.0667	3.87000	39.333	168.95	7.15	10.5
33	63.5	24.0	9.35	47.50	90.5667	4.48000	107.333	128.00	15.60	12.0
34	67.0	22.5	11.70	35.35	86.0000	4.73667	125.667	47.45	10.95	9.0
35	61.5	25.0	8.15	44.25	86.7333	4.67667	53.333	99.05	12.55	8.0
36	55.0	31.0	7.25	48.05	83.0333	5.20333	70.667	101.85	7.05	4.0
37	76.5	12.0	6.45	27.30	95.6333	4.69667	96.000	46.25	8.55	14.0
38	63.0	23.0	6.75	41.40	90.1333	4.87333	122.333	156.00	11.45	7.0
39	72.0	15.5	17.25	37.60	91.1333	4.61333	91.000	99.50	14.65	5.0
40	67.0	18.5	21.55	64.75	89.5667	4.90667	98.333	123.80	10.70	5.0
41	75.0	8.5	15.10	75.25	95.8000	4.13000	148.000	294.00	22.50	12.0
42	67.0	18.5	19.50	51.70	87.8667	4.65333	97.333	209.00	9.95	3.0
43	57.0	31.5	9.45	48.15	83.6000	4.79333	66.333	57.10	9.40	6.0
44	44.5	45.0	10.85	60.40	92.0333	4.54333	102.000	38.10	16.00	4.0
45	64.0	23.0	10.35	48.70	95.4000	4.51333	113.000	155.40	9.00	12.0
46	65.0	20.5	17.00	64.60	89.8333	4.90667	102.667	56.25	7.35	5.0
47	64.5	17.5	17.30	61.10	87.8667	4.73000	81.500	250.00	14.65	7.5
48	60.0	25.0	14.50	102.35	90.3667	4.29000	63.000	254.50	9.60	2.5
49	50.5	28.5	8.05	58.95	94.7333	4.07667	79.667	71.35	10.50	15.0
50	52.0	31.0	11.25	81.50	89.6333	4.48333	97.000	182.00	16.65	5.0
51	59.5	28.0	6.60	59.40	87.0000	4.85667	95.333	133.90	7.65	8.0
52	53.5	32.5	11.70	73.20	78.9000	4.42667	43.000	317.00	8.10	10.0

## Susie &amp; Lucy Blood Values for Anova 5/92 (ALL) 10:57 Friday, December 4, 1992 3

OBS	SUBJ	GROUP	HGB	TBC	FEP	FER	TST	HCT	CAL	PPR	PPRT
53	53	3	13.9667	312.000	19.4667	29.8667	30.6667	41.3333	1058.5	21.5	0.48215
54	54	3	13.3667	349.667	14.9667	34.4000	24.6667	39.4333	1251.0	23.0	0.50018
55	55	3	14.2333	362.667	22.7000	23.1333	30.0000	41.7333	974.5	12.5	0.36137
56	56	3	11.8333	372.500	13.4333	38.6000	20.0000	34.3667	2881.5	11.5	0.34598
57	57	3	13.4667	385.667	21.0333	17.2333	29.6667	39.9000	1977.0	12.5	0.36137
58	58	3	13.3333	383.500	23.5333	21.2667	27.0000	39.8667	1580.5	18.0	0.43815
59	59	3	13.4333	274.667	18.1000	62.4000	29.6667	39.4667	1590.0	13.5	0.37624
60	60	3	11.9333	314.667	23.8667	12.7667	35.3333	36.1667	2235.5	13.0	0.36886
61	61	3	13.8333	371.000	17.7000	19.2333	29.6667	40.7333	2109.0	18.0	0.43815
62	62	3	13.7667	378.000	23.1333	17.9667	20.3333	40.8667	1406.0	15.5	0.40465
OBS	CHO	FAT	IRON	GPR	MCV	RBC	SFE	CC	FI	EX	
53	64.0	14.5	11.90	56.75	89.8667	4.60000	96.667	88.95	5.05	15.0	
54	57.5	11.0	10.20	72.00	91.2333	4.32333	87.667	113.95	14.40	33.0	
55	52.0	35.0	4.30	30.05	90.4667	4.61333	109.667	42.80	5.75	6.0	
56	62.5	24.5	15.60	85.00	89.7667	3.83000	77.000	582.00	8.40	10.0	
57	65.5	22.0	12.25	63.65	91.9000	4.33667	114.000	149.00	10.50	3.0	
58	57.0	25.0	9.70	70.50	92.7000	4.29667	105.500	140.95	6.10	4.0	
59	56.0	31.0	8.50	54.60	93.4667	4.22333	84.000	92.70	9.85	6.0	
60	60.0	27.0	19.30	75.25	81.4333	4.44333	92.333	100.15	26.10	12.5	
61	60.5	22.0	16.25	96.70	93.6000	4.35333	113.000	131.50	13.45	15.0	
62	45.5	38.5	6.75	56.50	90.3667	4.52333	79.333	53.30	7.70	7.5	

## VITA

Lucy Smith Garman was born on August 19, 1949 in Orlando, Florida. She became a Registered Dental Hygienist in May, 1970. In 1980 she completed a Bachelor of Science degree in Fisheries and Wildlife at Virginia Polytechnic Institute and State University. She completed the requirements for a Master of Science degree in Human Nutrition and Foods at Virginia Polytechnic Institute and State University in September, 1992 where she is currently participating in a dietetic internship program.

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