

ADEQUACY OF IRON NUTRITURE
OF PREADOLESCENT GIRLS

By

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CHAPTER I

INTRODUCTION

Significance of the Study

In recent years there has been much interest, research, and controversy in the area of iron nutrition and iron fortification of foods. Iron deficiency is regarded as one of America's most prevalent and serious nutritional problems. It has been found that a significant proportion of the population probably have inadequate intakes of this nutrient (11,14,25,26,29,48,64,65). The most vulnerable groups are pregnant women, menstruating women, and infants (14). Actual iron deficiency anemia in these groups is not uncommon (22,29). Iron deficiencies occur in all economic classes and at all educational levels but are more frequent among the lower income groups and in families with less education (26). All these observations have led to speculation about the iron nutrition of vulnerable age groups of females just before the onset of the menses. It has been speculated that these females enter the critical child-bearing years with less than adequate serum iron levels. It seems pertinent, therefore, to consider this population group in relation to iron intake and serum iron levels.

Iron is one of the most thoroughly researched nutrients yet there is much that is still not completely understood about the absorption, utilization, and metabolism of this vital element. The Food and Nutrition Board of the National Research Council, National Academy of Sciences,

recommended in 1968 that the dietary allowance for iron be increased from 15 to 18 milligrams for females from the age of ten years through the child-bearing years (45). It is quite difficult to obtain this amount of iron from dietary sources. The long term effect of inadequate amounts of iron is anemia. Literally interpreted, the word anemia means without blood. Clinically, anemia may be defined as a condition in which the blood is reduced in amount or is deficient in red blood cells or in hemoglobin. The result is diminution in the capacity of the blood to combine with and transport oxygen to the peripheral tissues. Some of the clinical symptoms of anemia include general fatigue and lassitude, breathlessness on exertion, headache, insomnia, and skin pallor. Anemia can be due to many different factors. The type of anemia can be diagnosed quickly by blood tests. The most common anemia is due to an inadequate intake of iron. This nutritional anemia will arise slowly and take months or years to develop. Periods of stress such as pregnancy or lactation or periods of growth increase this tendency towards anemia. Girls between the ages of nine and 11 years need extra iron due to the loss of blood iron at the beginning of menstruation and also because of the growth needs for iron.

Statement of the Problem

The purpose of this study is to determine the adequacy of iron intake in nine- to eleven-year-old Girl Scouts in Stillwater, Oklahoma.

The hypotheses of the study are:

- a. This group of nine- to 11-year-old girls have adequate hemoglobin and hematocrit levels.
- b. This group of nine- to 11-year-old girls have adequate iron

intakes according to the Recommended Dietary Allowances, 1968.

c. These girls do not need improvement in their diets.

A three-day dietary record was kept by each subject in order to assess the adequacy of calories and eight nutrients. Finger prick blood was used to determine hemoglobin levels and hematocrit values for each subject.

CHAPTER II

REVIEW OF LITERATURE

The Metabolism of Iron

Iron is one of the trace minerals essential to human life. Iron, in the body, is usually complexed with protein molecules. Practically no ionic iron exists in the animal body. The iron protein complexes function as enzymes such as cytochromes, catalase, peroxidase, and as myoglobin and hemoglobin (23). Most of these complexes function in processes involving the transfer of oxygen. Hemoglobin is the most researched and understood of these compounds and is an essential constituent of the red blood cell.

The function of hemoglobin in the body is to carry oxygen to the tissues from the lungs and to return carbon dioxide from the cells to the lungs. Most of the essential body iron is in the form of hemoglobin. Hemoglobin is a conjugated protein composed of the iron-containing pigment, heme and the protein, globin. A single red blood cell contains about 280 million molecules of hemoglobin. Hemoglobin contains 10,000 atoms, four of which are iron atoms. Hemoglobin formation is then dependent on iron. Protein, of course, is also necessary for the formation of hemoglobin. Most of the B-complex vitamins are also needed because of their functions as coenzymes in protein production and red blood cell formation. For example, two B-complex vitamins necessary for hematopoiesis are folic acid which is necessary for the formation of

heme, and vitamin B₁₂, or cobalamin, which is necessary for red blood cell production and is the treatment for pernicious anemia. Copper, which functions much like iron in the body, is also essential for the formation of hemoglobin (17,59,78).

The essential body iron is 70 per cent of the approximately 35-50 mg. of iron per kilogram of body weight in females. About 20 per cent of the iron is in the protein-iron compound, ferritin, which is a storage or nonessential form. The main storage organs are the liver, spleen, and bone marrow. A small amount of the iron is also found in the plasma. This iron is bound to one of the plasma β -globulins, transferrin, and is in the process of being transferred from one place to another. The iron-binding capacity of serum is the quantitative ability of transferrin to retain iron. Another five per cent of body iron is found in the oxidative enzyme systems of all cells which produce energy. In humans, iron is severely conserved. It is estimated that under normal conditions, the human loses only about one mg. of iron per day. Average daily intake of iron is about 10-20 mg. from food. It would appear from these facts that iron deficiency would be rare. However, this is not the case and is due to many factors. One of these is a change in the need for iron (17,23,46,47,59,78).

Since most of the essential iron is in the blood, bleeding of any sort results in iron loss. During pregnancy, there is an increase in blood volume as well as iron transfer to the fetus. There is a blood loss at delivery also. Lactation requires increased iron as do periods of growth. Another factor affecting iron needs is the absorption of iron across the intestinal mucosa. The human absorbs only about ten per cent of food iron. The rest passes through the body by means of the

large intestine (14,17,24,46,47,59,78).

Absorption varies among individuals. Absorption is affected by the body iron levels. The lower these levels, the more iron is absorbed. Severe infection can hinder iron absorption. Certain substances in the body affect absorption. For example, ascorbic acid enhances iron absorption while phytates decrease absorption. An adequate amount of calcium helps to bind the phytates and other substances which would otherwise combine with the iron and inhibit its absorption. The oxidation state of the iron affects absorption-- Fe^{++} (ferrous) forms are easily absorbed while the Fe^{+++} (ferric) forms are not absorbed. However, high gastric acidity can reduce the ferric forms making them available for absorption. Iron salts are more readily absorbed than food iron while iron from different foods is absorbed at different rates. About ten to 30 per cent of iron from animal proteins can be absorbed while only two to ten per cent of iron in vegetables can be absorbed (14,15,23,24,56,69,78). Iron salts are given by mouth for the treatment and prevention of iron deficiency anemia. These salts are effective and inexpensive. The ferrous sulfate compounds are preferred over ferrous gluconate, ferrous succinate, and ferrous fumarate because of the lower price of the ferrous sulfate. All are equally effective unless the anemia or deficiency is caused by a malabsorption syndrome. In this case, saccharated oxide of iron may be given intravenously (8,59,60).

Toxic levels of iron have been found. Accidental poisoning, especially of children, with ferrous sulfate has caused death. Hemosiderosis, a condition in which large amounts of the iron storage compound, hemosiderin, are deposited in the body, especially in the liver and spleen, has been noted in Bantu natives who consume iron in excess of 100 mg.

per day. These high levels are due to the use of iron pots and utensils for cooking and beer brewing (17,19,59).

There are approximately six mg. of iron per 1000 calories in the average American diet. This represents up to 0.9 mg. of absorbable iron. Girls in the age group for this study need to retain approximately 1.5 to 1.6 mg. of iron per day since they are in a rapid growth phase (47). In the recommended dietary allowances these figures were used to determine the value set as a guide for interpretation of food consumption records and a goal for planning food supplies for females in the age group of nine to 11 years. The recommended daily allowances are regularly reviewed and changes are made according to new information about nutrients from recent research. The recommended allowances for iron for this age group have been increased recently to 18 mg. per day (24,25). This amount is extremely hard to satisfy in a regular diet because "iron is so widely distributed in the diet that manipulation of foods to modify iron intake is impractical" (24, p. 58).

History of the Recommended Dietary

Allowance for Iron

The Recommended Dietary Allowances were established in 1943 to be guides for planning for adequate nutrition for the civilian population of the United States. They gave amounts of specific nutrients that allowed a margin of safety above the average physiologic needs so that the variation of practically all individuals in the population would be covered. These allowances have been revised six times since then as scientific knowledge advanced sufficiently to allow better estimates to be made (45,66). The Recommended Dietary Allowances can be used as

guides for the requirements of individuals only if one realizes that the amounts may be in excess for a given individual. Because they are planned to cover the needs of practically everyone, they may well be considerably higher than some persons need. Therefore, it is erroneous to say an individual is malnourished or deficient if he does not meet the recommended allowances.

The recommended allowances for iron have seen much change since the inception of the Recommended Dietary Allowances because of the nature of the nutrient. That is, the question of the availability of iron from food and the fluctuation in capabilities of absorption by individuals make recommendations for needs difficult. The whole history of the recommended allowance for iron has been one of "...compromise between the opinion that more is needed than is recommended and the principle that the Recommended Dietary Allowances should be provided by foods in ordinary diets" (45, p. 115).

In the last two revisions of the Recommended Dietary Allowances made in 1964 and 1968, the iron requirement was increased for certain population groups. In 1964, the allowance for children from nine to 18 years was increased from 12 to 15 mg. daily (45). The research in the next four years and the evidence of the widespread occurrence of iron deficiency anemia in the United States led to re-evaluation and further changes in 1968. At this time the allowance was increased to 18 mg. per day for females from age ten through child-bearing years (24,66). It was pointed out that this amount would permit sufficient accumulation of iron stores to avoid the need for iron therapy during pregnancy. It was also noted that this amount would be difficult to obtain from the average 2000 calorie diet and that further iron fortification of breads and

cereals might be required to allow females on an ordinary diet to obtain the recommended allowance (66).

Hemoglobin and Hematocrit Levels

In order to discuss the deficiency groups, the limits of the hemoglobin and hematocrit values must be set. There is controversy over "at just what level" there is anemia and what values indicate low or deficient amounts of hemoglobin. Most of the arbitrarily set values today are based on values found from surveys on normal, healthy persons. Hence, as the number of normal values were compiled, standards were set from them. The trends in hemoglobin and hematocrit values are clear (35). At birth the hemoglobin values are high and are followed by a rapid decline. From the third to the fifth months there is a gradual decline with a minimum reached between one and two years. Variation in hemoglobin levels with age is most marked under three years. Hemoglobin values increase throughout childhood. Adult men usually have higher hemoglobin values than women. Hemoglobin values vary during different phases of pregnancy. Hawkins et al. (30) in their survey of 1308 male and 1424 female subjects between the ages of nine and 98 years in Canada, found that up until age 12 years there was a tendency for higher hemoglobin values among the females but that after that they were higher in the boys. Between the ages of 14 and 20 years, the hemoglobin values for the females decreased slightly but increased markedly for the males. After age 20, the values gradually decreased with age in the men but remained fairly constant in the women.

Two of the most often used parameters of hemoglobin status are those of Wintrobe and of the Interdepartmental Committee on Nutrition

for National Defense (ICNND). Wintrobe (80) gives values which are higher but are used as an ultimate guide (see Table 1). The ICNND Manual (35) states that most surveys have found lower values than those used by Wintrobe and they used the guidelines given in Table 2.

Table 1: Guide to interpreting hemoglobin levels--Wintrobe's recommendations

sex	age	normal hemoglobin concentrations
	yr.	gm./100 ml.
male and female	6-10	12.9
male and female	11-15	13.4
female	over 15	14±2
male	over 15	16±2

Table 2: Guide to interpreting hemoglobin levels--ICNND recommendations

	hemoglobin, gm./100 ml.			
	deficient	low	acceptable	high
men	< 12.0	12.0-13.9	14.0-14.9	≥ 15.0
women	< 10.0	10.0-10.9	11.0-14.4	≥ 14.5
children, 3 to 12 yrs.	< 10.0	10.0-10.9	11.0-12.4	≥ 12.5
	hematocrit, volume %			
	deficient	low	acceptable	high
men	< 36	36 - 41	42 - 44	≥ 45
women	< 30	30 - 37	38 - 42	≥ 43
children, 3 to 12 yrs.	< 30	30.0-33.9	34.0-36.9	≥ 37.0

These values established by ICNND were used in the recent ten-state nutrition survey, 1968-1970 (32). The Committee on Iron Deficiency of the American Medical Association (14) gives lower limits of normal hemoglobin values as presented in Table 3.

Table 3: Guide to interpreting hemoglobin levels--American Medical Association recommendations

age yrs.	sex	lower limits of normal	
		hemoglobin gm./100 ml.	hematocrit volume %
0.6-4	both sexes	11	33
5 -9	both sexes	11.5	34.5
10--14	both sexes	12	36
adults	men	14	42
adults	women	12	36
	pregnant women	11	33

Davis, Gershoff, and Gamble (18) in their review of the studies of vitamin and mineral nutrition in the United States from 1950 to 1968, stated that most of the biochemical studies prior to 1960 used Bessey and Lowry standards (7) while most studies after 1960 used the ICNND standards (35).

The Iron Deficiency Groups

The National Nutrition Survey (64,65) preliminary reports in 1969 indicated that between five and ten per cent of the population examined had iron deficiency anemia. The later Ten-State Nutrition Survey final results (33) verified this. This survey was established to identify the incidence, magnitude, and location of malnutrition and related health problems in the United States. The Public Health Service carried out the survey in ten states selected on the basis of geographic representation of the major areas of the country and a diversity of economic and socio-cultural composition of the population as well as availability of resources for conducting the study. The ten states were: Texas, Louisiana, New York, Massachusetts, Kentucky, West Virginia, Michigan,

Washington, California, and South Carolina. The primary persons sampled were from the lower economic groups. Over 86,000 persons were surveyed. The survey included a 24-hour dietary recall, a clinical evaluation, and a biochemical examination. It was concluded that iron deficiency anemia, as evidenced by a high prevalence of low levels of hemoglobin, is a widespread problem within the population surveyed. They found that the low levels of hemoglobin were associated with low levels of serum iron and serum transferrin saturation. They also found that the lower hemoglobin levels were usually associated with low iron intakes. The results revealed that generally there was increasing evidence of low and deficient hemoglobin values as income level decreased. The educational attainment of the person buying and preparing food was found to be related to the nutritional status of children under the age of 17 years. As the education of the homemaker increased, the evidence of low and deficient hemoglobin values of the children decreased. There was a positive relationship between the education and the hemoglobin status of adults. They also found that hemoglobin status was not age dependent--that both sexes in all age groups had about the same incidence of deficient and low hemoglobin values. The mean hemoglobin values of all age groups were consistently lower than generally accepted mean values. It was also shown that the black population had the poorest hemoglobin status while the white population had the best. For all ethnic groups, hemoglobin status was less satisfactory in the low-income-ratio states than in the high-income-ratio states.

Iron intake, as revealed by 24-hour dietary recall, was below dietary standards for many of the age groups. For infants 0-36 months of age, iron was the only nutrient for which the mean intakes were generally

below the dietary standard. In the group of ten- to 16-year-olds, it was found that all females had mean iron intakes that were less than 75 per cent of recommended dietary allowances. It was also found in this age group that the mean iron intakes per kilogram of body weight for both males and females decreased with age; values for 15- to 16-year-olds were approximately 30 per cent lower than those of the ten- to 11-year-olds. More than half of the pregnant women surveyed had iron intakes of less than 11.0 mg. of iron per day.

In the Household Food Consumption Survey of 1965 (2,3) a sample of 14,500 representative men, women, and children in the United States was used. It was found that calcium and iron were the nutrients most often found to be below the recommended allowances and that diets of girls and women provided iron at more than 30 per cent less than recommended. The diets of children under three years provided iron at about 50 per cent below recommended amounts. It was also discovered that iron intake was lower for persons in the income class under \$3000.

Davis and his coworkers (18) reviewed the studies of vitamin and mineral nutrition status done in the United States from 1950 to 1968. They determined that a total of 25 per cent of the persons in the studies had below acceptable hemoglobin and hematocrit levels and another 25 per cent had low levels. About five per cent had deficient hemoglobin and hematocrit levels. Approximately 60 per cent of the persons studied had below the recommended dietary allowance (at the time of the study) for iron. About 15 per cent had below two-thirds of the allowance and almost five per cent had below one-half of the allowance. In all age groups from age 12 years and above, the females had a greater percentage of the low and deficient values for both the biochemical indices and the

iron intakes. Davis et al. also determined that the dietary habits of the American public have become worse, especially since 1960.

The primary populations studied with regard to iron status have been the most nutritionally vulnerable groups--pregnant women, menstruating women, and infants (14).

Infants, because of their rapid rate of growth, have a greater iron requirement in proportion to food intake than any other age group. The healthy infant is born with a three to six-month supply of iron stored in the liver. Milk contains very little iron. Therefore, the diet must begin to include iron very early in life. The infant with severe iron deficiency is anorexic, irritable, and inactive (62). Beal (4) in her study of 58 children during their first five years of life collected 795 nutrition histories and found that there was a sharp rise of iron intake during the first year, due primarily to high iron content of commercially prepared infant cereals used during this time. This was followed by a decrease in iron intake as those foods were replaced in the diet. After three years, the intake levels increased but from age two and one-half years to five years, more than 75 per cent of the intakes remain below the recommended dietary allowances. Beal's research was done in 1954. In 1971, Fox et al. (25) in a survey of over 2000 preschool children in the North Central Region found that the amounts of iron, calcium, and phosphorus consumed were similar to those Beal had found earlier. The recommended dietary allowance for iron had been increased since Beal's study and the later investigation revealed that 95 per cent of the children between the ages of one and three years and 75 per cent of those between three and six years did not meet the higher recommendations. Fox and her coworkers also found that the amount of money spent

for food was more important than income of the family or education of the mother in influencing the intake of the child. Haughton (29) in a study of 283 preschool children randomly chosen from three child health stations in a low income district of New York City found that almost 20 per cent had hemoglobin values of less than 10 gm. per cent. Most of these children were under age two years. Haughton feels that while ten gm. per cent levels of hemoglobin may not be accepted by some as constituting anemia in children under age two years, his study does indicate a significant prevalence of nutritional anemia among underprivileged preschool children. Burroughs and Huenemann (11) conducted a survey of 168 rural, lower socio-economic groups of children from six months to nine years of age from the Coachella Valley of Southern California in 1970. Of these children, 52 per cent had hemoglobin values below normal acceptable standards, 88 per cent had serum iron levels below normal and latent iron binding capacities above normal. This would indicate iron deficiency, if not anemia. The dietary histories taken indicated that only a few of the children were receiving sufficient iron to meet the recommended dietary allowances and that infants between 12 and 24 months had the lowest mean iron intake and the highest incidence of low hemoglobin levels. Owen and Kram (56) in a 1969 study of 558 Mississippi preschool children reported that on the average, children from the lowest income groups had twice the incidence of low hemoglobin values than did children from more affluent homes. Futrell and coworkers (26) in a 1971 nutritional status study of 139 Negro preschool children in Mississippi evaluated intake of the children with the education of the mother. They found that, in general, the average daily intake of calories, protein, calcium, iron, vitamin A, and ascorbic acid paralleled the educa-

tion of the mother; as the grade level increased, so did the intake of these nutrients. Hemoglobin values were low for about 20 per cent of the children. They also found that the highest intake of nutrients fell within the families with incomes above \$750 per person per year. The 1970 Ross Conference on Pediatric Research--Iron Nutrition in Infancy concluded that

...data at hand concerning iron balance in the first year of life, plus the demonstrated high incidence of iron deficiency anemia, make it highly desirable that the prevention of iron deficiency be recognized as a public health problem (62, p. 45).

Menstruating, pregnant and lactating women have been studied because of their increased needs for iron. They have been found to be the groups with the greatest incidence of anemia and low hemoglobin values. They need more iron than the average American diet usually supplies. Pregnant women have a greater blood volume as well as a need to provide iron for the fetus. The lactating mother uses as much iron per day in the milk as the menstruating woman loses per day during the monthly blood loss (14).

White (74) in a review of a number of published surveys of the iron status of females from ages nine to 90 years, found that in no study was the mean iron intake of 15 mg. per day (the recommended allowance at the time of this review) fulfilled. Yet the mean hemoglobin values did not indicate a high prevalence of anemia. She concluded that much research needs to be done in this area to determine actual iron content of foods, actual absorption of iron, and the needs (including iron stores) of this group.

White and Gynne (76) in a later study on the utilization of inor-

ganic elements by young women eating iron-fortified foods, used nine university women. The subjects were given blood tests before a 30 day balance study began and it was found that eight of the nine women had little or no detectable marrow iron stores but that all had normal hemoglobin levels. The foods given during the study contained an average of 22.9 mg. of iron with almost half this amount coming from breads prepared from flour fortified with ferrous sulfate. The subjects absorbed an average of 16.3 per cent or 3.7 mg. of the dietary iron. This increased absorption was probably to compensate for earlier body losses as evidenced by the lack of iron stores. There was considerable variation among the subjects in the absorption and retention of the iron but the average amount of iron retained was more than enough to balance the estimated losses. The authors concluded from their study that if the dietary iron of girls and women were to approximate the recommended allowance of 18 mg. per day, and if a significant proportion of this were provided in a readily available form, iron would be absorbed in amounts sufficient to prevent depletion. This study reinforced the early findings that iron is more readily absorbed when the body is depleted and answered the question of whether 18 mg. per day of iron would prevent depletion (69).

Monson and his coworkers (48) in their study on 13 University of Washington students found that only four were considered to be normal in regard to iron status. While none exhibited iron-deficiency anemia, five exhibited iron deficiency as reflected by no iron stores, increased iron absorption, and a decreased level of saturation of transferrin. Two of the four subjects considered normal were supplementing their diets with iron enriched foods or medicinal iron. The authors concluded

that iron intake adequate to meet the needs of the entire female population can be met only by some form of supplementation or fortification.

In a report on the extent and meanings of iron deficiency in the United States by the Food and Nutrition Board of the National Academy of Sciences (15) it was pointed out that during periods of stress the incidence of iron deficiency anemia increase. Periods of growth, especially the adolescent growth spurt which is often accompanied by the onset of menstruation in females can cause anemia especially if it comes after a life long pattern of poor iron intake that does not allow significant iron storage. In a study of 401 Vermont Junior High School children made by Morse et al. (49) it was found that boys rated better than girls with respect to hemoglobin and hematocrit values. Twice as many of the girls compared to the boys had values below 14.0 gm. per 100 ml. blood. Two per cent of the girls had hemoglobin levels of less than 12.0 gm. per 100 ml. blood. Another 11.4 per cent of the girls had hemoglobin levels of less than 13.0 gm. per cent. When compared to the ICNND standards for young men, almost 65 per cent of the girls had low or deficient hemoglobin levels and almost 70 per cent had low or deficient hematocrit values. Dribble et al. (20) designed a study to compare 400 New York adolescents between the ages of 12 and 15 years from several different socio-economic groups with regard to various biochemical indices of nutritional status. Three schools representing different racial, social, and economic backgrounds were used. Although hemoglobin values were not taken, hematocrits were used and it was found that there were more females than males in the low hematocrit classification and that more adolescents from the school with the lowest economic status had low hematocrit values. Daniel and Roland (16) in a study of 268 adolescent

patients at the University of Alabama Medical Center found only four out of 123 boys and six of 145 girls had hemoglobin values less than 11 gm./100 ml. of blood. These authors concluded iron deficiency anemia was not related, in this case, to either ethnic or economic factors and that iron deficiency anemia is not a problem of this age group.

Lantz and Wood (40,41,42) however, in studies of New Mexico adolescents did find ethnic and economic differences in iron status of Spanish-American and "Anglo" adolescents. The hemoglobin and hematocrit levels were significantly lower in the Spanish-American subjects and they had a lower mean iron intake than their Caucasian counterparts.

Iron Status of the Preadolescent Girl

There has been little nutritional research done on the preadolescent girl. She is not at the most vulnerable age although she is between two vulnerable ages--infancy and adolescence. Because of the high incidence of anemia in young women, the purpose of this study is to determine something of the iron status of this group.

In 1950, McBee, Mochette, and Tucker (44) studied 515 white children between the ages of eight and 13 years in Louisiana. They found that 86.9 per cent of the subjects had a normal hemoglobin levels of above 12.0 gm. per 100 ml. and 13.1 per cent of the subjects were below normal with 3.5 per cent having less than 11.1 gm. per 100 ml. levels. In 1952, Moschette et al. (50) in a study of 487 Louisiana school children between the ages of eight and eleven years, found a mean hemoglobin level of 12.8 gm. per 100 ml. of blood. About five per cent of the children had levels of 10.9 gm. per 100 ml. or less and were given a hemoglobin rating of "poor". In 1956, Marlatt et al. (43) reported on a

study of school children from Iowa, Kansas, and Ohio. Hemoglobin concentrations were determined for 242 children. Only three of the 242 had hemoglobin concentrations of less than 11.0 gm. per 100 ml. of blood. Eppright et al. (21) reported in 1957 on the nutritional status of nine- to eleven-year-old children in Iowa, Kansas, and Ohio. Ninety-three per cent of the subjects in Iowa, 95 per cent of the subjects in Ohio, and only 39 per cent of the subjects in Kansas had hemoglobin levels above 11.5 gm. per 100 ml. of blood which was set as the lower limit of normal for this study. The children in rural areas of Iowa had lower hemoglobin values than those living in urban areas; however, in Kansas, the opposite was true. In a more recent report in 1963 by Roderuck (61) on the nutrition and growth of about 200 Iowa girls from the ages of eight to 16 years, hemoglobin values were taken at seven or eight different times over a period of four years (1953-1957). The values within six months of each birthday were averaged to give one mean value for each girl for each year of age. About five per cent of the girls at each age had hemoglobin concentrations below 11 gm. per cent although only two girls had consistently low hemoglobin levels from one reading to the next. Roderuck concluded that low concentrations of hemoglobin were not consistent findings in this group. In a nutritional study of 642 New York school children between the ages of ten and 13 years made in 1965, Christakes et al. (12) found over 90 per cent of the children had hemoglobin levels of 12.0 gm. per cent or above when their dietary histories had been rated excellent. This compared with only 84 per cent of those with a dietary history rated as poor with hemoglobin levels of above 12 gm. per cent. The minority ethnic groups studied (Negro, Puerto Rican, and Chinese) all had more subjects in the below ten gm. per 100 ml. of

blood hemoglobin level than did the Caucasians studied. However, the mean hemoglobin level was about the same for every group. The hematocrit values showed that the Caucasian children had a significantly greater proportion of values above 40 per cent and significantly fewer below 35 per cent.

Myers and her coworkers (51,52) conducted a nutrition study of 322 school children aged nine to 13 years in a depressed section of Boston. The children were considered to be part of a society which was underprivileged sociologically, economically, educationally, and medically. Twenty-two per cent of the children were found to have low or deficient hemoglobin levels (based on ICNND standards for adult males). More girls than boys fell into this group and more Negro than Caucasian children. Thirteen per cent of the children were in the low range for hematocrit levels. They also found that the older children had poorer evaluations for meals and food groups than did younger children, with the critical point being between ten and 11 years of age. Negro children scored lower in every dietary evaluation than the Caucasian children. Negro children also showed a sharper deterioration in food habits with age.

It can be seen that the literature dealing with the iron nutriture of this age group is sparse and conflicting. However, it seems that only the most recent studies have shown anemia and iron deficiency to be problems of this group. Iron intake is usually found to be below recommended allowances in all economic groups with the lower income groups having less than the higher income groups.

Means of Assessing the Adequacy of Iron Intake

Biochemical Methods

In order to best determine the adequacy of iron intake of the individual, it is necessary to use biochemical methods. Although anemia may be due to principally one of three causes--inadequate iron intake, faulty absorption of iron due to any of many factors, or body loss of iron as from bleeding--it is most often due to poor diet. Pearson states that while hemoglobin levels cannot be interpreted solely on a nutritional basis, "it remains the best single biochemical index of the general state of health of a population" (57, p. 576-78).

There are several biochemical indices used to determine the state of iron nutriture. One of these is the hemoglobin determination. The iron content of hemoglobin is 0.340 per cent and hemoglobin iron represents about 98 per cent of total blood iron. About three-fourths of all body iron is found in the two compounds hemoglobin and myoglobin. Most of the rest of the iron is in storage form in the liver, spleen, and bone marrow. Anemia is a slow developing disease that occurs only when iron stores are depleted and the body has a lowered capacity to provide oxygen because there is a lack of iron for production of the vital compounds of oxygen transport and the enzymes in the cellular electron transport system (17). In this way the hemoglobin determination is a measure of the adequacy of iron intake. There are several methods of determining the hemoglobin content of the blood. The method recommended by the Interdepartmental Committee on Nutrition for National Defense (35) and most often used by researchers today is the cyanmethemoglobin method. In this procedure, a cyanmethemoglobin reagent added to blood

causes the hemolysis of the erythrocytes and the reaction of hemoglobin to methemoglobin. This reaction is essentially the change of the iron in the hemoglobin from the ferrous form (Fe^{++}) to the ferric form (Fe^{+++}). The reagent then causes a color reaction with CN^- to form cyanmethemoglobin from methemoglobin. The optical density of cyanmethemoglobin can then be measured spectrophotometrically and plotted on a Beer-Lambert curve obtained from standard cyanmethemoglobin solutions to determine the gm. per cent of hemoglobin of the unknown sample (28,35, 55).

Another method of determining hemoglobin levels is the acid hematin method. In this procedure the blood is treated with dilute hydrochloric acid to produce the brown "acid hematin" color which is compared with the color produced by similar treatment of blood of known hemoglobin content. This method is not entirely satisfactory however because it is difficult to obtain an accurate standard and color is affected by non-hemoglobin substances (53,55). There is also an alkaline hematin method that is similar but less accurate. Both of these methods measure total hemoglobin. That is, active hemoglobin and its derivatives as methemoglobin, carboxyhemoglobin, and sulfhemoglobin are all found in one measurement (53,55).

Until recently the determination of hemoglobin was frequently made indirectly from specific gravity of whole blood. The specific gravity measurements are made by observing the rise or fall of drops of blood, plasma, or serum dropped into a graded series of copper sulfate solutions of known specific gravities. The hemoglobin concentration may then be found by calculations using established formulas or may simply be read from charts based on those formulas (34). This method is pre-

cise and actually can be done in the field. However, as Wilson et al. (79) point out, the method is almost as time consuming as the more precise spectrophotometric techniques and is subject to more operator error. For these reasons, the cyanmethemoglobin method is now more commonly used.

Still another method that can be used is the determination of the total iron content of the blood (55). In this method, concentrated sulfuric acid in the presence of potassium persulfate is used to detach the iron from the hemoglobin molecule. The proteins are removed by tungstic acid and the amount of iron in the filtrate can be determined colorimetrically. The amount of hemoglobin can then be calculated using the optical density of a sample of known iron content. This method is subject to operator error and the several reagents that must be used may make the procedure cumbersome for field work.

A biochemical measurement usually taken along with the hemoglobin determination is the hematocrit (28,35,53,55,63). This procedure gives the packed red blood cell volume. This is done by centrifuging whole blood to separate the red and white blood cells and the plasma. The percentage of red blood cells is indicative of the nature of the anemia. Pernicious anemia, one of the primary anemias, has a large volume of average red cells while the anemias caused by dietary deficiencies have red cells which are decreased in size and hemoglobin content. Hence, the iron deficiency anemia is characterized by microcytosis (small red cells) and hypochromia (lack of iron). The hematocrit will indicate the former condition and along with the hemoglobin values give a true indication of anemia. The hematocrit values are also a check on the hemoglobin values. Strumia, Sample, and Hart (67) have determined the rela-

tionship of the two values and have suggested that one hematocrit point equals 0.34 hemoglobin points. This gives the researcher some basis for determining the accuracy of his procedures.

Unfortunately, hemoglobin and hematocrit values do not give a clear indication of iron stores. Determination of the serum iron gives some indication of the earlier stages of iron depletion before the signs of anemia appear. There are several methods for this determination, however, Pearson (57) feels that this parameter of iron status has not been extensively explored and Krehl and Hodges (39) state that there is still debate about whether serum iron levels are sufficiently valuable to merit the time and difficulty of taking them. And as the National Academy of Sciences survey manual points out, low serum iron is not a reliable sign of dietary iron deficiency since there is also a diminished serum iron concentration during infection (53).

The determination of hemosiderin in the bone marrow is a good measure of iron stores although it is an impractical procedure for field work (37,38,57). White (75) feels that the estimation of bone marrow iron may be the most reliable method for evaluating iron stores. Her findings indicate that many healthy, nonanemic women and girls may be iron deficient. She feels that more research is needed in this area to define "adequate iron stores" and the actual prevalence of iron deficiency.

Another measure of early iron depletion is the determination of the serum total iron binding capacity (38,57,63). This is increased during iron deficiency (unless protein synthesis is depressed) because of the body's increased uptake and reserve of iron when little is available from dietary sources. This procedure will also help to distinguish be-

tween the anemia of iron deficiency and the anemia of infection since only in the case of the former condition is the iron binding capacity increased.

Dietary Survey Methods

Ideally the dietary survey is part of a nutritional study that includes a clinical examination and biochemical assays in addition to the dietary survey (57). It can be used alone but, of course, gives best information when supported by the clinical and biochemical findings. The dietary information is gathered to determine the kinds and amounts of foods an individual or a population is consuming. This information may be gained by several means but the seven-day record, the dietary history, and the 24-hour recall are most commonly used in the United States. The methods to be used are determined by the information needed (group or individual), the number to be surveyed, the competency and number of researchers, the reliability of the subjects and other related factors (79).

If information about an individual is needed, the dietary history is the most valuable method since it reveals the individual's food practices over a long period of time. Seasonal and other variations can be noted also. It does require the skills of a trained interviewer so as not to bias the report. Burke states in her explanation of this method that the dietary history method cannot be exact but that it does have value for many studies (10).

Young and coworkers (81) found when they compared the 24-hour recall, the seven-day record, and the dietary history that for the individual the 24-hour recall did not correlate significantly with either

the dietary history or the seven-day record. However, the means for all groups studied were about the same for both the seven-day record and the 24-hour recall. These researchers concluded that these two methods could be used interchangeably when obtaining information about a group of subjects. The mean for the dietary history gave higher values than the other two methods. Trulson's studies gave similar information. She found that for the individual, the seven-day record and the dietary history gave similar information but that three or more 24-hour recalls did not give the same information as the other two methods. Therefore, she recommended using only one method in the dietary survey or using a correction factor when more than one method has been used so that results will be consistent (70). Adelson, in her study on adult men concluded that the recall method was as satisfactory as the record method and that one week for the study was as satisfactory as two consecutive weeks (1). The most accurate dietary information is gained from balance studies where both intake and excretions are chemically analyzed to determine exact composition. However, these studies are time consuming and expensive (19).

Trulson and McCann point out that no one technique suitable for field work is completely reliable (71). Beal also feels that none of the techniques now used for dietary studies are completely reliable (5,6). She feels that weighing and then analyzing aliquot samples of all foods eaten would allow greater accuracy but points out that this would require a great deal of money and time making the method infeasible for most studies. Trulson and McCann in their study involving weighing of food to insure greater accuracy found that the method created an inconvenience that might alter eating patterns in the subjects

(71).

Hence, the 24-hour recall method does not give information about long term food habits and is reliable only for determining intakes of a large group. However, it is easy to use. The seven-day record must be completed by reliable, intelligent subjects and is also limited in the information about the long term food habits of the individual although not as much so as the 24-hour recall. The dietary history must be recorded by a skilled interviewer which makes this method expensive and time consuming (53). The Interdepartmental Committee on Nutrition for National Defense feels that a three-day record may be a compromise suitable for some field work (35,57).

Once the dietary information is gathered, determination of the different nutrients is used to assess the diet. This can be obtained by analyzing aliquots of the foods or by calculation from tables of amounts of nutrients commonly found in foods. The difference between the methods has been the subject for a study by Whiting and Leverton (77). They compared the values from laboratory analysis with the calculated values taken from Agriculture Handbook 8 tables (73) based on weighed food intake. They found that protein and calorie content of the diets calculated by both methods were within ten per cent of either side of each other but that fat content was considerably higher if calculated from Handbook 8. Monsen, Kuhn, and Finch (48) found a high correlation between the iron content of the diets of 13 college women when both calculated from food composition tables and determined chemically.

There are several food value tables in existence used for the determination of nutrients from diets. Watt and Merrill in Agriculture Handbook 8 (73) present food values for 2,500 food items in two tables.

One of these gives the nutritive content of 100 gm. edible portions of food while the other gives the nutrient values for the edible portion of one pound of food "as purchased." The values given were derived chiefly by chemical analysis. Another food nutrient table sometimes used to determine the nutritive value of foods is Bowes and Church Food Values of Portions Commonly Used (13). This table presents values for 26 nutrients found in common portions of food. The United States Department of Agriculture also prepares a pamphlet in addition to Agriculture Handbook 8 which gives the nutritive value of foods based on common household measurements. The revised Nutritive Value of Foods (54) is currently being used as the basis for a computer program at Oklahoma State University.

The use of the computer in determining the amounts of nutrients in diets is well documented, is in practice in hospitals and nutrition clinics (9,72) and has been used in dietary surveys (27,31). The computer calculations can be done more quickly and efficiently than by hand and the possibility of error is greatly diminished. The computer has made it possible to evaluate diets more quickly and can also give a routine statistical analysis of the results (58,68). In addition to saving time, Thompson and Tucker (68) found that using the computer to calculate nutrients rather than calculation by hand actually saved money.

CHAPTER III

EXPERIMENTAL METHOD

Research Design

Fifty-four Girl Scout volunteers, ages nine to 11 years, from four different troops in Stillwater were the subjects for this study. Three dietary records and finger prick blood samples from each girl were taken from October to December of 1972. Permission for using human subjects was obtained from the Committee on Research, Experimentation or Demonstration Involving Human Subjects. See Appendix A. After acquiring the permission of the Girl Scout Chairman of the Stillwater Neighborhood of the Magic Empire Council and the leaders in the Stillwater area, leaders suggested by the Chairman were contacted and the interviewer attended the various troop meetings. At the initial meeting, each girl in the troop was given a set of papers. These included a letter of explanation of the study, a permission slip to be signed by the parents, an instruction sheet on how to keep their dietary record, a dietary record sheet, and an information sheet to be filled out giving health and dietary information. The information sheet included special diets, if used, use of dietary supplements, frequency of meals, educational attainment of the parents, and income level of the family. See Appendix A. The researcher explained the purpose of the study, what would be expected of the volunteers, and how to fill out the dietary records at this initial meeting. At the second and third meetings, the dietary records, which

had been completed by the girls on the day following their last meeting, were collected and another record sheet for that week was passed out to the girls whose parents had consented to their being in the study. At the third or fourth meeting, a laboratory technician accompanied the interviewer and collected the finger prick blood samples. Two heparinized hematocrit tubes were filled and placed in the carrying tray of the Adams Autocrit Centrifuge.¹ Enough blood was also taken from the finger prick for two hemoglobin determinations. A Unopette² disposable pipetting system was used for this. The reservoirs were labelled and the blood was diluted with the reagent as it was taken. Within two hours, all samples were returned to the Nutrition Laboratory at Oklahoma State University where the centrifuging and spectroscopic work were done. The cyanmethemoglobin samples were read in the Coleman Junior II spectrophotometer at 540 m μ . The amount of hemoglobin was then read from a Beer-Lambert graph made by using a cyanmethemoglobin standard. Although a new graph was made each time, they were all essentially the same. A copy of the graph is shown in Figure 1. At one of their meetings, the heights and weights of the girls were taken. The weights were taken using household scales calibrated by the Health-O-meter professional

¹This is the Adams Autocrit model CT-2905, 115 volts, 60 cycles AC, 1.2 amps. serial no. AD8785A which has a nominal speed of 11,500 R.P.M. and a relative centrifugal force of 13,000. Three minutes operating time will give complete cell packing. The timer dial is calibrated in one minute intervals up to 30 minutes.

²The Unopette Disposable Pipetting System consists of a plastic automatic disposable pipette and a plastic reservoir containing a modified stable cyanmethemoglobin reagent. These No. 5857 Unopettes can be obtained from Beckton-Dickinson, Division of Beckton, Dickinson and Company, Rutherford, New Jersey 07070.

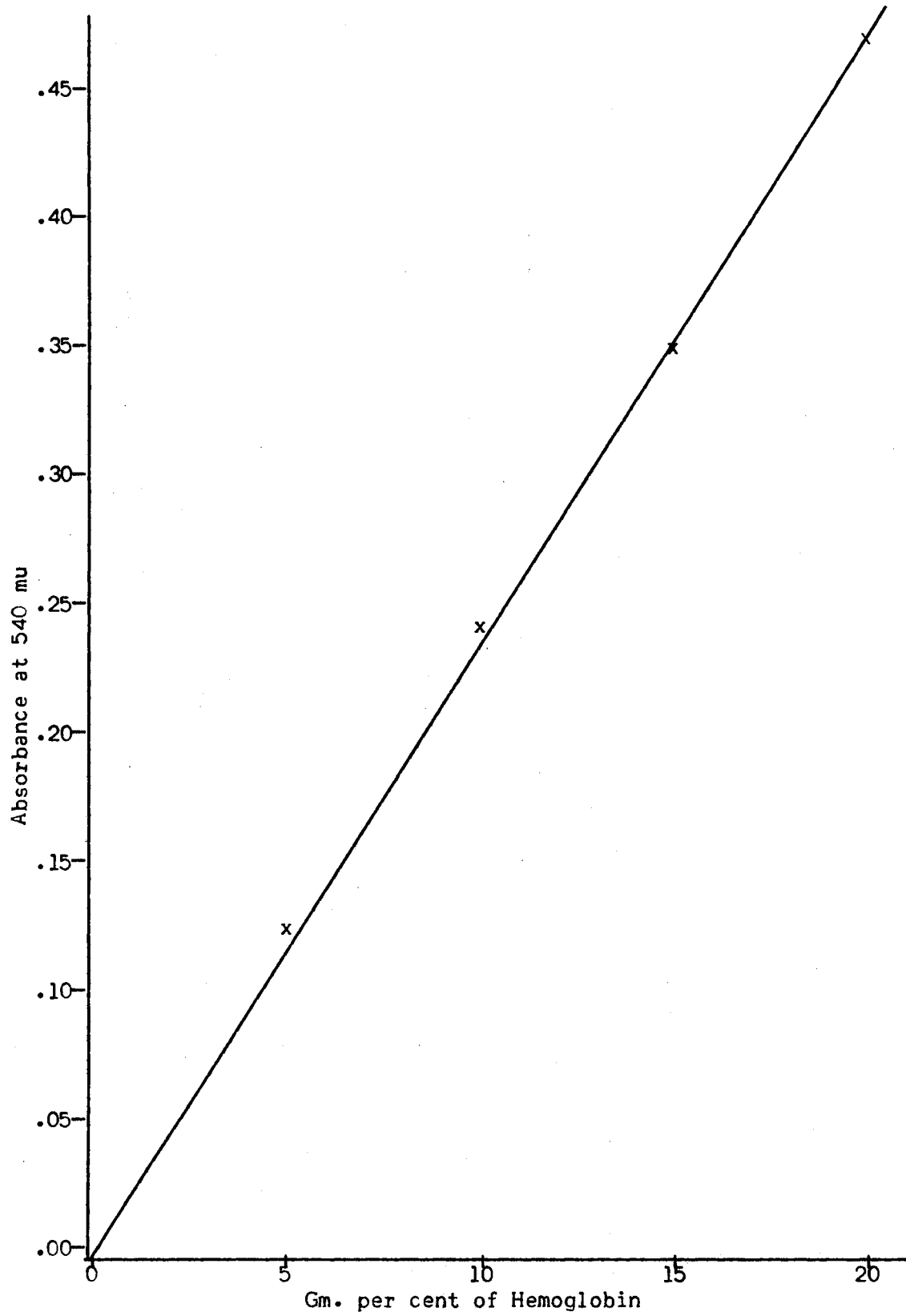


FIG. 1. Beer-Lambert plot for cyanmethemoglobin determination.

springless scales by Continental in the Nutrition Laboratory at Oklahoma State University. The heights were taken using yard sticks placed next to a wall. Another stick was used to place atop the heads of the girls to read the exact height to the closest one-half inch.

Analysis of Three-Day Dietary Records

The dietary records were analyzed by the IBM 360 computer at Oklahoma State University. The information from the records was transferred to 80 column computer cards using food codes and amounts from the Nutritive Value of Foods (54) as a reference. The amounts of the calories and following nutrients were found: protein, calcium, iron, vitamin A, thiamin, riboflavin, niacin, and ascorbic acid. Also given on the computer cards was the weight of each girl, a code number for each, her age, and the day of the dietary. Because the computer program was one already in the library of the Computer Center, basic changes had to be made in the program which was originally designed to determine calorie needs based on desirable weight and amount of exercise of men and women ages 18 to 22 years as well as the calculation of nutrients and percentage of the Recommended Dietary Allowances, 1968 (24). The changes permitted the computation of the percentage of the recommended allowances for each nutrient for nine- to 11-year-old girls and for comparing the calories eaten with the recommended allowances rather than computing them based on needs and activity. The recommended allowances are different for nine- to ten-year-olds and for ten- to 11-year-olds. The girls in this study were divided into two groups--those who were nine years to ten years and six months and those who were ten years, six months and older. For the younger group, the recommended allowances for children ages

eight to ten years were used. For the older group, comparisons were made with the recommended allowances for girls ages ten to 12 years.

Statistical Analysis of Results

A statistical analysis system from North Carolina State University³ was used for analyzing the data. This program gave correlation coefficients and other statistical information. The variables in this analysis included nutrient amounts for each day for calories, protein, calcium, iron, vitamin A, thiamin, riboflavin, niacin, and ascorbic acid; an average of the two hemoglobin determinations; an average of the two hematocrit readings; whether the subject took a vitamin or mineral supplement; height and weight; and age for each subject.

³Statistical Analysis System (Copyright Institute of State, Raleigh Division, 1972).

CHAPTER IV

RESULTS AND DISCUSSION

Biochemical and Dietary Findings

Fifty-four Girl Scouts between the ages of nine and 11 years were the subjects for this study. Hemoglobin and hematocrit values were established for each subject. In addition, a three-day dietary record was kept by each girl and an information sheet about dietary habits of each subject was completed by the parents. Height and weight for each subject was also determined.

The Interdepartmental Committee on Nutrition for National Defense (35) guidelines given in Table 2 for hemoglobin and hematocrit values were used to evaluate these parameters. The Recommended Dietary Allowances, 1968 (24) for iron for females in age groups eight to ten years and ten to 12 years were used as the standard for nutrient amounts. The recommended allowance for the former group is ten mg. per day and 18 mg. per day for the latter group. Amounts of seven other nutrients and calories were also found although iron was the primary nutrient studied. The mean percentages of the recommended allowances for calories, protein, calcium, vitamin A, thiamin, riboflavin, niacin, and ascorbic acid are given in Table 8 in Appendix B. Because the recommended allowances are different for eight- to ten-year-old females for ten- to twelve-year-old females, the subjects were divided into two groups--those from age nine to ten and one-half years and those ten and one-half years and older--so

that comparisons could be made between the two.

Table 4 gives the hemoglobin and hematocrit values for the number of subjects from the two age groups in each category.

Table 4: Hemoglobin and hematocrit values of two age groups

	deficient (< 10.0 gm. %)	low ($10.0-10.9$ gm. %)	acceptable ($11.0-12.4$ gm. %)	high (≥ 12.5 gm. %)
hemoglobin:				
9-year-olds	0	0	6	27
11-year-olds	0	0	5	16
hematocrit:	($< 30\%$)	($30.0-33.9\%$)	($34.0-36.9\%$)	($\geq 37.0\%$)
9-year-olds	0	3	6	24
11-year-olds	0	2	6	13

Since the hemoglobin and hematocrit values are related, the correlation of these factors is of interest. In this study there was a correlation coefficient of 0.902 between the hemoglobin and hematocrit values. This was significant at the 0.0001 level. Because of the high significance, it can be assumed that these two values were closely associated in this sample. There was a relationship between height and weight with a coefficient of correlation of 0.569 with an observed significance level of 0.0001. The study also showed a correlation between the height of the subject and iron intake. The correlation coefficient here was -0.184 with an observed significance level of 0.018. There was a positive significant correlation between most of the nutrients studied with only three cases where the intake of one nutrient was not related to the intake of another at the 0.05 level of significance. The consump-

tion of niacin was not related to the intake of vitamin A, ascorbic acid, or calcium.

The hemoglobin and hematocrit levels of these subjects were, for the most part, within the normal range for this age group. When compared with the ICNND (35) guidelines for this age group, 11 subjects had hemoglobin levels in the "acceptable" range. The remainder of the subjects had hemoglobin values in the "high" range. Five of the subjects had hematocrit values in the "low" range, 12 subjects had values in the "acceptable" range, and 37 subjects had "high" hematocrit values. In all cases, there was a greater proportion of the group of older girls in the low and acceptable ranges. When compared to Wintrobe's values for normal hemoglobin given in Table 1, 30 of the 54 subjects met his guidelines for normal hemoglobin values. The mean hemoglobin value for the group was 13.2 gm. per cent and the mean hematocrit value for the subjects studied was 37.9 per cent. When the subjects were studied according to the two age groups it was found that there was no significant difference between the two groups in either hemoglobin or hematocrit values. However, a joint analysis of variance of the hemoglobin and hematocrit values did reveal a significant difference between the two groups.

The scattergram presented in Figure 2 helps to show this difference. The least square lines drawn for both the younger girls and the older ones on the scattergram of hemoglobin and hematocrit values for all subjects shows the difference between the two age groups. The least square line for the nine-year-olds as compared to the 11-year-olds indicates higher hematocrit values for corresponding hemoglobin values. The mean hemoglobin value for the younger group was 13.21 gm. per cent and 13.26

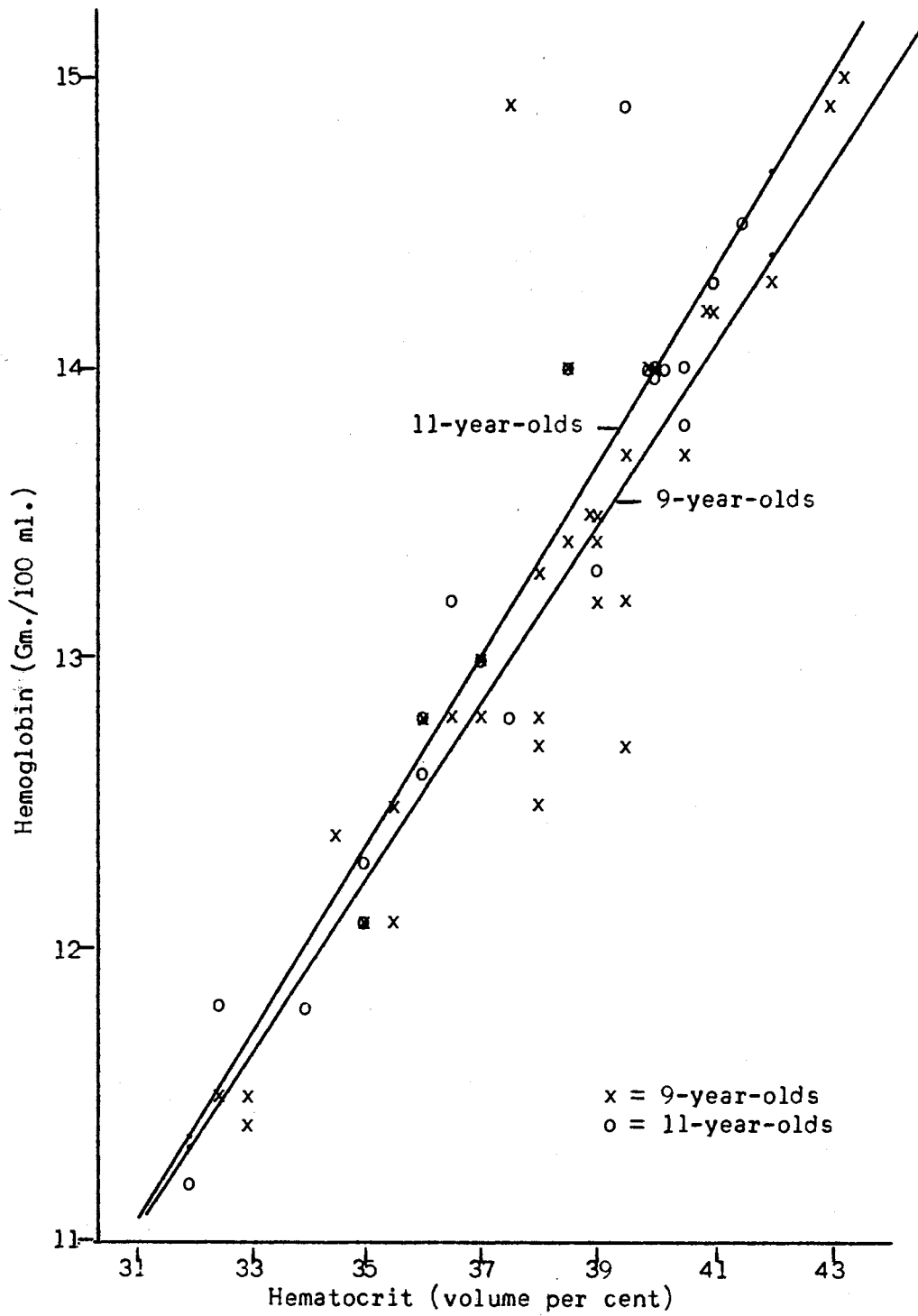


FIG. 2. Scattergram of hemoglobin and hematocrit values for nine- and eleven-year-olds.

gm. per cent for the older girls. The mean hematocrit value was 38.0 per cent for the younger girls and 37.7 per cent for the older ones. The mean values for height, weight, hemoglobin, hematocrit, and intake of iron in percentage of the recommended allowance for the two groups is given in Table 5. This shows that the older girls were heavier and taller than the younger subjects. The girls in both age groups were taller and heavier than the averages given by the 1968 Recommended Dietary Allowances (24) presented in Table 7 in Appendix B. The older girls also had a much smaller percentage of the recommended allowance for iron than did the younger girls. However, the recommended allowance is only ten mg. of iron per day for the younger group and is 18 mg. per day for the older group.

The actual mean iron intake was 12.4 mg. for the younger girls and 11.2 mg. for the older girls. The older girls who need more iron to meet the recommended allowance than the younger ones are actually getting less iron than the younger girls. The older group is getting, on the average, less than two-thirds of the recommended amount of iron. However, in this study, there was no significant correlation between iron intake and either hemoglobin or hematocrit values. There was a positive relationship significant at the 0.009 level between the use of a mineral and/or vitamin supplement and both hemoglobin and hematocrit values. There was also a correlation between the intake of thiamin and the use of a vitamin supplement which was significant at the 0.04 level.

Education and Income Levels

Although information about income was collected by means of checking categories of above or below \$4000 per year per family, it was found

Table 5: Mean values for height, weight, hemoglobin, hematocrit, and iron intake for the two age groups

subjects	number of subjects	height (inches)	weight (pounds)	hemoglobin (gm. per cent)	hematocrit (% of total)	iron intake (% of R.D.A.)
all subjects	54	56.5	84.6	13.22	37.94	100
9 to 10½ years	33	55.39	81.8	13.21	38.09	124
10½ years and older	21	58.28	89.2	13.26	37.71	62

that 48 of the 54 subjects had incomes above \$4000 which was set as the national poverty level for a family of four at the time of the study. Only two families had incomes below \$4000 per year and four subjects chose not to answer this question. Although more information about family income would have been helpful, it was felt that too specific questions about income might have limited the number of subjects willing to participate in the study. Since the main goal of the study was to determine iron status, it was felt that only broad definitions of income should be sought. Of course, no correlations could be found when there was no division of income level.

The education of the parents of the subjects in the study was quite high. This might help to account for the large number of higher incomes in the group. Only two mothers and one father had less than a high school education. Twenty-six of the mothers and 14 of the fathers had completed high school. Nineteen of the mothers and 14 of the fathers had completed a college degree and five more mothers and 28 fathers had schooling above the college degree. Two mothers were widows and two fathers were wifeless. Because the educational attainment of the parents was so high and no clear cut divisions in the groups could be made, it was decided not to use this information except to report it.

Frequency of Meals

Presented in Table 6 is the frequency of meals and snacks eaten by all subjects.

When asked about the frequency of meals--breakfast, lunch, dinner, and snacks--most of the subjects always had all these meals. Forty of the subjects always had breakfast. Nine of the subjects stated they

frequently had breakfast, four seldom had breakfast, and only one never had breakfast. Nineteen of the subjects frequently had snacks and four seldom had snacks while 31 of the girls always had snacks. Two subjects stated they had lunch frequently; all the rest of the girls always had lunch.

Table 6: Frequency of meals and snacks eaten by all subjects

meal	number of subjects in each category			
	always (7 times a week)	frequently (4 or more times a week)	seldom (less than 3 times a week)	never
breakfast	40	9	4	1
lunch	52	2	0	0
dinner	54	0	0	0
snacks	31	19	4	0

CHAPTER V

SUMMARY AND CONCLUSIONS

Fifty-four volunteer Girl Scouts between the ages of nine and 11 years from four different troops in Stillwater, Oklahoma, were the subjects for this study. After permission slips were obtained from the parents, each subject completed a questionnaire which gave information about food habits, education of the parents, and income of the family. In addition, each subject kept a three-day dietary record recording all food eaten in one day from each of three weeks. Finger prick blood was obtained from each girl for duplicate hemoglobin and hematocrit determinations. The hemoglobin determinations were made using the cyanmethemoglobin method. The dietary information was compiled by computer and results from this, the biochemical data, and the questionnaire were statistically analyzed.

It was found that this group of nine- to 11-year-old girls did have adequate hemoglobin and hematocrit levels. The correlation of 0.902 between the hemoglobins and hematocrits was significant at the 0.0001 level. When judged by the ICNND (35) guidelines, most of the subjects had hemoglobin levels that were in the "high" classification. The remainder of the girls had hemoglobin levels in the "acceptable" range. Thirty-seven of the 54 subjects had hematocrit values that were in the "high" classification; 12 subjects had "acceptable" hematocrit values; and, five subjects had hematocrit levels in the "low" range. When the

girls were divided into two groups based on nutrient needs as defined by the 1968 Recommended Dietary Allowances (24) it was found that a greater proportion of the older girls had hemoglobin and hematocrit values in the "low" and "acceptable" ranges.

There was a positive relationship that was significant at the 0.009 level between the use of a vitamin and/or mineral supplement and the hemoglobin and hematocrit values of the subjects. There was also a negative correlation between the height and weight of the subjects and their iron intake which was significant at the 0.03 level.

Not all these subjects had an adequate iron intake. That is, the younger group of girls from age nine to ten and one-half years had a mean iron intake of 12.4 mg. or 124 per cent of the recommended allowance which, for this age group, is ten mg. However, the older girls from age ten and one-half years and older had a mean iron intake of only 11.2 mg. per day which is only 62 per cent of the recommended allowance of 18 mg. for this age group. This is less than two-thirds of the recommended allowance.

There was no significant correlation between hemoglobin and hematocrit values and iron intake in this study. There was also no significant difference in the hemoglobin or hematocrit values between the two groups. Therefore, the lack of correlation between iron intake and hemoglobin and hematocrit values appears to be due to the recommended allowances for the older girls being high. That is, although the older girls are not receiving an adequate iron intake according to the recommended allowances, they do have adequate hemoglobin and hematocrit levels. This study did not assess iron stores and it may be that a difference between the age groups may be found here. It may be that the sample was not

large enough to adequately determine the relationship between the dietary and biochemical findings.

These girls do need some improvement in their diets. The mean percentages of the recommended allowances for calories, protein, calcium, iron, vitamin A, thiamin, riboflavin, niacin, and ascorbic acid are given in Appendix B. The younger girls in the study had at least 102 per cent of the recommended allowance of every nutrient except calories of which they received only 88 per cent of the recommended allowance. The older girls for whom the recommended allowances were higher for every nutrient except thiamin, niacin, and ascorbic acid had only 62 per cent of the iron, 78 per cent of the calcium, 81 per cent of the calories, 89 per cent of the thiamin, and 97 per cent of the niacin required according to the 1968 Recommended Dietary Allowances (24). Protein, vitamin A, riboflavin, and ascorbic acid were consumed in amounts greater than 100 per cent of the recommended allowances for the older girls. All the subjects received at least two-thirds of the recommended amounts of all nutrients except for iron for the older girls. This would indicate that the diets as a whole are probably adequate, except for the iron intake, for the older girls. The frequency of meals and snacks tends to reflect these high nutrient intakes. Almost all the subjects always had three meals per day plus snacks. The education of the parents and the family income were also above average for most of these subjects. This probably influences the high intake of nutrients of this group. It should be pointed out here that nutrients important for hematopoiesis other than iron (protein and the B-complex vitamins), are present in adequate amounts in these diets.

If this study of iron nutrition were to be conducted again, this

researcher feels that some measure of iron stores might be made to determine the effect of the adolescent growth spurt and beginning of menstruation on the iron stores of the oldest girls in this age group who are consuming less than the recommended amounts. It is also suggested that a follow-up study be made on the same subjects during their adolescence to determine any changes in their iron nutriture.

This researcher also suggests the use of the Unopette cyanmethemoglobin system because of the ease with which these can be used, especially if used in the fieldwork of a survey. It was also valuable to have an experienced technician to collect the blood samples since a less skilled person might encounter difficulty in obtaining an adequate sample for duplicate hemoglobin and hematocrit determinations.

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APPENDIX A

PERMISSION FROM COMMITTEE ON RESEARCH, EXPERI-
MENTATION OR DEMONSTRATION INVOLVING
HUMAN SUBJECTS

OKLAHOMA STATE UNIVERSITY

MEMORANDUM

DATE: August 22, 1972
TO: Dr. Esther Winterfeldt
FROM: Marvin T. Edmison

The Committee on Research, Experimentation or Demonstration Involving Human Subjects has unanimously agreed that there is no appreciable risk to subjects who will be involved in the project "Determination of Iron Nutritional Status in Preadolescent Girls in Stillwater." However, the Committee does wish to caution that no subject should be used who has had any history of bleeding difficulty. That is, the subject who might have some difficulty from the taking of a blood sample. Also, it is understood that informed consent in writing will be obtained from each subject and her parent, as indicated in the summary of the project. The University expects, of course, that major advisors as full time employees of the University will be responsible for the supervision of projects; this certainly is in no way meant to be an aspersion concerning the incompetence and/or responsibility of the student.

MTE:jd

cc: J. Edene Riggs

Consent Form:

Name of subject: _____

I will permit my daughter to participate in the study concerning
iron nutrition of girls between the ages of nine and eleven years.

Signed:

Parents Name

LETTER OF EXPLANATION TO PARENTS



Oklahoma State University

Department of Food, Nutrition and Institution Administration

STILLWATER, OKLAHOMA 74074
(405) 372-6211, Exts. 6007, 6091

September 18, 1972

Dear Girl Scout Parents:

As a graduate student at Oklahoma State University, I am involved in research regarding the iron nutrition of girls between the ages of nine and eleven years and the relation of that nutrition to the income and educational level of the parents. As I'm sure you are aware, iron deficiency is a major health problem today. It is hoped that this study will help to alleviate that problem.

Through the Girl Scouts of Stillwater and with the cooperation of the troop leaders, we would like to have your daughter serve as one of the subjects in our study. Her participation would involve the recording of all foods that she eats for three different 24-hour periods. A finger prick drop of blood for biochemical analysis will be needed and will be taken by trained technologists at the Food and Nutrition Department Research Laboratory on the O.S.U. Campus. This procedure has been approved by Dr. Marvin Edmison, Director of the Research Foundation. In addition, we need to ask you, her parents, to complete an information sheet concerning the health of your daughter, her regular food habits, your educational level, and a check in one of two categories of your income level.

We would appreciate your help in our study. Please have your daughter return the attached consent form to me at her next Girl Scout Meeting if you are willing to allow her to participate.

Very Sincerely,

Edene Riggs

Edene Riggs, Graduate Student
Department of Food, Nutrition
and Institution Administration
Oklahoma State University

Helen F. Barbour

Helen F. Barbour, Professor
Thesis Advisor
Department of Food, Nutrition &
Institution Administration
Oklahoma State University

P.S. If you have any questions, I would be glad to answer them. Please call Edene Riggs at 372-6211, Ext. 457 or 372-4038.

DIRECTIONS FOR FILLING OUT DIETARY STUDY SHEETS:

1. Record everything put into the mouth and swallowed.
2. Record the food eaten as frequently as necessary to include an accurate account of all the food which you consumed each day. Suggestion: Record at least once every day, more often if possible.
3. Record the time of day when you consumed the meals and snacks you listed.
4. For each food consumed:
 - a. Describe the type of food eaten. Example: fried, poached, baked, steamed, broiled, roasted, creamed, buttered, raw, peeled, skim, 2%, whole, instant non-fat dry, etc.
 - b. Record amount of food ingested:
 1. Record liquids according to standard 8 oz. measuring cup.
 cooked vegetables (e.g. $\frac{1}{2}$ c. buttered green beans)
 canned fruits
 cooked cereals
 dry cereals
 mixed dishes such as casseroles, salads
 cottage cheese
 ice cream, puddings, etc.
 liquids (e.g. 6 oz. orange juice, 4 oz. 2% milk)
 2. Record according to dimensions:
 cooked meat (e.g. 1 hamburger patty 3" diam., $\frac{1}{2}$ " thick)
 cakes
 pies
 crackers
 hot breads (muffins, biscuits, rolls) diameter and thickness
 3. Record according to portions commonly served:
 fresh fruit (e.g. 1 navel orange, 2" diam.)
 raw vegetables
 sliced bread (e.g. 1 sl. enriched white)
 sliced cheese, lunchmeat (e.g. 1 sl. bologna)
 eggs, frankfurters, number eaten
 4. Record according to teaspoons or tablespoons:
 butter or margarine
 mayonnaise
 sugar
 mustard
 catsup, gravies, whipped cream and all other condiments
 - c. In addition to amount and description of food, record the brand names for the following:
 candy
 soft drinks
 crackers, snacks such as daisies, pretzels
 any food fortified in addition to regular nutritive value, such as Hi-C fruit drinks, milk with added skim milk solids, Tang
 - d. If the food is in combination with other foods, such as a casserole, sandwich, describe the amount of each food in the serving.

DIETARY INFORMATION SHEET

To the Parents: This information sheet is designed to give us information about your daughter so that a study of iron nutrition of 9-to 11-year-old girls can be made. All answers will be kept confidential. Please do not place names on this or the dietary record sheet.

PLEASE RECORD ALL INFORMATION ABOUT YOUR DAUGHTER AS ACCURATELY AS POSSIBLE

1. Age: _____ years. Birthdate: Day _____
Month _____
Year _____
2. Height: _____
3. Weight: _____
4. Please check the highest grade or degree level:
- Father 6th grade _____ 8th grade _____ H.S. grad _____
college grad _____ above _____
- Mother 6th grade _____ 8th grade _____ H.S. grad _____
college grad _____ above _____
5. Income level of family (please check): above \$4000/year _____
below \$4000/year _____
How many members in family _____
6. Health:
- a. Is she on a special diet or medication prescribed by your doctor?
Yes _____ No _____
If yes, briefly describe the diet or medication _____

- b. List food allergies, if any _____
- c. Describe the kind and amount of food supplements which she is now taking,
such as vitamin pills (see label on bottle) _____

7. Check the following categories according to number of meals usually eaten:

	ALWAYS (7 times a week)	FREQUENTLY (4 or more times a week)	SELDOM (less than 3 times a week)	NEVER
BREAKFAST				
LUNCH				
DINNER				
SNACKS				

RECORD OF FOOD INTAKE

DATE _____

Food - Name and description**Amount**

BREAKFAST:

LUNCH:

DINNER:

SNACKS:

APPENDIX B

Table 7: Recommended Dietary Allowances, 1968 (11)

nutrients	recommended amounts for each age group of females	
	8- to 10-year-olds	10- to 12-year-olds
height	52 inches	56 inches
weight	62 lbs.	77 lbs.
calories	2200 Kcal.	2250 Kcal.
protein	40 gm.	50 gm.
calcium	1.0 gm.	1.2 gm.
iron	10 mg.	18 mg.
vitamin A	3500 I.U.	4500 I.U.
thiamin	1.1 mg.	1.1 mg.
riboflavin	1.2 mg.	1.3 mg.
niacin	15 mg.	15 mg.
ascorbic acid	40 mg.	40 mg.

Table 8: Per cent of R.D.A. (11) for nutrients for two age groups

subjects	number in group	nutrient in per cent of R.D.A.								
		<u>calories</u>	<u>protein</u>	<u>calcium</u>	<u>iron</u>	<u>vitamin A</u>	<u>thiamin</u>	<u>riboflavin</u>	<u>niacin</u>	<u>ascorbic acid</u>
all	54	86	184	93	100	126	100	152	105	157
9-10½ years	33	88	204	102	125	142	107	159	110	166
10½ years and older	21	81	153	78	62	101	89	143	97	144

VITA^r

Janice Edene Riggs

Candidate for the Degree of

Master of Science

Thesis: ADEQUACY OF IRON NUTRITURE OF PREADOLESCENT GIRLS

Major Field: Food, Nutrition and Institution Administration

Biographical:

Personal Data: Born in Weatherford, Oklahoma, November 19, 1947, the daughter of Mr. and Mrs. Eddie Short. Married to Charles Lee Riggs, May 27, 1967.

Education: Graduated from Weatherford High School, Weatherford, Oklahoma, in 1966; graduated from Southwestern State College (Weatherford, Oklahoma) in 1970 with a Bachelor of Science degree in Home Economics; studied at Oklahoma State University from 1970-1973 and completed the requirements for the Master of Science degree in July, 1973.

Professional Experience: Graduate Teaching Assistantship at Oklahoma State University, Department of Food, Nutrition and Institution Administration, from 1970-1973.

Professional Organizations and Honors: Member of Gamma Delta Kappa, Omicron Nu, and Phi Upsilon Omicron honor societies; member American Home Economics Association; recipient of the General Foods Fund Fellowship.