THE RIBOFLAVIN METABOLISM OF COLLEGE WOMEN ON A WEIGHT REDUCTION DIET

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Irene Hwei-lin Chang

1951

This is to certify that the

thesis entitled

The Riboflavin Metabolism of College Women on a Weight Reduction Diet.

presented by

Irene Hwei-lin Chang

has been accepted towards fulfillment of the requirements for

M. S. degree in Nutrition

Thelma L. Brewer
Major professor

Date August 10, 1951

THE RIBOFLAVIN METABOLISM OF COLLEGE WOMEN ON A WEIGHT REDUCTION DIET

By

Irene Hwei-lin Chang

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Food and Nutrition
School of Home Economics

1951

THESIS

ACKNOWLEDGMENT

The writer wishes to express her sincere thanks to Dr. Wilma D. Brewer, under whose constant supervision, and unfailing interest this investigation was undertaken and to whom the results are herewith dedicated.

The writer also deeply appreciates the material and financial supports of the Food and Nutrition Department, School of Home Economics at Michigan State College, which made it possible to complete this investigation.

****
****
******
****
**
*

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
REVIEW OF LITERATURE	3
Riboflavin Metabolism of College Women	3
Influence of Diet High in Fat on Riboflavin Metabolism	8
Methods of Determination of Riboflavin	10
EXPERIMENTAL PROCEDURE	13
Subjects	13
Experimental Plan	13
Experimental Diet	14
Sampling of Food and Urine for Riboflavin Determinations	14
Urinary Excretion of Riboflavin Following a Test Dose of Riboflavin	16
Chemical Methods	17
RESULT AND DISCUSSION	19
Riboflavin Metabolism of Overweight College Women on Self-selected Diets	21
Riboflavin Metabolism of Seven Overweight Women on Weight Reduction Diets	24
Riboflavin Metabolism of Three Subjects on Weight Reduction and Weight Maintenance	29
The Variation of Riboflavin Content of Seven Day Diets	32
SUMMARY AND CONCLUSION	34
REFERENCES CITED	36

LIST OF TABLES AND FIGURES

TABLES PAGE

I Typical Day's Diet for the Self-selected Diet Period and For the Weight Reduction Diet..... 15

II Height, Weight and Age of the Nine Overweight College Women. 20

III The Intake and Excretion of Riboflavin by Seven Overweight College Women on Self-selected Diets..... 22

IV The Average Intake and Excretion of Riboflavin By Overweight College Women on Self-selected Diets and During Twelve Weeks of Weight Reduction..... 25

V The Average Intake and Excretion of Riboflavin of Three Subjects on Weight Reduction Diets and Weight Maintenance Diets..... 30

VI The Riboflavin Content of Seven Day Composites of a Control Diet..... 33

FIGURES

1 The Average Daily Intake and Urinary Excretion of Riboflavin of Seven Overweight College Women on Self-selected Diets and During Twelve Weeks on a Weight Reduction Diet..... 27

2 The Average Daily Intake and the Urinary Excretion of Riboflavin of Three Subjects for Ten Weeks on a Weight Reduction Diet and Twelve Weeks on Weight Maintenance Diet..... 31

INTRODUCTION

INTRODUCTION

Riboflavin has long been recognized as an essential nutrient in animal and human nutrition. The influence of riboflavin on growth was demonstrated among others by Bourquin and Sherman (1931), Clarke et al (1940) and MacLeod and Taylor (1944) who reported that the growth rate of animals was directly dependent upon the riboflavin intake. The slowing of the growth rate which resulted from a suboptimal intake of riboflavin was accompanied by a corresponding retardation of general development. If shortage of riboflavin in the food supply was continued throughout the period of growth, the development of the individual remained permanently below his native potentiality.

Other studies which have demonstrated the physiological functions of riboflavin include those of Oden and Sebrell (1939), and Kruse et al (1940) who reported that riboflavin influenced the condition of the skin, mouth, and eyes, and Haas (1940) and Krebs (1935) who demonstrated that riboflavin took part in enzyme systems which regulated cellular oxidation.

Riboflavin also may be related to some extent to fat metabolism. Tange (1941), Mannering (1941) and Czacjkes (1946) reported that increasing amounts of fat in the diet apparently increased the riboflavin requirement of rats.

Obesity is considered to be a condition in which an abnormally large amount of adipose tissue is present. Since riboflavin has been demonstrated to have a physiological function in cellular oxidation and since there has been some indication that riboflavin and fat metabolism may be interrelated,

the possibility exists that riboflavin metabolism may be disturbed in obesity. In the present study, the riboflavin metabolism of overweight college women on a weight reduction diet which was relatively high in fat and protein has been investigated.

RIBOFLAVIN METABOLISM OF COLLEGE WOMEN

One of the earliest attempts to evaluate the riboflavin needs of women was reported by Strong (1941) who found that the twenty-four hour urinary riboflavin excretions for four young college women on unrestricted diets ranged from 500 to 800 micrograms on a daily intake of one to two milligrams of riboflavin. Strong concluded that an intake of one to two milligrams of riboflavin daily was no more than marginal and perhaps inadequate to supply the daily requirement.

Sebrell (1941) studied the metabolism of ten women on a basal diet containing 0.5 milligrams of riboflavin per 2400 calories. After 89 to 232 days on this diet, six out of the ten subjects developed symptoms of riboflavin deficiencies which were cured by adding additional riboflavin to the diet. Sebrell concluded that the intake of 0.05 milligrams of riboflavin per kilogram of body weight or three milligrams per day exceeded the requirement of the adult women and that an intake of 0.025 milligram per kilogram was insufficient. The average daily urinary excretion of these subjects was 77 micrograms.

Williams (1943) reported that when the intake of riboflavin was 0.8 milligrams per 1000 calories, there was no evidence of depletion of tissue stores, whereas an intake of 0.35 milligrams per 1000 calories produced depletion of the tissues. Williams concluded that an intake of 0.5 milligram per 1000 calories appeared to be the minimal daily requirement for riboflavin. This worker suggested that the riboflavin requirement of women was not less than 0.5 milligram and not more than 0.8 milligram per 1000

calories, or a total of 1.0 to 1.6 milligram per day, and considered that the recommendation of 2.2 milligrams per day which was made by the Food and Nutrition Board of the National Research Council (1943) for moderately active women provided a liberal margin of safety.

Davis (1946) studied 12 young women between the ages of 19 and 32 years on diets in which the amount of riboflavin progressively increased for eight months, and estimated that the riboflavin requirement of young women was between 0.49 and 0.66 milligram per 1000 calories. The average daily urinary excretion of these subjects during the preliminary period on self-selected diet was 433 micrograms. Brewer et al (1946) compared data reported in the literature with observations on young college women and reported that dietary intakes of 1.3 to 1.5 milligrams riboflavin appeared to represent the upper limit of economical utilization of this vitamin when the caloric intake was from 2100 to 2300 calories per day. A similar estimate of the riboflavin requirement of adults was reported by Horwitt (1949) whose studies indicated that the daily requirement was between 1.1 and 1.6 milligrams daily. This estimation of the riboflavin requirement approximated the recommended intake of 1.5 milligrams riboflavin for women which was reported in the last revision of recommended daily allowances of the Food and Nutrition Board of the National Research Council in 1948. Harris (1949) found that the daily riboflavin excretion of young women on self-selected diets ranged from 463 to 1157 milligrams. A similar range was reported by Ingalls (1945) and Everson (1948).

The use of the 24 hour urinary excretion of riboflavin to study the nutritional status of an individual has been questioned by Najjar (1941)

and Keys (1945). These workers found that it was not possible to define a limit of twenty-four hour riboflavin excretion which was characteristic of riboflavin deficiency, since the urinary riboflavin varied with the immediate intake and therefore did not indicate the state of the body stores of this vitamin. Additional factors which should be considered when the urinary excretion of riboflavin is used as an index to the metabolism of the vitamin include possible intestinal synthesis (Najjar, 1944; Keys, 1945; Hathaway, 1946), tissue destruction (Sure and Ford, 1943), poor absorption and impaired kidney function (Seyle, 1943; Mannering, Orsini and Elvehjem, 1944; Unna, 1944), and interrelationships with other nutrients (Ferrebee, 1945 and Sure and Ford, 1942).

The urinary excretion of riboflavin following a test dose has been considered closely related to the previous intake of this vitamin and an index to the nutritional status of the individual with respect to riboflavin by Najjar and Holt (1941), Williams (1943), Parsons (1944) and Copping (1945). The quantity of the test dose administered, the path of administration and the length of time of urinary collection after administration of the test dose have varied among workers.

A test dose of one milligram of riboflavin in distilled water injected intravenously was used by Najjar and Holt (1941). These workers reported that individuals who had had an adequate dietary intake of riboflavin retained 32 to 72 per cent of the test dose at the end of a four hour period, while riboflavin-deficient individuals retained 81 to 93 per cent. In this study the excretion of test dose appeared to vary inversely with the weight of the subject. Najjar and Holt suggested that if the test dose had been

based on the weight of the subject, the amount of variation observed for the group of normal subjects could be considerably reduced.

Williams (1943) suggested that a test dose of one milligram of sodium riboflavin injected subcutaneously would be a satisfactory test dose for the adult whose minimal requirement varied between 1.5 and three milligrams of riboflavin.

In studies with children, Oldham et al (1944) used a test dose of 75 micrograms per kilogram body weight and found that the excretion of 20 per cent of the test dose in a four hour urine sample or the presence of nine milligrams of riboflavin in an one hour fasting urinary sample showed satisfactory nutritional status. Oldham indicated that the urinary riboflavin in the one hour fasting sample and four hour and 24 hour periods following the test dose could be used equally well in determining the nutritional status with respect to riboflavin and thiamine. Brewer (1946) also found that there was a significant relationship between the one hour fasting excretion and the twenty hour urinary excretion of riboflavin after a test dose of three milligrams which was given orally. On the other hand, Berryman and French (1947) reported that the load test response was more significant than the fasting urinary excretion in the appraisal of nutritional status and Ingalls (1945) reported that the one hour fasting excretion of riboflavin was a satisfactory measurement of riboflavin nutrition when a group was studied but inadequate for evaluation of a single individual.

Feder, Lewis and Alden (1944) used a test dose of 0.016 milligram of riboflavin per kilogram of body weight given intramuscularly and reported

that an excretion of less than 35 per cent of the test dose indicated a deficiency state and that the saturation dose seldom gave more information than could be obtained by analysis of a fasting hour specimen.

The administration of several combinations of water soluble vitamins has been studied by Melnick (1945) who used a test dose of 10 milligrams of riboflavin and showed that extra urinary excretions of the water soluble vitamins were the same regardless of whether the vitamin was taken alone or in various combinations. This fact also has been reported by Ingalls (1945) and Johnson and Robinson (1945).

Johnson and Robinson (1945) observed that increased urinary excretion following an intravenous dose of riboflavin and ascorbic acid was no longer apparent after four hours and that the excretion of oral doses of these vitamins occurred in 12.6 and eight hours respectively. These workers suggested that the collection of urine for one hour after an intravenous dose and during the second hour after an oral dose might yield results directly comparable with those obtained after collection periods of four to ten hours.

Davis (1946) used a test dose of 0.02 milligram of riboflavin per kilogram body weight and observed that the values for excretion per hour were more constant than the value for excretion per unit volume contrary to the finding of Hathaway (1946) and Feder (1944).

INFLUENCE OF DIET HIGH IN FAT ON RIBOFLAVIN METABOLISM

The literature concerning the effect of fat in the diet on riboflavin metabolism is controversial. Guerrant and Dutcher (1934) found that the fat content of the diet of albino rats did not bear any relation to the rats' requirement for vitamin G, and when restricted daily supplements of both vitamin B and G were fed to rats, greater growth rates were obtained for animals which received diets containing from 15 to 20 per cent of fat than for similar animals which received diets of low fat content.

Mannering, Lipton and Elvehjem (1941) fed diets containing 25 to 40 per cent of fat to growing rats and reported that the riboflavin requirement increased when the fat level in the diet was increased. In these experiments fat isocalorically replaced dextrin.

Tange (1941) indicated that young rats which were fed a diet containing 25 per cent fat over a period of seven weeks showed little difference in growth from those fed a diet containing only five per cent fat when both diets were supplemented with 50 micrograms riboflavin. However when the riboflavin supplement was limited to less than 15 micrograms daily, the rats on the high fat diet showed impairment of growth after 12 days and a condition of the fur which resembled seborrhea. Additional intake of riboflavin cured these symptoms.

Potter, Axelrod and Elvehjem (1942) reported that the isocaloric substitution of lard for sucrose did not increase the riboflavin requirement of the growing dog and therefore considered that the riboflavin requirement of the dog was not related to the fat content of the diet.

Mannering, Orsini and Elvehjem (1944) reported that the growth of rats which received a diet containing seven per cent fat was superior to that of rats which received a diet containing 40 per cent fat. Furthermore the high fat diet produced spastic paralysis and decreased the survival time of the riboflavin deficient rats. These workers considered that the effect was due to the influence of fat on decreasing the intestinal synthesis of riboflavin.

Czaczkes and Guggenheim (1946) reported that rats kept on high protein and high fat diets needed at least twice as much riboflavin as rats kept on the normal diet for the maintenance of the same concentration of riboflavin in the organs and urine; the high fat diets apparently diminished the number of viable bacteria in the flora. These workers confirmed the finding of Mannering et al (1944) that fat decreased the intestinal synthesis of riboflavin.

Reiser and Pearson (1949) extended studies of this nature to the avian species. Pearson reported that lard did not retard the rate of growth of chicks nor did a commercial hydrogenated vegetable fat. When 20 per cent cottonseed oil was used instead of lard in the diet, the chicks made less efficient gains than those chicks on the diets with no added fat. Here again, the author suggested that the unsaturated acids in the cottonseed oil interfered with the intestinal synthesis of riboflavin.

These studies would appear to indicate that the riboflavin requirement of animals may be increased by a diet which is relatively high in fat content. No studies of the interrelationship of fat and riboflavin in the metabolism of humans have been found in the available literature.

METHODS OF DETERMINATION OF RIBOFLAVIN

Riboflavin is determined both biologically and chemically. Biological methods for the determination of riboflavin have been based upon measurements of the effects of small quantities of the vitamin on the growth of rats or chicks or micro-organisms. In this connection, the bacteriological techniques of Snell and Strong (1937, 1939) and the rat growth procedure of Bourquin and Sherman (1931) have been quite extensively used.

Chemical methods of determination of riboflavin have been based upon measurements of the light absorption of riboflavin or of the fluorescence exhibited by riboflavin in solution. Kuhn and Kaschars (1935) proposed a method based on measurement of the light absorption of the vitamin extract. The intensity of the color was measured either by a step photometer or by a polarograph. Kemmerer (1940) and Lingane (1941) have used this method to determine the amount of riboflavin in foodstuffs.

The fluorometric method for determination of riboflavin has been the most commonly used method for determining the amount of riboflavin in urine, foodstuffs and other biological materials. This method is based upon the observation that riboflavin in solution possesses yellow green fluorescence as a result of excitation by light of certain wave lengths. The accuracy of this method depends upon the complete removal of certain interfering substances so that the fluorescence measured will be only that of riboflavin.

Adsorption procedures have been suggested by many workers to remove the interfering pigments in urine and foodstuffs. Emmett (1917), Narayanan

and Drummond (1930) indicated that fuller earth absorbed the B vitamins. Neuwieler and Bierry (1936) adsorbed riboflavin in finally divided foodstuffs on fuller's earth. Ferrebee (1940) introduced two fuller's earth preparations, Florisil, and Supersorb, for the adsorption of riboflavin in urine. Other workers as Conner and Straub (1941) and Keys (1944) have employed this procedure to extract riboflavin from urine and foodstuffs.

Some pigments in normal urine and foodstuffs have been found to interfere with the determination of riboflavin. Hodson and Norris (1939) used stannous chloride to reduce the interfering pigments, and Sure and Ford (1942) oxidized the interfering pigments with potassium permanganate and destroyed the excess potassium permanganate with hydrogen peroxide.

A sample blank has been used by many workers to eliminate the errors due to interfering substances which were not removed by adsorption and by oxidation. Najjar (1941) and Keys (1944) prepared blanks by exposure of the samples to ultra violet light or direct sunlight for one to two hours; this brought about photolytic destruction of riboflavin. Sure and Ford (1942) obtained sample blanks by adding sodium hydrosulfite to the sample to reduce the riboflavin to a non-fluorescent leuco form after the reading had been taken. Morell (1946) reported that urine contained not only apparent riboflavin but also precursors of apparent riboflavin which could be destroyed by stannous chloride reduction, by potassium permanganate oxidation or during the process of adsorption on florisil. Morell used an internal blank to compensate for interfering substances.

The use of acid hydrolysis and of enzymatic hydrolysis to liberate combined riboflavin in foodstuffs has been reported by some workers.

Conner and Straub (1941) recommended that samples should be incubated with clarase at a temperature of 45° C. for 24 hours. McLaren and Pearson (1944) extracted riboflavin from meat with a combination of papain and takadiastase enzymes at a pH of 4.0 for two hours. Klocke (1947) reported that enzymatic digestion with polidase -S for 16 hours at 45° C. was considered adequate and practical for extraction of riboflavin from tissues.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

Subjects

The subjects were overweight college women who acted as subjects for a research project in the Foods and Nutrition Department of the School of Home Economics at Michigan State College. This research was concerned with the utilization of calories and protein by overweight college women on a weight reduction diet. The subjects carried full college programs and engaged in the usual activities of the school. Physical examinations at the beginning of the study indicated that the subjects were healthy except for the condition of excess weight. The ages of the subjects varied from 18 to 23 years.

Experimental Plan

The study was begun in September, 1950 with two subjects, Sr. and Pe., who were given a weight reduction diet and continued on this diet, with the exception of the official school vacation period, until March 1951. In March, the subjects reached the desired weight for the body build and were given diets planned to maintain their body weight.

In January 1951, seven subjects were added to the study. During the first two weeks, these subjects were permitted to eat without restriction from diets typical of this region. All servings were weighed and served in the small apartment located in the Home Economics building. Food and urine collections were made during the second week of this period. After the preliminary period, the subjects were given a weight reduction diet and were continued on the diet until satisfactory loss of weight resulted or

until the end of the school year. One of these subjects Wo reached the desired weight for her body build after 10 weeks of weight reduction and was given a diet planned to maintain a body weight. One subject, Se, left the experiment after four weeks on the weight reduction diet.

Every third week on the weight reduction diet, seven day composites of food were analyzed for the riboflavin content and the riboflavin content of the urine was determined.

Experimental Diet

The diet supplied approximately 1500 calories and 100 grams of protein daily. The calories of the diet were supplied chiefly by protein and fat. A typical day's diet for the preliminary period when the subjects ate without restriction in amounts of food and a typical day's diet during weight reduction are given in Table I. Seven menus were planned for the weight reduction diet, and these menus were repeated each week. All foods eaten were weighed on a Hansen dietetics scale. After eight weeks on the weight reduction diet, one subject, So, was given reduced quantities of food in order to increase the weight losses for this subject. The reduction in the diet was approximately 10 per cent. However, on the lowered intake the rate of weight loss for the subject appeared to be not more than desirable, so she was returned to the same food intake as the rest of the group received.

Sampling of Food and Urine for Riboflavin Determinations

Aliquots of one-fifth of the serving of food were weighed on a trip balance and frozen after each meal. At the end of the seven day period, a

TABLE I

TYPICAL DAY'S DIET FOR THE SELF-SELECTED DIET PERIOD AND FOR THE WEIGHT REDUCTION DIET

Self-selected Diet	Weight Reduction Diet	
	Food	Amount Gm.
Grape fruit section	Orange juice	100
Egg, poached	Eggs	100
Bread, whole wheat	Butter	20
Milk	Bread	20
Jelly	Lamb patties	125
Coffee	Pears, canned	100
Cream	Lettuce	15
Bread	Roast veal	125
Butter	Peas	75
Cream of tomato soup	Applesauce	100
Saltines	Milk	450
Lettuce		
Pears, canned		
Cookies		
Bread		
Butter		
Milk		
Steak, cubed		
Potatoes, boiled		
Green beans, buttered		
Lettuce		
Tomatoes		
Cottage cheese		
Bread		
Butter		
Milk		
Apple crisp		

composite was made by blending the aliquots together in a Waring blender and making to a volume of 2000 milliliters. Aliquots were taken for riboflavin analysis. During the first three month period, the food intake for each week was determined; following this, only food samples of the self-selection period and balance periods were analyzed. Aliquots which could not be analyzed immediately after the composites were made were stored in brown bottles and frozen.

Daily twenty-four hour urine collections were made during the balance period. The urine was collected in a brown glass bottle containing three milliliters of glacial acetic acid as a preservative and kept in the refrigerator during the period of collection. When the twenty-four hour collection was completed, the urine was transferred to a two liter glass stopped graduate cylinder and thoroughly mixed. The urine was then diluted to a volume corresponding to the nearest 500 milliliters with distilled water. The creatinine of the urine was determined daily as a check of the accuracy of the completed collection of the twenty-four hour period. One fifth of the daily sample was transferred to a three liter brown glass bottle and stored in the refrigerator. An analysis of riboflavin was made on composites of urine from the first three days, the second three days and the last day of the balance period.

Urinary Excretion of Riboflavin Following a Test Dose of Riboflavin

On the morning of the seventh day of the balance period, a test dose containing three milligrams of riboflavin was given orally. The urine excreted during the following 24 hours was collected in a brown glass bottle

containing three milliliters of glacial acetic acid as a preservative. Analysis of riboflavin was made. The percentage increment in riboflavin excretion of the 24 hours urine collected after the test dose over the average daily excretion of the six days preceding the test dose was calculated to give the percentage excretion of riboflavin of the test dose.

Chemical Methods

Aliquots of food were acid hydrolyzed for one hour on a steam bath, then adjusted to pH 4.5 with sodium hydroxide using nitrazine paper as an indicator and incubated over night with Polidase S-enzyme (Klocke, 1947). The riboflavin content was determined by the Conner and Straub (1941) modification of the fluorometric method. This included adsorption of riboflavin on activated florisil, elution of the adsorbed riboflavin with 20 per cent pyridine-acetic acid solution and subsequent oxidation with potassium permanganate with the excess permanganate decolorized by treatment with hydrogen peroxide. The fluorescence of the riboflavin in solution was measured in a Coleman photofluorometer against a standard solution containing 0.1 milligram of riboflavin per milliliter.

The riboflavin content of urine was determined by the same procedure as modified by Keys (1944) and Demerre and Brown (1944). Corrections for interfering substances were made by using a separate blank for each sample. The sample blanks were obtained by photolytic destruction of riboflavin by exposure of urine aliquots under ultra violet light for two hours. After the second month of the experiment there was a failure of the mercury lamp and for the rest of the period, sample blanks were obtained by destruction

of riboflavin by sodium hydrosulfite which was added to the sample after the initial measurement of fluorescence was made (Assoc. of Vitamin Chemists, 1947).

RESULT AND DISCUSSION

RESULT AND DISCUSSION

The description of the subjects who participated in the weight reduction study is given in Table II. The subjects ranged from 18 to 23 years old and were from seven to 51 per cent overweight. After ten weeks on the weight reduction diet, one subject, Wo, reached her desired weight and after 12 weeks two more subjects Sr and Pe reached their desired weights also. The subjects were considered healthy; however, Go had received thyroid therapy for several years preceding the study.

Obesity is considered to be a condition in which an abnormally large amount of adipose tissue is present. The condition of overweight is considered to be the result of an intake of energy which exceeds the output and this disproportion results from an abnormal appetite (Newburgh 1943). According to Newburgh a decrease of the calorie intake of obese individuals below their metabolic needs causes a burning of body tissues which results in loss of weight. The caloric intake of these overweight college women during the weight reduction diet was approximately 1500 calories which was lower than the 2000 calories recommended by the Food and Nutrition Board of the National Research Council in 1948 for women of moderate activity. A satisfactory weight loss of these subjects on this reduced calorie diet should be expected.

The average weekly weight loss during this experimental period ranged from 0.4 to 1.4 kilogram with an average of one kilogram per week. It may be considered that these subjects lost weight at a satisfactory rate. However, the weights of some subjects highly exceeded their desirable

TABLE II

HEIGHT, WEIGHT AND AGE OF THE NINE OVERWEIGHT COLLEGE WOMEN

Subject	Age (Yr)	Height (cm)	Desirable[1] Weight (kg)	Weight at the beginning of the Experiment (kg)	Weight at the end of the Experiment (kg)	Weight change/ week (kg/wk)
Go	18	176.1	65.9	83.6	73.5	0.8
Ha	18	161.0	59.0	89.3	74.5	1.2
Jo	19	168.9	68.1	83.4	72.5	0.9
Se	22	176.5	72.7	78.5	73.2	1.4
So	22	156.0	54.5	59.2	54.6	0.4
Va	18	166.0	65.9	84.6	70.9	1.1
Wo	18	168.0	65.9	79.6	65.9	1.4
Pe	22	155.0	54.5	60.3	51.5	0.7
Sr	23	166.2	61.3	73.4	63.8	0.8

1 Desirable weight for body build predicted from anthropometric measurements by Dr. Margaret A. Ohlson.

weights at the beginning of the experiment; therefore at the end of the experiment two subjects Ha and Go still were 15 kilograms and eight kilograms overweight respectively and two other subjects Va and Jo were five kilograms overweight.

Riboflavin Metabolism of Overweight College Women On Self-selected Diets

The average intake of riboflavin of these subjects during the period in which the subjects selected their food from the diet table without restrictions in amounts are given in Table III. The urinary excretions of riboflavin during the self-selected diet period and the urinary excretion of riboflavin during the twenty-four hour period following the administration of a test dose of three milligrams of riboflavin are also given. The percentage of test dose excreted represented the increment in the urinary excretion of riboflavin during the twenty-four hour period following the test dose over the average daily excretion of riboflavin preceding the test dose. The average intake of riboflavin during the period of self-selected diet ranged from 1.58 to 2.06 milligrams per day, and the average for the seven subjects was 1.76 milligrams. The riboflavin intakes of all subjects exceeded the daily allowance of 1.5 milligrams per day which was recommended by the Food and Nutrition Board of the National Research Council (1948). The average daily urinary excretion of the seven subjects ranged from 0.59 to 0.94 milligram per twenty-four hours, with an average daily excretion of 0.74 milligram. This range is slightly higher than the values of 0.5 to 0.8 milligrams per day which were reported by Strong (1941) for four average young women on unrestricted diets and within the range of

TABLE III

THE INTAKE AND EXCRETION OF RIBOFLAVIN BY SEVEN OVERWEIGHT COLLEGE WOMEN ON SELF-SELECTED DIETS

Subject	Calorie Intake (per 24 Hr)	Protein Intake (gms/24 hr)	Riboflavin Intake	Urinary Excretion of Riboflavin			
				Daily Urinary Excretion (Mg./24 hr)	Percent of Intake	Excretion after Test Dose (Mg./24 hr)	Percent of Test Dose Excreted (24 hr)
Go	2004	75.3	1.78	0.63	35.1	1.02	34.1
Ha	2170	79.1	2.06	0.66	32.1	0.72	24.2
Jo	1740	70.0	1.65	0.80	48.6	1.06	35.3
Se	1906	76.0	1.58	0.74	47.1	1.29	43.1
So	1846	73.5	1.80	0.83	46.2	0.90	30.0
Va	1800	70.1	1.78	0.59	33.4	0.73	24.4
Wo	1920	77.2	1.68	0.94	56.1	1.12	37.4
Average	1942	74.2	1.76	0.74	42.7	0.98	32.6
Range	1740-2004	70-79.1	1.58-2.06	0.59-0.94	32.1-56.1	0.72-1.29	24.2-43.1
S. D.	42.3	7.4	0.15	0.13	9.17	0.21	6.93

463 to 1153 micrograms per day which was reported by Harris in 1949. Similar ranges in daily urinary excretion of riboflavin were reported by Ingalls (1945) and Everson (1948) for young healthy women on self-selected diets. The average daily urinary excretion of riboflavin was higher than the average values of 0.43, 0.49, 0.44 milligrams of riboflavin per day which were reported by Davis (1946), Brewer (1946) and Ingalls (1945), respectively, and similar to the value of 0.71 milligram which was reported by Harris (1949) for young healthy women on self-selected diets. The percentage of riboflavin intake which was excreted in the urine ranged from 32 to 56 with an average of 43 per cent. This range is similar to the range of 25 to 55 per cent which was reported by Everson (1948) and that of 36 to 51 per cent which was reported by Harris (1949).

The percentage of a three milligram test dose of riboflavin which was excreted at the end of the period on self-selected diets ranged from 24 to 43 per cent with an average of 32 per cent. According to Feder, Lewis and Alden (1944), the excretion of less than 35 per cent of the test dose would indicate a deficiency state with respect to riboflavin. Harris (1948) reported that the percentage excretion of a five milligrams test dose given orally for nine young women on unrestricted diets ranged from 24 to 44 per cent with an average of 34 per cent. The amount of test dose which is administered and the path of administration may have an effect on the percentage excretion (Melnick, 1945). Previous studies from this laboratory have reported the per cent excretion of a three milligrams test dose given under comparable circumstances to those of the present study. Ingalls (1945) reported that the urinary excretion of a three milligrams test dose

given orally ranged from 10.7 to 69.5 per cent with an average of 51 per cent for 20 subjects on self-selected diets. Brewer (1946) reported that the percentage excretion of nine subjects on an intake of 1.6 milligrams daily was 31 per cent and, for four subjects on an intake of 2.2 milligrams riboflavin daily was 55 per cent.

Since the average daily urinary excretion of riboflavin by these subjects was comparable to values reported in the literature for healthy young women and since the percentage excretion of a test dose by these subjects also was comparable to that of healthy young women, it would appear that the utilization of riboflavin by these subjects was similar to that of healthy young women and that the extent of overweight of these subjects did not affect their riboflavin metabolism. Moreover, it may be considered that these young women were in a good nutritional status with respect to riboflavin at the beginning of the experiment.

Riboflavin Metabolism of Seven Overweight Women On Weight Reduction Diets

The daily urinary excretion of riboflavin by the seven subjects who were studied from January, 1951 until the end of the school term and the excretion of riboflavin in the urine following a test dose of three milligrams of riboflavin are given in Table IV. The average riboflavin content of the weight reduction diet was 1.64 milligrams per day. This represented an average of intakes which ranged from 1.61 to 1.78 milligrams per day during the various periods of weight reduction. Since the diet was kept constant during this period, this range of intake represented the natural variation in riboflavin content of foods and the error of sampling aliquots

TABLE IV

THE AVERAGE INTAKE AND EXCRETION OF RIBOFLAVIN BY OVERWEIGHT COLLEGE WOMEN ON SELF-SELECTED DIETS AND DURING TWELVE WEEKS OF WEIGHT REDUCTION

Period	Number of Subjects	Riboflavin Intake (Mg./24 hr)	Urinary Excretion of Riboflavin: Daily Urinary Riboflavin (Mg./24 hr)	Urinary Excretion of Riboflavin: Percent of Intake	Urinary Excretion of Riboflavin: Excretion After Test Dose (Mg./24 hr)	Urinary Excretion of Riboflavin: Percent of Test Dose Excreted (24 hr)
Self-selected	7	1.76 ± 0.15* (1.58-2.06)ᵀ	0.74 ± 0.13 (0.59-0.94)	42.7 ± 9.17 (32.1-56.1)	0.98 ± 0.21 (0.72-1.29)	32.6 ± 6.9 (24.1-43.1)
Weight Reduction						
After 2 weeks	7	1.53	0.69 ± 0.10 (0.57-0.86)	45.3 ± 6.29 (37-56.2)	1.17 ± 0.4 (0.51-1.55)	39.1 ± 13.5 (17-58.6)
After 4 weeks	7	1.78	0.89 ± 0.20 (0.66-1.17)	49.8 ± 11.3 (37.2-65.6)	0.73 ± 0.34 (0.39-1.34)	24.5 ± 31.6 (12.9-44.6)
After 10 weeks	6	1.61 ± 0.44 (1.52-1.63)	0.65 ± 0.07 (0.54-0.74)	40.4 ± 4.3 (32.9-45.7)	1.07 ± 0.33 (0.64-1.47)	35.7 ± 11.2 (21.4-48.9)
After 12 weeks	5	1.61	0.72 ± 0.22 (0.44-1.04)	44.3 ± 13.3 (27.4-64.5)	1.08 ± 0.44 (0.82-1.42)	36.1 ± 4.2 (27.4-47.4)

* Standard Deviation of the items

ᵀ Range of values

of food for the composites. The average daily excretion of riboflavin by these subjects during successive periods of weight reduction were 0.69, 0.89, 0.65 and 0.72 milligrams per day. These values are quite similar and closely approximate the average daily urinary excretion of riboflavin (0.74 milligrams per day) of the subjects during the period of self-selected diet. The range of values for urinary riboflavin and the standard deviations of the values also were similar during the successive periods of weight reduction.

The relationship between the average riboflavin intake and the urinary excretion of riboflavin during the periods of weight reduction is shown graphically in Figure 1. The constancy of riboflavin excretions during the twelve weeks of weight reduction is apparent in the graph.

The average urinary excretions of riboflavin following the test dose of three milligrams of riboflavin were similar after two, ten and twelve weeks on the weight reduction diet. These values were 1.17, 1.07 and 1.08 milligrams of riboflavin, respectively. The average excretion of riboflavin was lowered to 0.73 milligram per day after four weeks of weight reduction. Since the excretion was increased after 10 weeks and after twelve weeks of weight reduction, the lowered excretion of riboflavin after four weeks of weight reduction probably was not significant. The percentage of the test dose excreted in twenty-four hours were 39, 25, 36 and 36 per cent after two, four, ten and twelve weeks of weight reduction, respectively. These values are closely similar to the average percentage test dose excretion of 33 per cent by the subjects on the self-selected diet and also are similar in range to the average value of 31 per cent which was reported by

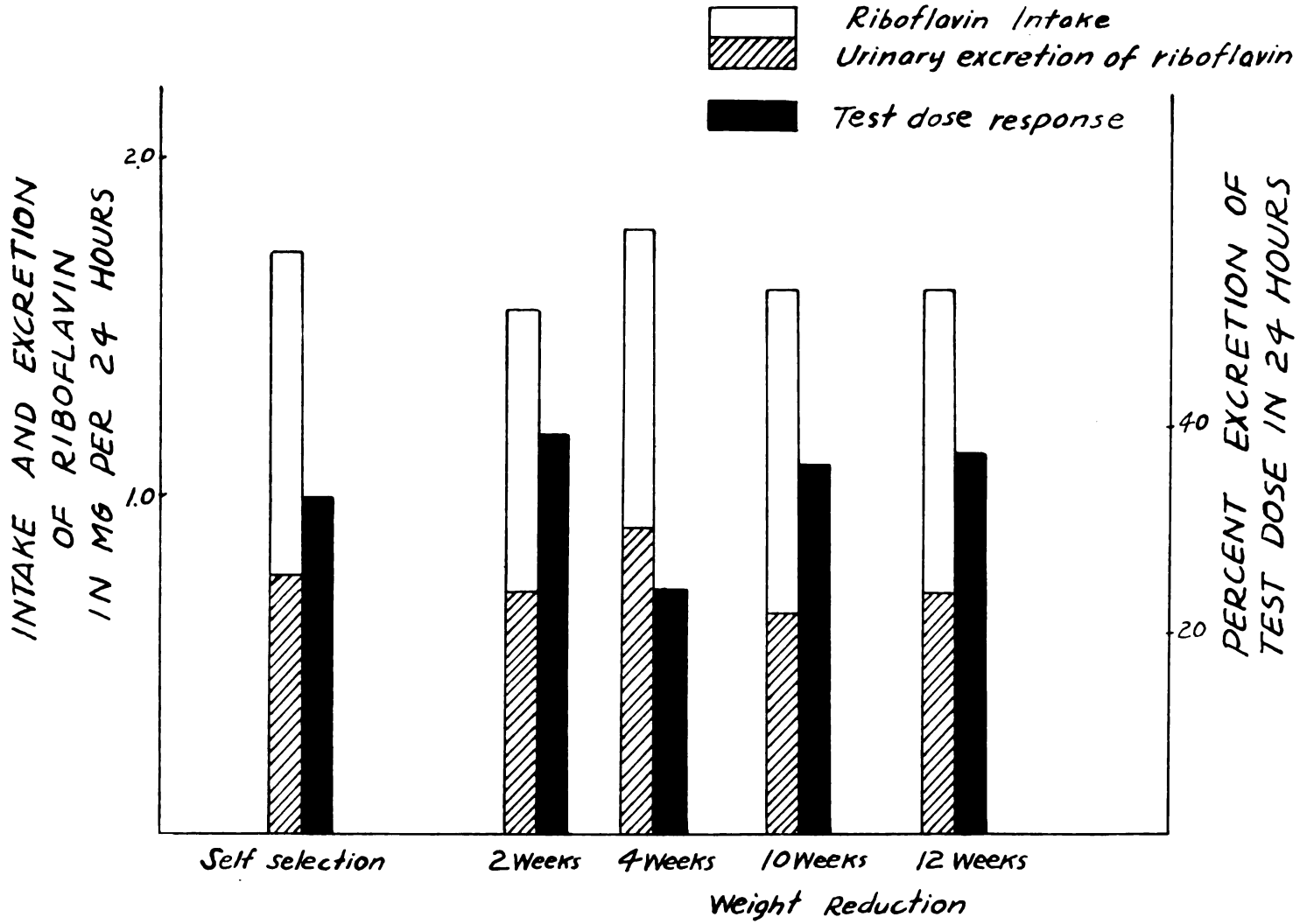

Figure 1

The average daily intake and urinary excretion of riboflavin of 7 overweight college women on self selected diets and during twelve weeks on a weight reduction diet.

Brewer (1946) for healthy young women on a riboflavin intake of 1.6 milligrams per day.

Since the metabolic data given in Table IV for the subjects on weight reduction are similar to the data obtained for these subjects during the self-selected diet and also are similar to values reported in the literature for healthy young women, it would seem that the intake of 1.64 milligrams of riboflavin daily was adequate for these subjects during the process of weight reduction. Furthermore, it would seem that the weight reduction diet used in this study did not affect the utilization of riboflavin by these subjects. Since this diet contained approximately 90 grams of fat, it would seem also that the presence of a relatively high amount of fat in the diet did not affect the riboflavin metabolism. This is contrary to the observations reported by Mannering, Lipton and Elvahjem (1941) and Tange (1941) and Reiser and Pearson (1949) that a high fat content of the diet increases the riboflavin requirements of rats and chicks; however it supports the findings of Axelrod and Elvahjem (1942) that the fat content of the diet did not bear any significant relationship to the riboflavin requirement of the dog. The fecal excretion of riboflavin of these subjects was not determined. Therefore whether or not the relatively high proportion of fat in the diet would decrease the intestinal synthesis of riboflavin as suggested by Czaczkes (1946) and Mannering (1944) is not known.

Riboflavin Metabolism of Three Subjects on Weight Reduction and Weight Maintenance

Three subjects, Sr, Pe and Wo reached the desired weight for their body builds during the progress of the experiment and were given diets planned to maintain their desired weights. Unfortunately the urinary excretion of riboflavin by subjects Sr and Pe was not studied when these subjects were on self-selected diets. However these subjects were observed for twelve weeks on weight reduction and for twelve weeks on weight maintenance diets. Subject Wo reached her desired weight shortly before the end of the experiment and was observed on a weight maintenance diet for three weeks. The metabolic data for these subjects are given in Table V, and shown graphically in Figure 2.

The riboflavin intake was increased to an average of 1.88 milligrams per day on the weight maintenance diets. The added riboflavin was supplied chiefly by the addition of fruits and vegetables to the weight reduction diet. Since the average daily intake of riboflavin on the weight maintenance diet was higher than that of the weight reduction diet, it would be expected that the urinary excretion of riboflavin also would be higher. That this was true is shown in Figure 2. The percentage excretion of the test dose which was excreted during the weight maintenance period was comparable to the percentage excretion during the weight reduction period.

TABLE V

THE AVERAGE INTAKE AND EXCRETION OF RIBOFLAVIN OF THREE SUBJECTS ON WEIGHT REDUCTION DIETS AND WEIGHT MAINTENANCE DIETS

Period	Subject	Riboflavin Intake	Urinary Excretion of Riboflavin			
			Daily Urinary Excretion	Percent of Intake	Excretion After Test Dose	Percent of Test Dose Excreted in 24 hr
Weight Reduction						
After 2 weeks	Wo	1.53 ± 0.00	0.60 ± 0.00	39.3 ± 0.00	1.04 ± 0.00	34.8 ± 0.00
After 4 weeks	Wo	1.78 ± 0.00	0.98 ± 0.00	55.0 ± 0.00	0.45 ± 0.00	15.1 ± 0.00
After 10 weeks	Wo	1.63 ± 0.00	0.69 ± 0.00	42.1 ± 0.00	1.24 ± 0.00	41.3 ± 0.00
After 12 weeks	Pe, Sr	1.53 ± 0.00	0.74 ± 0.03 (0.72-0.76)	48.3 ± 2.10 (46.7-49.8)	1.50 ± 0.11 (1.42-1.57)	50.0 ± 3.21 (47.7-52.3)
Weight Maintenance						
After 2 weeks	Wo,Pe,Sr	1.89 ± 0.00 (1.89-1.90)	0.86 ± 0.19 (0.67-1.06)	45.8 ± 10.5 (35.4-56.5)	1.00 ± 0.09 (0.89-1.06)	33.29 ± 3.14 (29.7-35.5)
After 10 weeks	Pe, Sr	1.87 ± 0.00	0.87 ± 0.07 (0.82-0.91)	46.3 ± 3.21 (44.0-48.5)	1.09 ± 0.05 (1.05-1.12)	36.1 ± 1.29 (34.9-37.2)
After 12 weeks	Pe, Sr	1.90 ± 0.00	0.75 ± 0.11 (0.67-0.82)	39.1 ± 5.51 (35.2-43.0)	1.02 ± 0.36 (0.77-1.27)	34.05 ± 11.62 (25.8-42.3)

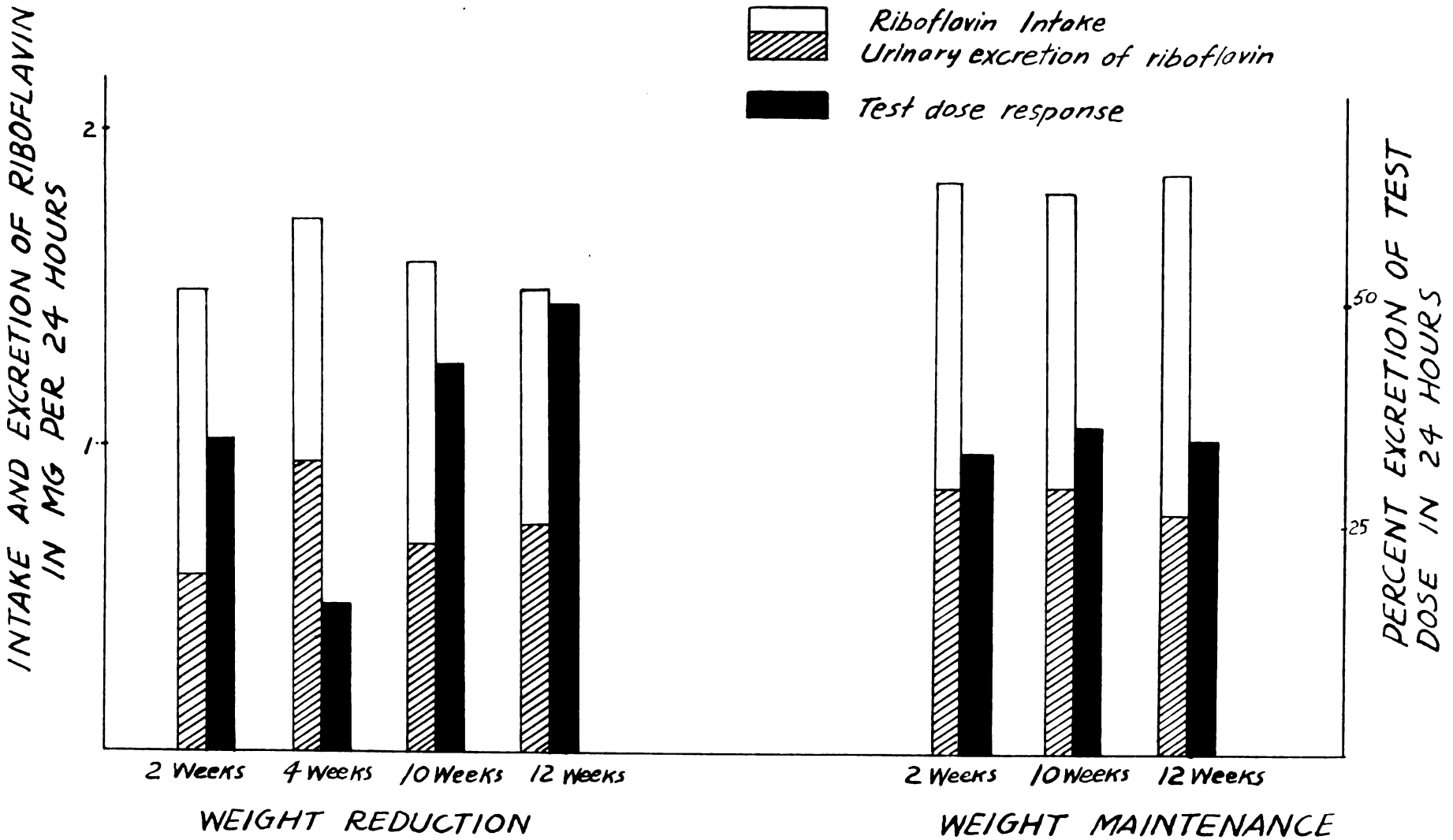

FIGURE 2

The average daily intake and the urinary excretion of reboflavin of 3 subjects for 10 weeks on a weight reduction diet and 12 weeks on weight maintenance diet.

The Variation of Riboflavin Content of Seven Day Diets

The riboflavin contents of the food samples collected during this experiment is shown in Table VI. The average amount of riboflavin in the diets used from September to December 1950 ranged from 2.10 to 2.34 milligrams riboflavin per day with an average of 2.16 milligrams per day. The average amount of riboflavin in the diets used from January to June 1951 ranged from 1.53 to 1.78 milligrams riboflavin per day with an average of 1.64 milligrams riboflavin per day. Since the diets used in the above two periods were the same within each period, the small differences in riboflavin content of the seven day composites represent the variation which may be expected from week to week for a constant diet. This variation includes the variability in food, the effect of cooking on the riboflavin content of foods, the sampling errors in preparing the food composites and the technical errors in riboflavin analysis.

The diets used during the two periods were planned to provide the same intake of calorie and other nutrients. But at the beginning of January 1951, the diet had been modified slightly in selection of vegetables and fruits; moreover, fewer fresh citrus fruits were used during the period from January to June 1951. These may have accounted for the lowered riboflavin intake during the months from January to June 1951, than the months from September to December 1950. It is possible that the decrease also reflects a seasonal variation in riboflavin content of foods.

TABLE VI

THE RIBOFLAVIN CONTENT OF SEVEN DAY COMPOSITES OF A CONTROL DIET

Weight Reduction Diet		Riboflavin Content of Diet (mg/day)
September to December 1950		
Sample	1	2.11
	2	2.34
	3	2.22
	4	2.10
	5	2.10
	6	2.10
	Average	2.16 ± 0.10
	Range	2.10-2.34
January to June 1951		
Sample	1	1.53
	2	1.78
	3	1.63
	4	1.62
	Average	1.64 ± 0.10
	Range	1.53-1.78

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The riboflavin metabolism of nine overweight college women, who acted as subjects for a research project in the Food and Nutrition Department of the School of Home Economics at Michigan State College was studied. This experiment was started in September 1950 with two subjects and later on in January 1951, seven more subjects were added to the study. The subjects were seven to 51 per cent overweight and the age of the subjects ranged from 18 to 23 years old. After ten weeks on the weight reduction diet one subject, and after 12 weeks on the weight reduction diet two more subjects reached their desirable weights and they were placed on a weight maintenance diet for a period of two weeks and 12 weeks respectively. One subject left the experiment after four weeks on the weight reduction diet. Five subjects were five to 15 kilograms overweight at the end of the experiment. The average weekly loss of weight during this period ranged from 0.4 to 1.4 kilograms per week with an average of one kilogram per week per person.

The weight reduction diet contained 1400 calories, 100 grams of protein, 90 grams of fat and 1.61 to 1.78 milligrams of riboflavin per day.

The average daily urinary excretion of the seven subjects on the self-selected diets ranged from 0.59 to 0.94 milligram per day with an average of 0.74 milligram per day. The average per cent of daily intake of riboflavin excreted ranged from 32.1 to 56.1 per cent with an average of 42.7 per cent. The per cent of three milligrams test dose given orally excreted in

24 hours ranged from 24.1 to 43.1 per cent with an average of 32.6 per cent. The average daily urinary excretion of the same seven subjects after two, four, ten and twelve weeks on the weight reduction diet were 0.69, 0.89, 0.65, 0.72 milligram riboflavin per day. The average per cent of daily riboflavin intake excreted were 45.3, 49.8, 40.4 and 44.3 per cent respectively, and the per cent of test dose excreted in 24 hours were 39.1, 24.5, 35.7 and 36.1 per cent respectively.

The riboflavin metabolism of three subjects on weight maintenance diets appeared to be similar to that of the subjects on the weight reduction diets.

The riboflavin metabolism of the overweight college women on a reduction diet was similar to that of healthy women.

The relatively high fat content of the reduction diet did not appear to interfer with the riboflavin metabolism of the nine overweight college women.

Under the conditions of this experiment an intake of 1.6 milligrams of riboflavin per day appeared to be adequate for these subjects.

REFERENCES CITED

REFERENCES CITED

Association of Vitamin Chemist, 1947.

Axelrod, A. E., T. D. Spies and C. A. Elvehjem 1941 A study of urinary riboflavin excretion in man. J. Clin. Invest. vol. 20, p. 229.

Berryman, G. H., C. E. French, H. R. Baldwin, S. L. Bell and C. R. Henderson 1947 The relation of load test response and fasting excretion levels to tissue content of thiamine and riboflavin. Am. J. Physiol., vol. 149, p. 254.

Brewer, W. D., T. Porter, R. Ingalls and M. A. Ohlson 1946 The urinary excretion of riboflavin by college women. J. Nutrition, vol. 32, p. 583.

Bourquin, A. and H. C. Sherman 1931 Quantitative determination of vitamin G (B_2). J. Am. chem. Soc. vol. 53, p. 3501.

Clarke, M. F., M. Lechycka and C. A. Cook 1940 The biological assay of riboflavin. J. Nutrition 20, p. 133.

Conner, R. T. and G. J. Straub 1941 Combined determination of riboflavin and thiamine in food products. Ind. Eng. Chem. (Anal. Ed.), vol. 13, p. 385.

Copping, A. M. 1945 Some aspects of riboflavin nutrition in man. Nutrition Absts. and Revs., vol. 14, p. 433.

Czaczkes, J. W. and K. Guggenheim 1946 The influence of diet on the riboflavin metabolism of the rat. J. Biol. Chem., vol. 162, p. 267.

Davis, M. V., H. G. Oldham and L. J. Roberts 1946 Riboflavin excretions of young women on diets containing varying levels of the B vitamins. J. Nutrition, vol. 32, p. 143.

DeMerre, L. J. and W. S. Brown 1944 Effect of various lighting conditions on riboflavin solutions. Arch. of Biochem., vol. 5, p. 181.

Emmett, A. D. and L. H. McKim 1917 The value of the yeast vitamin fraction as a supplement to a rice diet. J. Biol. Chem., vol. 32, p. 409.

Everson, G., E. W., H. Walker and W. J. Caulfield 1948 Availability of riboflavin of ice cream, peas, and almonds judged by urinary excretion of the vitamin by women subjects. J. Nutrition, vol. 35, p. 209.

Feder, V. H., G. T. Lewis and H. S. Alden 1944 Studies on the urinary excretion of riboflavin. J. Nutrition, vol. 27, p. 347.

Ferrebee, J. W. 1940 The urinary excretion of riboflavin, fluorometric methods of its estimation. J. Clin. Invest., vol. 19, p. 251.

Ferrebee, J. W. and N. Weissman 1943 Riboflavin and Thiamine interrelationships in rats and in man. J. Nutrition, vol. 26, p. 459.

Food and Nutrition Board, National Research Council 1943 Recommended dietary allowances. National Research Council Reprint and Circular Series no. 115.

Food and Nutrition Board, National Research Council 1948 Recommended dietary allowances. National Research Council Reprint and Circular Series no. 129.

Guerrant, N. B. and R. A. Dutcher 1934 Some effects of the composition of the diet on the vitamin B and vitamin G requirement of the growing rat. J. Nutrition, vol. 8, p. 397.

Haas, E., B. L., Horecker, and T. R. Hogness 1940 The enzymatic reduction of cytochrome C, cytochrome c reductase. J. Biol. Chem., vol. 136, p. 747.

Harris, J. N. and F. I. Scoular 1949 Riboflavin metabolism of young women on self-selected diets. J. Nutrition, vol. 38, p. 435.

Hathaway, M. L. and D. E. Lobb 1946 A comparison of riboflavin synthesis and excretion in human subjects on synthetic and natural diets. J. Nutrition, vol. 32, p. 9.

Hodson, A. F. and L. C. Norris 1939 A fluorometric method for determining the riboflavin content of foodstuffs. J. Biol. Chem., vol. 131, p. 621.

Horwitt, M. K., O. W. Hills, C. C. Harvey, E. Liebert and D. L. Steinberg 1949 Effects of dietary depletion of riboflavin. J. Nutrition, vol. 39, p. 357.

Ingalls, R. L. 1945 Urinary excretion of riboflavin of college women on a self chosen diet and the response to a saturation dose of riboflavin and thiamine. Thesis, M.S.C.

Johnson, R. E., L. A. Contreras, F. C. Consolazio and P. F. Robinson 1945 A comparison of intravenous and oral vitamin tolerance tests. Am. J. Physiol., vol. 144, p. 58.

Kemmerer, A. R. 1940 Report on riboflavin. J. Assoc. Off. Agri. Chem., vol. 23, p. 346.

Keys, A., A. F. Henschel, O. Mickelsen, J. M. Brozek, and J. H. Crawford 1944 Physiological and biochemical functions in normal young men on a diet restricted in riboflavin. J. Nutrition, Vol. 27, p. 165.

Keys, A., A. F. Henschel, H. L. Taylor, O. Mickelsen and J. M. Brozek 1945 Experimental studies on man with a restricted intake of the B vitamins Am. J. Physiol., vol. 144, p. 5.

Klocke, J. F., P. I. Tack, M. A. Ohlson, R. Mitchals, E. Leffler and N. S. Henry 1947 Nutritive value of fish from Michigan water, II. Thiamin of lake herring, carp, common sucker, burbot and smelt. Food Res., vol. 12, p. 36.

Krebs, H. A. 1935 Metabolism of Amino acids III. Determination of Amino acids Biochem. J., vol. 29, p. 1620.

Kruse, H. D., V. P. Sydenstricker, W. H. Sebrell and H. M. Cleckley 1940 Ocular manifestations of ariboflavinosis. U. S. Pub. Health Repts., vol. 55, p. 157.

Lingane, J. J. and O. L. Davis 1941 Polarographic determination of riboflavin (vitamin B_2) and other vitamin B factors. J. Biol. Chem., vol. 137, p. 567.

Macleod, G. and C. M. Taylor 1944 Rose's Foundations of Nutrition, 4th Ed.

Mannering, G. J., M. A. Lipton and C. A. Elvehjem 1941 relation of dietary fat to riboflavin requirement of growing rats. Pro. Soc. Exp. Biol. Med., vol. 46, p. 100.

Mannering, G. J., D. Orsini and C. A. Elvehjem 1944 Effect of the composition of the diet on the riboflavin requirement of the rat. J. Nutrition, vol. 28, p. 141.

McLaren, B. A., S. Cover and P. B. Pearson 1944 A simplified fluorometric method for riboflavin in meat. Arch. Biochem., vol. 4, p. 1.

Melnick, D., M. Hockberg, and B. L. Oser 1945 Physiological availability of the vitamins. I. The Human Bio-assay technic. J. Nutrition, vol. 30, p. 67.

Morell, D. B. and E. C. Slater 1946 The Fluorometric determination of riboflavin in urine. Biochem. J., vol. 40, p. 652.

Najjar, V. A. 1941 The fluorometric determination of riboflavin in urine and other biological fluids. J. Biol. Chem., vol. 141, p. 355.

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

Najjar, V. A., G. A. Johns, G. C. Medairy, G. Fleischmann and L. E. Holt Jr. 1944 The biosynthesis of riboflavin in man. J. Am. Med. Assn., vol. 126, p. 357.

Newburgh, L. H. 1942 Obesity Arch. Inter. Med., vol. 70, p. 1033.

Narayanan, B. T., and J. C. Drummond 1930 The concentration of vitamin B_2. Biochem. J., vol. 24, p. 19.

Oden, J. W., L. H. Oden, Jr. and W. H. Sebrell 1939 Report of three cases of ariboflavinosis. U. S. Pub. Health Repts., vol. 54, p. 790.

Oldham, H. F., J. S. Kleiger and H. H. Arismendi 1944 A study of the riboflavin and thiamine requirements of children of pre-school age. J. Nutrition, vol. 27, p. 435.

Parsons, H. T. 1944 Further studies on human requirements for riboflavin. Fed. Proc., vol. 3, p. 162.

Peterson, W. J., D. E. Brady, and A. O. Shaw 1943 Fluorometric determination of riboflavin in pork products. Ind. Eng. Chem. (Anal. Ed.), vol. 15, p. 634.

Potter, R. L., A. E. Axelrod and C. A. Elvehjem 1942 The riboflavin requirement of the dog. J. Nutrition, vol. 24, p. 449.

Reiser, R. and P. B. Pearson 1949 The influence of high levels of fat with sub-optimum levels of riboflavin on the growth of chicks. J. Nutrition, vol. 38, p. 247.

Sebrell, W. H., R. E. Butler, J. G. Wooley and H. Isbell 1941 Human riboflavin requirement estimated by urinary excretion of subjects on controlled intake. U. S. Pub. Health Repts., vol. 56, p. 510.

Selye, H. 1943 The role played by the gastrointestinal tract in the absorption and excretion of riboflavin. J. Nutrition, vol. 25, p. 137.

Snell, E. E., F. M. Strong and W. H. Peterson 1937 Growth factors for bacteria VI Fractionation and properties of an accessory factor for lactic acid bacteria. Biochem. J., vol. 31, p. 1789.

Snell, E. E. and F. M. Strong 1939 A Microbiological Assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.), vol. 11, p. 346.

Strong, F. M., R. E. Feeney, B. Moore and H. T. Pearsons 1941 The riboflavin content of blood and urine. J. Biol. Chem., vol. 137, p. 363.

Sure, B. and Z. W. Ford, Jr., 1942 Vitamin interrelationships II. Thiamine and riboflavin interrelationships in metabolism. J. Biol. Chem., vol. 146, p. 241.

Sure, B. and Z. W. Ford, Jr. 1943 Influence of increasing doses of thiamine and riboflavin on efficiency of their utilization. J. Nutrition, vol. 26, p. 659.

Ume, Tango 1947 Relation between fat and riboflavin requirement of growing rats. Chem. Abstract, vol. 41, p. 4550. (From J. Agr. Chem. Soc. Japan, vol. 17, p. 1050, 1941).

Unna, K., H. C. Singher, C. J. Kensler, H. C. Taylor and G. P. Rhoads 1944 Effect of dietary protein on liver riboflavin levels and on inactivation of Estradiol by liver. Pro. Soc. Exp. Biol. Med., vol. 55, p. 254.

Williams, R. O., H. L. Mason, P. L. Cusick and R. H. Wilder 1943 Observations on induced riboflavin deficiency and the riboflavin requirement of man. J. Nutrition, vol. 25, p. 361.