ANALYZING THE RIBOFLAVIN STATUS OF ADOLESCENT GIRLS

By

MARGARET ANNE HAYDEN FRISTOE

Bachelor of Science

Mississippi University for Women

Columbus, Mississippi

1980

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1982

ANALYZING THE RIBOFLAVIN STATUS

OF ADOLESCENT GIRLS

Thesis Approved:

Mary Alice Kenney
Thesis Adviser

Eldon C. Nelson

N. Sue Knight

Norman N. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to extend my sincere gratitude and appreciation to Dr. Mary Alice Kenney, my major thesis adviser, Dr. Sue Knight, and Dr. E. C. Nelson, committee members, for all the guidance, assistance, encouragement, and patience extended to me throughout the duration of this research project.

Thanks are extended to all the graduate students, faculty, and staff involved in the adolescent study; their hard work on this study is greatly appreciated. I would also like to thank Sharon Phillips, who typed the final copy of this manuscript.

Gratitude is expressed to my husband, Conner, for his love, patience, support, encouragement, and pleasant distractions throughout my graduate studies.

Appreciation is extended also to my family: Maggie (Mom), Lee (Dad), Jan, Holly, Mike, Patty, Nathan, Leej, Dan, and Michael; and to my friends for their never ending support and encouragement during the past two years.

It is to my husband and family that I wish to dedicate this thesis.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

CHAPTER I

INTRODUCTION

The nutritional status of all populations needs to be monitored as changes in lifestyle and culture cause changes in eating habits (1). Monitoring the nutritional status of all age groups allows the researcher to uncover deficiencies of nutrients due to changing eating habits or to lack of knowledge about nutrient needs. By uncovering and treating deficiencies of nutrients through supplementation and nutrition education, clinical deficiencies and other medical problems can be prevented. Nutritional status studies have also been recognized for their value in developing nutrition policies, because these studies identify problem areas of nutrition and trends in nutritional status. Monitoring nutritional status is a preventative form of health care. Due to the rising cost of health care, there should be more emphasis on the prevention rather than the treatment of diseases (2, 3, 4).

In order for studies of nutritional status to be valid, standards for assessing status and methods of data collection need to be evaluated and improved, if necessary (4). There is a need for research in methodology for determining nutritional status. There is a hesitation

among the general population to participate in nutritional studies (5, 6), and the hesitation to become involved is greater when biochemical or clinical data are required (7); therefore, less invasive methods of determining nutritional status need to be evaluated. This study will evaluate three methods used to judge riboflavin status.

Adolescents, as a group, have many characteristics which encourage examination of their nutritional status. Adolescents grow at different rates from each other, and have different physiological needs than children or adults (8). Because nutrient recommendations are based on chronological rather than physiological age, they can only be regarded as rough approximations for adolescents (9). Recommendations for adolescent nutrient needs are based largely on fragmented, little, or no data. Nutrient requirements for adolescents have often been based on the requirements of younger children or adults (10). Previous nutritional surveys have provided biochemical evidence of riboflavin deficiency in adolescents (7, 11, 12, 13). For these reasons, the riboflavin nutritional status of adolescents needs to be monitored.

This study will examine the riboflavin status of adolescent girls through various methods. It is hoped that this research will help those examining the nutritional status of adolescents and help those who seek to improve the methods used to examine nutritional status.

Purpose and Objectives

The purpose of this study was to assess the extent to which urinary excretion of riboflavin reflects riboflavin intake, as recalled the next day, and riboflavin saturation of the body, as indicated by erythrocyte glutathione reductase (EGR) stimulation.

The following objectives were developed to guide the study:

1. To determine if urinary excretion of riboflavin in a rising urine specimen, relative to creatinine excretion, reflects recent riboflavin intake;

2. To determine if urinary riboflavin, relative to creatinine, reflects the riboflavin state in the body, as shown through erythrocyte glutathione reductase stimulation.

Hypotheses of the Study

The following null hypotheses were examined:

1. There is no significant relationship between the riboflavin status of members of the population as determined by riboflavin excretion and the status suggested by dietary intake of riboflavin.

2. There is no significant relationship between the riboflavin status of members of the population as determined by the degree of erythrocyte glutathione reductase stimulation by riboflavin containing cofactor, an accepted index of body state.

Assumptions

The following assumptions were recognized in the study:

1. It is assumed that the respondents were accurate in recalling all food items eaten the preceeding 24 hours and in estimating amounts eaten.

2. It is assumed that the respondents' 24-hour dietary recall was typical of their daily intake.

3. It is assumed that the riboflavin relative to creatinine excretion in the urine samples was not affected by variables that may affect urine composition, such as work, sleep, heat, and diuresis.

Definition

The following concept is defined for clarity in how it was used in the study. Riboflavin status is the degree to which an individual's physiological need for riboflavin is met by the intake of riboflavin. It is the result of a balance in the individual between riboflavin intake and riboflavin catabolism or excretion. The riboflavin status of a subject is reflected in several measurements such as the excretion of riboflavin and an activity coefficient, which represents the degree of erythrocyte glutathione reductase stimulation by an in vitro addition of riboflavin containing cofactor in the form of flavin adenine dinucleotide (FAD).

CHAPTER II

REVIEW OF LITERATURE

Sources of Riboflavin

Riboflavin belongs to a group of yellow fluorescent pigments called flavins. Riboflavin combines in the tissues with phosphoric acid to become part of the structure of two flavin coenzymes, flavin monomucleotide (FMN), and flavin adenine dinucleotide (FAD) (14).

Riboflavin is found in a wide variety of foods, though it is usually found in small quantities. The best dietary sources are milk and other dairy products. Organ meats also contain high concentrations of riboflavin. Lean meats, eggs, and green vegetables are also important daily sources of riboflavin. Fortified breads and cereals contribute to the total daily riboflavin intake (14). A mixed diet with milk and meat products is not likely to be low in riboflavin, but with the omission of dairy and animal foods the diet may easily become deficient in riboflavin (15).

Riboflavin is a B vitamin and is sparingly water soluble. It is relatively stable to heat, oxidation, and acid. It disintegrates in the presence of alkaline substances (14). The rate of riboflavin destruction increases as pH and temperature increase (16). It is very sensitive

to light, especially ultraviolet light (14). Exposure to the sunlight affects the riboflavin content greatly. When milk was widely delivered in glass bottles, this characteristic of riboflavin was significant. Milk, on exposure to sunlight in glass bottles, loses 60 percent of the riboflavin content in two hours (17). The improvement of milk containers has helped decrease the riboflavin lost (18). Riboflavin also acts as a sensitizer of the destruction of other vitamins (17). Luminflavin, the product resulting from riboflavin destruction, destroys the ascorbic acid in milk. Another study (19) showed that summer commercial milk contained 20 percent more riboflavin than winter milk. This suggests that the summer pasture provides the cow with a better riboflavin source than winter grazing or feeds may provide.

The content of riboflavin in foods is variable. When processing, storing, and preparing foods, the factors which cause riboflavin content to vary should be considered. The use of good food preparation and storage techniques along with the saving or use of thaw and cooking liquids helps to keep riboflavin loss low. Riboflavin is found in a wide variety of foods and is relatively stable to most environmental factors except alkaline substances and ultraviolet light.

Riboflavin Metabolism

Riboflavin combines in the intestinal mucosa with phosphoric acid to become part of two flavin coenzymes,

RAD and FMN. These coenzymes catalize oxidation and reduction reactions in cells as hydrogen carriers in the mitochondrial electron system (14). The enzymes with which they function include the amino acid oxidases, xanthine oxidase, the succinic dehydrogenase complex, glutathione reductase, and others (15). Cellular growth cannot evolve in the absence of riboflavin (14).

Most studies in the area of absorption and elimination have used large doses of riboflavin and are therefore more useful when examining mechanisms of these processes in regard to riboflavin, rather than investigating the quantitative aspects of these mechanisms when riboflavin is from normal dietary sources (20). Riboflavin is absorbed by a specialized transport process that is localized in the proximal small intestine (20). Two studies featured support this theory. One study (21) showed that when a 30 mg dose of riboflavin was given, 50 percent of the oral dose was recovered. Rapid absorption of riboflavin is reflected by the early appearance of peak excretion rate (21). Such rapid absorption indicates that specialized transport may be used in absorption. Specialized transport is also indicated through site specificity. A study in this area (22), comparing riboflavin administration by various routes, revealed that riboflavin absorption occurred mostly in the small intestine.

Riboflavin is eliminated from the body through renal excretion, biliary excretion, minor losses with various

body secretions and metabolic conversions to other flavins, coenzymes, and flavoproteins (23). The condition of the subject and size of the riboflavin dose determines the amount of riboflavin to be recovered and how quickly it will be recovered (23). The renal excretion of riboflavin involves glomerular filtration, tubular secretion, and tubular reabsorption. The contribution of each to the renal output depends on the amount of vitamin in the body. For example, in normal subjects intravenous doses of 30 mg riboflavin could be almost completely recovered in the 24-hour urine collection (23), whereas only 39 percent of a 1 mg intravenous dose was found in the urine. In subjects with ariboflavinosis, only 15 percent of a 1 mg dose was recovered

Administration of either riboflavin or FMN results in the urinary excretion of mainly riboflavin (23). No significant amount of other urinary excretion products are produced in man after large intravenous doses of riboflavin (23). Degradation products of riboflavin have been found in the urine after a 1 mg oral dose of riboflavin. Part of the unabsorbed vitamin was degraded by bacteria in the gastrointestinal tract to hydroethylflavin which was then absorbed and appeared in the urine 24 hours or more after the dose (25). Riboflavin is absorbed in the small intestine, combines to become part of two coenzymes, and is excreted as mainly riboflavin without other significant excretion products.

Dietary Intake of Riboflavin

The nutritional status of an individual cannot be adequately judged by the nutritive value of the nation's food supply, as in food consumption surveys, or of an individual food intake alone. Even knowledge about the food consumption of families does not reveal the intake of family members because certain members may eat well or poorly due to family food patterns or individual food habits (26). Individual food habits can be evaluated through the use of 24-hour dietary recalls, dietary histories, or various other questionnaires. Through the use of food samples, representing different size portions of food, trained personnel, and detailed questions, it is possible to get data on the kinds and amounts of food eaten on the previous day (22). The meal-by-meal estimation and recording of food gives a view of all food items eaten, but the amount recorded may be just approximate. Measurement of food intake can help correct this disadvantage, but this is impractical in large research studies. The recording or disclosing of food intake can cause an individual to deviate from normal eating habits (20).

One-day diet records show close agreement with seven-day food records in a large population (28). In small samples, however, there is much variation in the nutrient means obtained from each record. If one-day intake values are held valid for an individual's intake, problems may

arise. A person may eat far more or less on a given day than usual, so an individual's intake of nutrients may be grossly different from a one-day intake than from, say, a seven-day average (28).

A 24-hour dietary recall is a verbal or written recall of food eaten in the past 24 hours. Such recalls, because they depend on memory, are subject to human error. One-day dietary recalls, carefully taken and analyzed, have a real value in determining the intake of a population. One-day dietary records should not include feast days, fast days, or days of illness because of dietary variations, unless these days occur frequently and are usual for the individual. In females, the dietary intakes on Sundays was higher than on weekdays (29). There are also seasonal variations in the dietary intake in regard to some nutrients such as vitamin C, calcium, and riboflavin (30). One study (31) examined the validity of the 24-hour recall. No significant difference was found in regard to riboflavin and most nutrients except kilocalories between the 24-hour recall and the actual intake. Milk and milk products as a group are remembered well in nutritional recalls (32). Since this food group contains good sources of riboflavin, the recall of riboflavin is believed to be of value.

Replications of the 24-hour dietary recall have a special and potentially important use in studies which require high levels of accuracy for individual data.

Repeated recalls should be used more frequently in studies where there is not an extreme range of variation in nutrient intake among the population. When daily variation is extreme, the recalls will reveal this situation and indicate that no method short of extensive daily sampling is likely to describe the dietary intake in those individuals (34). The longer the interval between measurements, the greater the chance for changes in food habits. Recollection of the first interview should not influence the second interview. Repeated recalls for individuals correlated poorly with each other, but at the group level the results of the repeated 24-hour recall were very similar (34). Many points are raised to emphasize the hazards of placing too much weight on nutrient intake data. However, such data do provide guides for evaluating the nutrient intake within reasonable limits for various population groups (20, 26).

The Recommended Dietary Allowances of the Food and Nutrition Board of the National Research Council are designed for the maintenance of good nutrition in all healthy people of the United States (35). The RDA are set at levels above the physiological requirements to cover variations in the needs of all population groups. Requirements differ with age, body size, sex, and stage of growth and are also influenced by activity and environment. The RDA, when used wisely, have much value in providing goals for population groups that will assure them good nutritional

status (26). Analyzing the methods of recording dietary intakes and recognizing their advantages and disadvantages helps the researcher to select a method to accurately recall dietary intake.

Urinary Excretion as an Indicator of Status

The body metabolizes little riboflavin so that urinary excretion appears to correlate well with reserves and intakes of the vitamin (36, 37). However, in conditions of negative nitrogen balance, fasting, bed rest, heat stress, and starvation, deceptively high excretion levels of riboflavin may be found. On the other hand, sleep and short periods of heavy work decrease riboflavin excretion (38).

If urine is to be used for an assay of nutritional status, the researcher must determine when and what size urine samples will be collected. The 24-hour urine specimen provides the most accurate estimate of the daily excreted nutrient amount (38). This method is hard to use in field surveys and has the disadvantage of being physically difficult and sometimes embarrassing to collect. It is difficult also to assess the completeness of the sample, since a portion of the sample could have been missed. A random specimen is easier to obtain, but does not provide the accuracy found in a timed or 24-hour urine specimen. This is because it has the disadvantage of varying sizes of samples, physical activity, liquid consumption

prior to collection, and the time of day when the sample is collected (38). All these factors may affect the riboflavin concentration. A timed urine sample also shows many of these disadvantages and is not practical to collect in many nutritional studies (27). The overnight or first voided morning specimen has merit because there is some standardization in the physical activity and the liquid consumption during the collection period (38). This specimen is also more convenient for individuals to give because most people experience a need to urinate upon waking.

If nonfasting samples are collected, urinary riboflavin levels may be elevated, reflecting recent riboflavin intake. However, this effect is minimal in individuals with marginal or inadequate intakes of ribflavin or in those whose body stores are depleted or not saturated. Under these conditions, any riboflavin eaten would be largely retained rather than excreted. The errors associated with random or overnight urine samples are of reduced concern, since they would be largest in those with adequate intakes of riboflavin (38, 39).

In many nutritional surveys, the amount of a nutrient excreted has been expressed in relation to the amount of creatinine excreted. Creatinine is a urinary constituent resulting from tissue catabolism and is eliminated at a fairly constant rate by an individual in amounts roughly proportional to lean body weight (5). Comparison

of creatinine to other urine nutrients is based on the assumption that creatinine excretion is relatively constant in the amount and rate of excretion and that the concentration in any random urine sample could be used to derive a factor applicable to analyzing other nutrients (40). Expressing the urinary riboflavin per gram of creatinine has the advantage of correcting for variation due to the dilution of urine as the solute load and body size vary (39). Creatinine excretion does vary with diet, body size, age, sex, time of day, and meat intake, but varies less than most other components of urine. Urine creatinine differs from one individual to another, but the excretion for any given individual may be reasonably constant (27).

Clark (39) concluded that riboflavin-to-creatinine ratios would be useful in evaluating the nutritional status of population groups but not of individuals, based on studies of adolescent girls and boys. One study (41) showed that the urinary excretion of riboflavin per six hours can be predicted from the excretion per gram of creatinine within limits of 30 to 40 percent. The predictability is affected by variations in body size, creatinine excretion, and intake of creatinine. The predictability is more accurate with a fasting urine sample (41). Plough and Consolazio (41) concluded that for surveys of large groups of individuals, the measurement of vitamin excretion per gram of creatinine in

casual urine samples is a satisfactory procedure in the biochemical evaluation of nutritional status.

Despite the criticisms against the use of creatinine as a basis for determining the excretion of vitamins in casual specimens of urine, it is still a helpful tool in nutrition surveys of large groups. Urinary vitamin-to-creatinine ratios are useful indicators of the nutritional intake of a population. When a large percentage of the population shows low excretions, it may be concluded that the intake of that nutrient is substandard and clinical signs of deficiency may be anticipated if such practices continue (27). Analyzing the urine for riboflavin and relating that to creatinine gives the researcher a good index on which to judge riboflavin status.

Blood Constituents as an Indicator of Status

Plasma levels of nutrients reflect, to a large degree, recent dietary history rather than long term nutritional status. The use of fasting samples will reduce some of the variance in nutrients in the body due to variation in recent dietary intake (38). Riboflavin, FMN, and FAD have been measured in blood, plasma, and erythrocytes (38, 42). Under conditions of restricted riboflavin intake, the riboflavin content of erythrocytes and plasma decreased (42). Plasma levels of riboflavin may be more related to the riboflavin consumed just before sampling

than to the riboflavin saturation of body tissues. The riboflavin content of the erythrocytes was less sensitive to dietary influences than was that of plasma, as shown by the slow recovery of erythrocyte riboflavin content during repletion (36). The erythrocyte should therefore be a better index of riboflavin saturation of body tissues than plasma (36).

A recently developed method of evaluating riboflavin is based on the measurement of erythrocyte glutathione reductase. Erythrocyte glutathione reductase (EGR) is one of two flavoproteins in the erythrocyte requiring FAD. Protein-bound riboflavin, such as that in EGR, is not markedly affected by daily fluctuations of dietary riboflavin intakes because tissue riboflavin, in the form of FMN and FAD, is not mobilized until the circulating free riboflavin has been depleted. The degree of activity of EGR, with and without riboflavin containing cofactor, FAD, is expressed by an activity coefficients (AC). The activity coefficient was related to riboflavin status in individuals during partial riboflavin depletion and repletion. Urinary riboflavin was less useful in measuring the severity of the riboflavin deficiency than EGR activity. The sensitive response of AGR to FAD addition is assumed to be representative of the response of other tissues to riboflavin and to reflect the degree to which an enzyme is saturated with vitamin containing cofactor. High activity coefficients of EGR identified those individuals

receiving marginal amounts of riboflavin for long duration as well as those suffering from severe ariboflavinosis. Activity coefficients should be useful in nutritional surveys for the evaluation of riboflavin status of individuals. The activity coefficients of EGR are a sensitive measure of the riboflavin metabolic status in humans (43).

Riboflavin Status of Adolescents

The riboflavin status of adolescents as examined through dietary, urinary, and blood constituents will be reviewed. Riboflavin deficiency has been reported in adolescent populations in the United States. Riboflavin intakes of 31 percent of the white and 24 percent of the black adolescent girls of one such population were below 67 percent of the Recommended Dietary Allowance (RDA) (45). The greatest prevalence of riboflavin deficiency in adolescents (12), as shown by EGR activity coefficients, was among those who consumed less than one cup of milk per week, and the lowest prevalence, in those who consumed more than three cups a day.

Urinary excretion of riboflavin by one adolescent sample of over 400 averaged 369 $\pm$ 22 µg riboflavin/g creatinine (13). Thirty percent of all girls, including 27 percent of the white girls and 47 percent of the black girls, excreted less than the desirable level (13). Riboflavin deficiency occurred in 17 percent of male and female adolescents at the poverty level in a survey based on excretion of riboflavin (7).

Based on EGR stimulation, 11 percent of over 400 adolescents (13) had riboflavin deficiency, while 16 percent of all girls and 38 percent of the black girls were deficient. Another study (11) revealed that 11 of 100 subjects, ranging in age from 2-1/2 days to 14 years, had EGR stimulation indicating riboflavin deficiency. In another adolescent population (12) 23 percent of the sample, including 15 percent of the whites, 20 percent of the Hispanics, and 34 percent of the blacks were deficient. Due to the small number and variation of the studies in this area, it is hard to make definite conclusions about the riboflavin status according to sex, race, age, location, or time of the study. However, examination of intakes reveal that white males had the best riboflavin intakes followed by black females, white females, and black males (45). The riboflavin excretion and EGR stimulation show that white males again had the best status, followed by white females, black males, and black females (13). Generally, males and whites appear to have the best riboflavin status.

There is much discussion as to the possible causes of riboflavin deficiency in adolescents. The cause may be dietary habits which lead to low riboflavin intakes, as suggested by several studies (12, 44, 46, 47). Apparent riboflavin deficiency may in some cases be due to lack of adequate research upon which to establish guidelines for judging riboflavin nutriture (9, 48). Any effect of oral

contraceptives on riboflavin nutriture may have some effect on the status of adolescents who become sexually active. Riboflavin excretion was not significantly different between the oral contraceptives (OC) and the non-OC users (49). Oral contraceptive users appeared to have lower EGR levels than non-OC users, although this difference was not significant. This led the researchers to suggest that there may be a risk of depletion of riboflavin body stores when marginal dietary intake of riboflavin is coupled with OC use. The riboflavin status of adolescents and the methods used to determine that status have been reviewed. This study will examine the riboflavin status of a sample of female adolescents using three methods to determine riboflavin status.

CHAPTER III

PROCEDURES

This research was conducted as an ancillary study in connection with the Southern Regional Research Project S-150 "Nutritional Health of Adolescent Females," funded by the United States Department of Agriculture. This study involves the states of Oklahoma, Arkansas, Virginia, Tennessee, Louisiana, North Carolina, South Carolina, and Alabama. Only the data from Oklahoma were used in this study. The subjects included all S-150 subjects and additional girls who failed to meet age intervals specified for the regional study. Girls were studied between February and May of 1981, and analytical work was completed between February and December of 1981.

Sample

The population was adolescent girls enrolled in selected public schools and youth groups in various sites in north-central Oklahoma. The girls who volunteered for the research and their parents were required to give informed consent for participation. The volunteers were given special directions and instructed to meet at the data collection sites on designated days, usually Saturdays,

where dietary, sociological, psychological, anthropometric, biochemical, and clinical data were gathered from each subject.

One hundred and fifty girls participated in the study. Their distribution by age, race, and income is shown in Table I. Seventy-seven percent of the population were of the white race and 23 percent were of the black race. Two of the girls classified as black were part American Indian. The mean, standard deviation, and range of the variables of age, income, and body weight is shown in Table II. The average age of the girls was 13 years and 10 months. The ages ranged from 11 years and 8 months to 16 years and 6 months. The average per capita income was $5,925, with a range of $800 to $20,000. The average body weight was 114 pounds, with a range of 72 to 195 pounds. Thirty-one percent of the sample was pre-menarcheal; 69 percent was post-menarcheal. The girls were broken into three age categories. The 12-year-old category included all subjects less than 13.5 years. The 14-year-old category included all those girls between 13.5 to 15.5 years. The 16-year-old category included those girls older than 15.5 years. Urine and blood samples were not available from all subjects.

Data Collection

Dietary

Information was taken through an interview which

TABLE I

POPULATION DISTRIBUTION BY AGE, RACE, AND INCOME

Variable	Age Categories (yrs.)			Total
	12	14	16	
Race				
White	41 (28)*	44 (30)	27 (18)	112 (77)
Black	8 (5)	20 (17)	6 (4)	34 (23)
Total	49 (34)	64 (44)	33 (23)	146 (100)
Per Capita Income Categories				
< $5,500	25 (17)	31 (21)	17 (11)	73 (49)
≥ $5,500	27 (18)	33 (22)	15 (10)	75 (51)

*Parentheses designated percentage of total sample.

TABLE II

MEAN, STANDARD DEVIATION, MINIMUM, AND MAXIMUM VALUES OF THREE VARIABLES

Variable	n	Mean	SD	Min. Value	Max. Value
Age, months	150	166	17	140	198
Per Capita Income, Dollars	148	5,925	3,180	800	20,000
Body Weight, Kilograms	147	51.6	10.3	32.8	88.4

included a 24-hour recall and questions about meal patterns and food habits. The subjects were asked to recall all that was eaten after waking on the previous day. Trained nutritionists interviewed the subjects. A set of food models made of paraffin-coated rice resembling the shapes of food on a plate, a set of calibrated beverage glasses, a card with various circles and squares, and a ruler were used as aids for estimating the sizes of food servings. Food items and amounts were coded. Nutrient totals were calculated by the Nutritional Analysis System in Baton Rouge, Louisiana. The riboflavin intakes of the subjects were evaluated by comparison with the Recommended Dietary Allowances for riboflavin for their age. Two dietary recalls were taken. The dietary data from the day previous to the urine and blood collection was used for analyses relating riboflavin excretion and intake, since this intake might have more influence on the urine reading. Nutrients from a dietary recall taken two or more weeks after the first recall were averaged with those from the first recall for relating intake to EGR stimulation.

Urine

The subjects were instructed to bring with them to the research site a urine sample collected from the first void of the day. They had fasted from 10:00 p.m. the previous night until blood was drawn after 8:00 or 9:00 p.m. Urine was protected from light and was acidified

with 1 ml of glacial acetic acid per 25 ml of urine. It was stored frozen until it was analyzed by this researcher. Riboflavin excretion was determined by a modification of the fluorometric technique which measures the fluorescence of the riboflavin after oxidation-H_2O_2 and after extraction with a butanol-pyridine solution. An internal standard is used and the blank is determined by destruction of the riboflavin by irridation (6, 50). Creatinine was determined by the alkaline picrate procedure in which creatinine reacts with picrate to produce a stable, intense, orange color which is measured spectrophotometrically (6, 51). The riboflavin excretion will be expressed as micrograms of riboflavin per gram of creatinine; values below 70 µg/g creatinine are considered indications of riboflavin deficiency for 10 to 15 year olds (7, 38, 39, 45).

Erythrocytes

A blood sample was taken from each subject by a medical technologist. Heparized blood was centrifuged. Plasma and white cells were removed and red cells were washed twice in two volumes of 0.9 percent sodium chloride. A final 1:20 dilution of cells in water was stored frozen until it was analyzed by a team researcher. Erythrocyte glutathione reductase was determined spectrophotometrically, with and without added riboflavin-containing cofactor, by the method of Tillotson and Baker (43). The rate of enzyme reaction is measured by loss of the

substrate color. The assay results were expressed in terms of activity coefficients (AC) representing the degree of stimulation resulting from the in vitro addition of FAD.

The activity coefficient is expressed as:

$$AC=\frac{\text{reduction of absorbance with added FAD/10 minutes}}{\text{reduction of absorbance without added FAD/10 minutes}}$$

Since the EGR activity coefficient appeared to be unrelated to age and sex, a single value of 1.20 has been used for all ages to indicate the upper limit of normal. Activity coefficients about this value, indicating a 20 percent increase in stimulation, have identified those deficient in riboflavin (38, 45).

Analysis of Data

The data were analyzed to examine how the status determined by riboflavin excretion was related to the status that was suggested by dietary riboflavin and that was determined by EGR stimulation. In order to relate variables or account for the effects of riboflavin intake, riboflavin excretion, and EGR stimulation, Pearson correlation coefficients (r), analysis of variance, and analysis of covariance were calculated. The effects of other variables such as age, race, income, calcium intake, protein intake, weight, day of data collection, menstruation, and vitamin usage were examined against the three methods of

determining riboflavin status using regression and Pearson correlation. The variables were tested both as categorical and as continuous data when possible, to identify either linear or non-linear effects. To account for the effects of more than one variable simultaneously, an analysis of covariance was carried out using GLM in the Statistical Analysis System (SAS) computer program (52). For the three methods of determining riboflavin status, categories were developed to identify good and poor values. These methods were compared against each other using chi-square tests for distribution for all pairs of variables. A level of significance for determining relationship or differences was established as $p \leq 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

Results

The results can be divided into three categories of data: dietary, riboflavin excretion, and erythrocyte glutathione reductase (EGR) stimulation. First, each of the three types of data will be presented and discussed along with the results of other studies. Then the results will be applied to the acceptance or rejection of the hypotheses. Finally, the population status and the methods used to determine status will be discussed. The population has been described according to general characteristics in Tables I and II.

Riboflavin Intake

The mean riboflavin intake was 1.96 mg when determined from averaging two days' intakes (Table III). The least squares means and standard errors, based on an analysis of variance describing the effects of race, age, and income on the previous day's riboflavin intake and on the two-day average riboflavin intake are shown by Table IV. All groups had mean riboflavin intakes above the 1.3 mg recommended by the National Research Council

(36). None of the probabilities for the effects of the variables were significant.

TABLE III

MEAN, STANDARD DEVIATION, MINIMUM, AND MAXIMUM VALUES OF SEVERAL VARIABLES

Variable	n	Mean	SD	Min. Value	Max. Value
Riboflavin intake, prev. day, mg	145	2.17	2.44	0.36	16.83
Riboflavin intake, avg., mg	150	1.96	1.99	0.35	17.09
Calcium intake, avg., mg	150	826	384	198	2291
Protein intake, avg., g	150	64	24	17	184
Riboflavin excretion, µg/g creatinine	140	577.8	436.8	1.2	2573.4
EGR, % stimulation	146	16.7	12.6	-9.0	61.0

Note: avg.=average; prev.=previous; min.=minimum; max.=maximum.

Despite the fact that mean intakes exceeded recommendations, 31 percent of the population had intakes below the recommended allowances for that age group, and 12 percent consumed below 67 percent of the RDA. Twenty-seven

percent of a group of teenagers, ranging in age from 12-19 years, in Kentucky (44), had riboflavin intakes below 67 percent of the RDA. Perhaps the Oklahoma girls in this study showed better intakes because they were younger than the Kentucky population and their dietary intake may be more influenced by their families. Those who consumed more than three cups of milk a day (12) showed the lowest prevalence of riboflavin deficiency. Whites had the best milk consumption, followed by Hispanics and blacks.

TABLE IV

LEAST SQUARES MEANS AND STANDARD ERRORS OF RIBOFLAVIN INTAKE, FROM ANALYSIS OF VARIANCE INCLUDING RACE, AGE, AND INCOME

Variables	Riboflavin Intake, mg Previous Day	Two-Day Average
Race		
White	2.11 ± 0.25*	1.93 ± 0.21
Black	2.46 ± 0.48	2.06 ± 0.39
Age Categories		
12	2.51 ± 0.41	2.33 ± 0.34
14	2.32 ± 0.35	1.99 ± 0.28
16	2.01 ± 0.51	1.66 ± 0.42
Per Capita Income Categories		
< $5,500	2.13 ± 0.33	1.94 ± 0.27
≥ $5,500	2.44 ± 0.37	2.05 ± 0.30

*SEM

Correlations calculated for pairs of variables are shown in Table V. The significant correlations with riboflavin intake of the preceding day and average riboflavin intake are average calcium intake and average protein intake. There was a significant and strong positive correlation between average riboflavin intake and previous day riboflavin intake, which was one component of the average. Riboflavin intakes varied with intakes of other nutrients, probably because many foods that are high in riboflavin are also good sources of calcium and protein.

Riboflavin intakes were related to those of other nutrients such as protein and calcium. The calcium intake was examined because several food sources that are high in riboflavin are also high in calcium; 85 percent of the population had calcium intakes below the 1200 mg recommended (36) for this age group. The average calcium intake was 826 mg, with a range of 198 mg to 2291 mg. Among Kentucky teens (44), 24 percent had intakes of at least 67 percent of the RDA, compared to 46 percent in that range in this study. Thus, the calcium intake of a larger portion of the population studied here met that standard than of Kentucky teens. Twenty-two percent of the population had protein intakes below the 46 g recommended for this age group (36). The average protein intake was 64 g, with a range of 17 to 184 g.

Riboflavin Excretion

The urinary excretion of riboflavin is presented as

TABLE V

CORRELATIONS COEFFICIENTS, PROBABILITIES, AND THE NUMBER OF OBSERVATIONS EXAMINED FOR PAIRS OF VARIABLES

Variable	Previous Day Ribo. Intake	Average Ribo. Intake	Ribo. Excretion	EGR Stim.	Age
Previous day ribo. intake	--- --- ---	.9494* .0001 145	.1869* .0281 138	-.0895 .2863 144	-.1372 .0999 145
Average ribo. intake	.9494* .0001 145	--- --- ---	.1472 .0826 140	-.0406 .6266 146	-.1509 .0653 150
Average calcium intake	.2622* .0014 145	.2921* .0003 150	.1497 .0694 148	.1171 .1593 146	-.0259 .0013 150
Average protein intake	.1836* .0271 145	.2239 .0057 150	-.2213 .0069 148	.0204 .8066 146	-.2544* .0017 150
Ribo. excretion	.1869* .0281 138	.1472 .0826 140	--- --- ---	-.0644 .4512 139	-.0272 .0011 140
EGR stim.	.0895 .2836 144	-.0406 .6266 146	-.0644 .4512 139	--- --- ---	-.0081 .9226 146

*p < 0.05. Note: ribo.=riboflavin; stim.=stimulation.

the riboflavin-to-creatinine ratio. The mean ratio was 578 µg riboflavin/g creatinine, with a range of 1.2 to 2573 (Table III). Guidelines based on research (7, 38, 45) show that riboflavin excretion less than 70 µg riboflavin/g creatinine indicated deficient status in 10 to 15 year olds. The least squares means and standard errors on an analysis of variance which included demographic variables and previous day's riboflavin intake or EGR stimulation are shown in Table VI. The highest excretion levels were by the 12 year old category, those with per capita incomes below $5,500, whites, and those with riboflavin intakes greater than 1.3 mg. The 12 year old category was the only group with mean excretion above 600; it presumably had the lowest creatinine excretion because of the smaller size of girls in that age group. Riboflavin excretion, relative to creatinine, as a function of those variables, shows only one of the variables, age, which significantly affected riboflavin excretion.

Riboflavin excretion was significant and positively correlated with previous day's riboflavin intake and average calcium intake, and negatively related to average protein intake and age (Table V). The correlation between riboflavin excretion and previous day's riboflavin intake was weak, as indicated by the small r value. The relationship between these two variables was examined further in analysis of covariance, as will be shown later in this chapter. Riboflavin excretion varied significantly

TABLE VI

LEAST SQUARE MEANS AND STANDARD ERRORS OF RIBOFLAVIN EXCRETION THROUGH AN ANALYSIS OF VARIANCE WITH DEMOGRAPHIC VARIABLES AND RIBOFLAVIN STATUS SHOWN THROUGH EGR STIMULATION OR RIBOFLAVIN INTAKE

Variable	Riboflavin Excretion, µg Ribo./g Creatinine	
	EGR Model	Diet Model
Race		
White	599 ± 43*	590 ± 42
Black	523 ± 81	501 ± 80
Age Categories		
12	759 ± 70[a]	734 ± 68[c]
14	448 ± 56[b]	429 ± 58[d]
16	477 ± 87[b]	473 ± 85[d]
Per Capita Income Categories		
< $5,500	597 ± 55	576 ± 55
≥ $5,500	525 ± 64	515 ± 62
EGR Stimulation Range		
< 20	572 ± 52	---#
≥ 20	551 ± 68	---
Riboflavin Intake Range, Previous Day, mg		
< 1.3 mg	---#	497 ± 68
≥ 1.3 mg	---	594 ± 54

*SEM
#Not included in the analysis.

Note: Those variables sharing a common superscript are not different from each other ($p \leq 0.05$).

Note: When the variables above were examined as continuous covariables to riboflavin excretion, none of the probabilities for the linear models were significant except age, with a probability for that linear slope of 0.026.

with the previous day's riboflavin intake, as shown through correlation. The effects of calcium and of protein intake on riboflavin excretion are possibly related to riboflavin intake and foods eaten that are high in all three nutrients: riboflavin, calcium, and protein. The effect of age may be due to the expression of riboflavin excretion relative to creatinine. Creatinine excretion increases with age in children and with increasing body size (40). Guidelines (7, 38, 45) for riboflavin excretion acknowledge these influences and are modified according to age. Modifications for age do not consider, however, that individuals of any age before adulthood vary widely in size.

A chi-square test (Table VII) examining the distribution of high and low values for riboflavin excretion and the riboflavin intake of the previous day show that about an equal proportion of those with good intakes of riboflavin had high or moderate riboflavin excretion. Those with moderate excretions are more likely to have adequate intakes than low intakes. Those with low intakes are more likely to have moderate excretions than high excretions. The dividing line for high and moderate excretion was 600 µg/g creatinine. This value was selected because it is near the mean excretion for the sample. Excretions above this value are high for both the sample population and the age group. Excretions below this value are somewhat more normal for the age group.

TABLE VII

INCIDENCE AND PERCENTAGE OF RIBOFLAVIN EXCRETION RANGE BY RIBOFLAVIN INTAKE RANGE

Riboflavin Excretion µg/g Creatinine		Riboflavin Intake, Previous Day		
		< 1.3 mg	≥ 1.3 mg	All Intakes
< 600 (Moderate)	n	38	46	84
	percent	27.5	33.3	60.9
≥ 600 (High)	n	12	42	54
	percent	8.7	30.4	39.1
All Excretions	n	50	88	138
	percent	36.2	63.8	100.0

Note: chi-square=7.536; p=0.0060

Riboflavin excretion of other high school students (13) averaged 369 ± 22 µg riboflavin/g creatinine, in contrast to the mean excretion of 578 found in this study. Two percent of the population had excretion levels below 70 µg riboflavin/g creatinine. The high school students (13) had 22 percent with excretion levels below 100 µg/g creatinine, which they considered less than desirable for that age group. In this study, three percent of the population had excretions in that range. This shows a very low level of poor riboflavin status according to the riboflavin excretion values.

EGR Stimulation

The tissue concentration of riboflavin is estimated by

measuring the stimulation of erythrocyte glutathione reductase (EGR) by a riboflavin-containing cofactor and is expressed as an activity coefficient. Activity coefficients showing higher than 20 percent stimulation indicate riboflavin deficiency (43). The mean percentage stimulation of EGR was 17, with a range of -9 to 61 (Table III). Thirty-seven percent of the population had values of stimulation above 20. The least squares means by race, age, income, and riboflavin intake are shown by Table VIII. Stimulation was lowest in those with per capita incomes greater than $5,500, 16 year old category, and those with riboflavin intakes below 1.3 mg. None of the variables had significant effects. Correlations (Table V) show that none of the variables examined were significantly related to EGR stimulation.

The mean EGR stimulation for the population was 17; 37 percent of the population had values above 20, indicating riboflavin deficiency. A study of 210 adolescents ranging in age from 13 to 19 years (12) showed 23 percent to be deficient by the same criterion. There appeared to be no difference in the occurrence of deficiency according to sex or age in this study. Fifteen percent of the whites, 20 percent of the Hispanics, and 35 percent of the blacks were deficient. The present study revealed 33 percent of the blacks and 36 percent of the whites to be deficient. In a pediatric study, with 100 subjects ranging in age from 2-1/2 days to 14 years (13), 11 percent

of the sample was deficient. With over 400 subjects ranging in age from 14 to 17 (11), the incidence of riboflavin deficiency was also 11 percent. Eleven percent of the white and 30 percent of the black girls had stimulation greater than 20 percent. Two studies observed sexual difference in the occurrence of riboflavin deficiency (12, 13). Females were deficient more often than males in one study (11). Two studies (11, 12) observed a higher frequency of riboflavin deficiency in blacks, although that was not found in this study.

TABLE VIII

LEAST SQUARE MEANS AND STANDARD ERRORS OF EGR STIMULATION THROUGH AN ANALYSIS OF VARIANCE WITH DEMOGRAPHIC VARIABLES AND RIBOFLAVIN INTAKE

Variable	EGR Percentage Stimulation
Race	
White	15.4 ± 1.3*
Black	15.3 ± 2.5
Age Categories	
12	15.3 ± 2.2
14	17.0 ± 1.8
16	13.7 ± 2.6
Per Capita Income Categories	
$5,500	17.0 ± 1.7
$5,500	13.7 ± 1.9
Riboflavin Intake Range Average, mg	
1.3 mg	14.1 ± 2.2
1.3 mg	16.6 ± 1.5

*SEM

A chi-square test (Table IX) examining the distribution of high and low values for EGR stimulation and riboflavin excretion shows those with either normal or abnormal EGR stimulation are more likely to have moderate riboflavin excretions than high excretions. All girls appeared to be more likely to have normal EGR stimulation than abnormal EGR stimulation.

TABLE IX

INCIDENCE AND PERCENTAGE OF RIBOFLAVIN EXCRETION RANGE BY EGR STIMULATION RANGE

Riboflavin Excretions µg/g Creatinine		EGR Percentage Stimulation		
		< 20	≥ 20	All Stimulations
< 600 (Moderate)	n	56	29	85
	percent	40.3	20.9	61.2
≥ 600 (High)	n	34	20	54
	percent	24.5	14.4	38.9
All Excretions	n	90	49	139
	percent	64.8	35.3	100.0

Note: chi-square=0.123; p=0.7255

The possible effects of menstruation and day of data collection were examined in analysis of variance and found not to be significant. The possible effect of vitamin

usage was examined in an analysis of variance with riboflavin intake, riboflavin excretion, and EGR stimulation. Twenty-three subjects, or 16 percent of the population, used vitamin supplements containing riboflavin. Vitamin usage did significantly affect the two day average and previous day's riboflavin intake. This was because the nutrient composition of the vitamin was included as part of the intake. The vitamin usage did not significantly affect the riboflavin excretion or EGR stimulation, although the probabilities approached the level of significance ($p \leq 0.05$) Chi-square test for categories of riboflavin intake and vitamin usage showed all vitamin users consumed 1.3 mg of riboflavin. The population distribution for vitamin usage and ranges of riboflavin excretion shows that 59 percent of those taking vitamins had high riboflavin excretion, while 43 percent had moderate excretion. High and moderate excretions are divided at 600 µg/g creatinine ratio. Chi-square test for vitamin usage and EGR stimulation show 74 percent of the vitamin users to have normal EGR stimulation, while 26 percent have high EGR stimulation indicative of riboflavin deficiency. It is not known why so many vitamin users would have high EGR stimulation, unless these subjects are not usually vitamin users but took the vitamin only before coming in for testing.

Hypothesis 1

The first hypothesis stated that there was no significant relationship between the riboflavin status of the population as determined by the excretion of riboflavin and the status suggested by dietary intake of riboflavin. This hypothesis was tested by several analyses. Analysis of variance, examining categorial data (Table VI), shows age as the only variable that significantly affected riboflavin excretion. Analyses of covariance (Tables X and XI) examined the slopes of the regressions of riboflavin excretion on different continuous variables. In both these analyses (Tables X and XI), the dependent variable of riboflavin excretion was significantly affected by age. Previous day's riboflavin intake did not show a significant effect. The effect of age probably detracts from the possible effect of riboflavin intake, since intake decreased with age (Table IV). When riboflavin intake alone was examined in analysis of covariance, the t for the slope comparing the regression with zero was 2.22, with a probability of 0.0281, which is significant. Riboflavin excretion and riboflavin intake were positively correlated (Table V). The significant effects shown by analysis of covariance between riboflavin excretion and intake and the correlation between these two, led to the rejection of the null hypothesis, that there is no significant relationship between the riboflavin status of

TABLE X

COMPUTED VALUES OF T AND LEVELS OF PROBABILITY AS SHOWN THROUGH ANALYSIS OF COVARIANCE FOR RIBOFLAVIN EXCRETION

Covariables	T for Ho	Probability
Riboflavin intake, previous day, mg	1.89	0.0621
Age, months	-3.05	0.0028

Note: df=136

TABLE XI

COMPUTED VALUES OF T AND LEVELS OF PROBABILITY AS SHOWN THROUGH ANALYSIS OF COVARIANCE OF FIVE VARIABLES FOR RIBOFLAVIN EXCRETION

Covariables	T for Ho	Probability
Riboflavin intake, previous day, mg	1.74	0.0841
Age, months	-3.07	0.0026

Note: df=129

Note: The variables of per capita income, race, and EGR stimulation were included in the analysis but did not show a significant effect on riboflavin excretion.

the population as determined by the urinary riboflavin excretion and riboflavin dietary intake.

Hypothesis 2

The second hypothesis examined the relationship between EGR stimulation and riboflavin excretion. The correlation coefficient (Table V) was small, negative, and is not significant. Analysis of variance, examining categorial data (Table VIII), show none of the variables significantly affected EGR stimulation. Analysis of covariance (Table XII) examined the slopes of the regression of EGR stimulation on different continuous variables. Again, none of the variables examined significantly affected EGR stimulation. Due to the lack of any significant effects between EGR stimulation and riboflavin excretion through all the above analyses, the test hypothesis must be accepted. This hypothesis states that there is no significant relationship between the riboflavin status as determined by riboflavin excretion and the status estimated from EGR stimulation. In a similar study (13), the coefficient of correlation between riboflavin excretion and EGR stimulation was -0.308. In this study, the correlation coefficient between these two variables was -0.0644 (Table V). Low correlation is not unlikely, in view of high variability of riboflavin excretion.

TABLE XII

COMPUTED VALUES OF T AND LEVELS OF PROBABILITIES AS SHOWN THROUGH ANALYSIS OF COVARIANCE FOR EGR STIMULATION

Covariables	T for Ho	Probability
Riboflavin excretion µg/g creatinine	-0.90	0.3708
Age, months	-0.46	0.6435
Per capita income	-1.36	0.1749

Note: df=1.33

Population Status

A review of the status of the population shows the following results:

Thirty-one percent of the population had riboflavin intakes lower than 1.3 mg recommended, indicating that they may be at some risk of developing riboflavin deficiency if that intake is typical and continues.

Two percent of the population had riboflavin excretion below 70 µg riboflavin/g creatinine, indicating high risk of riboflavin deficiency.

Thirty-five percent of the population had EGR activity coefficients higher than 20, indicating high risk of riboflavin deficiency.

The status suggested by riboflavin excretion shows fewer adolescents at high risk of developing riboflavin deficiency than is shown by EGR stimulation or than is suggested by riboflavin intake. In comparison with other studies of individuals of similar ages, this sample showed better riboflavin status when evaluated by riboflavin excretion and riboflavin intake, but had poorer EGR stimulation than other populations examined (11, 12, 13, 44). The variability of riboflavin excretion is indicated by its large standard deviation (Table III). Because of inconsistency between riboflavin intakes and excretion, and lack of correlation with an accepted measure of riboflavin status (EGR stimulation), riboflavin excretion in the first void is not recommended as the sole index upon to base an assessment of riboflavin status.

CHAPTER V

SUMMARY

The aim of this study was to contribute to knowledge of nutritional status of adolescents and of the methods used to examine nutritional status with regard to riboflavin. The purpose of this study was to assess the extent to which excretion of riboflavin reflects riboflavin intake and EGR stimulation. The following null hypotheses were examined:

H_1: There is no significant relationship between the riboflavin status of members of the population as determined by excretion of riboflavin and the status suggested by dietary intake of riboflavin.

H_2: There is no significant relationship between the riboflavin status of members of the population as determined by excretion of riboflavin and by EGR stimulation.

Dietary and biochemical data were taken from 150 adolescent girls in Oklahoma. The data were examined in regression, correlation, and chi-square tests. The dietary intake of riboflavin, riboflavin excretion, and EGR stimulation were examined in relationship to each other and to the variables of age, race, income, calcium intake, and protein intake.

Results show the following significant relationships:

1. Riboflavin excretion increased with increases in previous day's riboflavin intake.

2. Riboflavin intakes and riboflavin excretion increased with increases in average calcium intake and average protein intake.

3. Average riboflavin intake varied significantly with previous day's riboflavin intake.

4. Riboflavin excretion declined with age.

A review of the status of the population shows the following results:

Thirty-one percent of the population had riboflavin intakes lower than 1.3 mg recommended, indicating they may be at some risk of developing riboflavin deficiency if that intake is typical and continues.

Two percent of the population had riboflavin excretion below 70 µg riboflavin/g creatinine, indicating high risk of riboflavin deficiency.

Thirty-seven percent of the population had EGR activity coefficients higher than 20, indicating high risk of riboflavin deficiency.

The status suggested by riboflavin excretion shows fewer adolescents at high risk of developing riboflavin deficiency than does EGR stimulation or than is suggested by the riboflavin intake.

One null hypothesis was rejected, because riboflavin excretion was related to the previous day's intake. There

was, however, not a significant relationship between the status shown by riboflavin excretion and the status suggested by EGR stimulation. Because of the variability of riboflavin excretion, it should not be the only index on which to judge riboflavin status.

A SELECTED BIBLIOGRAPHY

1. McGinnis, J. M.: Prevention--today's dietary challenges. J. Am. Dietet. A. 77:129, 1980.

2. Kristein, M. M., Arnold, C. B., Wynder, E. L.: Health economics and preventive care. Science. 195:457, 1977.

3. Peterson, R. W.: Impact of technology. Am. Sci. 60:30, 1979.

4. Quelch, J. A.: The resource allocation process in nutrition policy planning. Am. J. Clin. Nutr. 32:1058, 1979.

5. Matwr, K., Loudhe, S. R., Poe, C. D., and Funchess, W.: Nutritional status of rural people in Hampton county. Orangeburg, SC: South Carolina State College, 1978.

6. Interdepartmental Committee on Nutrition for National Defense: Manual for nutritional surveys. 2nd ed. Bethesda, MD: National Institute of Health, 1963.

7. Ten State Nutrition Survey, 1968-1970, Washington, D.C.: Department of Health, Education, and Welfare, Publication no. (HSM) 72-8134, Vols. I-V, 1971.

8. Katchadourian, H.: The biology of adolescence. San Francisco: W. H. Freeman and Co., 1977.

9. Heald, F. P.: Adolescent nutrition. Med. Clin. No. Am. 59:1329, 1975.

10. Heald, F. P.: The adolescent. In Human Nutrition--A Comprehensive Treatise, Vol. 2, Nutrition and Growth, Eds. Jelliffe, D. B. and Jelliffe, E. F. P. New York: Plenum Press, 1979.

11. Lopez, R., Cole, H. S., Montoya, M. F., and Cooperman, J.: Riboflavin deficiency in a pediatric population of low socioeconomic status in New York City. J. Pediatr. 87:420, 1975.

12. Lopez, R., Schwartz, J. V., and Cooperman, J.: Riboflavin deficiency in an adolescent population in New York City. Am. J. Clin. Nutr. 33:1283, 1980.

13. Sauberlich, H. E., Judd, J. H., Nichoalds, G. E., Darby, W. J., and Broquist, H. P.: Application of the EGR assay in evaluating riboflavin nutritional status in a high school population. Am. J. Clin. Nutr. 25:756, 1972.

14. Krause, M. V. and Mahan, L. K.: Food, Nutrition and Diet Therapy. 6th ed. Philadelphia: W. B. Saunder Co., 1979.

15. Goodhart, R. S. and Shils, M. E.: Modern Nutrition in Health and Disease. 8th ed. Philadelphia: Lea and Febiger Co., 1980.

16. Harris, R. S. and Karmas, E.: Nutritional Evaluation of Food Processing. 2nd ed. Westport, CN: A. V. I. Publishing Co., Inc., 1975.

17. Holmes, A. D. and Jones, C. P.: Effect of sunshine upon the ascorbic acid and riboflavin of milk. J. Nutr. 29:201, 1944.

18. Nordlund, J., Junkkarien, L., and Kneula, M.: The effect of the packaging method on the quality of beverage milk. XVIII Intern. Dairy Cong. 1E:167, 1970.

19. Hand, D. B. and Sharp, P. F.: The riboflavin content of cow's milk. J. Dairy Sci. 22:779, 1939.

20. Rivlin, R. S.: Riboflavin. New York: Plenum Press, 1975.

21. Jusko, W. J. and Levy, G.: Absorption, metabolism and excretion of riboflavin-5-phosphate in man. J. Pharm. Sci. 56:58, 1967.

22. Levy, G. and Jusko, W. J.: Factors affecting the absorption of riboflavin in man. J. Pharm. Sci. 55:285, 1966.

23. Stripp, B.: Intestinal absorption of riboflavin in man. Acta. Pharmacol. et Toxicol. 22:353, 1965.

24. Najjar, V. A. and Holt, L. E.: A riboflavin excretion test as a measure of riboflavin deficiency in man. Bull. Johns Hopkins Hosp. 69:476, 1941.

25. West, D. W. and Owen, E. C.: Urinary excretion of metabolites of riboflavin by man. Brit. J. Nutr. 23:889, 1969.

26. Krehl, W. A. and Hodges, R. E.: The interpretation of national survey data. Am. J. Clin. Nutr. 17:191, 1965.

27. Schaefer, A. E.: Assessment of nutritional status. In Nutrition, Eds. Beaton, G. H. and McHenry, E. W. New York: Academic Press, 1966.

28. Garn, S. M., Larkin, F. A., and Cole, P. E.: The real problems with 1-day diet recall. Am. J. Clin. Nutr. 31:1114, 1979.

29. Beaton, G. H. and Milner, J.: Sources of variance in 24-hour diet recall. Am. J. Clin. Nutr. 32:2546, 1979.

30. Young, C. M., Smudski, V. L., and Steel, B. F.: Fall and spring diets of school children in New York State. J. Am. Dietet. A. 27:289, 1951.

31. Madden, J. P., Goodman, S. J., and Guthrie, H. A.: Validity of the 24-hour recall. J. Am. Dietet. A. 68:143, 1976.

32. Greger, J. L. and Etnyre, G. M.: Validity of 24-hour dietary results by adolescent females. Am. J. Pub. Health. 68:70, 1978.

33. Balogh, M., Kahn, H. A., and Medalie, J. A.: Random repeat in dietary recall. Am. J. Clin. Nutr. 24:304, 1971.

34. Rasanen, L.: Nutritional surveys of Finnish rural children, methodological study comparing the 24-hour recall and the diet history review. Am. J. Clin. Nutr. 39:2560, 1979.

35. National Research Council: Recommended Dietary Allowances. National Academy of Sciences, Washington, D.C., 1980.

36. Pearson, W. N.: Blood and urine vitamin levels as potential indices of body stores. Am. J. Clin. Nutr. 20:514, 1967.

37. Tucker, R. G., Mickelson, O., and Keys, A.: The influence of sleep, work, diuresis, heat, acute starvation, thiamin intake, and bedrest on human riboflavin excretion. J. Nutr. 72:251, 1960.

38. Sauberlich, H. E., Dowdy, R. P., and Skala, J. H.: Laboratory Test for the Assessment of Nutritional Status. Cleveland: C.R.C. Press, 1976.

39. Clark, R. P., Cosgrove, L. G., Morse, E. H.: Vitamin to creatinine ratios: Variability in separate voiding of urine in adolescents during a 24-hour period. Am. J. Clin. Nutr. 19:335, 1966.

40. Pollack, H.: Creatinine excretion as an index for estimating urinary excretion of micronutrients on their metabolic end products. Am. J. Clin. Nutr. 23:865, 1970.

41. Plough, I. C. and Consolazio, C. F.: The use of casual urine specimens in the evaluation of the excretion rates of thiamin, riboflavin, and n-methylniamide. J. Nutr. 69:365, 1959.

42. Burch, H. B., Bessey, O. A., and Lowry, O. H.: Fluorometric measurements of riboflavin and its natural derivatives in small quantities of blood, serum, and cells. J. Biol. Chem. 175:457, 1948.

43. Tillotson, J. A. and Baker, E. M.: An enzymatic measurement of the riboflavin status in man. Am. J. Clin. Nutr. 25:425, 1972.

44. Lee, C. J.: Nutritional status of selected teenagers in Kentucky. Am. J. Clin. Nutr. 31:1453, 1978.

45. Pearson, W. N.: Biochemical appraisal of nutritional status in man. Am. J. Clin. Nutr. 11:462, 1962.

46. Huenmann, R. L., Shapiro, L. R., Hampton, M. C., and Mitchell, B. W.: Food and eating practices of teenagers. J. Am. Dietet. A. 53:17, 1968.

47. Kaufmann, N. A., Poznanski, R., and Guggenheim, K.: Eating habits and opinions of teenagers on nutrition and obesity. J. Am. Dietet. A. 66:264, 1975.

48. Leverton, R. M.: The paradox of teenage nutrition. J. Am. Dietet. A. 53:13, 1968.

49. Carrigan, P. J., Machinist, J., and Kershner, R. P.: Riboflavin nutritional status and absorption in oral contraceptive users and nonusers. Am. J. Clin. Nutr. 32:2047, 1979.

50. Slater, E. C. and Morell, D. B.: A modification of the fluorometric method of determining riboflavin in biochemical materials. Biochem. J. 40:644, 1946.

51. Folin, O. and Wu, H.: A system of blood analysis-determination of creatinine and creatine. J. Biochem. 38:98, 1919.

52. Helwig, J. T. and Council, K. A.: SAS Users Guide. Raleigh: SAS Institute Inc., 1979.

APPENDIX

ABBREVIATIONS USED IN APPENDIX TABLE

SUBJ: subject number

AGE: age in months

RACE: 1=white; 2=black

PCINC: per capita income of family, $

RBX: previous day's riboflavin intake, mg

RB: two day average riboflavin intake, mg

NRBCR: µg riboflavin/g creatinine

EGR: percentage stimulation of EGR

CA: two day average calcium intake, mg

PRO: two day average protein intake, g

USAGE: 1=usage of vitamin supplement containing riboflavin
2=non-usage of vitamin supplement

WT: body weight, kg

MENS: 1=postmenarcheal
2=premenarcheal

DAY: day of data collection

TABLE XIII

VALUES OF ALL VARIABLES EXAMINED FOR ALL SUBJECTS

SUBJ	AGE	RACE	PCINC	RBX	RB	NRBCR	EGR	CA	PRO	USAGE	WT	MENS	DAY
1	146	1	2200	1.42	1.54	415.09	7	731	84	2	38.5	2	2
2	189	1	6580	4.12	2.30	463.14	25	1207	61	1	54.1	1	1
3	168	1	1833	2.56	1.98	510.70	30	1397	90	2	56.7	2	1
4	187	1	9333	1.30	1.83	261.07	10	884	65	2	56.6	1	1
5	162	1	8500	0.93	1.10	191.36	9	634	47	2	54.5	1	1
6	189	1	4080	5.09	3.34	2.98	4	1131	60	1	46.9	1	1
7	166	1	846	2.86	2.20	431.95	14	1187	109	2	53.8	2	1
8	193	1	4167	1.11	0.66	158.69	0	300	33	2	57.1	1	1
9	174	1	3800	1.35	1.62	.	5	772	67	2	43.1	2	1
10	163	1	20000	0.47	0.59	222.87	0	250	32	2	67.2	1	1
11	189	1	10000	0.74	0.75	255.91	16	326	32	2	88.4	1	1
12	193	1	2500	0.96	0.81	354.23	8	300	62	2	54.3	1	1
13	172	1	6400	1.75	1.42	285.65	17	934	53	2	57.8	1	1
14	165	1	10000	4.96	3.56	557.28	-1	1781	94	1	41.2	1	1
15	164	1	10000	1.89	2.01	741.30	2	1395	90	2	56.0	1	1
16	162	1	10354	2.01	1.70	132.49	26	1011	69	2	52.4	1	1
17	175	1	1580	1.54	1.65	411.14	22	1007	105	2	53.6	1	1
18	16:	1	8000	0.79	0.62	346.00	8	283	36	2	46.2	1	1
19	149	1	3500	0.74	1.76	.	32	832	59	2	38.2	2	3
20	165	1	3240	3.63	3.51	600.86	17	1418	102	1	45.0	2	2
21	193	1	4167	0.87	0.96	380.17	8	528	39	2	54.9	1	1
22	193	1	7200	1.34	2.35	485.62	5	680	80	1	47.8	1	1
23	179	1	5200	.	0.79	225.07	6	903	34	2	51.6	1	1
24	174	1	5750	1.03	0.73	450.34	2	270	34	2	51.0	1	1
25	173	1	5040	0.80	0.61	212.88	15	217	36	2	54.0	1	1
26	198	1	6000	4.50	3.65	661.85	12	1238	85	1	44.1	1	2
27	173	1	8800	0.67	1.10	901.07	37	721	42	2	70.1	1	1
28	195	1	3960	1.28	1.14	561.40	2	641	70	2	70.0	1	1
29	164	1	8667	2.54	1.51	793.85	7	311	49	1	58.7	1	1
30	173	1	1167	1.49	1.17	306.69	23	541	56	2	55.9	1	1
31	143	1	6000	2.40	2.14	1441.44	7	1178	97	2	39.8	2	2
32	188	1	11250	2.41	2.33	1094.22	19	1325	82	2	53.2	1	3
33	148	1	3725	2.34	2.04	1119.48	28	1363	96	2	41.7	2	7
34	188	1	8571	1.15	1.46	702.99	21	551	64	2	50.2	1	8
35	172	1	6052	1.91	1.78	240.93	30	868	59	2	51.9	1	3
36	144	1	5000	3.67	2.79	2183.61	9	1168	83	2	41.4	2	3
37	189	1	2857	1.88	1.88	289.33	21	878	61	2	59.4	1	3
38	146	2	1349	1.23	1.94	379.69	23	1200	74	2	45.4	2	2
39	168	2	1349	1.81	1.87	253.89	8	944	104	2	51.7	1	2
40	140	1	5100	1.48	1.60	848.11	40	979	50	2	48.2	2	8

TABLE XIII (Continued)

SUBJ	AGE	RACE	PCINC	RBX	RB	NRBCR	EGR	CA	PRO	USAGE	WT	MENS	DAY
41	147	1	4398	16.83	17.09	422.19	-1	1139	72	1	68.8	1	2
42	169	1	4667	1.02	1.13	187.94	10	540	75	2	85.9	1	2
43	140	1	8000	3.09	2.63	621.62	20	1633	98	2	39.9	2	2
44	150	1	2667	3.60	3.67	795.46	44	2291	98	2	36.7	2	8
45	188	1	7000	1.24	0.91	381.78	8	501	53	2	56.4	1	2
46	171	1	8600	1.40	1.36	230.95	49	789	66	2	52.6	1	2
47	191	1	8600	1.20	1.76	360.64	23	961	68	2	60.1	1	2
48	190	1	5917	0.59	0.51	.	14	297	32	2	55.5	1	2
49	196	1	5000	0.71	0.61	.	11	434	20	2	47.3	1	3
50	174	1	5800	0.98	0.67	367.83	8	444	27	2	63.8	1	3
51	150	1	9667	2.75	3.04	959.37	9	1140	60	1	45.8	2	3
52	148	1	6667	2.64	2.64	258.77	31	1690	163	2	37.0	2	3
53	190	1	6250	1.44	1.65	657.82	1	792	59	2	62.5	1	3
54	186	1	5000	0.61	0.82	565.34	2	456	38	2	44.6	1	3
55	150	1	8000	3.65	3.41	726.18	4	1646	82	1	55.7	2	3
56	187	1	8000	4.61	2.95	749.43	11	993	74	1	62.0	1	3
57	149	1	6000	1.33	1.34	226.33	18	832	62	2	32.8	2	7
58	141	1	7500	3.11	2.51	844.43	3	876	70	2	39.4	2	7
59	144	1	6250	1.06	1.26	662.81	34	761	50	2	59.6	2	8
60	168	1	7000	3.34	2.85	924.68	29	1526	80	2	45.0	1	8
63	141	1	7000	1.25	0.90	736.80	18	483	42	2	47.1	2	7
64	168	1	7000	0.85	1.08	412.17	26	594	49	2	45.4	1	7
65	142	1	5000	3.34	2.63	571.98	7	2070	104	2	34.6	2	7
66	150	1	2229	0.86	1.21	619.48	0	813	44	2	55.2	1	7
67	173	1	9000	0.89	1.04	1878.61	24	499	45	2	62.6	1	7
68	174	1	5520	2.21	2.21	858.95	16	868	56	2	54.1	1	7
69	148	1	4600	1.54	1.88	2195.80	16	1069	50	2	46.0	1	7
70	149	1	17500	.	0.59	.	.	198	46	2	.	.	.
71	143	1	17500	0.81	1.00	135.93	32	337	71	2	45.3	2	7
72	198	1	12500	0.37	0.35	434.61	21	199	17	2	51.7	1	7
73	142	2	1047	.	1.46	.	.	1207	54	2	.	.	3
74	141	2	4800	2.32	2.76	1043.71	44	700	49	1	46.0	1	7
75	197	1	3200	1.96	1.34	679.35	35	759	60	2	58.0	1	8

TABLE XIII (Continued)

SUBJ	AGE	RACE	PCINC	RBX	RB	NRBCR	EGR	CA	PRO	USAGE	WT	MENS	DAY
76	147	1	5520	2.49	2.55	293.11	11	1271	71	2	38.2	2	7
77	166	1	4000	1.97	1.56	279.85	10	760	42	2	50.1	1	7
78	192	1	7200	0.84	1.02	.	30	263	60	2	60.9	1	8
79	144	1	1776	1.21	0.95	680.72	12	459	45	2	36.2	2	8
80	142	1	6000	1.69	1.43	1129.60	13	938	57	2	63.3	2	7
81	163	2	3429	1.54	1.47	477.55	14	709	72	2	42.6	1	4
82	170	2	4000	2.17	1.53	321.68	38	622	54	2	67.0	1	4
83	171	1	3600	2.27	2.32	420.76	29	1222	72	2	51.8	1	4
84	145	1	2600	2.06	2.98	789.55	8	1012	184	2	37.2	2	4
85	157	2	3500	1.00	1.11	66.28	17	534	69	2	59.1	1	4
86	170	2	3500	1.34	1.66	305.45	15	718	67	2	69.8	1	4
87	168	1	9250	1.56	1.35	247.97	32	787	48	2	54.8	1	4
88	171	1	3600	0.65	0.95	145.48	15	509	45	2	49.6	2	4
89	171	1	3840	0.51	1.41	734.83	21	910	68	2	47.9	2	4
90	140	2	2057	1.09	1.05	286.56	.	373	43	2	41.9	2	4
91	144	2	6000	12.69	6.89	1712.87	0	1125	99	1	37.2	2	4
92	168	2	3333	1.65	2.24	361.02	30	796	66	1	50.1	1	4
93	142	1	11250	1.25	1.63	368.85	4	809	78	2	40.5	1	4
94	140	1	8250	1.97	1.64	1.21	7	941	58	2	43.0	1	4
95	146	1	2375	1.72	1.18	453.89	12	398	53	2	74.2	2	4
96	149	2	4800	0.47	1.43	195.05	17	763	68	2	54.7	1	4
97	151	2	6600	0.76	0.93	84.32	6	339	47	2	76.8	1	4
98	146	1	4750	2.45	2.67	2083.27	17	1470	96	2	42.8	2	4
99	170	2	5333	1.69	1.55	314.05	15	526	51	2	50.3	1	4
100	140	1	5000	3.33	3.08	801.34	19	1615	93	2	37.4	2	5
101	170	1	5000	1.34	1.33	565.73	22	802	62	2	59.1	1	5
102	151	1	.	1.67	2.00	733.82	24	1121	85	2	43.7	2	5
103	144	1	6800	1.58	1.53	438.19	12	687	70	2	42.3	2	5
104	168	2	1538	1.79	1.62	440.73	2	982	80	2	53.9	1	5
105	150	1	6500	1.60	1.92	628.75	17	1542	113	2	51.2	2	5
106	165	1	6250	0.52	0.87	363.46	39	494	50	2	55.1	1	5

TABLE XIII (Continued)

SUBJ	AGE	RACE	PCINC	RBX	RB	NRBCR	EGR	CA	PRO	USAGE	WT	MENS	DAY
107	150	2	7167	2.02	1.56	636.86	0	959	66	2	42.0	2	5
108	144	1	10000	0.73	0.73	904.27	9	484	19	2	54.8	2	5
109	149	1	4500	1.98	1.42	643.15	24	792	61	2	34.1	2	5
110	163	1	10250	1.99	1.90	1189.29	-9	1208	79	2	53.5	1	5
111	164	2	2517	1.11	1.54	298.63	39	899	62	2	45.6	1	5
112	171	1	5340	1.09	1.39	278.13	6	772	66	2	36.6	1	5
113	168	2	5000	1.85	1.44	190.65	12	724	71	2	43.7	1	5
114	151	1	9000	1.23	1.13	404.37	19	563	44	2	43.0	2	5
115	149	1	6760	1.20	1.54	577.90	14	848	61	2	34.8	2	5
117	146	1	3750	2.47	2.57	729.98	4	447	66	1	35.3	2	5
118	153	2	5400	0.84	0.89	2573.29	6	588	57	2	52.5	1	5
119	144	1	5100	1.48	2.53	974.25	23	524	91	1	40.0	2	6
120	193	2	1478	1.31	1.15	642.19	9	365	58	2	52.2	1	6
121	190	2	1500	2.18	1.68	1210.05	11	871	60	2	50.7	1	8
142	196	2	5000	1.37	1.38	195.49	61	959	69	2	59.4	1	6
143	162	2	13000		1.47	226.71	16	761	39	2	76.1	1	6
144	180	2	4572	1.20	1.20	215.08	22	772	55	2	57.5	1	6
145	158	2	3840	2.34	2.27	707.34	29	1127	74	2	64.1	1	6
146	166	2	6800	1.83	1.59	198.32	-3	654	70	2	45.5	1	6
147	169	1	5714	11.84	11.61	719.84	23	818	56	1	57.3	2	6
148	175	2	1606	1.00	0.80	522.94	20	510	30	2	53.8	1	6
149	167	2	6250	2.03	2.11	.	40	912	63	2	60.4	1	6
150	169	1	13500	3.37	2.27	996.31	0	768	54	1	51.6	1	6
151	170	1	5750	3.00	2.10	393.24	8	635	71	1	70.5	1	6
152	172	2	7000	0.36	0.51	200.93	31	218	25	2	59.1	1	6
153	177	2	5730	1.78	1.74	289.39	8	989	84	2	43.1	1	6
154	173	2	5833	16.20	13.60	405.09	27	580	31	1	54.8	1	6
155	189	2	800	1.98	2.43	.	39	1324	107	2	48.3	1	6
156	173	2	4509	2.42	1.68	325.02	11	955	72	2	52.1	1	6
157	175	2	9220	2.15	1.66	367.01	13	297	41	1	55.0	1	6
158	171	1	2443	1.83	1.56	370.99	42	1006	62	2	56.5	1	6
159	192	2	.	0.94	1.07	354.00	15	479	60	2	59.9	1	6
161	143	1	7875	2.19	1.66	840.54	46	1112	50	2	34.1	2	7
162	191	1	8250	0.90	1.14	415.36	-5	647	50	2	53.7	1	8
163	146	1	6900	0.98	1.28	296.13	19	698	66	2	54.7	1	7
164	150	1	7000	.	1.20	.	.	469	32	2	.	.	8
165	.	1	.	.	2.47	.	.	1149	67	2	.	.	.
166	156	1	8000	1.24	1.82	703.95	9	1017	72	2	51.8	1	8
167	166	1	5000	1.26	1.34	805.60	30	677	70	2	48.8	1	8
168	140	1	3327	11.39	6.49	1387.46	15	440	80	1	44.2	1	8
169	172	1	8100	2.35	1.44	703.99	22	1107	64	2	48.8	1	8
170	168	1	4333	1.47	1.29	294.71	27	587	83	2	44.8	1	8
171	194	1	5400	3.37	2.28	1035.66	8	810	63	1	57.4	1	8
172	178	1	7500	3.10	1.86	143.67	6	556	41	2	69.4	1	8
173	190	1	5000	1.07	0.97	380.60	23	753	53	2	56.2	1	8
174	191	1	4000	0.70	0.78	349.76	16	543	38	2	48.3	1	8

VITA 2

Margaret Anne Hayden Fristoe

Candidate for the Degree of

Master of Science

Thesis: ANALYZING THE RIBOFLAVIN STATUS OF ADOLESCENT GIRLS

Major Field: Food, Nutrition and Institution Administration

Biographical:

Personal Data: Born in Norman, Oklahoma, July 6, 1958, the fourth child of six, daughter of Margaret Dantagnan Hayden and Forrest Lee Hayden. Married on June 19, 1982, to Thomas Conner Fristoe.

Education: Graduated from Bay High School, Bay St. Louis, Mississippi, in May, 1976; received the Bachelor of Science degree in Foods and Nutrition from Mississippi University for Women, Columbus, Mississippi, in May, 1980; completed requirements for the Master of Science degree at Oklahoma State University in December, 1982.

Professional Experience: Graduate Research Assistant in Food, Nutrition and Institution Administration at Oklahoma State University, Fall, 1981. Dietetic Supervisor, Oktibbeha County Hospital, Spring, 1981. Food Service Worker, Summers of 1976-1979, Common's Cafeteria, OCCE, Norman, Oklahoma.