ASSESSMENT OF VITAMIN B-6 STATUS AND INTAKE OF FEMALE ADOLESCENTS IN OKLAHOMA

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The importance of vitamin B-6 as a dietary essential has been widely recognized (1, 2, 3, 4, 5, 6, 7). As an active form of this vitamin, pyridoxal phosphate (PLP) acts as a coenzyme in the catalysis of a large number of biochemical reactions in the metabolism of amino acids, fats, and carbohydrates (3). Although clinical signs of vitamin B-6 deficiency are seldom seen in humans, the body retains only a small amount of this vitamin which can be quickly depleted (8, 9).

Vitamin B-6 nutritional status has been assessed in several population groups. Few studies, however, which have reported adolescent nutritional status, have included an assessment of vitamin B-6 nutriture (10, 11, 12). Adolescence includes a period of rapid physical growth when most nutrient needs, including vitamin B-6, increase in relation to body size (13, 14). Because adolescent females in our weight conscious society may limit the number of food items they consume in order to lose weight or for other reasons, they may decrease intake of some essential nutrients, including vitamin B-6 (11, 15, 16). Concern has also developed regarding the adequacy of the average diet in fulfilling the human requirement for B-6 due to losses during food processing and storage (17) and the variable bioavailability of the vitamin (18).

Teenage pregnancy is a growing problem (19), and pregnant teenage girls as a group exhibit considerable evidence of nutritional deficiency (20). A greatly increased requirement for vitamin B-6 has been demonstrated when pregnancy is superimposed on growth (21). Evidence has accumulated that vitamin B-6 inadequacy may affect the outcome of pregnancy in human subjects (20, 22, 23), and numerous animal studies have demonstrated the harmful effects on the offspring of maternal vitamin B-6 deficiency during pregnancy (24, 25, 26, 27, 28). Therefore, it would seem to be of importance to investigate the vitamin B-6 nutritional status of growing adolescent girls entering the age when pregnancy can occur.

Determination of dietary intake is one of the procedures that is used in the estimation of nutritional status; however, a thorough evaluation requires an assessment of metabolic adequacy by biochemical measurement, such as coenzyme stimulation of erythrocyte alanine aminotransferase (% ALAT), as a vitamin B-6 status parameter. In this study the increase in the activity of the PLP-dependent enzyme erythrocyte alanine aminotransferase (E-ALAT) caused by the addition of PLP in vitro was used as the biochemical measure to evaluate vitamin B-6 status, and was related to dietary intake and selected background variables.

Purpose and Objectives

The purposes of this study were: (1) to assess the vitamin B-6 nutritional status of a group of female adolescents by measurement of % ALAT, (2) to assess dietary intake, and (3) to determine if these measurements were affected by age, age level, race, income, menarcheal

state, or stage of the menstrual cycle. The objectives of the study were:

1. To relate vitamin B-6 intake to % ALAT.

2. To assess the effects on vitamin B-6 intake of age, age level, race, income, and menarcheal state.

3. To assess the effects on % ALAT of age, age level, race, income, menarcheal state, or stage of the menstrual cycle.

Hypotheses

The hypotheses postulated for the study were as follows:

1. There will be no significant relationship between vitamin B-6 intake and % ALAT.

2. There will be no significant relationship between vitamin B-6 intake and age, age level, race, income, or menarcheal state.

3. There will be no significant relationship between % ALAT and age, age level, race, income, menarcheal state, or state of the menstrual cycle.

Assumptions and Limitations

The following assumptions are recognized in the study:

1. The participants answered all questions truthfully and accurately.

2. The participants fasted from 10:00 p.m. the evening before blood was drawn.

3. The dietary information collected was accurate, complete, and representative of usual eating practices.

4. Percent ALAT is a satisfactory indicator of vitamin B-6 status.

The study is most directly applicable to the population sampled, which was female volunteers, 11.5 through 16.5 years of age in north central and northeast Oklahoma. Care should be taken in extrapolating to other population groups.

CHAPTER II

REVIEW OF LITERATURE

The active form of vitamin B-6 is pyridoxal-5-phosphate (PLP), which acts as a coenzyme in numerous biochemical reactions. As this coenzyme, vitamin B-6 has been shown to catalyze a large number and variety of enzyme reactions in the metabolism of proteins, fats, and carbohydrates (5). It functions in nearly all reactions involved with the metabolism of amino acids, including transamination, desulfhydration, decarboxylation, amine oxidation and deamination. PLP is required for many specific reactions of individual amino acids, including the metabolism of tryptophan and its conversion to niacin. Seldom, however, have clinical signs of vitamin B-6 deficiency been seen in humans, although the body retains only a small amount of this vitamin and can be quickly depleted of it (8, 9). An active interest continues in the nutritional status assessment of vitamin B-6.

Methods of Biochemical Assessment of Vitamin B-6 Status

Biochemical diagnosis of vitamin B-6 deficiency in man has been considered essential due to the many possible slowly appearing symptoms which are not specific for the deficiency (30). This makes diagnosis by clinical means alone normally impossible.

Many biochemical tests have been used in the assessment of vitamin B-6 nutritional status in humans. The majority of the tests used may be divided into six categories: (1) measurement of urinary vitamin B-6; (2) measurement of urinary metabolites of vitamin B-6; (3) measurement of metabolic products such as xanthurenic acid in blood or urine following a tryptophan load; (4) measurement of blood levels of vitamin B-6; (5) measurement of activities of blood transaminases dependent on PLP as a coenzyme; and (6) measurement of in vitro stimulation of blood transaminases by PLP.

The first three of the above methods are not practical for use in most nutrition surveys, due to the desirability of 24-hour urine collections. For urinary measurement of vitamin B-6, timed six-hour urine collections have been reported in most instances to be reasonably indicative of 24-hour excretion (31). The urinary level of vitamin B-6 reflects recent intake of the vitamin and is of limited value as an indicator of the severity of a B-6 deficiency in an individual. Studies with adult subjects have suggested that urinary excretions of less than 20 μg/g creatinine indicate marginal or inadequate consumption of vitamin B-6 (8). Age differences exist in the creatinine excretion per kilogram of body weight with children excreting considerably more vitamin B-6 per gram of creatinine than do adults. Sauberlich et al. (31) gave a tentative guide for the interpretation of urinary B-6 levels in children. An unacceptable level of excretion was listed to be < 40 μg/g creatinine for 10- to 12-year old children and < 30 μg/g creatinine for 13- to 15-year olds.

The urinary metabolite usually measured in the assessment of vitamin B-6 is 4-pyridoxic acid. The relationship of B-6 intake to

the excretion level of 4-pyridoxic acid has not been well established; however, normal subjects appear to excrete 0.5 to 1.3 mg 4-pyridoxic acid per day (31).

Urinary metabolites of tryptophan, with xanthurenic acid the easiest to measure, have increased in a vitamin B-6 deficiency following a tryptophan load (32, 33). A test load of 2-5 g tryptophan results in little or no increase in the metabolite excretion in subjects receiving adequate levels of vitamin B-6 (6, 31, 33, 34). Tryptophan load results may be misleading with respect to long-term vitamin B-6 status (32). If the individual is in a vitamin depleted state, the test appears to be a measure of B-6 intake during the previous 24 hours.

Concentration of blood vitamin B-6 is also highly dependent upon dietary composition and appears to reflect recent intake and not vitamin B-6 status (9). Plasma levels of vitamin B-6 in apparently healthy adult subjects have been > 50 ng/ml. In subjects with biochemical evidence of vitamin B-6 deficiency, levels fell below 25 ng/ml.

Transaminase activities in blood components have been utilized to assess vitamin B-6 status in man (32, 35, 36, 37, 38, 39, 40, 41). Measurements of two transaminases, alanine aminotransferase (ALAT) or glutamate-pyruvate transaminase (GPT) and aspartate aminotransferase (ASPAT) or glutamate-oxaloacetate transaminase (GOT) represent functional biochemical tests that have been investigated as useful indices in evaluating the nutritional status of vitamin B-6 in humans. PLP serves as the coenzyme in these transamination reactions. Transaminase measurements may provide information regarding the degree of

vitamin B-6 depletion. Transaminase reactions have been studied using whole blood, plasma, serum, leukocytes, and erythrocytes. Erythrocyte alanine aminotransferase (E-ALAT) activity has been reported to be a better index of vitamin status than serum, plasma, or leukocyte ALAT (37, 39, 42) in spite of the fact that erythrocyte and whole blood ALAT activities are only one-tenth that of ASPAT or less (39, 43, 44, 45). However, activities of both enzymes have decreased with an inadequate intake of vitamin B-6, with a simultaneous increase in the percentage stimulation following the in vitro addition of PLP (32, 39, 43, 46). Considerable individual variation exists among normal individuals as to transaminase activities (6, 9, 31, 43).

Several investigators have suggested that the change in percentage stimulation of E-ALAT activity after the in vitro addition of PLP (% ALAT) is a more reliable measure of the nutritional adequacy of vitamin B-6 than measurement of basal enzyme activity (31, 32, 35, 41). Percent ALAT has varied among normal adults (8, 32, 35, 39, 43, 45, 47). Cinnamon and Beaton (32) noted values of 15, 23, and 26% ALAT for three normal subjects. Cheney et al. (39) found the average % ALAT to be 25 in seven apparently healthy adults. Percent ALAT for a group of female subjects ranged from 0 to 14 (35). Other investigators have reported average % ALAT for females of 8 (29), 18 (45), 17 (46), and 5 (47). In a study investigating the vitamin B-6 status of 127 12- and 14-year old girls (10), a mean of 6.5 was found for 87 subjects with % ALAT < 16. The mean was 26.0 for 40 subjects with % ALAT ≥ 16. A range of 0-56.5 was noted in the study for % ALAT for all subjects.

Values for % ALAT of < 16 have been considered indicative of normal vitamin B-6 status and values $\geq$ 16, high and indicative of some inadequacy of vitamin B-6 in this study. This criterion was based on several previous studies (29, 32, 35, 47) where average % ALAT for apparently healthy subjects was approximately 16 or less. In one study, status of three subjects was evaluated from a tryptophan blood test before vitamin B-6 depletion and was found to be normal (32). Brown et al. (47) found nine control subjects had normal 4-pyridoxic acid excretion and E-ALAT and E-ASPAT activities but low plasma B-6 levels before vitamin B-6 depletion. Vitamin B-6 status was evaluated by Driskell et al. (29) and Kirksey et al. (10) using only % ALAT as the status indicator. In the study by Kirksey et al., subjects were classified on the basis of % ALAT < 16 or $\geq$ 16.

Percent ALAT has been considered indicative of long-term vitamin B-6 status (32, 48). One month or longer may be required for vitamin B-6 supplementation to increase E-ALAT activity and to decrease % ALAT and restore these measurements to normal, pre-experimental levels in other-wise healthy, B-6 depleted subjects (32, 47, 48).

Methods for Assessing Dietary Intake

Dietary assessment is an integral part of most evaluations of nutritional status. The most common techniques for the collection of dietary data of individuals are: the dietary history interview method which measures intake for a period of one year or less; the dietary record which may cover varying lengths of times typically from one to seven days; and the 24-hour recall which may be given once or repeated

for varying numbers of days. Of these techniques, the 24-hour recall requires the least time, money, and number of subjects (49, 50).

Greger and Etnyre (51) tested the validity of the 24-hour dietary recall as a tool to estimate nutrient intakes of female adolescents by comparing the recalled intake of the subjects to their actual intake during a metabolic study. It was concluded that the dietary recalls used were a valid estimate of the dietary intake of energy, protein, calcium, and zinc of the group studied. Young et al. (50) found that for population groups the 24-hour and seven-day record gave approximately the same estimates when used to figure the mean intake of a group of 50 or more people when errors of 10% could be tolerated. Similar results were not found on an individual basis. Twenty-four hour recalls obtained from children 10 to 15 years of age have been reported to be as accurate as data obtained from weighed food records (52). Emmons and Hayes (53) found the ability of children ranging in age from six to twelve years to correctly recall foods eaten improved with age. A similar study with children ranging in age from nine to eighteen years, evaluated how well children recalled what they had eaten (54). The kind and amount of weighed and measured foods taken and eaten during one school lunch by 94 children was recorded individually. Later that day the children were asked to recall what they had eaten. The criteria used for agreement between the meal eaten and the recall required that the children recall correctly both the food and the amount eaten. The recall and actual intake were not considered to be in agreement if one piece of celery was omitted or if three-fourths cup was the estimated amount of a two-thirds cup serving. Five percent recalled the same numbers and kinds of food items as were eaten,

but were incorrect in reporting the quantities for one to three items. There appeared to be a tendency for greater under-reporting of food items eaten as the number of foods increased.

The validity of the 24-hour recall was studied by Madden et al. (55). For three (energy, protein, and vitamin A) of the eight nutrients considered, small intakes tended to be over-reported, and large intakes tended to be under-reported. The 24-hour dietary recall for these three nutrients would seldom, if ever, indicate a difference between recalled and actual intakes for the group if no difference existed, due to this conservative reporting by subjects. However, a false negative could occur, where no significant difference between recalled and actual intakes might be found when a difference did exist. Chalmers et al. (56) found that a dietary record needed to consist of only one day to be most efficient when characterizing the mean intake of a group. The number of subjects needed in the group depended on the precision required for the estimate. To obtain greater precision for the intake estimate of a group, increasing the size of the group was more efficient than increasing the number of days of the dietary record. A graph was presented from which the size of the group necessary for a given level of confidence could be estimated. From this graph it was estimated that to obtain a 0.05 level of confidence for either side of the mean equal to 5%, 10%, or 15% of the recommended dietary allowance (RDA) for a nutrient, a group of 250, 60, or 30 subjects, respectively, would be needed. The number of subjects required was reduced by 5% when the group included women only. by 5% when the group included women only. It was assumed that a one-day record and a 24-hour recall would be essentially equivalent.

Factors Affecting the Vitamin B-6 Requirement

Protein Intake

The level of protein in the diet has been found to alter the requirement of vitamin B-6. When high protein diets were consumed, the requirement of man for vitamin B-6 was increased (33, 34, 43). Normalization of xanthurenic acid excretion in young men following a tryptophan load required 1.25 mg/day of vitamin B-6 at 30 g protein/day and 1.5 mg/day at 100 g protein (33). Optimum B-6 intakes of 1.25-1.5 mg/day and 1.75-2 mg/day were recommended on the low and high protein diets, respectively. In another study 0.76 mg/day of pyridoxine prevented the excretion of abnormal metabolities of tryptophan and methionine in male students fed 54 g protein (34). When 150 g protein per day were fed, however, the same amount of vitamin B-6 was insufficient to prevent abnormal metabolite excretion. On a diet providing only 57 g protein, the vitamin B-6 requirement of eight young women was estimated as 1.5 mg/day (8). This dietary requirement was based on excretion rates of 4-pyridoxic acid and vitamin B-6 when a basal diet containing 0.34 mg vitamin B-6 was supplemented with 0.6, 1.2, and 30.0 mg/day pyridoxine hydrochloride (HCL) for 7, 3, and 1 days, respectively.

Bioavailability of Vitamin B-6

The term "bioavailability" has been defined by Gregory and Kirk (18, p. 1) as "the net utilization of the vitamin" and refers in this context to "the fraction of the total dietary vitamin which undergoes

intestinal absorption and functions qualitatively as vitamin B-6." Although many researchers have studied the bioavailability of vitamin B-6 under varying circumstances, few generalizations can be made concerning the overall bioavailability of the B-6 vitamins or factors which affect them (18).

The first indication that the bioavailability of vitamin B-6 in foods might not be complete was reported by Sarma et al. (57, 58). Rat growth and Saccharomyces uvarum assay results were compared for a variety of materials (57). The incomplete utilization by the rat could not be explained by the differing activities of pyridoxamine, pyridoxal, and pyridoxine when mixed in the diet (58).

Other research has been directed toward investigating the bioavailability of vitamin B-6 in foods. In studies of the nutritional quality of canned combat rations in the early 1950's, the vitamin B-6 available to support rat growth in diets based on rations was less than that available to test microorganisms (59, 60). The bioavailability of the vitamin in fresh, cooked, and processed meats was examined by Lushbough et al. (61). Values derived from rat bioassays were consistently greater than those from the microbiological yeast assay employed.

The bioavailability for man of vitamin B-6 from whole wheat and white bread was studied by Leklem et al. (62). Urinary B-6 and E-ALAT indicated no difference in the bioavailability of the B-6 vitamins from the breads. However, fecal vitamin B-6 increased and urinary 4-pyridoxic acid excretion decreased with whole wheat bread consumption, suggesting utilization of only 90-95% as much of the vitamin from the whole wheat bread as from the white bread. A daily intake of only 1.5

mg of vitamin B-6 was maintained during this study. This marginal intake could affect vitamin B-6 status indicators. Another human bioassay suggested that vitamin B-6 was less available from soybeans than from beef (63).

Tarr et al. (64) examined the availability to man of vitamin B-6 in a typical American mixed diet. The B-6 availability ranged from 61 to 81%, with a mean of 71%, using plasma PLP data, and ranged from 73 to 92%, with a mean of 79%, according to urinary vitamin B-6 data. These percentages were relative to the bioavailability of pyridoxine HCL, which was assumed to be 100%. Thermal processing may have been partially responsible for the incomplete bioavailability of the vitamin, since much of the diet was composed of canned goods.

Ascorbic Acid Intake

An interaction of ascorbic acid and vitamin B-6 has been suggested by Baker et al. (33, 65). Plasma ascorbic acid decreased in subjects during vitamin B-6 depletion, even though ascorbic acid intake was adequate and constant throughout the study (33). Upon repletion, plasma ascorbic acid returned to control levels. During the depletion phase of an ascorbic acid metabolism study, a 3.5 fold increase in urinary vitamin B-6 occurred even though subjects' intake of 2.5 mg of pyridoxine HCL/day remained constant (65). During repletion of ascorbic acid, urinary B-6 values progressively decreased although vitamin B-6 intake did not vary. The mechanism of the interrelationship of these two nutrients was not determined in these studies.

The short-term influence of ascorbic acid intake on vitamin B-6 metabolism was studied further by Schultz and Leklem (66). The major vitamin B-6 excretory metabolite, urinary 4-pyridoxic acid, was measured in order to assess metabolic changes in excretion caused by short-term doses of ascorbic acid. During the third part of the study, which lasted 10 days, the eight subjects were fed all their meals, which contained 1.06 mg of vitamin B-6 per day, in a metabolic unit for days 2, 3, 9, and 10. For the remaining days, subjects consumed self-selected diets and recorded daily food intakes. A 1-g dose of ascorbic acid was given the mornings of days 4-10. A 2-mg dose of pyridoxine HCL was given the mornings of days 3 and 10. Complete 24-hour urines were collected on days 1 to 4 and days 9 and 10. The mean urinary excretion level of 4-pyridoxic acid for the eight subjects was not significantly different when 1 g ascorbic acid plus 2 mg pyridoxine HCL values were compared to the pre-ascorbic acid plus 2 mg pyridoxine HCL control values. It was concluded that short-term ascorbic acid supplementation did not alter vitamin B-6 metabolite excretion. Excretion of B-6 itself, however, was not measured in this part of the study.

Elliot (67) studied the effects of vitamin C loading on serum components. During a 12 week period, serum ASPAT decreased with 3.0 g/day vitamin C loading as compared with values for a two-week period of individual baseline intake. This finding is in contrast to variable responses in serum ASPAT reported by Van Steirteghem et al. (68), who also reported a consistent decrease in serum ALAT following the administration of 3 g of ascorbic acid for 18 days.

Hormone Concentrations

Numerous studies have shown changes in vitamin B-6 status indicators during pregnancy, when estrogen and progesterone levels are high, and during the use of oral contraceptives (OC) containing synthetic estrogen and progesterone combinations. The changes suggest an increased requirement of vitamin B-6 (69, 70, 71, 72, 73, 74, 75). Based on low plasma PLP levels (42, 69, 70, 71), decreased activities of PLP-dependent aminotransferases in erythrocytes (42, 70, 72), and increased stimulation values of these enzymes (22, 70), a relative vitamin B-6 deficiency may have been established in pregnancy. Excretion of increased levels of tryptophan metabolites after a tryptophan load has been found in OC users (72, 73, 74, 75). Decreased 4-pyridoxic acid excretion (73), as well as decreased E-ALAT and increased % ALAT (29, 45, 75) have also been found in OC users in comparison to control subjects not using OC.

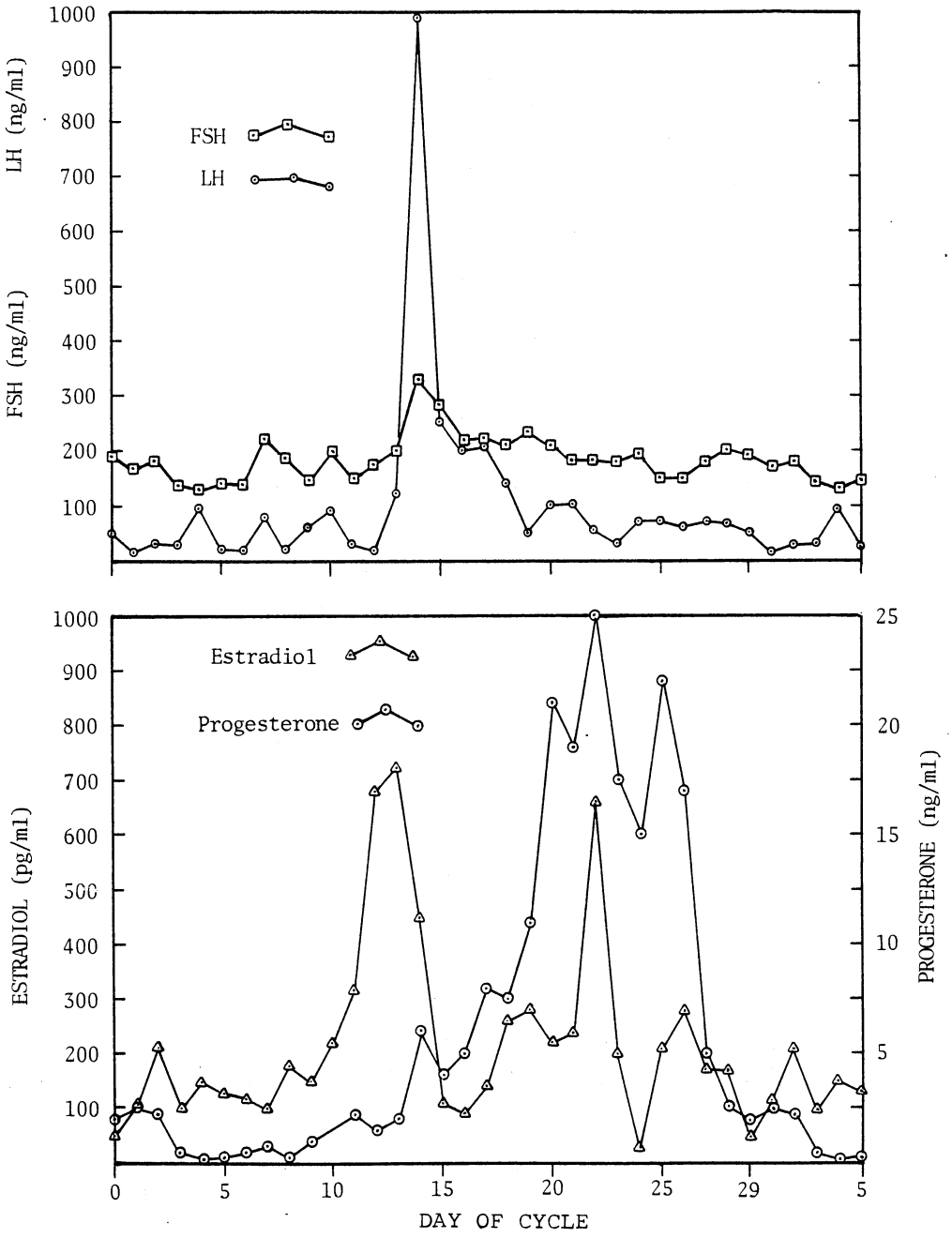
A possible mechanism responsible for the apparent increase in the requirement for vitamin B-6 may be that the estrogen component induces an increased turnover rate of PLP-dependent amino acid-metabolizing pathways, thereby increasing the B-6 requirement (46). Total nonessential amino acids in the plasma were significantly lower for OC users than for non-users (75). Total essential amino acids were not significantly different between the groups, however. Based on previous research, one might anticipate a decrease in vitamin B-6 status (increasing % ALAT) in a stage of the menstrual cycle when both estrogen and progesterone blood levels are highest.

Greep et al. (76) characterized the female menstrual cycle by recurring changes in the levels of the four primary reproductive hormones:

estrogen, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). The concentrations of these hormones in the blood as shown by Van de Wiele (Figure 1) determine the course of the menstrual cycle. Blood levels of FSH and LH remain low and relatively constant during the entire cycle, except for a sharp preovulatory spike for LH and lesser one for FSH. Blood estradiol concentration, the most important of the estrogens secreted by the ovary, rises fairly steadily during the early part of the menstrual cycle, and reaches a peak at midcycle. Blood progesterone levels remain very low throughout the early part of the menstrual cycle. During the last half of the cycle, large quantities of progesterone are secreted. Just previous to and at the onset of the menstrual flow, a sharp decline of blood estradiol and progesterone occurs, which levels out by the termination of the menstrual flow (76). Boots et al. (78) could not relate serum vitamin B-6 concentration and endogenous estrogen and progesterone levels in the baboon.

Factors Affecting Vitamin B-6 Intake

Interest has been directed toward the adequacy of the average American diet in fulfilling the human requirement for vitamin B-6. Concern exists as to the adequacy of intake of the vitamin due to considerable loss during food processing (17, 61, 79, 80, 81, 82, 83), variable loss during storage (17, 83, 84), and the frequent consumption of amounts less than the RDA (10, 17, 29, 85, 86).



[Redrawn from Van de Wiele et al., (77).]

Figure 1. Plasma LH, FSH, Estradiol and Progesterone During the Normal Menstrual Cycle

Losses of Vitamin B-6 in Food Processing and Storage

A great interest in causes of vitamin B-6 destruction began in the early 1950's when convulsions responsive to vitamin B-6 were observed in some infants fed an unfortified, heat-sterilized canned infant formula (87). The loss of available vitamin B-6 was believed to have been due to extensive heat processing. Ensuing research on heat-processed dairy products showed varying results (79, 80, 88). Hassinen et al. (79) demonstrated that pyridoxal and pyridoxamine, which comprise the majority of the naturally occurring vitamin B-6 in milk, were much less stable to heat than added pyridoxine. Therefore, pyridoxine HCL has been used for fortification processes. Pyridoxine HCL usually has been assumed to be stable during processing and storage when used to fortify products (89). However, according to recent research, pyridoxine added during food fortification may be susceptible to significant losses under some conditions such as heating, and the remaining forms of vitamins may not be consistently available (81, 90, 91, 92).

Schroeder (17) ascertained food sources providing the recommended daily intake of 2.0 mg or more of vitamin B-6 and analyzed the effect of processing and storage on the vitamin content of the foods. The processes of canning and freezing vegetables reduced the vitamin B-6 content by 57-77% and 37-56%, respectively, compared with the raw counterparts. The canning losses of 10 seafoods amounted to 49% of the original B-6 content. Meat canning produced losses of 43%.

Schroeder (17) assumed that the daily diet was composed of 1 kg food (wet weight exclusive of fluids). Therefore, the RDA of 2 mg would be attained if all foods contained 2 ppm vitamin B-6. Corn, rice, rye, and wheat products, all canned fruits and dairy products, 7 of 11 canned meats, raw fruit (except bananas), and fruit juices had concentrations below the recommended vitamin B-6 density level of 2 ppm. Thirty-one percent of a total of 552 foods analyzed had concentrations of 2 ppm or more, and 14% had concentrations of 2.5 ppm or more.

Vitamin B-6 losses were 50-70% from dry heat processing of breakfast cereals, which is a more severe process than toasting, when measured by Gregory and Kirk (81). Meyer et al. (82) determined that the retention of B-6 in roasted beef averaged 72%, with 16% recovered in the drip. An average of 49% was retained in oven-braised beef, with 34% transferred to the drip. An average retention of 54% of the original amount of vitamin B-6 remained after samples of beef, lamb, veal, pork, and several processed meat products were cooked (61). The vitamin B-6 content in 81 food items sampled from the serving lines at two military installations varied widely (83). For example, three samples of rib roast averaged 1.43 ppm, with a range of 0.71 to 2.37 ppm.

Richardson et al. (93) observed losses of available vitamin B-6 in a variety of products during storage after being frozen, thermally processed, or irradiated using rat growth assay. The storage of lima beans and sweet potatoes apparently increased the available vitamin B-6 after each processing treatment.

Adolescent Dietary Practices

Adolescence includes a period of rapid growth when nutrient needs increase rapidly in relation to body size (14). Some adolescents may have less than optimal nutrient intake due to attempts to reduce caloric intake or limit choices of food items (11, 15, 16). Children 10 to 15 years of age have consumed poorer diets relative to the recommended recommended allowances than younger children (14, 94, 95). Of children studied from 10 to 15 years, those aged 13 to 15 had the poorest nutrient intakes (14). Of 421 teenagers from three schools in southern Illinois studied by Wharton (96), 80% consumed less than the recommended allowances for energy.

Dwyer et al. (97) examined participation of adolescents in weight reduction programs. Sixty percent of 464 senior high school girls from a middle and upper-middle class suburban community had been on a reducing diet, even though only 15% were obese.

Recommended Vitamin B-6 Allowance for Adolescent Females

The American Academy of Pediatrics, Committee on Nutrition (98), estimated the vitamin B-6 requirement of the adolescent to be from 1.5 to 2.0 mg per day. The estimate was based on the absence of deficiency symptoms in populations with probable intakes in that range. The Food and Nutrition Board of the National Academy of Sciences (99) recommended in 1980 a daily vitamin B-6 allowance of 1.8 and 2.0 mg per day for females for age ranges of 11-14 and 15-18, respectively, based on 0.02 mg of vitamin B-6 per gram of expected protein intake, as estimated

from food consumption surveys. The board indicated, however, that the data were not sufficient to permit a satisfactory evaluation of the requirement for adolescents.

Vitamin B-6 Status and Intake of Females

Nonpregnant Adolescents

Only a few of the studies reporting adolescent nutritional status have been concerned with or included an assessment of vitamin B-6 nutriture (10, 11, 12). Hodges and Krehl (11), in a study of the nutritional status of 622 Iowa teenagers (grades 9-12), determined urinary vitamin B-6. On the whole, the values fell within the normal range, averaging 211 μg pyridoxine/100 ml urine for the girls studied. In a study of 642 10- to 13-year old New York City school children (12), blood levels of 12 vitamins, including vitamin B-6, were determined. A mean of 36 ng vitamin B-6/ml blood for 576 apparently healthy subjects was considered by the authors to represent a normal value for this group, due to the absence of deficiency symptoms in the population studied.

A mean daily intake of 1.24 $\pm$ 0.07 mg of vitamin B-6 was reported by Kirksey et al. (10) for 12- to 14-year old females. Approximately one-half of the subjects studied consumed less than two-thirds of the recommended dietary allowance for the vitamin. The mean vitamin B-6 intake from self-selected diets of two groups of older female students, 18 to 25 years of age, studied by Driskell et al. (29), was approximately 62% of the RDA. Almost one-third of the girls consumed less than 70% of the RDA for vitamin B-6.

Pregnant Adolescents

As a group, adolescent girls are entering the age when pregnancy may occur. Pregnant adolescents must not only satisfy the vitamin B-6 needs of their own growing bodies but that of the fetus as well (21). This multiple demand heightens the need for vitamin B-6 and increases the possibility of developing a deficiency. If a great many adolescent girls are in a deficient vitamin B-6 nutritional state before pregnancy, most will probably remain in a deficient state if pregnancy occurs. This could result in undesirable effects to the fetus.

It has been recognized that the requirement for vitamin B-6 increases during pregnancy (23, 42, 100, 101, 102). The vitamin is essential for normal fetal (25, 26, 27, 28, 103, 104) and postnatal growth and development (24, 28, 105). A vitamin B-6 inadequacy may affect the outcome of pregnancy in human (20, 22, 23) and animal subjects (24, 25, 26, 27, 28). When pregnancy was superimposed on a rapid phase of growth and dietary vitamin B-6 was restricted, less body weight was gained by pregnant rats and fetuses were smaller when compared with values for pregnant rats fed the same level of B-6 but mated after the rapid growth phase (21).

A group of pregnant teenage girls, ages 13 to 17, who received prenatal care at a special clinic facility in Newark, New Jersey, were studied by Kaminetzky et al. (20). The group studied exhibited considerable evidence of nutritional deficiency based on overt physical signs, low blood vitamin and other chemical values, and dietary evaluations. Blood vitamin levels were low for approximately 20% or more of the subjects for vitamin B-6, niacin, folate, vitamin A, and thiamin.

Of the 77 patients studied in the third trimester of pregnancy, 58% had low blood vitamin B-6 levels. Prescribed oral vitamin supplements appeared to be effective in raising low blood levels of folic acid and thiamin only; the blood levels of the other vitamins studied, including vitamin B-6, appeared unaffected or only slightly improved by supplement intake. Of the patients who developed preeclampsia or delivered low birth weight babies, all had low or borderline protein intake, low energy intake, and low circulating vitamin B-6 levels.

Vitamin B-6 Intake and Status Studies

In vitamin B-6 status studies in which both coenzyme stimulation and dietary vitamin B-6 intake were considered, vitamin B-6 intake failed to predict biochemical vitamin status (22, 29, 48). Chrisley and Driskell (106) reported apparently adequate vitamin B-6 status, as indicated by % ALAT, for all but three of 89 subjects between the ages of 19 and 59. Subjects consuming more than 0.8 mg of B-6 daily (40% RDA) appeared to have adequate vitamin B-6 status, as indicated by % ALAT. Kirksey et al. (10) found female adolescents with high % ALAT tended to have lower mean intakes for all nutrients, and significantly lower mean intakes for vitamin B-6, compared to subjects with normal values.

CHAPTER III

METHODS AND PROCEDURES

Sample

The subjects of this study were 149 healthy, female adolescents between the ages of 11 years, 8 months and 16 years, 5 months. The subjects were volunteers from selected northeastern and north central Oklahoma public schools which had agreed to participate in a regional study entitled "Nutritional Health of Adolescent Females."

Dietary data were available for all subjects. Red cell preparations were lost for all subjects examined on one of the eight days of data collection, and a few additional % ALAT values were deleted because of poor agreement or apparent loss of activity during storage (% ALAT $\geq$ 50). Therefore, % ALAT values were available for 119 girls.

The subjects were divided into age level categories of 12 years, which consisted of girls 11.5 through 13.4 years of age; 14 years, which consisted of girls 13.5 through 15.4 years of age; and 16 years, which consisted of girls 15.5 through 16.5 years of age. A description of the sample appears in Table I.

Eight days (mostly Saturdays) were designated as data collection days during the study. On these days, girls from designated schools came, fasted, to a common location, to bring urine samples, have blood drawn, be given a dental and physical examination, and provide dietary and medical information. Information concerning income, race,

TABLE I

DESCRIPTION OF THE SAMPLE

Age Level	Subjects With Dietary Information					Subjects With % ALAT[1] Values				
		Race		Menarcheal State			Race		Menarcheal State	
	Total	White	Black	Pre-	Post-	Total	White	Black	Pre-	Post-
12[2]	52	43	9	38	11	45	40	5	36	9
14[3]	64	44	20	7	56	57	38	9	5	42
16[4]	33	27	6	0	33	27	26	1	0	27
Total	149	114	35	45	100	119	104	15	41	78

[1]% ALAT = Percentage of stimulation of erythrocyte alanine aminotransferase

[2]Age 12 = 11.5 yr. - 13.4 yr.

[3]Age 14 = 13.5 yr. - 15.4 yr.

[4]Age 16 = 15.5 yr. - 16.5 yr.

and family background was obtained by interview with each subject's mother. Information concerning age and menarcheal state of each subject was obtained by interview with the subject. Each subject and a parent or guardian provided written, informed consent to participate.

Dietary Assessment

Trained dietary interviewers, using a standard set of models to aid in estimating portion sizes, obtained two 24-hour recalls of food consumption from the subjects. One recall was obtained the morning blood was drawn and the other recall was recorded a minimum of two weeks earlier or later. Nutrient intakes were calculated from the Nutritional Analysis System (NAS) at Louisiana State University, Baton Rouge, Louisiana. Dietary data were examined for the average of the two recalls.

Biochemical Assessment

Blood Sampling

Blood samples were taken by venipuncture from fasting subjects. Samples were collected in sodium heparinized evacuated tubes. Blood samples were kept on ice in the dark until hemolysates of erythrocytes were prepared the same day.

Erythrocyte Hemolysate Preparation

The heparinized blood samples were centrifuged at 800 x g and 5°C for 10 minutes. A Pasteur pipette was used to remove the plasma and the buffy coat. A 0.9% NaCl solution equal to twice the volume

of the erythrocytes was used to wash the erythrocytes, mixing by inversion. The mixture was centrifuged again in the cold for 10 minutes at 800 x g and the supernatant removed by suction and discarded. This washing was repeated. Cold phosphate buffer, pH 7.4, was used to dilute an aliquot of the erythrocytes to a 10% (vol/vol) solution. The blood was kept cold during this entire procedure. The diluted, buffered samples were then kept frozen at -20°C until analyzed. Driskell et al. (39) reported this method.

Stimulation of Alanine Aminotransferase Activity in Erythrocytes

Alanine aminotransferase (ALAT) (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2) (107), an enzyme formerly known as glutamic-pyruvate transaminase, catalyzes the transferring of the α-amino group of L-alanine to α-ketoglutarate in the reaction which results in the formation of glutamate and pyruvate. The coenzyme in this reaction is PLP.

In the assay for this enzyme in erythrocytes the amount of pyruvate formed is determined. The pyruvate combines with dinitrophenylhydrazine to yield dinitrophenylpyruvate hydrazone, which is then extracted with toluene. The addition of alkali to the toluene solution results in the formation of a colored compound which is measured in a spectrophotometer. The method used was essentially that of Tonhazy et al. (108) as modified by Heddle et al. (109).

Pyridoxal phosphate was added to other tubes containing erythrocytes for determining the PLP stimulation responses as recommended by Raica and Sauberlich (43). The only change made to the above methods was a reduction in the quantities of all reagents used.

Reagents

A standard pyruvate stock solution (0.50 mg pyruvate/ml solution) was made by dissolving 25 mg pyruvic acid in distilled water and bringing it to a volume of 50 ml. Working standards of 12.5, 25, and 50 μg pyruvate/0.5 ml solution were made from the stock solution.

Aqueous alanine reagent contained 1.78 g L-alanine, 2.00 g potassium monophosphate, and 0.60 g α-ketoglutaric acid in 100 ml. The pH was adjusted to 7.4 using 10% potassium hydroxide.

Dinitrophenylhydrazine (DNPH) reagent was prepared by dissolving 50 mg 2,4-dinitrophenylhydrazine in 10 ml concentrated hydrochloric acid. This mixture was brought to a total volume of 50 ml with distilled water. The mixture was heated covered in a dry bath at 45-50°C to accelerate the solution of DNPH. For later use, the clear solution was decanted, avoiding any undissolved residue.

Other reagents were prepared as follows: Alcoholic potassium hydroxide (KOH) solution consisted of 2.5 g potassium hydroxide plus 100 ml of 95% ethanol. To this was added 20 ml of distilled water. Pyridoxal phosphate solution contained 50 mg of pyridoxal phosphate in 10 ml distilled water and was protected from light. One hundred percent trichloroacetic acid (TCA) solution was purchased as such.

Assay Procedure

For the first part of this procedure, tubes containing erythrocytes were kept in the dark in an ice cold salt brine consisting of crushed ice, salt, and water. Each analysis was performed in duplicate.

Two tubes were used for zero activity determinations, two for basal activity determinations, and two for in vitro stimulation determinations ("+PLP"). Therefore, six tubes were needed per sample. Daily, each of the three working pyruvate standards was also processed in duplicate, along with duplicate blanks.

Five microliters of pyridoxal phosphate solution were added to the bottom of two tubes per sample. To these two "+PLP" tubes and four others (two designated as "basal" tubes and two designated as "zero" tubes) was added 0.125 ml cold buffered erythrocytes. For each standard solution, 0.125 ml of that standard was added to two tubes. To two blank tubes, 0.125 ml of distilled water was added.

The "+PLP" tubes containing erythrocytes and vitamin B-6 were mixed and placed in a 37°C water bath for 20 minutes. They were then returned to the ice water brine. One drop of 100% TCA was added to the "zero" tubes before mixing. To all tubes, 0.125 ml of cold alanine reagent was added. All tubes were mixed and placed in a 37°C water bath for exactly 10 minutes. Upon removal, they were again placed in the ice water brine. One drop of 100% TCA was added to each tube, except for the two "zero" tubes, to which TCA had been added before incubation. All tubes were mixed. The tubes were then kept at room temperature for a minimum of 20 minutes. At this point, it was possible to freeze the contents of the tubes and complete the analyses at a later time. The lights were turned on at this point.

To each tube, 0.25 ml DNPH was added. The contents of the tubes were mixed and permitted to stand at room temperature for exactly five minutes. One-half milliliter of toluene was added to the tubes. Each tube was covered with plastic film and shaken vigorously. The

tubes were centrifuged for 10 minutes. From each centrifuge tube, 0.25 ml of the toluene layer was removed and transferred to another centrifuge tube. This was done by timed intervals as before to allow extractiom from each tube of the pyruvate hydrazone into the toluene for an equal period of time. One and one-half milliliters of alcoholic KOH was added to each aliquot of the extract. These tubes were then covered with plastic film and the contents mixed by inversion. The absorbances of the contents of the tubes were read on a spectrophotometer at 430 nm.

The % ALAT was calculated as follows from absorbances:

$$\frac{(+PLP - Zero) - (Basal - Zero)}{(Basal - Zero)} \times 100 = \% \text{ ALAT}$$

Statistical Analysis

Subjects were classified on the basis of normal or high values for % ALAT, < 16 and ≥ 16, respectively, as having adequate or deficient vitamin B-6 nutritional status. Subjects classified on the basis of normal or high % ALAT were further divided according to whether their intakes of vitamin B-6 were $<$ or $\geq$ 67% of the RDA.

F tests were used to examine the main effects of selected classification and continuous variables on % ALAT in an analysis of variance. Classification variables included age levels (12 yr., 14 yr., 16 yr.), race (white, black) menarcheal state (premenarcheal, postmenarcheal), stage of the menstrual cycle (stage 1, days 5-15; stage 2, days 15-25; stage 3, days 0-5 and 25-29), and use or lack of nutritional supplements. Continuous variables were nutrient intakes, age in months, and

per capita income. The analyses also included the examination of the effects of age, age level, race, menarcheal state, and per capita income on nutrient intakes. All analyses performed on % ALAT for all days of data collection also included the variable "day of data collection" to minimize the effect of significantly higher % ALAT values on days 1 and 4 of data collection.

Chi-square tests were used to evaluate the frequencies of supplemented and unsupplemented subjects and of individuals consuming two ranges of vitamin B-6 within normal and high % ALAT groups. Maximum R^2 improvement stepwise regression analyses were used to create best models to identify the major factors in the study which correlated with % ALAT and unsupplemented vitamin B-6 consumption.

CHAPTER IV

RESULTS

Percentage Stimulation of ALAT (% ALAT)

Throughout this report, two sets of % ALAT values will be presented, in most cases. One set will be values of subjects who participated on any of the seven days of data collection (119 subjects), for whom data are available; a second set will be the values of subjects who participated on only one of those five days (days 2, 3, 5, 7, and 8) of data collection (81 subjects). Percent ALAT values of subjects participating on days 1 or 4 were excluded from the latter set due to significantly higher mean % ALAT. Listed in Table II are mean % ALAT for all days of data collection. The mean of 21 for day 1 was significantly higher than the means for days 2, 7, and 8. A mean of 17 for day 4 was significantly higher than the mean for day 8. This difference may have been due to vitamin B-6 loss during the 2- to 3-hour longer holding of the blood samples before processing on days 1 and 4. There were no values for day 6 of data collection due to an error in making a reagent which led to loss of all samples for that day. All analyses investigating effects of variables on % ALAT for all days included the variable day of data collection to account for the influence of that effect on the analyses.

Subjects were classified on the basis of normal (< 16) or high values (≥ 16) for % ALAT. For all days of data collection, 75 subjects

had normal % ALAT values, with a mean of 4, and 44 subjects had high % ALAT values, with a mean of 27. Means of 4 and 26 were determined for 60 subjects with normal and 21 subjects with high values of % ALAT, respectively, participating on five data collection days. Only 26% had high % ALAT on the five similar days, while 67% of the girls on days 1 and 4 had high % ALAT. The range found for normal values was -9 through 15 and for high values, 16 through 44. Overall means of 13 and 9 were found for % ALAT for all days and five days of data collection, respectively.

TABLE II

LEAST SQUARES MEAN % ALAT FOR ALL DAYS OF DATA COLLECTION

Day	No. of Subjects	% ALAT[1]
1	24	21 ± 3[2a]
2	13	11 ± 3[bc]
3	13	11 ± 3[abc]
4	15	17 ± 3[ab]
5	17	13 ± 3[ab]
6	--	--
7	18	10 ± 3[bc]
8	19	4 ± 3[c]

Note: Means with the same letter are not significantly different from each other ($p > 0.05$).

[1]% ALAT = Percentage stimulation of alanine aminotransferase.

[2]Mean ± SE

Relationship of Selected Variables to % ALAT

From a series of analyses of variables singly evaluated, the effects of categories for age level, race, menarche, and stage of the menstrual cycle and of the covariables age and per capita income on % ALAT are shown in Table III. Race and income did not affect % ALAT. The subjects were divided into age level categories of 11.5 - 13.4 years, 13.5 - 15.4 years, and 15.5 - 16.5 years of age. This was done to identify effects that might not be progressive and linear over the entire range of ages. When five days of analysis were considered, age level (but not age as a continuum) did have a significant influence on the % ALAT for subjects evaluated on those days. The least squares mean % ALAT for 12-year old subjects was significantly higher than the 14-year mean. The 16-year olds did not differ significantly from the 12- or 14-year olds. Menarche also affected % ALAT, when values for subjects participating on five days were considered. Postmenarcheal subjects had a lower mean % ALAT than premenarcheal subjects.

The menstrual cycle was divided into three stages roughly corresponding to changes in the levels of the four primary reproductive hormones in the blood. Stage one was defined to be days 5 to 15 of the menstrual cycle, and stage two, days 15 to 25. Stage three was days 0 to 5, and days 25 to 29 of the cycle. The stage of the cycle was based on days since the start of the last menstrual period (day 0), as reported by subjects on the day blood was drawn. Stage of the menstrual cycle was not related to % ALAT.

Nutrient Intake

Presented in Table IV are the mean intakes of selected nutrients

TABLE III

LEAST SQUARES MEANS AND PROBABILITIES OF EFFECTS OF SELECTED VARIABLES EXAMINED INDIVIDUALLY BY ANALYSES OF VARIANCE ON % ALAT

Class Variable	Value	% ALAT All Days			% ALAT Five Days		
		n	Mean	P	n	Mean	P
Age Level	12	45	15 ± 2[2]	0.22	39	13 ± 2[a]	0.03
	14	47	10 ± 2		23	5 ± 2[b]	
	16	27	12 ± 3		19	8 ± 3[ab]	
Race	White	104	12 ± 1	0.44	72	9 ± 1	0.43
	Black	15	15 ± 4		9	12 ± 4	
Menarche	Pre-	41	12 ± 2	0.64	33	12 ± 2[a]	0.05
	Post-	78	13 ± 1		47	7 ± 2[b]	
Stage of the Menstrual Cycle[3]	1	23	11 ± 2	0.42	16	6 ± 2	0.76
	2	21	9 ± 3		15	8 ± 3	
	3	18	15 ± 3		6	9 ± 4	

TABLE III (Continued)

Covariable	Value	% ALAT All Days			% ALAT Five Days		
		n	Mean	P	n	Mean	P
Age				0.68			0.14
Per Capita Income				0.86			0.50

Note: Means with the same letter are not significantly different ($p > 0.05$).

[1]Age 12 = 11.5 yr. - 13.4 yr.; age 14 = 13.5 yr. - 15.4 yr.; age 16 - 15.5 yr. - 16.5 yr.

[2]Mean $\pm$ SE

[3]Stage 1 = Days 5 to 15 of the menstrual cycle; Stage 2 = Days 15 to 25 of the menstrual cycle; Stage 3 = Days 0 to 5 and days 25 to 29 of the menstrual cycle. (Day 0 was the first day of menstrual flow.)

TABLE IV

MEAN NUTRIENT INTAKES OF ALL SUBJECTS AND PERCENTAGES OF SUBJECTS INGESTING ≤ 67% RDA

Nutrient	RDA[1]	Mean	Subjects With ≤ 67% RDA	
			n	%
Energy, kcal	2100	1845 ± 626[2]	38	25
Protein, g	46	64 ± 24	7	5
Vitamin B-6, mg	2.0	1.25 ± 0.91	113	75
Vitamin C, mg	60	102 ± 106	43	29
Vitamin A, IU	4000[3]	4023 ± 2738	59	39
Iron, mg	18.0	12.2 ± 6.3	88	59

[1]Taken from 1980 revised RDA for 15-18 year old females.

[2]Mean ± SD.

[3]Taken from 1974 revised RDA for 15-18 year old females.

Between 25 and 50% of the subjects consumed < 67% of the RDA for energy, vitamin C, and vitamin A, and less than 25% consumed < 67% of the RDA for protein.

The RDA for 15- to 18-year old females have been used in all cases in this report, even though the majority of the subjects in this study were younger than 15. The RDA for the nutrients examined in this report are the same for 11- to 14- and 15- to 18-year old females, except in the cases of vitamins B-6 and C. The larger recommendations for these two nutrients were chosen since the requirement for these two vitamins during adolescence is uncertain (48).

Use of Supplements

Supplements taken by subjects have been included in the calculations of nutrient intakes unless otherwise specified. Subjects were divided into three groups with regard to supplementation: subjects not taking a supplement containing either vitamin B-6 or C, subjects taking supplements containing both vitamins B-6 and C, and subjects taking supplements containing vitamin C and not vitamin B-6. A subject was classified as taking a supplement if its use was reported on at least one of the two 24-hour dietary recalls. Four subjects took supplements containing neither vitamin B-6 nor vitamin C and were considered unsupplemented in this classification.

The largest number of subjects, 97, were unsupplemented. Of these, 67% had normal % ALAT. Fifty-three percent of the 17 subjects taking a supplement containing vitamins B-6 and C had normal % ALAT, but only 20% of the five subjects taking a supplement containing only vitamin C had normal % ALAT. Four of the five subjects taking a

supplement containing only vitamin C had high % ALAT. This suggests that high intakes of vitamin C could have increased % ALAT. Due to the small number of subjects taking only vitamin C supplements, however, this possibility could not be investigated conclusively. Since no subjects consumed a B-6 containing supplement without vitamin C, the effect of vitamin B-6 alone could not be evaluated.

A chi-square test was used to examine the distribution of normal and high values of % ALAT for the unsupplemented subjects and subjects taking supplements containing vitamin B-6 and vitamin C. Subjects taking only vitamin C supplements were excluded from this test due to the small number in this group. The observed chi-square of 1.26 for one degree of freedom was not significant at the 0.05 level ($P = 0.26$). Therefore, the observed proportions of supplemented subjects classified as normal and high % ALAT do not differ significantly from those for unsupplemented subjects.

Relationship of Selected Variables to Nutrient Intake

The least squares means were determined, and probabilities of effects of each of the variables age level, race, and menarche, and the covariables age and per capita income on nutrient intake were determined in analyses of variance. The findings are reported in Table V. Age levels significantly differed in protein and energy consumption and possibly in vitamin C consumption. The mean consumption of all nutrients except vitamin B-6 decreased with increases in the covariable age. Protein, energy, and iron intake were also influenced by menarche. The consumption of these three nutrients decreased significantly after

TABLE V

LEAST SQUARES MEANS AND PROBABILITIES OF EFFECTS IN SELECTED VARIABLES ON THE NUTRIENT INTAKES OF ALL SUBJECTS

Class Variables	Value	Protein (g)		Energy (kcal)		Vitamin B-6 (mg)		Vitamin C (mg)		Vitamin A (IU)		Iron (mg)	
		Mean	P	Mean	P	Mean	P	Mean	P	Mean	P	Mean	P
Age Level[1]	12	71 ± 3[2a]	0.01	2007 ± 86[a]	0.05	1.39 ± 0.13	0.33	126 ± 15	0.06	4198 ± 381	0.42	13.5 ± 0.9	0.18
	14	61 ± 3[b]		1790 ± 77[ab]		1.20 ± 0.11		98 ± 13		4162 ± 344		11.7 ± 0.8	
	16	57 ± 4[b]		1689 ± 107[b]		1.11 ± 0.16		71 ± 18		3462 ± 478		11.2 ± 1.1	
Race	White	64 ± 2	0.77	1813 ± 58	0.25	1.24 ± 0.08	0.76	100 ± 10	0.77	4095 ± 256	0.56	12.4 ± 0.6	0.68
	Black	63 ± 4		1952 ± 106		1.29 ± 0.15		106 ± 18		3787 ± 464		11.8 ± 1.1	
Menarche	Pre-	77 ± 3[a]	0.01	2140 ± 90[a]	0.01	1.42 ± 0.14	0.15	117 ± 16	0.26	4439 ± 411	0.27	14.6 ± 0.9[a]	0.01
	Post-	58 ± 2[b]		1726 ± 60[b]		1.18 ± 0.09		95 ± 11		3893 ± 274		11.3 ± 0.6[b]	
Covariables													
Age			0.01-[3]		0.01-		0.15		0.01-		0.02-		0.05-
Per Capita Income			0.01-		0.05-		0.32		0.99		0.72		0.14

Note: Means with the same letter are not significantly different ($p > 0.05$).

[1]Age 12 = 11.5 yr. - 13.4 yr.; age 14 = 13.5 yr. - 15.4 yr.; age 16 = 15.5 yr. = 16.5 yr.

[2]Mean ± SE.

[3]Slope of regression (positive or negative).

the onset of menarche. Per capita income inversely affected protein and energy consumption.

The effects of the variables considered in Table V are also in Table VI for the nutrient intakes of supplemented and unsupplemented subjects considered separately. Age level affected protein, energy, and iron intakes of unsupplemented girls. The consumption of all nutrients also decreased with increasing age. Race had no effect, but menarche influenced intake of protein, energy, vitamin B-6, and iron by unsupplemented girls. Premenarcheal girls had higher intakes of these nutrients than postmenarcheal girls. Per capita income negatively influenced protein consumption. Per capita income may also have been inversely related ($P \leq .06$) to vitamin C intake of supplemented subjects and to iron intake of unsupplemented subjects. No significant relationship was observed among the smaller group of unsupplemented girls.

Relationship of % ALAT to Nutrient Intakes

Least squares mean nutrient intakes for subjects with normal and high % ALAT are given in Table VII. Subjects with normal and high values had similar mean intakes for energy, protein, vitamin B-6, vitamin A, and iron. There was a significant difference, however, between the intakes of vitamin C for the two groups. Subjects with normal % ALAT on the average consumed 80 mg of vitamin C, while subjects with high % ALAT consumed 136 mg.

A relationship between vitamin B-6 intake and the vitamin B-6 status indicator, % ALAT, was sought in this study. Subjects classified on the basis of normal or high values for % ALAT were further

TABLE VI

LEAST SQUARES MEANS AND PROBABILITIES OF EFFECTS OF SELECTED VARIABLES ON NUTRIENT INTAKES DIVIDED BY SUPPLEMENTATION

Class Variables	Value	Protein				Energy				Vitamin B-6				Vitamin C			
		Unsuppl.[1]		Suppl.[2]		Unsuppl.		Suppl.		Unsuppl.		Suppl.		Unsuppl.		Suppl.	
		Mean	P	Mean	P	Mean	P	Mean	P	Mean	P	Mean	P	Mean	P	Mean	P
Age Level[3]	12	71 ± 4[4a]	0.02	75 ± 7	0.51	2036 ± 96[a]	0.03	1961 ± 218	0.99	1.04 ± 0.06	0.13	3.28 ± 0.45	0.45	88 ± 9	0.19	231 ± 51	0.31
	14	60 ± 3[b]		64 ± 7		1770 ± 86[b]		1976 ± 218		0.96 ± 0.05		2.87 ± 0.45		73 ± 8		201 ± 51	
	16	56 ± 5[b]		70 ± 8		1654 ± 122[b]		1986 ± 252		0.84 ± 0.07		2.39 ± 0.52		62 ± 12		112 ± 59	
Race	White	63 ± 3	0.84	73 ± 4	0.09	1815 ± 66	0.44	1922 ± 144	0.47	0.97 ± 0.04	0.96	2.79 ± 0.31	0.49	72 ± 6	0.20	180 ± 36	0.66
	Black	64 ± 5		57 ± 8		1919 ± 118		2145 ± 266		0.97 ± 0.07		3.24 ± 0.57		88 ± 11		213 ± 66	
Menarche	Pre-	77 ± 4[a]	0.01	79 ± 7	0.09	2182 ± 101[a]	0.01	2078 ± 226	0.58	1.10 ± 0.06[a]	0.01	3.10 ± 0.49	0.62	85 ± 10	0.23	171 ± 56	0.73
	Post-	58 ± 3[b]		65 ± 5		1703 ± 67[b]		1924 ± 154		0.90 ± 0.04[b]		2.79 ± 0.33		71 ± 7		195 ± 38	
Covariables																	
Age			0.01-[5]		0.47		0.01-		0.94		0.02-		0.26		0.04-		0.18
Per Capita Income			0.01-		0.55		0.12		0.13		0.12		0.11		0.90		0.06-

TABLE VI (Continued)

		Vitamin A				Iron			
		Unsuppl.		Suppl.		Unsuppl.		Suppl.	
	Value	Mean	P	Mean	P	Mean	P	Mean	P
Age Level	12	3920 ± 402	0.22	5765 ± 1010	0.85	12.4 ± 0.7[a]	0.01	19.9 ± 3.2	0.87
	14	3846 ± 358		6525 ± 1010		10.5 ± 0.6[b]		20.1 ± 3.2	
	16	2868 ± 511		6419 ± 1166		9.0 ± 0.9[b]		22.3 ± 3.7	
Race	White	3673 ± 272	0.97	6611 ± 656	0.23	10.9 ± 0.5	0.99	21.5 ± 2.1	0.42
	Black	3655 ± 487		4890 ± 1209		10.9 ± 0.9		17.8 ± 3.9	
Menarche	Pre-	3928 ± 440	0.54	7263 ± 1023	0.23	13.0 ± 0.7[a]	0.01	19.1 ± 2.2	0.23
	Post-	3606 ± 292		5733 ± 699		10.0 ± 0.5[b]		24.0 ± 3.2	
Covariables									
Age			0.06-		0.72		0.01-		0.60
Per Capita Income			0.49		0.64		0.04-		0.48

Note: Means with the same letter are not significantly different ($P > 0.05$).

[1]Unsupplemented.

[2]Supplemented.

[3]Age 12 = 11.5 yr. - 13.4 yr.; age 14 = 13.5 yr. - 15.4 yr.; age 16 = 15.5 yr. - 16.5 yr.

[4]Mean ± SE.

[5]Slope of regression (positive or negative).

divided according to vitamin B-6 intakes. A chi-square test was used to examine the distribution of % ALAT for vitamin B-6 intake < or ≥ 67% of the RDA. Fourteen percent of all subjects studied consumed ≥ 67% of the RDA and had normal % ALAT. Ten percent had vitamin B-6 intakes ≥ 67% of the RDA and had high % ALAT. Forty-nine and 27% of the subjects had vitamin B-6 intakes of < 67% of the RDA and normal and high % ALAT, respectively. At the 0.05 level of significance, the observed chi-square of 0.32 for one degree of freedom was not significant (P = 0.57). The observed frequencies for subjects with vitamin B-6 intakes < or ≥ 67% of the RDA and classified as having normal and high % ALAT do not differ significantly from the frequencies that would be expected to occur in these categories.

TABLE VII

LEAST SQUARES MEAN NUTRIENT INTAKES FOR NORMAL AND HIGH % ALAT FOR ALL DAYS OF DATA COLLECTION

Nutrient	Normal % ALAT (< 16) (n=75)	High % ALAT (≥ 16) (n=44)
Energy, kcal	1898 ± 75[1]	1894 ± 103
Protein, g	66 ± 3	66 ± 4
Vitamin B-6,	1.20 ± 0.10	1.35 ± 0.13
Vitamin C, mg	80 ± 13[2]	136 ± 17
Vitamin A, IU	4261 ± 307	3557 ± 422
Iron, mg	12.4 ± 0.8	12.6 ± 1.1

[1]Mean ± SE

[2]Significantly different from mean for subjects with high values (P < 0.05).

No significant relationship was found between vitamin B-6 intake and % ALAT for all subjects on all or only five data collection days or for subjects divided into supplemented and unsupplemented or pre- and postmenarcheal groups (Table VIII). A possible exception was with postmenarcheal subjects for five days of data collection where the probability of effect was 0.08. Even in this case, the direction of the relationship was the opposite of what was expected, with % ALAT tending to increase with increasing vitamin B-6 intake. Presented in Figure 2 is a graphic representation of % ALAT for all days of data collection versus vitamin B-6 consumption.

The probabilities of effects of nutrient intakes on % ALAT for all days and for five data collection days were determined individually from analyses of variance and the results are shown in Table VIII. Analyses of variance were also used to determine the effect of nutrient intakes on % ALAT for vitamin supplemented and unsupplemented subjects and for pre- and postmenarcheal subjects. Of all the nutrients considered, vitamin C intake most consistently influenced % ALAT. When all days of data collection were considered, ALAT values for all subjects and for supplemented subjects were positively affected by vitamin C consumed.

As with seven days' data, when % ALAT for only five days of data collection was considered, vitamin C intake was effective. This same positive relationship between vitamin C and five-day % ALAT was also found for postmenarcheal subjects. Vitamin C may also have increased five days' % ALAT for unsupplemented girls ($P < 0.10$). Thus, % ALAT tended to increase as vitamin C intake increased. The energy intake of the supplemented subjects for those days may have had a negative

TABLE VIII

PROBABILITIES OF EFFECTS OF INDIVIDUAL NUTRIENT INTAKES ON % ALAT FOR ALL SUBJECTS AND FOR SUBGROUPS OF SUBJECTS

Group Analyzed	n	% ALAT Mean	Energy	Protein	Vitamin B-6	Vitamin C	Vitamin A	Iron
For All Days:								
Total	119	13	0.77	0.64	0.27	0.02+	0.45	0.51
Unsupplemented	97	11	0.79	0.61	0.29	0.18	0.20	0.46
Supplemented	17	17	0.40	0.51	0.12	0.02+	0.93	0.61
Premenarcheal	41	11	0.49	0.74	0.98	0.12	0.94	0.43
Postmenarcheal	78	13	0.29	0.53	0.15	0.14	0.12	0.66
For Five Days:								
Total	80	9	0.61	0.60	0.21	0.01+	0.58	0.27
Unsupplemented	67	9	0.24	0.40	0.83	0.09+	0.41	0.51
Supplemented	10	11	0.07-[1]	0.17	0.13	0.11	0.92	0.55
Premenarcheal	33	12	0.49	0.78	0.92	0.11	0.94	0.24
Postmenarcheal	47	7	0.13	0.47	0.08+	0.01+	0.26	0.26

[1]Slope of regression (positive or negative).

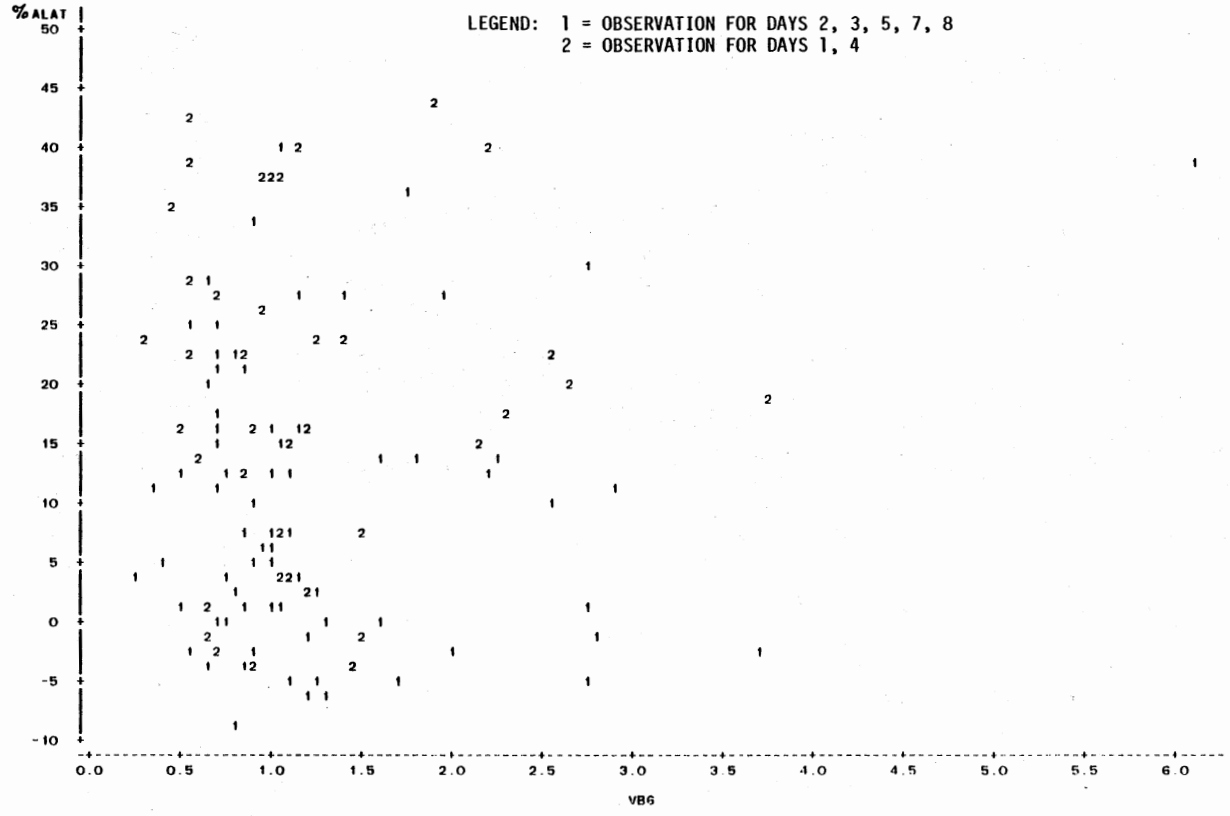


Figure 2. Percent ALAT for All Days Versus Vitamin B-6 Consumption (VB6), in mg

effect on % ALAT of those subjects ($P < 0.10$). The % ALAT for five days tended to increase with the vitamin B-6 intake of the postmenarcheal subjects, but there was no indication of a relationship in the smaller group of premenarcheal subjects.

Graphic representations of % ALAT for all days and for five days of data collection versus vitamin C consumption are shown in Figures 3 and 4.

Subjects were divided according to whether they ingested less than or as much as the RDA for vitamin C. The % ALAT for all days and for five days was significantly greater for those exceeding the RDA than for those not meeting it. Means for all days were of 9% ALAT for those not meeting the RDA and of 15% ALAT for those exceeding the RDA were found. Means of 7 and 12% ALAT were found for five days of data collection for subjects consuming less than or greater than or equal to the RDA, respectively.

The probabilities of effects of nutrient intakes on % ALAT in three stages of the menstrual cycle are given in Table IX. As can be seen in the table, none of the nutrients examined consistently influenced % ALAT in these stages. Vitamin B-6 intake increased with increasing % ALAT for five days in stage two of the cycle. Percent ALAT for all days of data collection increased with increasing vitamin C consumption in stage three of the cycle, and also for stage two when % ALAT for only five days was considered. The small number of subjects in stage three of the cycle for five days may have obscured any effect of vitamin C intake on % ALAT during this stage, such as occurred when all data collection days were considered.

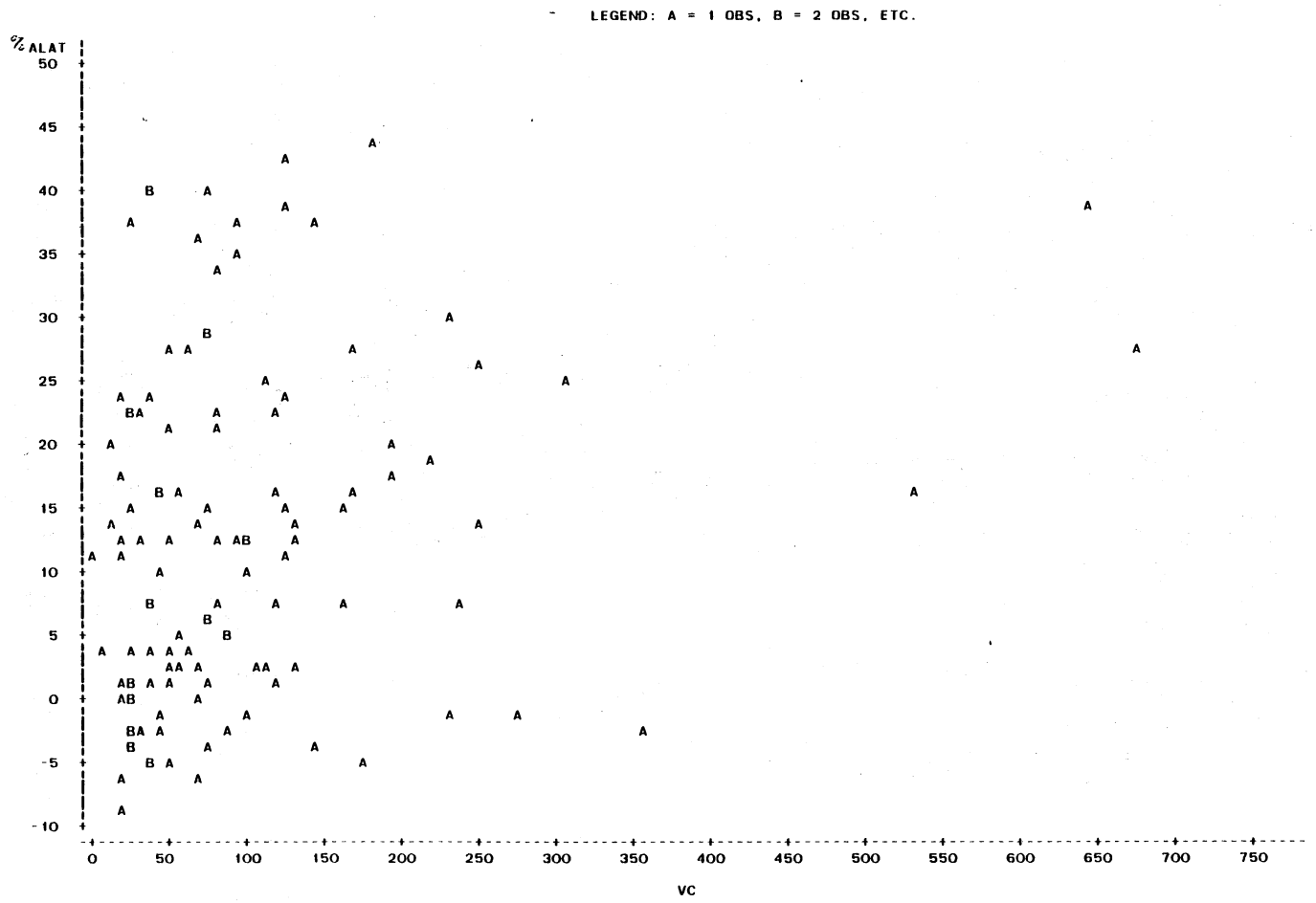


Figure 3. Percent ALAT for All Days Versus Vitamin C Consumption (VC), in mg

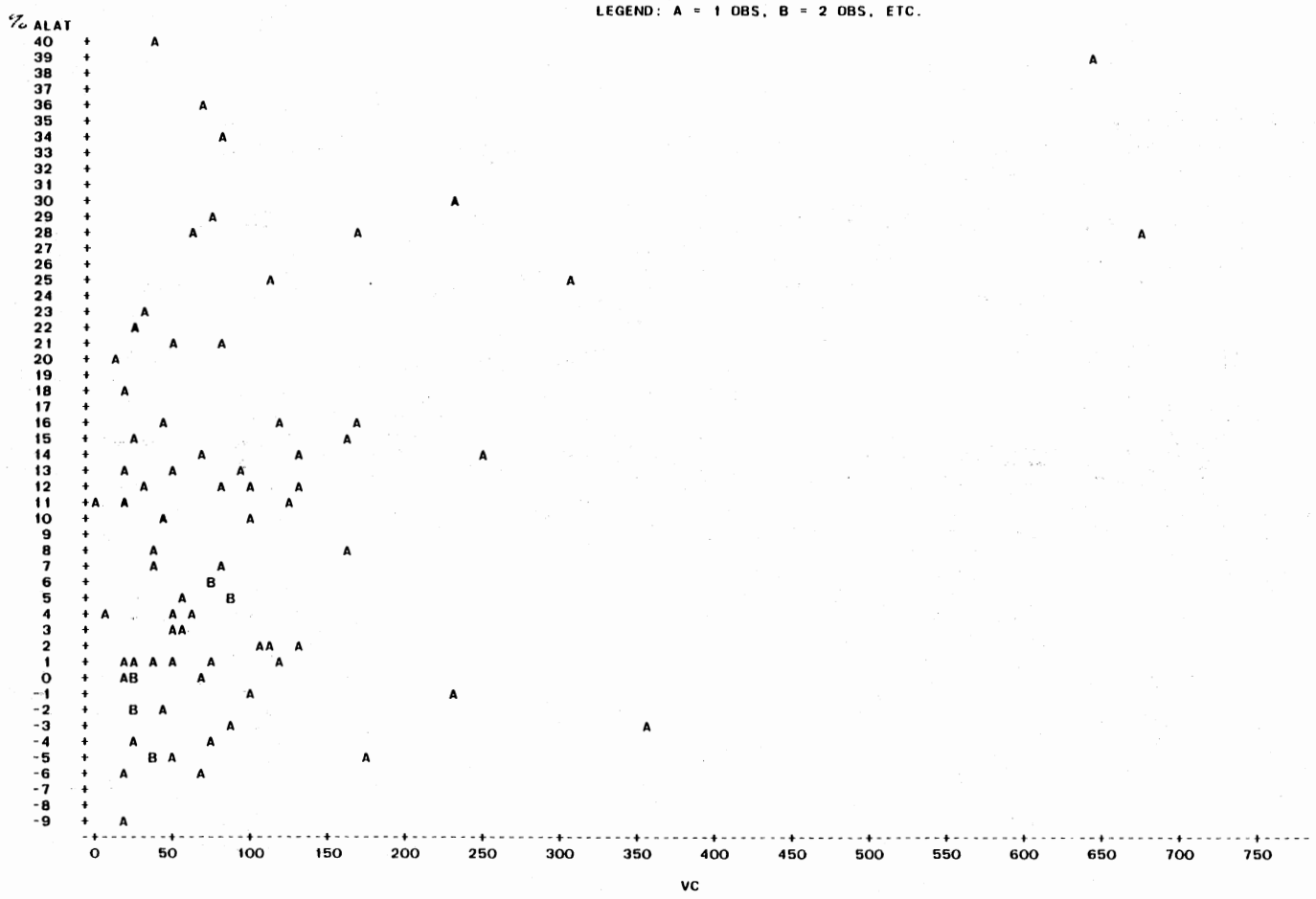


Figure 4. Percent ALAT for Five Days Versus Vitamin C Consumption (VC), in mg

TABLE IX

PROBABILITIES OF EFFECTS OF NUTRIENT INTAKES ON
% ALAT IN STAGES OF THE MENSTRUAL CYCLE

Nutrients	% ALAT All Days			% ALAT Five Days		
	Stage 1 of Cycle,[1] n=23	Stage 2 of Cycle,[2] n=21	Stage 3 of Cycle,[3] n=18	Stage 1 of Cycle, n=16	Stage 2 of Cycle, n=15	Stage 3 of Cycle, n=6
Energy	0.77	0.41	0.44	0.47	0.79	0.14
Protein	0.70	0.70	0.44	0.34	0.74	0.64
Vitamin B-6	0.29	0.14	0.79	0.28	0.01+	0.60
Vitamin C	0.25	0.41	0.01+[4]	0.45	0.01+	0.36
Vitamin A	0.26	0.29	0.14	0.92	0.32	0.50
Iron	0.32	0.32	0.98	0.61	0.71	0.31

[1]Stage 1 of the cycle = days 5 to 15 of the menstrual cycle.

[2]Stage 2 of the cycle = days 15 to 25 of the menstrual cycle.

[3]Stage 3 of the cycle = days 0 to 5 and days 25 to 29 of the menstrual cycle. (Day 0 was the first day of menstrual flow.)

[4]Slope of regression (positive or negative).

Best Models for Predictive Measurements of Vitamin B-6 Nutritional Status

To summarize the results and to determine the extent to which % ALAT for all days, % ALAT for five days, and unsupplemented vitamin B-6 consumption could be predicted by the factors identified, a single model was created using Maximum R^2 Improvement Stepwise Regression Analysis for each of the above measurements. Each of these models identified the major factors examined in this study which influenced that measurement. These models are presented in Table X.

The variables which were found to best predict % ALAT for all days of analysis were day of data collection, consumption of vitamin A, vitamin B-6, and protein. These variables accounted for 25 % of variance in % ALAT. Percent ALAT for five days of data collection could be predicted with an R^2 of 0.25 when the variables vitamin C consumption, protein consumption, and per capita income were considered. The vitamin B-6 intake of the unsupplemented subjects was related most closely to the variables energy consumption, iron consumption, and age, with an R^2 of 0.66.

TABLE X

BEST MODELS FOR PREDICTIVE MEASUREMENTS OF VITAMIN B-6 STATUS AND INTAKE

Predictive Measurements	Variables	Overall P for Model	R^2
% ALAT for All Days	Day of Data Collection	0.01	0.17
	Vitamin A Consumption		0.21
	Vitamin B-6 Consumption		0.23
	Protein Consumption		0.25
% ALAT for Five Days	Vitamin C Consumption	0.02	0.15
	Protein Consumption		0.22
	Per Capita Income		0.25
Vitamin B-6 Intake for Unsupplemented Subjects	Energy Consumption	0.01	0.55
	Iron Consumption		0.64
	Age		0.66

CHAPTER V

DISCUSSION

Percent ALAT

A primary purpose of this investigation was to classify subjects with regard to their vitamin B-6 nutritional status. Subjects were categorized on the basis of normal (< 16) or high ($\geq$ 16) % ALAT. This criterion was based on previous studies where average % ALAT for apparently healthy subjects were < 16 (29, 35, 47, 106). High values have generally been considered to indicate some degree of inadequacy of vitamin B-6 (10, 35, 45, 106). Percent ALAT for subjects in apparent good health has been found by others to seldom exceed 25 (31, 32).

In this study, % ALAT means were 4 for normal subjects and 27 for subjects showing an indication of B-6 deficiency. Ranges were -9 through 15% ALAT for normal subjects and 16 through 44 % ALAT for B-6 deficient subjects. Thirty-seven percent of the subjects in this investigation had high % ALAT and were therefore considered B-6 deficient. Kirksey et al. (10) reported, for 127 females between the ages of 12 to 14, high % ALAT for 31%. Ranges of 0-15.6 and 16-56.5 were considered by them to be normal and B-6 deficient, respectively. Mean values were 6.5 for normal and 26.0 for high % ALAT. Woodring and Storvick (35) observed a range of 0-14.6 for % ALAT and a mean of 4.4

for seven adult females. Driskell et al. (29) reported a mean % ALAT of 7.6 for 73 healthy females ages 18 to 25.

Using % ALAT values above 25 to indicate a vitamin B-6 deficiency, 21 (18%) of the subjects in this study would be classified as deficient. Kirksey et al. (10) found 13% of their subjects to be B-6 deficient using this parameter.

Determination of reference standards is a major problem when assessing nutritional status using biochemical indices. There may be a substantial overlap in the division of values between "normal" and "deficient" categories (110). The determination of a reference standard, above or below which values are classified as normal or deficient, is a matter of philosophy. The less stringent the standard, the greater the number of deficient subjects that will be classified as normal. The more stringent the parameter, the greater the number of normal subjects that will be classified as deficient. However, few deficient subjects will be classified as normal under this condition.

At the survey level of nutritional status assessment one usually wishes to err in the direction of increasing the sensitivity for detecting deficiency, even though some subjects classified as deficient may be from the normal population (111). Therefore, by choosing in this investigation the more stringent of the two parameters used when % ALAT is involved in the evaluation B-6 status, the probability is increased that some of the subjects classified as deficient are normal with regard to vitamin B-6 nutritional status.

Relationship of Background Variables to % ALAT

The third hypothesis of this study was:

3. There will be no significant relationship between % ALAT and age, age level, race, income, menarcheal state, or stage of the menstrual cycle.

No significant relationship was discovered between % ALAT and race, age, per capita income, or stage of menstrual cycle for all days or for only five days (Table III). In agreement with these findings were data presented by Driskell et al. (29), who found % ALAT was not significantly affected by race, age, or income. Chrisley and Driskell (106) also observed no significant effect of income or age under 59 years on % ALAT. No difference due to age was observed by Kirksey et al. (10) with adolescent girls. Schuster et al. (22) found no change in % ALAT with age in a study of low income, pregnant adolescents and adult females. A trend was noticed, however, for black women to have higher % ALAT than white women. There was no apparent race effect in this study; that may have been due to the small number of black participants. Only 15 of the 119 females with reportable % ALAT were black.

In this study, a significant effect of age level and menarche on % ALAT for five days of analysis was detected (Table III). This effect was not observed when all days of analysis were considered. No direct explanation could be found to account for these findings. Kirksey et al. (10) found no significant relationship between % ALAT and menarche.

With increasing age, a tendency was seen for a decrease in unsupplemented vitamin B-6 intake (Table VI). A significant effect on the B-6 status indicator, % ALAT, however, was found for age level and

menarche only when five days of data collection were considered (Table III). In neither case did % ALAT increase with age, as might be expected. The premenarcheal mean % ALAT was in fact higher than the postmenarcheal mean, and the 12 year age level mean was higher than the 14 or 16 year mean. None of these means were less than 16% ALAT, the criterion of vitamin B-6 inadequacy used in this study. Therefore, although B-6 intake tended to decrease with age, a corresponding increase in the vitamin B-6 status indicator was not found.

Relationship of Vitamin C Intake to % ALAT

A possible interaction between vitamin B-6 and vitamin C has been suggested in a few studies (33, 65, 67, 68). Some of the data presented in this study also indicate a possible connection between vitamin B-6 metabolism and vitamin C. Mean vitamin C intakes for subjects with normal and high % ALAT differed. The mean was 80 mg per day for subjects with normal % ALAT and 136 mg per day for subjects with high % ALAT (Table VII). Four of five subjects consuming a supplement containing only vitamin C showed high % ALAT. Vitamin C was the only nutrient considered in this study which showed a significant ($P \leq 0.05$) effect on % ALAT (Table VIII). As vitamin C intake increased, % ALAT tended to increase. This effect was seen with all subjects for all days of data collection and for five days. The effect was also observed for the subgroups of supplemented subjects for all days and postmenarcheal girls for five days. A possible effect ($0.05 < P \leq 0.10$) was observed on % ALAT of vitamin C intake by unsupplemented girls for five days. Percent ALAT increased as vitamin C intake increased. A significant difference was also noted for the mean % ALAT

for vitamin C intakes less than or greater than or equal to RDA for all days and for five days of data collection. The mean % ALAT was significantly higher in each case for vitamin C intake greater than or equal to RDA than for intake less than RDA. All data in this study indicate that as vitamin C intake increased, vitamin B-6 status became less adequate.

Others also have found indications that with vitamin C loading, vitamin B-6 status may become increasingly inadequate. Elliot (67) and Van Steirteghem et al. (68) reported decreases in serum ASPAT and ALAT activities, respectively, with vitamin C consumption of 3 g/day. A simultaneous increase in percentage stimulation for both enzymes has been reported with a decrease in activity regardless of cause (32, 39, 43, 46). In contrast, excretion of urinary vitamin B-6 increased during ascorbic acid depletion, even though vitamin B-6 intake remained at 2.5 mg/day (65). During repletion, urinary B-6 values progressively decreased, suggesting (normal) vitamin B-6 retention with normal ascorbic acid intake and decreased retention with decreasing ascorbic acid intake. Further, Schultz and Leklem (66) found no significant elevation of urinary 4-pyridoxic acid with short-term ascorbic acid supplementation of 1 g/day.

Therefore, this study and other studies reporting vitamin B-6 enzyme status indicators have found a tendency for less adequate vitamin B-6 status with supplemented vitamin C intake. However, studies reporting urinary excretion status parameters have indicated decreased B-6 retention with deficient ascorbic acid intake or no effect of short-term ascorbic acid supplementation on vitamin B-6 metabolism.

Relationship of Hormone Levels to % ALAT

Previous investigators have found that during pregnancy, when estrogen and progesterone levels are high, and with the use of oral contraceptives containing estrogen and progesterone, vitamin B-6 requirements are possibly increased (69, 70, 71, 72, 73, 74, 75). Due to these findings, the effect of stage of menstrual cycle, which was assumed to be related to different blood hormone levels, on % ALAT was investigated in this study. The menstrual cycle was divided into three stages roughly corresponding to the changes in the blood levels of the four primary reproductive hormones: estrogen, progesterone, FSH, and LH. Stage two, days 15 to 25 of the menstrual cycle, was the stage when combined estrogen and progesterone levels were highest during the cycle (Figure 1). The possibility of apparent vitamin B-6 deficiency (% ALAT $\geq$ 16) during this stage of the cycle was probably greater than at other times. Stage of the menstrual cycle did not, however, affect % ALAT for all days or for only five days of data collection (Table III). The mean % ALAT for stage two of the cycle was not significantly different from the mean for stage one or stage three. Failure to demonstrate a difference between stages of the cycle may relate to the nature of the % ALAT measurement. A relatively short-term increase of estrogen and progesterone in blood, as seen during the menstrual cycle, apparently was of insufficient duration to increase % ALAT significantly.

Nutrient Intake

Mean nutrient intakes (Table IV) of the subjects were assessed by

24-hour dietary recall. Supplements were included in the calculation of nutrient intakes. Mean intakes of energy, protein, vitamin B-6, vitamin C, vitamin A, and iron were less than two-thirds of the RDA for 25, 5, 75, 29, 39, and 59% of the subjects, respectively. Correspondingly, other investigations which have assessed adolescent nutritional status have found intakes of energy, vitamin C, vitamin A, and iron to be low (10, 11, 14, 15, 16, 96).

The mean intake of vitamin B-6 for the subjects in this study was 1.25 mg per day, which is less than the 2.0 mg per day recommended allowance for 15-18 year old females (99). The only previously reported intake of vitamin B-6 by adolescents was by Kirksey et al. (10), who reported an intake of 1.24 mg per day for 12 to 14 year old subjects. Studies of the dietary vitamin B-6 of adults have indicated low intakes of the vitamin ranging from 1.20 mg to 1.60 mg (29, 32, 85). The lowest mean intakes of vitamin B-6 for adults were similar to the mean intakes of the adolescents in this study.

Relationship of % ALAT to Vitamin B-6 Intake

The first hypothesis upon which this study was based was:

1. There will be no significant relationship between vitamin B-6 intake and % ALAT.

In this study, no significant relationship was found between the vitamin B-6 intake and % ALAT, on all days or on the five most consistent days of data collection (Table VIII). This confirmed previous reports of a lack of correlation between B-6 intake and % ALAT (22, 29, 48). These findings contrast with those of Kirksey et al. (10),

who observed a significant effect of adequacy of vitamin B-6 intakes (less than or greater than 2/3 RDA) on % ALAT when studying female adolescents. Vitamin B-6 intake for subjects with high % ALAT exceeded that for subjects with normal % ALAT (Table VII). This increase is the opposite (positive correlation) of what would be expected. It occurred only where a significant positive correlation of vitamin C intake with % ALAT also occurred and may have been secondary to this significant relationship.

A possible explanation for the lack of a significant correlation in this study between % ALAT and B-6 intake could be that erythrocyte transaminase activities reflect long-term vitamin B-6 status (32), which is not immediately influenced by daily consumption of the vitamin, while dietary data are based on only two days' intake. Johanson et al. (112) proposed a model for vitamin B-6 metabolism which consisted of a small, rapid, turnover compartment and a larger storage compartment. Percent ALAT could be considered a long-term vitamin B-6 status indicator which reflects the status of the larger proposed storage compartment, while recent diet probably affected the smaller compartment more directly.

There are also inherent difficulties when trying to correlate nutrient intakes from 24-hour dietary recalls and biochemical status indicator values (111). One problem is that the RDA is the usual intake reference standard. The use of this standard, which is normally greater than the "requirement" of most healthy persons (99), therefore guarantees that there will be subjects with intakes below that standard with normal biochemical values. There is also the problem in a population that biochemical nutritional status indicators such as

% ALAT, exhibit a wide range of values, with "normal" and "deficient" values overlapping. This presents difficulty in establishing a reference standard. Schutz (112) found, when attempting to predict measures of nutritional status, that nondietary intake variables, such as sociodemographic variables, could be important contributors to nutritional status.

Relationship of Background Variables to Vitamin B-6 Intake

The second hypothesis of this study was:

2. There will be no significant relationship between vitamin B-6 intake and age, age level, race, income, or menarcheal state.

No significant effect of age, age level, race, per capita income, or menarcheal state was observed on vitamin B-6 intake (Table V). However, when only unsupplemented vitamin B-6 intake was considered (Table VI), significant relationships appeared between age and B-6 intake, and between menarche and B-6 intake. Both correlations were negative. These data indicate that vitamin B-6 intake tended to decrease with increasing maturity.

Relationship of Age to Nutrient Intake

At least one of the variables age, age level, and menarche (a variable which can also be related to age) appeared to influence consumption of all the nutrients studied (Tables V and VI). When a relationship existed, nutrient intake decreased with increasing age. This might not be an unexpected trend, as the majority of the subjects participating in this study had probably passed their rapid

growth spurt and were experiencing a decreasing rate of growth. Some of the adolescents may also have been attempting to reduce caloric intake of limit food item choices, thereby decreasing food consumption to control weight. All of these factors suggest that as age increases, a likely trend may be for nutrient intake to decrease.

Best Models for Vitamin B-6 Status Indicator and Vitamin B-6 Intake

A single model was created to identify which of the variables explored in this study best predicted each of the dependent variables: % ALAT for all days, % ALAT for five days, and vitamin B-6 consumption for unsupplemented subjects. In all three instances, dietary intake variables appeared in the models as well as another non-intake variable. Others have also found that the "best" equation for the prediction of nutritional status indicators included non-nutrient factors (111, 113).

In this study, the small magnitude of the R^2 values for the nutrient variables found to best predict % ALAT indicate that short-term recalled intake is a poor predictor of the subject's biochemical status indicator. Recalled intake of other nutrients is a better indicator of vitamin B-6 intake than of % ALAT, as can be seen from the R^2 values listed in Table X. This may be expected, because foods high in other nutrients, such as iron, will often contain appreciable amounts of vitamin B-6.

CHAPTER VI

SUMMARY

The vitamin B-6 nutritional status of 119 females between the ages of 12 and 16 from northeastern and north central Oklahoma was evaluated using coenzyme stimulation of erythrocyte alanine aminotransferase (% ALAT) as the parameter. Intakes of energy, protein, vitamin B-6, vitamin C, vitamin A, and iron were calculated from two 24-hour dietary recalls. Possible relationships were examined between % ALAT and nutrient intakes, as well as between % ALAT and the variables age, age level, race, income, menarcheal state, or stage of the menstrual cycle, and between nutrient intakes and the variables age, age level, race, income, and menarcheal state.

Two sets of % ALAT values were reported. One set included values of subjects who participated on seven days of data collection, and a second set included values of subjects who participated on only five days of data collection. This was due to the significantly higher mean % ALAT values on days 1 and 4 of data collection than on other days; this was related to longer holding of the blood samples on those two days, and possibly to some vitamin B-6 loss.

Subjects were categorized on the basis of normal (< 16) or high (≥ 16) % ALAT. Thirty-seven percent of the subjects for all data collection days had high % ALAT, indicating some degree of vitamin B-6 inadequacy. For five days of data collection, 25% had high % ALAT.

The mean daily vitamin B-6 intake for all girls was 1.25 mg $\pm$ 0.91 (SD). Seventy-five percent of the subjects consumed $\leq$ 67% of the RDA for vitamin B-6.

Percent ALAT was not significantly affected by the intake of any nutrient except vitamin C. No significant influence was found on % ALAT for all days by age, age level, race, income, menarcheal state, or stage of the menstrual cycle. However, % ALAT for five days was significantly affected by age level and menarche.

No consistent influence of race or income was found on nutrient intakes. Intakes of all nutrients investigated, except vitamin B-6, were significantly and inversely related to the continuous variable age. When dietary consumption of only unsupplemented subjects was considered, intakes of all nutrients except possibly vitamin C were inversely related to age.

The findings of the study indicated vitamin B-6 inadequacy as evidenced by coenzyme stimulation of erythrocyte alanine aminotransaminase among some apparently healthy, female adolescents.

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APPENDIX

OBS	ALAT	DAY	RACE	TWOYR	AGE	MENS	PCINC	DCYC	PRO	E	VB6	VC	VA	FE
1	.	1	1	14	173	1	8800	3	42	818	0.37	18	1714	4.9
2	.	1	1	16	195	1	3960	6	70	2512	0.93	83	3256	12.4
3	.	.	1	12	149	.	17500	.	46	1306	0.74	29	1102	8.3
4	.	8	2	12	142	.	1047	.	54	1497	1.23	128	2797	7.6
5	.	4	2	14	157	1	3500	3	69	2353	0.83	61	2259	9.9
6	.	4	1	14	171	2	3600	.	45	1961	0.77	89	1393	9.5
7	.	4	2	12	140	2	2057	.	43	1049	0.78	27	1688	8.2
8	.	4	2	12	149	1	4800	4	68	2513	0.99	235	6947	12.9
9	.	5	2	12	153	1	5400	7	57	1315	0.55	16	1149	7.3
10	.	6	1	12	144	2	5100	.	91	1879	2.45	77	5725	26.2
11	.	6	2	16	193	1	1478	18	58	2074	1.07	48	2518	12.7
12	.	6	2	16	196	1	5000	29	69	1702	1.21	67	1800	12.1
13	.	6	2	14	162	1	13000	14	39	1288	0.60	43	1045	5.2
14	.	6	2	16	180	1	4572	10	55	2219	0.72	216	7480	8.3
15	.	6	2	14	158	1	3840	.	74	2187	0.97	25	2339	12.6
16	.	6	2	14	166	1	6800	7	70	2020	1.15	178	6359	12.4
17	.	6	1	14	169	2	5714	.	56	1418	4.99	225	12622	22.2
18	.	6	2	14	175	1	1606	5	30	1072	0.41	175	1751	6.2
19	.	6	2	14	167	1	6250	20	63	2099	1.37	65	4835	17.5
20	.	6	1	14	169	1	13500	16	54	2063	1.94	199	7306	8.5
21	.	6	1	14	170	1	5750	11	71	1745	2.01	187	10527	10.1
22	.	6	2	14	172	1	7000	19	25	748	0.46	66	2023	5.5
23	.	6	2	14	177	1	5730	8	84	2825	1.26	51	3335	12.3
24	.	6	2	14	173	1	5833	25	31	1214	5.88	483	1866	24.1
25	.	6	2	16	189	1	800	9	107	2482	1.29	50	3187	14.0
26	.	6	2	14	173	1	4509	.	72	1908	1.11	113	14058	14.0
27	.	6	2	14	175	1	9220	.	41	1331	1.71	71	5025	15.2
28	.	6	1	14	171	1	2443	10	62	1852	1.15	169	3920	10.1
29	.	6	2	16	192	1	.	.	60	1753	1.09	39	1384	10.9
30	.	8	1	12	150	.	7000	.	32	1300	0.96	78	2018	9.2
31	.	.	1	.	.	.	.	.	67	2085	1.80	86	4609	12.8
32	-9	8	1	12	144	2	6250	.	50	1280	0.82	20	2695	7.5
33	-6	2	2	14	168	1	1349	.	104	2538	1.30	16	2937	18.0
34	-6	5	2	14	164	1	2517	11	62	2467	1.18	67	3696	9.4
35	-5	2	1	16	198	1	6000	22	85	2488	2.74	175	6006	25.6
36	-5	2	1	12	143	2	6000	.	97	2020	1.11	37	4732	12.9
37	-5	8	1	12	150	2	2667	.	98	2727	1.69	49	7604	14.6
38	-5	8	1	14	166	1	5000	19	70	2007	1.24	36	16376	14.5
39	-4	1	1	14	166	2	846	.	109	2515	1.45	146	4088	15.9
40	-4	8	1	12	144	2	1776	.	45	1874	0.65	73	4130	8.7
41	-4	4	1	12	146	2	2375	.	53	1431	0.91	23	2545	10.4
42	-4	8	1	14	156	1	8000	.	72	2375	0.86	26	3069	11.6
43	-3	1	1	14	174	2	3800	.	67	1673	0.71	29	2326	10.3
44	-3	2	1	14	169	1	4667	10	75	1657	0.89	86	3179	11.5
45	-3	8	1	12	140	1	3327	14	80	2099	3.68	358	2420	12.6
46	-2	3	1	12	144	2	5000	.	83	2566	1.98	42	5056	20.9

47	-2	7	1	14	173	1	9000	.	45	1634	0.55	27	1986	8.5
48	-2	5	1	14	165	1	6250	18	50	1095	0.54	28	5136	11.8
49	-1	1	1	16	187	1	9333	24	65	2605	1.52	275	4173	13.8
50	-1	1	1	14	162	1	8500	4	47	1170	0.64	44	10156	7.9
51	-1	2	1	14	165	2	3240	.	102	2915	2.82	230	6704	24.7
52	-1	7	1	12	142	2	5000	.	104	3040	1.18	98	3852	11.8
53	0	2	1	16	190	1	5917	5	32	1418	0.74	21	4300	6.7
54	0	8	1	14	168	1	7000	6	80	2298	1.58	66	7563	18.4
55	0	7	1	14	174	1	5520	25	56	1749	1.32	28	4307	15.8
56	0	7	1	12	148	1	4600	18	50	1293	0.68	23	1679	6.1
57	1	1	1	16	193	1	2500	.	62	1271	0.67	27	1508	9.2
58	1	8	1	16	188	1	8571	.	64	2458	1.03	35	2902	11.2
59	1	8	1	12	140	2	5100	.	50	1382	0.99	72	3732	7.5
60	1	3	1	12	150	2	8000	.	82	1690	2.73	117	6399	7.8
61	1	7	1	14	166	1	4000	10	42	999	0.83	21	2252	7.5
62	1	8	1	16	192	1	7200	19	60	1195	1.05	27	1965	8.8
63	1	8	1	16	191	1	4000	14	38	1205	0.48	49	5882	7.2
64	2	1	1	14	168	2	1833	.	90	2281	1.21	66	3382	13.8
65	2	8	1	16	197	1	3200	17	60	1480	0.79	111	1504	8.8
66	2	7	1	12	143	2	7875	.	50	1118	0.81	130	4679	5.8
67	2	8	1	14	172	1	8100	21	64	2349	1.22	107	3261	8.7
68	3	3	1	16	188	1	11250	8	82	2067	1.27	58	4247	11.0
69	3	5	1	14	168	1	10250	26	29	2240	1.21	49	5653	12.9
70	4	3	1	16	189	1	2857	14	61	1976	0.73	6	1845	7.8
71	4	7	1	16	198	1	12500	.	17	483	0.25	47	691	2.7
72	4	4	1	14	171	1	3600	20	72	2074	1.11	36	3866	12.2
73	4	4	2	14	170	1	5333	23	51	1668	1.04	24	1906	11.1
74	4	5	1	12	149	2	4500	.	61	2058	1.16	62	4429	11.6
75	5	7	1	12	148	2	3725	.	96	2052	1.00	89	7426	11.8
76	5	3	1	16	196	1	5000	21	20	822	0.38	59	1182	3.8
77	5	5	1	12	150	2	6500	.	113	2777	0.91	88	4253	12.7
78	6	2	1	14	171	1	8600	29	66	1643	0.99	76	2527	9.9
79	6	7	1	12	141	2	7500	.	70	2123	0.96	76	5199	23.7
80	7	3	1	14	172	1	6052	10	59	1673	0.99	39	2374	10.8
81	7	4	1	12	146	2	4750	.	96	2806	1.50	119	3576	17.1
82	7	7	1	12	146	1	6900	8	66	1807	0.83	79	2888	11.5
83	8	3	1	16	190	1	6250	22	59	1551	1.08	36	3895	10.5
84	8	7	1	12	143	2	17500	.	71	2812	1.11	165	1792	12.1
85	8	4	2	14	163	1	3429	6	72	1751	1.06	239	8113	14.8
86	10	7	2	12	141	1	4800	25	49	1904	2.56	101	9344	16.3
87	10	5	1	12	144	2	6800	.	70	1996	0.91	43	1868	13.2
88	11	3	1	16	187	1	8000	22	74	1747	2.88	128	9356	10.9
89	11	5	1	12	144	2	10000	.	19	831	0.35	1	1634	2.8
90	11	8	1	16	190	1	5000	34	53	1330	0.70	16	3027	6.3
91	12	3	1	14	174	1	5800	20	27	948	0.48	84	1724	4.4
92	12	3	1	12	150	2	9667	.	60	2052	2.22	99	6823	26.4
93	12	7	1	12	149	2	6000	.	62	1419	0.76	132	1797	8.3
94	12	8	1	14	168	1	4333	35	83	1638	0.99	34	1907	13.0
95	13	2	1	12	140	2	8000	.	98	2479	1.09	20	2922	14.1

96	13	4	1	12	140	1	8250	6	58	1832	0.84	103	11085	18.6
97	13	5	2	14	168	1	5000	5	71	2206	0.98	93	2639	11.8
98	13	5	1	12	151	2	9000	.	44	1957	0.52	49	2510	7.9
99	14	1	1	14	174	1	5750	26	34	1198	0.58	15	833	5.5
100	14	5	1	12	140	2	5000	.	93	3032	2.26	253	11714	29.7
101	14	8	1	16	194	1	5400	20	63	1844	1.81	134	5076	27.4
102	14	8	1	14	178	1	7500	13	41	1481	1.59	69	9938	14.3
103	15	1	1	16	193	1	7200	20	80	2035	2.16	77	5096	22.6
104	15	2	1	16	191	1	8600	19	68	1739	1.05	24	3384	10.5
105	15	4	1	14	171	2	3840	.	68	1996	1.12	122	2348	8.2
106	15	5	2	12	150	2	7167	.	66	2455	0.72	165	2346	9.9
107	16	1	1	14	163	1	20000	32	32	1195	0.51	45	2616	5.2
108	16	1	1	14	164	1	10000	4	90	2124	1.22	56	3227	13.1
109	16	1	1	14	162	1	10354	18	69	1132	0.89	534	4330	9.0
110	16	3	1	16	186	1	5000	3	38	1081	0.68	45	825	6.0
111	16	5	1	14	170	1	5000	5	62	1824	1.14	166	6011	9.4
112	16	5	2	14	168	1	1538	.	80	2135	0.99	120	1951	12.1
113	18	4	2	14	168	1	3333	9	66	3676	2.30	192	3998	19.1
114	18	8	2	16	190	1	1500	.	60	1640	0.71	17	2886	7.7
115	19	4	2	12	144	2	6000	.	99	2600	3.77	220	4216	14.2
116	20	7	1	14	168	1	7000	13	49	1647	0.63	15	2300	9.4
117	20	4	1	12	145	2	2600	.	184	4171	2.65	194	6557	30.3
118	21	2	1	16	188	1	7000	30	53	1723	0.69	49	2236	10.7
119	21	7	1	12	150	1	2229	27	44	1429	0.83	82	2624	8.3
120	22	1	1	16	189	1	10000	11	32	1109	0.57	26	1897	6.0
121	22	5	1	14	171	1	5340	21	66	2002	0.82	28	2975	10.7
122	23	1	1	16	189	1	4080	3	60	1622	2.53	81	7065	27.2
123	23	1	1	14	172	1	6400	25	53	1572	0.84	116	3062	8.8
124	23	8	1	16	191	1	8250	9	50	1556	0.72	29	2360	6.6
125	24	1	1	14	179	1	5200	.	34	949	0.30	17	2062	2.7
126	24	1	1	14	164	1	8667	25	49	1138	1.39	37	3086	17.5
127	24	4	2	14	170	1	3500	8	67	2337	1.24	122	3680	10.9
128	25	7	1	12	141	2	7000	.	42	1261	0.54	306	2839	6.6
129	25	7	1	12	142	2	6000	.	57	1732	0.69	112	2410	10.2
130	26	4	1	12	142	1	11250	.	78	2119	0.95	250	2801	14.6
131	28	2	1	12	146	2	2200	.	84	2028	1.13	166	2960	14.9
132	28	7	1	12	147	2	5520	.	71	2432	1.40	61	5590	14.5
133	28	4	2	12	151	1	6600	3	47	965	0.68	52	1677	7.7
134	28	5	1	12	151	2	.	.	85	1935	1.95	677	4686	15.0
135	29	1	1	14	162	1	8000	26	36	1005	0.56	74	1561	6.6
136	29	5	1	12	149	2	6760	.	61	1991	0.66	78	3318	11.5
137	30	5	1	12	146	2	3750	.	66	1992	2.74	233	8353	46.2
138	34	3	1	12	149	2	3500	.	59	1628	0.92	82	3394	13.1
139	35	1	1	16	193	1	4167	26	33	1078	0.44	93	2071	5.5
140	36	3	1	12	148	2	6667	.	163	4478	1.77	68	4250	20.5
141	37	1	1	14	175	1	1580	12	105	2188	1.04	27	2438	15.7
142	37	4	2	14	170	1	4000	26	54	1959	0.99	95	2103	10.4
143	38	4	1	14	168	1	9250	14	48	1614	0.93	144	2401	8.3
144	39	1	1	16	193	1	4167	.	39	1291	0.57	125	3343	6.0

145	39	2	1	12	147	1	4398	23	72	1472	6.09	643	2839	9.7
146	40	1	1	16	189	1	6580	3	61	2178	2.22	78	5915	20.2
147	40	1	1	14	173	1	1167	23	56	1483	1.14	38	2278	7.9
148	40	2	2	12	146	2	1349	.	74	2373	1.07	39	7201	12.3
149	42	1	1	14	173	1	5040	4	36	1085	0.57	128	1297	6.3
150	44	1	1	14	165	1	10000	34	94	2368	1.89	184	8375	27.6

Note:

OBS = observation number
ALAT = % ALAT
DAY = day of data collection
RACE: white = 1; black = 2
TWOYR = age level
AGE = age in months
MENS = premenarcheal = 2; postmenarcheal = 1
PCINC = per capita income
PRO = protein intake (g)
E = energy intake (kcal)
VB6 = vitamin B-6 intake (mg)
VC = vitamin C intake (mg)
VA = vitamin A intake (IU)
FE = iron intake (mg)

VITA

Susan Elaine Bentley

Candidate for the Degree of

Master of Science

Thesis: ASSESSMENT OF VITAMIN B-6 STATUS AND INTAKE OF FEMALE ADOLESCENTS IN OKLAHOMA

Major Field: Food, Nutrition and Institution Administration

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