AN ABSTRACT OF THE THESIS OF

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Title: VITAMIN B_6 STATUS IN YOUNG WOMEN USING ORAL CONTRACEPTIVES

Abstract approved: _____

The vitamin B_6 status of young women using oral contraceptives was determined by evaluation of their dietary records and plasma levels of vitamin B_6. Women between the ages of 18 and 26 years served as subjects: 26 had been using an oral contraceptive agent (OCA) for five months or longer (OCA users), 25 had never used or had not used these drugs for five months or longer (non-OCA users).

The subjects' three-day dietary records were analyzed for vitamin B_6, protein and nine other nutrients. Most of the women were consuming self-chosen diets which contained 70 percent or more of the recommended dietary allowance (RDA) for all nutrients except iron, calories and vitamin B_6. The mean dietary intake of vitamin B_6 by the two groups was similar: 1.4 ± 0.5 mg/day for the OCA users and 1.6 ± 0.5 mg/day for the non-OCA users. The OCA users and non-OCA users were consuming diets containing a mean protein content of 72.6 ± 19.4 g/day and 66.9 ± 13.6 g/day, respectively, which is more than 100 percent of the RDA for protein. When the intake of vitamin B_6 was compared to that of protein, expressed as a mg/g ratio, both groups had ratios above 0.019, which is considered adequate by Donald (1978). However, the OCA users had a significantly lower ($p<0.05$) mean ratio (0.020 ± 0.004) of vitamin B_6 to protein than the non-OCA users (0.025 ± 0.01).

Blood, which was drawn from the fasting subjects, was analyzed for plasma vitamin B_6. Determined by a microbiological assay using Saccharomyces uvarum, the mean plasma vitamin B_6 in the OCA users (7.4 ± 2.5 ng/ml) was significantly lower (p 0.05) than that of the non-OCA users (10.1 ± 4.2 ng/ml). Plasma vitamin B_6 levels did not correlate with age, body mass (kg/m^2), B_6/protein ratio, alcohol intake, exercise, length of OCA therapy, or the estrogen content of the OCA. In general, the subjects' hematocrits, which were within the normal range, were similar for the two groups.

A questionnaire was used to compute a general health score, a vitamin B_6 diet history score and other pertinent data. According to our criteria, the general health score (25 possible points) indicated that the subjects were in normal health with no differences between the OCA users (20.1 ± 1.8) and the non-OCA users (21.0 ± 2.0). The vitamin B_6 diet history score served as a check for the dietary records. The questionnaire also showed that the OCA users had a significantly higher average alcohol intake than the non-OCA users.

Vitamin B_6 Status in Young Women Using Oral Contraceptives

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

VITAMIN B_6 STATUS IN YOUNG WOMEN USING ORAL CONTRACEPTIVES

INTRODUCTION

As early as 1798, Malthus postulated in his "Essay on the Principle of Population" that population growth would outrun the food supply. Most people agree that the key to improved food supply and nutrition lies more with limiting population growth than with improving agricultural technology, although both are important (Drosdoff, 1979).

When oral contraceptives were introduced in 1960 (Hodges, 1971), they seemed to be the ideal method to limit population growth. Since that time, however, considerable concern has been raised about the side effects of the hormones which make up "the pill." What was once praised is now being questioned.

In the last 15 years considerable interest has been generated in the effect of oral contraceptive agents (OCAs) on the nutritional status of women. Theuer (1972) and Briggs (1979) summarized these effects on vitamins B_6, A, C, and K, riboflavin, niacin and several minerals.

Rose (1966) was the first to observe the effect of OCAs on vitamin B_6 status. He reported abnormal tryptophan metabolism, which occurs in vitamin B_6 deficiency, in women receiving estrogens. Since this abnormality was corrected by pyridoxine, he suggested that OCAs affect the requirement for vitamin B_6. Since then, other indices have been used to measure the vitamin B_6 status in OCA users. These methods include: 1) transaminase activity (Rose 1973; Miller et al., 1975; Aly et al., 1971; Rose et al., 1972; Brown et al., 1975; and Leklem et al., 1975), 2) urinary vitamin B_6 and 4-pyridoxic acid (Leklem et al., 1975; Miller et al., 1974 and Brown et al., 1975), 3) blood or plasma pyridoxal phosphate (Lumeng et al., 1974; Miller et al., 1975; Shane and Contractor 1975; and Brown et al., 1975), 4) blood or plasma vitamin B_6 (Bosse and Donald 1979; and Miller et al., 1978), and 5) urinary

excretion of tryptophan-kynurenine metabolites (Rose 1966; Leklem et al., 1973; Miller et al., 1974; Leklem et al., 1975; and Donald and Bosse 1979).

In any dietary study the dietary intake as well as the nutritional status based on biochemical tests should be considered (Pike and Brown 1975). Most of the studies assessing the vitamin B_6 status of women using OCAs have not considered the subject's diet, which may influence the biochemical tests used to determine the vitamin B_6 nutritional status. There is some evidence to suggest that the vitamin B_6 content of self-selected diets may be marginal (Driskell et al., 1976 and Chrisley and Driskell 1979). The purpose of the research presented in this thesis was to determine the dietary intake and plasma levels of vitamin B_6 in young women using OCAs. Dietary vitamin B_6 was calculated from a three-day dietary record. The vitamin B_6 in plasma, a direct measure of vitamin B_6 status, was determined using the microbiological assay with Saccharomyces uvarum as the test organism.

REVIEW OF LITERATURE

Vitamin B_6

In 1934, György defined vitamin B_6 as a preventive or curative factor for rat acrodynia and alopecia (György, 1971). By 1938, vitamin B_6 was isolated and the three forms, pyridoxine, pyridoxamine and pyridoxal, were identified. In general, pyridoxine is the prevalent form in plants; pyridoxamine and pyridoxal occur mostly in animal tissues (Sauberlich and Canham, 1973).

The physiologically active form of the vitamin is pyridoxal-5-phosphate (PLP) (Sauberlich et al., 1972) into which all of the free forms can convert. These interconversions and phosphorylations are presented in Figure 1. Pyridoxal is oxidized to 4-pyridoxic acid by aldehyde oxidase in the liver. Four-pyridoxic acid cannot be reconverted to the active form, and is excreted in the urine (Brin, 1978).

Pyridoxal phosphate serves as a coenzyme for more than 60 enzymes, particularly in amino acid metabolism. PLP functions in transamination, racemization, decarboxylation, cleavage, synthesis, dehydration and desulfhydration. Pyridoxal phosphate also serves a structural or conformational role in phosphorylase. Pyridoxamine phosphate functions as a coenzyme for transaminases (Sauberlich and Canham, 1973).

Vitamin B_6 Status in Women Using Oral Contraceptive Agents

The methods for assessing vitamin B_6 status in humans can be divided into three classes. Indirect methods measure a product formed in a reaction which requires vitamin B_6; examples of which are the measurement of tryptophan-kynurenine metabolites in urine and transaminase activity in erythrocytes. The direct methods measure vitamin B_6 or PLP in blood or one of its components. Measuring urinary 4-pyridoxic acid, a metabolite of vitamin B_6, is a third way to assess vitamin B_6 status.

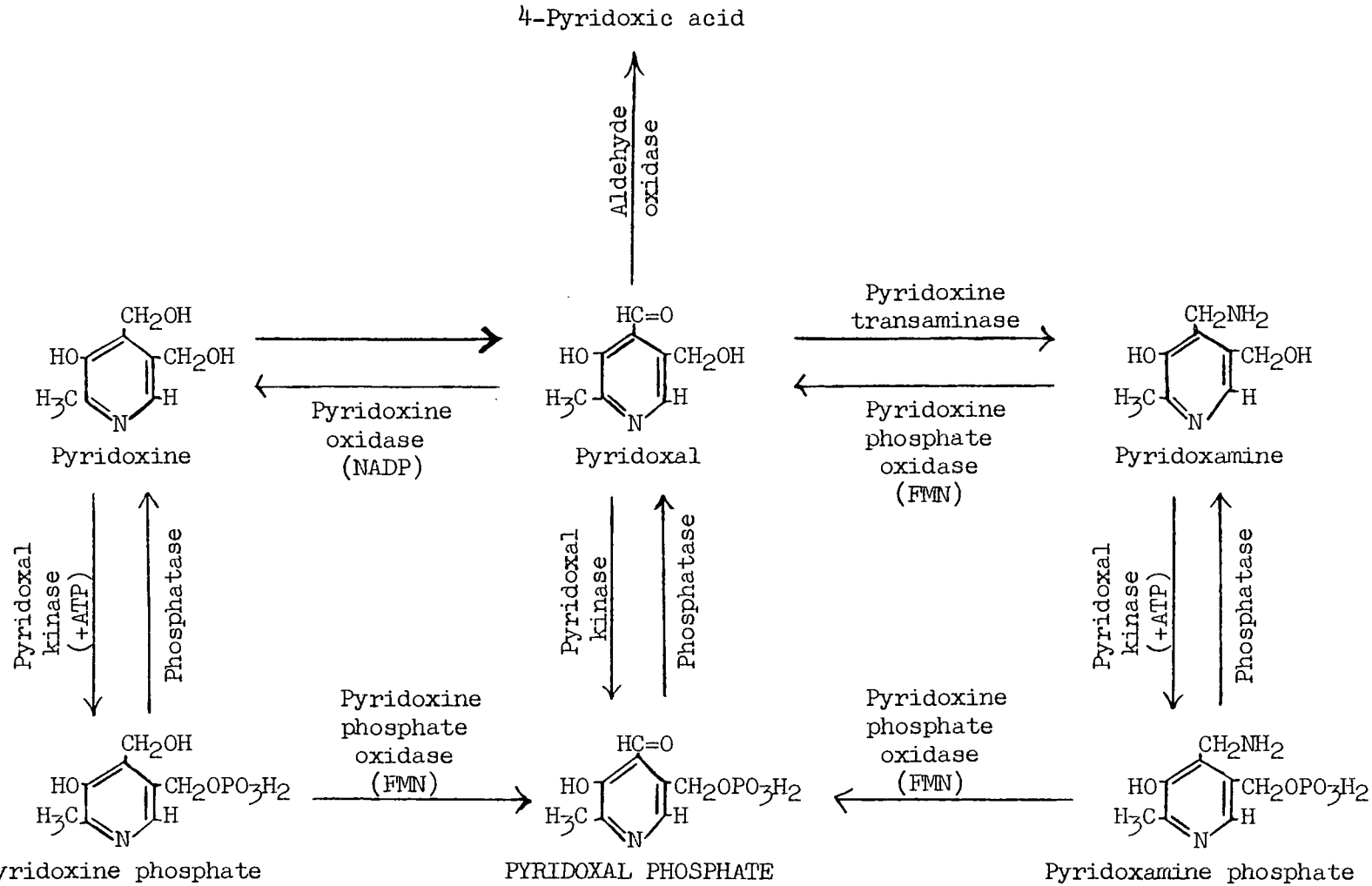

Figure 1. Interconversions and structures of vitamin B_6. (Adaption from Sebrell and Harris, 1968, pp. 32 and 34). NADP refers to nicotinamide adenine dinucleotide phosphate, ATP to adenosine triphosphate, and FMN to flavin mononucleotide.

These different ways have been used to determine vitamin B_6 status in OCA users.

Indirect Methods

Tryptophan Load Test. Tryptophan, an essential amino acid, can be converted to niacin through a series of steps, several of which require PLP (Figure 2). In response to a loading dose of L-tryptophan, a person deficient in vitamin B_6 will excrete increased amounts of xanthurenic acid (XA), kynurenine (K), and 3-hydroxykynurenine (HK) (Rose et al., 1972).

Abnormal tryptophan metabolism in women taking oral contraceptives was first reported by Rose (1966). In response to 5 gm of L-tryptophan, 18 women receiving an estrogen-containing preparation excreted elevated amounts of HK and XA. When three of the subjects were given pyridoxine, their excretion of these tryptophan metabolites returned to normal.

Increased excretion of tryptophan metabolites in the OCA users was also noted by Aly et al. (1971). Leklem et al. (1973) observed similar results when loading doses of L-tryptophan and L-kynurenine sulfate were given. Miller et al. (1974) also found increased excretion of tryptophan metabolites. Differences in the excretion were noted when the tryptophan load was given at different times of the day, which suggests a diurnal variation in tryptophan metabolism, or when the subjects discontinued the estrogen for the menses interval. Studying the effects of depletion and repletion of vitamin B_6 on the metabolism of tryptophan in OCA users, Leklem et al. (1975) and Donald and Bosse (1979) noted that tryptophan metabolism deteriorated faster during depletion and improved slower upon repletion in the OCA users than in the non-OCA users.

Three reasons have been suggested for the abnormal tryptophan metabolism in OCA users: 1) The direct effect of estrogens on tryptophan oxygenase activity may result in an increased flow of tryptophan into the kynurenine pathway (Brin, 1971). Green (1978) suggests, however, that the oxygenase activity is the same in OCA users and non-OCA users. 2) Indirect effect of estrogens may increase the activity of amino-

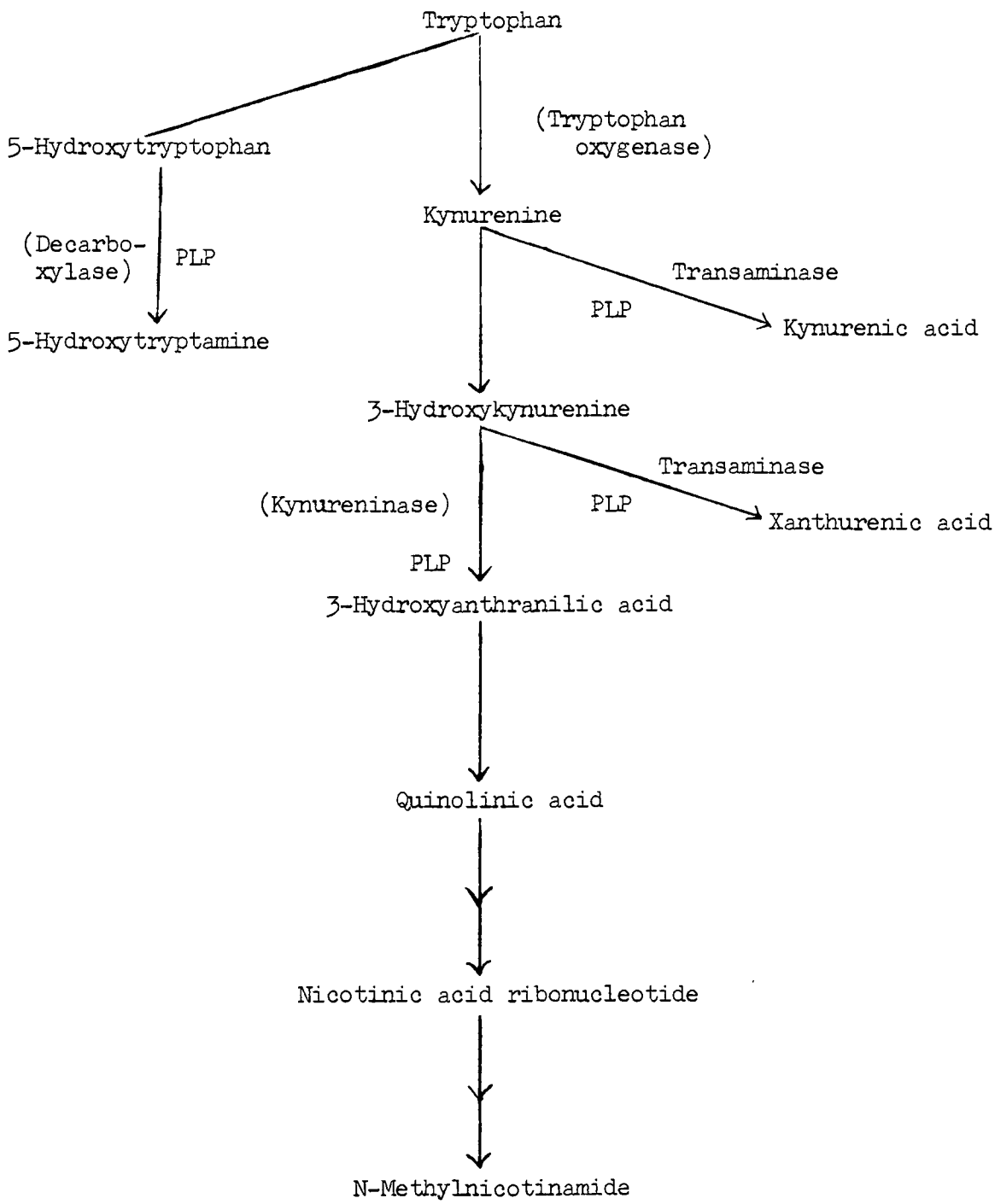

Figure 2. Tryptophan-kynurenine pathway (adapted from Rose et al., 1972). PLP indicates the known pyridoxal phosphate-dependent enzyme reactions.

transferases via elevated glucocorticoid levels, thus creating more binding sites for PLP. In this increased competition for PLP, the kynureninase may be left with a reduced amount of its cofactor (Brin, 1971). 3) Estrogen conjugates may inhibit PLP-dependent enzymes by competing with PLP for binding to apoenzymes (Mason et al., 1969).

Transaminase Activity. Since PLP is a cofactor for transaminases, vitamin B_6 status can be assessed by determining the activity of these enzymes. The activity of two transaminases in erythrocytes and their in vitro stimulation by PLP is commonly used to assess vitamin B_6 status. This is usually expressed as the ratio of total enzyme to holoenzyme or as an activation factor (Shane, 1978). The two enzymes used are aspartate (glutamic-oxaloacetic transaminase EC 2.6.1.1, hereafter referred to as GOT) and alanine (glutamic-pyruvic transaminase EC 2.6.1.2, hereafter referred to as GPT) transaminase (Rose, 1973). Transaminase activity can be measured in leukocytes, plasma or erythrocytes. Measuring activity in erythrocytes is the most common and most accurately reflects vitamin B_6 status (Sauberlich et al., 1972; Linkswiler, 1967). A decrease in activity of these two enzymes and a rise in the in vitro stimulation with pyridoxal phosphate are associated with vitamin B_6 deficiency (Ahmed and Bamji, 1976).

Altered vitamin B_6 metabolism in women using oral contraceptives as tested by transaminases proves very confusing. EGOT activity was found to be elevated in OCA users by Miller et al. (1975), Aly et al. (1971), and Rose et al. (1972). However, Doberenz et al. (1971), Salkeld et al. (1973) and Kishi et al. (1977) noted lower EGOT activity, while Leklem et al. (1975) and Brown et al. (1975) found no difference in either EGOT or EGPT activity between OCA users and non-OCA users.

That OCAs did not affect EGPT activity was also noted by Rose et al. (1972) and Aly et al. (1971). However, Driskell et al. (1976) noted a higher percentage stimulation of EGPT with PLP added in vitro in OCA users than in non-OCA users. A wide range of values were obtained, and EGPT levels did not correlate to dietary intake of vitamin B_6. Some investigators have suggested that transaminases are poor,

inconsistent indicators of vitamin B_6 status in women using OCAs (Shane and Contractor, 1975; Bosse and Donald, 1979).

Part of the problem with measuring vitamin B_6 status by transaminase activity is that it is an indirect method, and as noted under tryptophan load test, the estrogens may increase transaminase activity, via elevated glucocorticoid levels. Also, part of the problem is the lack of a uniform method for measuring the activity of the transaminases (Miller, personal communication).

Direct Methods

Plasma Pyridoxal Phosphate. As stated earlier, PLP is the physiologically active form of vitamin B_6 and is the predominant form in blood (Sauberlich et al., 1972). Plasma PLP, a direct measure of vitamin B_6 status, correlates appropriately with other biochemical indicators of vitamin B_6 status (Lumeng et al., 1974).

Prasad et al. (1975), who compared the plasma PLP values in OCA users and non-OCA users from two socioeconomic levels, found that at the high socioeconomic level OCA users had a significantly lower level of plasma PLP than non-OCA users. Shane and Contractor (1973) also noticed a lower level of PLP in whole blood among OCA users than non-OCA users. When the subjects received a daily supplement of 20 mg of pyridoxine hydrochloride (PN·HCl), there was no significant difference in the PLP levels of whole blood between the two groups. Miller et al. (1975) noted no significant difference between the plasma PLP levels of OCA users and non-OCA users.

Comparing 55 OCA users with 77 non-OCA users, Lumeng noted that OCA users had a significantly lower plasma PLP level than the non-OCA users. Lumeng et al. (1974) found no significant difference between OCA users and non-OCA users in the 20- to 24-year old group, while the plasma PLP levels of the 25- to 35-year old OCA users suggested a deficiency. They also followed women sequentially from two weeks before OCA therapy through six months of therapy. Plasma PLP decreased during the first three months and then returned to pre-treatment levels by six

months in most subjects. According to Lumeng et al., this change in plasma PLP is suggestive of this sequence of events: assuming a constant dietary intake, an increase in the amount of PLP-binding proteins caused by the estrogens may produce a redistribution of PLP in tissues and body fluids, resulting in a fall in plasma PLP concentration. For most women, dietary intake and economical utilization of PLP by the body are sufficient to adjust to the increased requirement, and the temporary deficiency corrects itself naturally.

Studying the effect of depletion and subsequent repletion of vitamin B_6, Brown et al. reported that before depletion the plasma PLP level of the non-OCA users was 11.7 ng/ml and that of the OCA users was 9.15 ng/ml; at the end of the 28-day depletion period these values were 3.18 ng/ml and 2.99 ng/ml, respectively. After the subsequent 28-day repletion with 0.8 mg/day supplementation of PN·HCl, the plasma PLP values were 4.50 ng/ml and 5.66 ng/ml, respectively. Similarly, with a repletion of 2.0 mg/day of PN·HCl the plasma PLP values were 15.0 ng/ml for the non-OCA users and 12.9 ng/ml for the OCA users. These apparent differences were not, however, statistically significant, perhaps because the number of subjects was so small, or because the women using oral contraceptive agents were not receiving identical steroid preparations (Brown et al., 1975).

Blood Vitamin B_6[1]. The level of vitamin B_6 in the blood is reflective of the intake of the vitamin (Baysal et al., 1966). Highest blood levels of vitamin B_6 were noted in men on a high vitamin B_6 and low protein intake, suggesting the interrelationship of these two nutrients (Kelsay et al., 1968). Studying young women, Donald et al. (1971) found that the vitamin B_6 content of erythrocytes declines dur-

[1] In preparation for assay, blood or one of its components are usually treated with acid which results in conversion of the phosphorylated forms into free forms which are then measured by microbiological assay. This assay measures free forms and phosphorylated forms (Wozenski et al., 1980).

ing depletion and rises during repletion with the vitamin, suggesting the measurement of blood vitamin B_6 is indicative of vitamin B_6 status.

Although not statistically significant, the erythrocyte vitamin B_6 levels in OCA users were lower than those in non-OCA users. During repletion with vitamin B_6 after a 30-day depletion period, OCA users required more pyridoxine than non-OCA users to achieve the same percentage increase in erythrocyte vitamin B_6 levels (Bosse and Donald, 1979).

Miller et al. (1974) observed that the whole blood vitamin B_6 values were lower in OCA users than in non-OCA users (6.0 vs 7.7 ng/ml). Although not statistically significant, the mean concentration of vitamin B_6 in blood was inversely related to length of OCA therapy. In another study, Miller et al. (1978) noted the plasma vitamin B_6 status in OCA users was lower than in non-OCA users (5.9 vs 9.9 ng/ml). The nine subjects were on self-chosen diets, which upon evaluation showed three out of five OCA users had intakes of vitamin B_6 which were 1.3 mg/day or lower. All but one of the non-OCA users had intakes of 1.6 or greater. Since the recommended dietary allowance (RDA) for vitamin B_6 is 2 mg/day, this may suggest a dietary inadequacy (Miller et al., 1978).

The response of OCA users and non-OCA users to a vitamin B_6 supplement was studied by Miller et al. (1975). Ten non-OCA users and 11 OCA users were tested before and after supplementation with 50 mg of pyridoxine for two days. The differences in level of vitamin B_6 in the blood between the two groups were not significant either before or after this supplement.

Metabolite Method

Urinary 4-Pyridoxic Acid and Vitamin B_6. The urinary excretion of 4-pyridoxic acid, a metabolite of vitamin B_6, is a reflection of the dietary intake of the vitamin and is thus another measure of vitamin B_6 status. In a review, Sauberlich et al. (1972) stated that between 40

to 50 percent of the vitamin B_6 in a normal diet is excreted in the urine as 4-pyridoxic acid, with the remainder of urinary excretion being various forms of vitamin B_6 and other unidentified metabolites. Urinary vitamin B_6 accounts for eight percent of the ingested vitamin B_6 (Leklem et al., 1980). Urinary 4-pyridoxic acid and vitamin B_6 decrease with depletion and increase with subsequent repletion with vitamin B_6, suggesting that these compounds are indicative of vitamin B_6 intake (Linkswiler, 1967; Baysal et al., 1966; Donald et al., 1971).

When comparing OCA users and non-OCA users, Leklem et al. (1975), Miller et al. (1974), Brown et al. (1975), Donald and Bosse (1979) and Aly et al. (1971) found that the use of oral contraceptives did not affect the excretion of 4-pyridoxic acid. In these studies, the concentration of vitamin B_6 in the urine was more reflective of dietary intake than of the use of oral contraceptives. In subjects receiving a diet containing 1.9 mg vitamin B_6, Miller et al. (1974), however, noted that OCA users excreted approximately 30 percent less total vitamin B_6 than non-OCA users.

Vitamin B_6 Requirements of Young Women, Including OCA Users

Most of the early work in determining vitamin B_6 requirements for adults was done on men. Kelsay et al. (1968) investigated the vitamin B_6 metabolism of young men on a diet containing either 54 or 150 gm of protein per day and 0.16 mg of vitamin B_6. Following depletion, the subjects were given an additional 0.6 mg of vitamin B_6. The concentration of vitamin B_6 in the blood and urine of the subjects consuming the low-protein diet increased slightly while that of subjects receiving the high-protein diet did not. This difference reflects the increased demand for vitamin B_6 in subjects consuming a high-protein diet. This interrelationship between vitamin B_6 and protein led to the recommended intake of 0.02 mg of vitamin B_6 for each gram of protein (Bureau of Nutritional Sciences, 1975).

Studies on the vitamin B_6 requirement of young women are few. To date, only two studies have been conducted which specifically studied the vitamin B_6 needs of young women. These studies were conducted in 1971 at Cornell University (Donald et al., 1971) and in 1974 at the University of Wisconsin (Shin and Linkswiler, 1974). Two other studies have been conducted to determine the vitamin B_6 needs of OCA users; one at the University of Wisconsin (Brown et al., 1975; Leklem et al., 1975), and the other at the University of Alberta (Donald and Bosse, 1979; Bosse and Donald, 1979). A summary of these investigations is given in Table 1. In general, these studies agree that 1.5 to 2.0 mg/day is an adequate intake of vitamin B_6 for young women. OCA users may need slightly more (Donald and Bosse, 1979). A recent article in Nutrition Reviews (Anonymous, 1979) suggests the vitamin B_6 requirement in some OCA users may be 5.0 mg/day; however, intakes of more than this are not advised.

In setting recommended dietary allowances for vitamin B_6, the National Research Council (NRC) recognized the difficulties involved:

> "Although a number of methods have been devised for determination of the various forms of the vitamin, data on the vitamin B_6 content of foods is insufficient, and adequate information on the availability of vitamin B_6 is lacking." (NRC, 1980, p. 97)

The Council, in 1974, recommended an allowance of 2.0 mg vitamin B_6/day for adults. This recommendation of 2.0 mg/day for women, including OCA users, is upheld in the 1980 revised RDA (NRC, 1980).

Dietary Intake of Vitamin B_6

The FAO/WHO guidelines contain no recommended allowance for vitamin B_6. It seemed unnecessary because this nutrient is, "...so widely distributed in foods that it is extremely rare for a human being to show signs of deficiency..." (Passmore et al., 1974, p. 24). While vitamin B_6 is widely distributed in food, it is important to note that vitamin B_6 may be destroyed in processing. The vitamin is freely soluable in water and is destroyed rapidly by ultraviolet light in neutral

Table 1. Summary of studies determining the vitamin B_6 requirement of young women, including OCA users.

	Donald et al., 1971	Shin and Linkswiler, 1974	Brown et al., 1975		Donald and Bosse, 1979	
			Non-OCA Users	OCA Users	Non-OCA Users	OCA Users
Subjects						
Number	8	5	10	15	8	8
Mean age, years	24.7	23.2	22.3	23.2	21	20
Mean weight, kg.	56.3	49.1	49.8	58.5	61.4	63.2
Diet						
Protein, g.	57	109	78		65	
Vitamin B_6, mg.	0.34	0.16	0.19		0.36	
Duration of Study						
Adjustment period, days	-	7	4		10	
Depletion period, days	43	14	28		32	
Repletion period, days	11	14	28		24	
Repletion Amounts						
Vitamin B_6 supplement, mg	0.6, 1.2, 30.0 PN·HCl	2.0 PN	0.8, 2.0 PN·HCl		0.6, 1.2, 4.7 PN·HCl	
"Recommendations"						
For vitamin B_6	1.5 mg/day with a moderate protein intake	2.0 mg/day	2.0 mg/day for both groups with perhaps some exceptions		Non-OCA users: 1.5 mg/day OCA users: 1.5 to 5.0 mg/day, probably closer to 1.5 mg/day	

or alkaline solutions. The vitamin also becomes less stable to heat at neutral or high pH (Sebrell and Harris, 1968).

Schroeder (1971) pointed out that while it is apparent that raw foods supply adequate amounts of vitamin B_6, persons subsisting on refined, processed and canned foods may not have adequate intakes of the vitamin. He noted that the milling of flour could reduce the vitamin B_6 content of wheat by as much as 72 percent. Canning and freezing of meats and vegetables could result in a 17 to 77 percent loss. Canning causes a greater loss than freezing.

The Food and Nutrition Board of the NRC has recommended a daily vitamin B_6 intake of 2.0 mg for young women; however, present evidence suggests that the population of young women in the U.S. may be ingesting only 1.3 to 1.6 mg of vitamin B_6 per day. Cinnamon and Beaton (1970) estimated the daily intake to be 1.5 mg of vitamin B_6 per day in three young men on a self-selected diet. A group of young women studied by Donald et al. (1971) received 1.6 mg of vitamin B_6. Driskell et al. (1974) in an extensive study of 33 males, 73 females and 46 females using oral contraceptives reported that the males met the RDA for vitamin B_6. The two groups of females, however, had a mean vitamin B_6 intake that was 60 percent of the RDA, and approximately one-third of them did not even consume 50 percent of the RDA for this vitamin. In a study of 11- to 14-year old female adolescents, Kirksey et al. (1978) noted a dietary intake of 1.24 mg/day, which is 79 percent of the RDA for this age group.

Oral Contraceptives

Faced with a world of ever growing numbers of people and limited resources, mankind has had to re-think some of his basic suppositions. Medical science which is in part responsible for our present population growth--due to a significant decline in mortality and an increase in life expectancy--has also provided an escape. Drugs in the form of hormones and their analogs have been developed to control human fertility (Goodman and Gilman, 1974).

What actually started out as a search for a control or regulator of the human menstrual cycle, and thus an enhancement of fertility, has become the most popular means of birth control. Rock, Pincus and Garcia experimenting with mixtures of estrins and progestins to regulate the human menstrual cycle noted that their subjects failed to become pregnant (Hodges, 1971). Soon the "side effect" became the most important characteristic of the new "pill."

Today in the United States, "the pill" is available in a combination form containing both estrogens and progestins. These tablets are taken in 28-day cycles; they are taken for 21 days and then discontinued for seven days. Some brands include seven iron supplements or inert tablets to bring the total tablets to 28, thus alleviating the need to count days.

In the United States, the recent trend is to reduce the amount of both estrogens and progestins contained in the tablets (Hodges, 1971). Briggs (1979) suggests that it is important to use the lowest possible dose, especially of estrogen, that still has high contraceptive effectiveness. This dose appears to be 30 μg ethinyl estradiol daily.

Oral contraceptives prevent ovulation, and interfere with implantation of the ovum. The estrogen inhibits the secretion of follicle-stimulating hormone and the progestin inhibits the release of luteinizing hormone. Thus either estrogens or progestins can prevent ovulation. These hormones may also alter the endometrium, interfering with implantation of the fertilized ovum (Goodman and Gilman, 1975).

That "the pill" causes undesirable side effects such as nausea, vomiting, dizziness, headaches, breast discomfort and weight gain, is generally known and accepted. The problems of most concern are the risks of cancer and thromboembolism (Goodman and Gilman, 1975). Also of particular concern to nutritionists are the effects of oral contraceptives on nutritional status, including that of vitamin B_6.

MATERIALS AND METHODS

Experimental Design

To determine the influence of self-chosen diets on the vitamin B_6 status of young women using OCAs, the dietary intake and the blood level of vitamin B_6 were measured in 26 OCA users and 25 non-OCA users. Subjects were asked to keep a three-day dietary record, answer a questionnaire and give 20 ml of venous blood. This study was planned and conducted with Judith Hoaglund. The data on whole blood and erythrocytes vitamin B_6 will be reported in her thesis (Hoaglund, 1980). The concentration of plasma vitamin B_6 and the dietary intake of the vitamin are reported in this thesis.

Subjects

Young women between the ages of 18 to 26 years served as subjects. Most of them were students at Oregon State University. Of the subjects, 25 were non-OCA users having never taken an OCA or having not used one for at least five months prior to the study. The other 26 subjects were OCA users who had been taking OCAs for at least five months. (See the Appendix for the composition of the OCAs used.) Subjects were asked to disqualify themselves if they used a nutritional supplement containing vitamin B_6 or had any known metabolic disorder. Descriptive data on the OCA and non-OCA users are presented in Tables 2 and 3, respectively.

Procedure

To eliminate any hormonal differences, the blood was drawn from the non-OCA users during the last half of the menstrual cycle (after the 14th day of a 28-day cycle) and from the OCA users between the 7th and 21st day of "pill" cycle. Blood was drawn between 8:00 and 9:00 A.M. at the Nutrition Research Laboratory of the Department of Foods

Table 2. Descriptive statistics of OCA users.

Subject no.	Age yrs.	Height inches	Weight pounds	Body Mass kg/m^2	Hematocrit percent	General Health Score[a]	Contraceptive[b] Used	Duration months
301	19	63	108	19.8	42.5	23	Ovral	5
302	21	65	145	25.0	42.5	19	Lo/Ovral	16
303	20	65	140	24.1	43.5	21	Ortho-Novum 1/50	7
305	22	61	113	22.1	38.0	18	Ortho-Novum 1/50	36
306	25	64	120	21.3	41.5	18	Norinyl 1 + 50	12
307	23	68	134	21.2	38.5	20	Lo/Ovral	12
308	19	65	130	22.4	39.0	23	Norlestrin 2.5	19
309	19	68	140	22.0	36.5	20	Modicon	18
310	20	66	113	18.9	48.5	19	Lo/Ovral	16
311	19	65	121	20.8	42.5	19	Norinyl 1 +50	28
312	21	71	167	24.1	41.0	22	Ovral	9
313	21	65	112	19.3	42.0	16	Lo/Ovral	20
314	23	65	127	21.9	44.0	20	Ovral	24
317	21	64	120	21.3	39.0	22	Lo/Ovral	9
318	25	65	125	21.5	40.0	22	Norinyl 1 + 50	14
319	19	67	130	21.1	42.5	23	Modicon	8
320	21	62	128	24.2	40.0	20	Lo/Ovral	15
322	24	62	115	21.8	41.0	20	Lo/Ovral	22
324	23	66	123	20.5	37.5	20	Demulen	10
325	22	70	145	21.5	36.0	19	Demulen	36
326	22	62	121	22.9	38.5	21	Demulen	34
327	21	64	130	23.1	40.5	18	Norlestrin	38
328	20	62	108	20.4	36.5	21	Ortho-Novum 1/50	48
330	21	69	140	21.4	40.0	21	Brevicon	24
334	18	64	120	21.3	39.0	19	Norinyl 1 + 50	18
335	22	63	120	22.0	41.5	18	Lo/Ovral	11
Mean	21	65	126.7	21.8	40.5	20.1		20
± SD	1.9	2.6	13.6	1.5	2.7	1.8		11

[a]See Appendix for questionnaire and calculation of score.

[b]See Appendix for composition of OCA.

Table 3. Descriptive statistics of non-OCA users.

Subject no.	Age yrs.	Height inches	Weight pounds	Body Mass kg/m^2	Hematocrit percent	General Health[a]
102	24	68	167	26.3	40.5	20
103	25	66	145	24.2	41.5	21
106	24	67	135	21.9	40.5	17
107	21	62	120	22.7	41.0	23
108	18	65	136	23.4	40.0	22
109	24	69	125	19.1	39.5	20
111	20	66	140	23.4	39.5	21
112	25	64	120	21.3	40.5	22
115	19	68	146	23.0	41.0	24
116	24	59	106	22.2	43.5	21
117	21	69	137	20.9	41.5	24
121	21	65	142	24.4	41.0	20
122	23	70	152	22.6	39.5	24
123	22	64	125	22.2	40.0	21
124	19	64	130	23.1	36.0	22
125	25	65	135	23.2	38.5	21
126	21	68	145	22.8	37.5	20
127	21	63	122	22.4	39.5	20
131	24	63	108	19.8	42.5	21
133	21	67	160	25.9	41.0	17
134	26	69	132	17.3	40.5	25
135	25	67	107	20.0	40.0	19
136	20	66	120	20.1	42.5	20
137	20	64	113	21.4	39.0	20
138	20	69	140	20.2	39.0	21
Mean	22	65.9	132.3	22.1	40.2	21
± SD	2.3	2.7	15.9	2.1	1.6	2.0

[a]See Appendix for questionnaire and calculation of score.

and Nutrition. A registered medical technologist drew approximately 20 ml of blood by venipuncture from the fasting subjects into heparenized vacutainer tubes.

During the 72 hours immediately proceeding the time of blood drawing, the subjects kept records of all foods and beverages that they consumed. Before participating in the study, subjects were shown food models and given verbal instructions. Written instructions were given at the time the subjects were ready to record their diets. All written instructions and the food record sheets were standardized for the entire study (see Appendix).

On the days that blood was drawn, one of the researchers reviewed the completed questionnaire and dietary record with each subject to spot any obvious problems or questions. The questionnaire and calculation of scores are given in the Appendix.

Methods

After the blood was drawn, it was immediately placed in the refrigerator and protected from light to avoid loss of vitamin B_6. Hematocrit was determined by a standard method from a portion of the blood. The remaining blood was then centrifuged for 15 minutes at 30,000 R.P.M. at approximately 0° C. After the plasma was removed, two samples of 2 ml each were measured into separate 40 ml conical centrifuge tubes. To precipitate the protein, 10 ml of 10% trichloroacetic acid (TCA) were added with stirring. These samples were refrigerated for 30 minutes during which time they were stirred thrice. Following 45,000 R.P.M. centrifugation at room temperature for 10 to 15 minutes, the supernatant was poured into a 50 ml beaker. To the precipitate, 5 ml of 10% TCA were added with stirring, followed by centrifugation and collection of the washing. The washings were added to the supernatant. This process was repeated once.

The supernatant and washings were then autoclaved at 15 pounds for 30 minutes to remove the TCA. When cool, the hydrolyzates were adjusted to pH 4.5 with potassium hydroxide, and diluted to 40 ml with redis-

tilled water. The hydrolyzates were refrigerated in brown bottles until assayed within two days. Vitamin B_6 in the hydrolyzate was measured by Saccharomyces uvarum (carlsbergensis) ATCC 9080 microbiological assay (Storvick et al., 1964).

A control sample was analyzed with six of the assays. The control was a large volume of plasma from one person which was divided into small sample portions and frozen. For each assay, one control sample was thawed and processed along with the other samples to determine the vitamin B_6 content. These samples had a mean value of 6.4 ng/ml with a range from 6.3 to 6.6 ng/ml. A known amount of pyridoxal (PL) was added to a sample and the percent of that amount which was recovered was determined. In the present study recovery was within 90 to 100 percent of the amount added.

The nutrient content of the diets was calculated by the computer using the Ohio State University data base (Schaun et al., 1973). To check the computer results, the protein and vitamin B_6 content of the diets were also calculated by the investigator (Watts and Merrill 1975).

Statistical analysis of the data and instructions on the proper programming for the computer were obtained through the counseling service of the Computer Center and the Statistics Department of Oregon State University. The SIPS computer package was used for most of the analysis.

Experimental Approval

The Human Subjects Committee of Oregon State University granted approval to Dr. Lorraine T. Miller and Dr. James E. Leklem as Program Directors for this study on May 4, 1979. In accord with the recommendation of the committee, each subject was asked to sign a consent form before participating in the study (see Appendix). Each subject was also informed of her right to withdraw at any time or abstain from any part of the study she found objectionable.

RESULTS AND DISCUSSION

General Health of Subjects

Two criteria were used in determining the general health of the subjects: hematocrit and responses to the questionnaire. These data along with other descriptive statistics for the OCA users and non-OCA users are given in Tables 2 and 3, respectively. While not comprehensive, these two criteria provided a means of spotting any glaring problems in the general health of the subjects. Hematocrit is the single most useful test available for determining anemia or polycythemia (Wintrobe, 1974). Normal hematocrit values for women are between 37 and 47 percent, with the average being 42 percent (Thomas, 1977). The second criterion used was a history-type questionnaire. From the questionnaire (see Appendix for questionnaire and calculation of scores), questions 2-10 and 16 were used to determine the general health of the subjects.

The mean hematocrit values for the OCA users and non-OCA users were 40.5 ± 2.7 and 40.2 ± 1.6, respectively. All except five subjects fell within the normal range. OCA users nos. 309, 328 and 325 and non-OCA user no. 124 had values slightly below normal. OCA user no. 310 had a value slightly above normal. These values, while not within the quoted normal range, were still close enough that the subjects were included in the experiment.

The mean General Health Scores for the OCA and non-OCA users were not significantly different. The mean score for the OCA users was 20.1 ± 1.8; that for the non-OCA users was 21.0 ± 2.0. A score of 15 or greater was considered to be satisfactory; all subjects scored above this.

The greatest differences between OCA users and non-OCA users were the questions relating to the subject's use of alcohol (no. 5) and the subject's medical history (nos. 7-10). The mean medical history score for OCA users was 3.96; whereas non-OCA users had a mean score of 4.85.

This relationship between the use of OCAs and lower medical history scores would be interesting to explore further, but is beyond the scope of this paper.

The OCA users consumed significantly more alcohol than non-OCA users ($p<0.05$). The mean alcohol consumption scores for the OCA and non-OCA users were $3.88 \pm .71$ and $4.36 \pm .56$, respectively. Lumeng (1978) has suggested that ethanol may have a deleterious effect on PLP. This being the case, an increased intake of alcohol by the OCA users could lower their vitamin B_6 status.

The mean height and weight for the OCA users were 65.0 ± 2.6 inches and 126.7 ± 13.6 pounds, respectively. Non-OCA users had a mean weight of 132.3 ± 15.9 lbs. and a mean height of 65.9 ± 2.7 inches. All of the subjects fell within $\pm$ 20 percent of their desirable weights. Most subjects were within 10 percent of their desirable weights. OCA user no. 310 and non-OCA users nos. 134 and 109 were within minus 10 to 20 percent of desirable weight. OCA user 302 and non-OCA users nos. 133 and 102 were within plus 10 to 20 percent of desirable weight.

Dietary Intake

The dietary intakes as assessed by the computer compared well with the hand calculations for protein and vitamin B_6, two nutrients which were selected to check the computer calculations. Also, the vitamin B_6 score from the questionnaire (see Appendix for questionnaire and calculation of scores) showed a slight positive correlation with the actual intake. No difference in this score was noticed between the OCA and non-OCA users. Thus we believe the vitamin B_6 intakes as recorded by the subjects and calculated by the computer are representative of the population surveyed. Unfortunately, any food record is prone to error because of the limitations of the food composition tables, error in food records, or interpretation of the records (Chrisley and Driskell, 1979).

The dietary intake, as the percent of the RDA of selected nutrients, of the OCA and non-OCA users is summarized in Tables 4 and 5.

Table 4. Nutrient intake of OCA users in percent RDA (NRC, 1974).

Subject No.	Energy	Thiamine	Protein	Phosphorus	Iron	Riboflavin	Vit. A	Calcium	Niacin	Vit. B_6	Vit. C
301	90.7	115.5	131.3	126.2	50.7	82.8	72.2	75.6	79.1	53.9	267.2
302	122.0	108.2	224.9	260.2	75.8	159.7	124.0	192.9	102.0	72.2	137.4
303	108.6	142.7	210.1	200.1	82.1	120.5	125.7	92.2	116.0	79.3	273.6
305	128.1	166.3	176.1	159.4	101.9	140.0	209.9	97.4	181.2	87.2	103.5
306	74.6	138.4	127.9	122.5	66.1	106.3	142.4	74.5	109.3	50.4	406.3
307	66.3	96.2	102.9	79.1	61.0	93.4	36.5	42.8	100.5	42.3	70.2
308	72.2	69.2	185.1	182.8	34.6	127.2	61.2	171.8	88.2	57.9	117.5
309	95.5	89.2	168.8	164.2	64.9	116.2	128.3	97.1	97.1	67.3	184.9
310	77.6	113.2	126.5	140.1	74.6	96.0	260.0	93.8	125.2	71.1	64.5
311	124.2	149.5	192.7	201.1	93.0	190.6	90.8	167.7	164.5	88.2	370.2
312	143.2	131.2	221.9	257.6	74.3	240.7	125.9	196.5	132.7	120.9	183.5
313	68.2	91.0	92.3	87.3	58.2	83.6	132.2	51.4	155.7	52.3	49.6
314	74.4	61.6	93.3	81.1	45.6	68.9	44.2	43.1	75.4	31.5	29.5
317	56.9	81.7	97.7	97.4	48.9	69.0	57.0	53.4	80.5	50.4	247.5
318	66.6	104.6	129.2	129.4	45.3	111.3	161.6	93.7	92.6	64.3	397.2
319	75.0	118.6	167.2	133.0	72.8	97.3	30.2	57.4	118.5	73.0	247.8
320	93.4	190.0	142.4	178.3	93.9	214.5	83.6	127.6	187.6	89.8	103.2
322	94.3	121.2	111.1	128.3	54.3	100.6	86.1	79.1	102.3	45.5	97.6
324	92.1	105.8	198.3	158.3	72.7	117.1	175.1	47.4	223.4	107.1	174.6
325	82.4	112.5	161.3	187.2	69.6	107.7	356.9	139.9	142.5	56.7	330.7
326	74.4	72.2	168.7	152.7	50.1	103.1	70.5	105.0	102.7	50.1	64.2
327	105.5	109.0	197.7	206.4	67.2	188.3	143.1	168.4	116.5	76.9	133.2
328	139.8	178.6	154.4	179.6	95.3	163.9	101.4	120.5	143.2	62.2	102.0
330	142.1	342.7	233.1	285	145.1	183.1	96.2	189.2	244.9	121.2	368.2
334	80.3	87.8	123.5	92.1	52.9	120.6	55.1	66.3	100.4	77.6	191.7
335	94.8	116.9	162.2	165.6	81.4	101.5	91.6	112.0	135.2	102.8	100.3
Mean	94.0	123.6	157.7	159.8	70.5	127.1	117.8	106.0	127.6	71.2	185.2
± SD	25.4	55.0	42.4	54.8	23.0	45.1	72.7	49.4	43.4	23.5	115.8

Table 5. Nutrient intake of non-OCA users in percent RDA (NRC, 1974).

Subject No.	Energy	Thiamine	Protein	Phosphorus	Iron	Riboflavin	Vit. A	Calcium	Niacin	Vit. B_6	Vit. C
102	74.5	148.2	95.8	114.7	59.1	90.4	382.3	70.0	105.7	117.2	512.1
103	61.0	121.5	149.8	138.0	53.5	137.3	81.2	86.6	208.9	107.1	320.7
106	137.0	151.4	148.1	143.6	70.1	146.3	64.2	70.4	192.0	94.0	359.9
107	57.8	129.3	87.8	112.8	74.6	89.9	1114.6	84.7	81.5	108.2	317.1
108	116.5	131.6	154.7	122.4	66.5	150.1	73.0	76.8	88.5	65.9	258.7
109	78.0	122.5	158.3	160.5	75.3	116.9	696.8	120.5	133.2	103.8	622.0
111	151.0	106.0	205.8	252.9	66.8	119.8	124.9	143.7	172.2	64.8	191.2
112	77.4	184.4	182.2	162.9	70.9	162.6	107.8	99.3	101.5	59.3	307.9
115	70.1	179.5	148.7	179.8	99.4	144.6	175.1	77.3	137.3	91.7	402.9
116	84.2	127.6	161.4	171.7	57.8	125.7	295.9	158.8	111.2	70.9	580.0
117	78.6	79.9	151.5	142.1	53.1	100.0	107.5	89.3	106.1	70.7	341.5
121	66.2	98.4	112.9	169.0	53.4	159.2	381.4	189.7	94.4	51.7	98.1
122	112.9	187.7	161.9	150.2	77.0	172.8	81.6	83.4	134.2	63.6	170.8
123	91.0	127.4	201.7	200.3	72.7	165.5	96.6	160.4	120.1	75.5	227.3
124	82.6	127.3	153.6	184.7	84.7	117.8	297.3	94.2	145.1	79.9	265.7
125	82	129.7	139.5	157.6	69.7	153.0	187.0	123.4	105.0	72.9	313.5
126	73	85.5	110.5	104.7	47.3	66.3	35.8	50.5	113.6	46.7	332.9
127	71.1	80.5	147.5	152.0	45.2	99.7	204.1	95.0	126.4	69.6	144.2
131	74.3	102.9	111.5	106.0	41.8	107.0	58.9	70.1	105.8	61.4	314.9
133	84.2	88.9	124.2	130.7	61.1	96.9	98.1	71.6	111.0	68.8	261.6
134	93.9	118.6	120.7	134.0	72.3	99.4	307.8	109.5	89.9	63.1	218.9
135	52.9	100.7	125.4	101.3	59.3	116.8	170.9	64.9	95.2	60.0	234.5
136	82.2	78.2	153.1	182.0	39.8	104.1	149.5	113.3	93.0	50.1	78.5
137	92.7	144.8	173.5	172.7	70.4	126.9	317.0	110.1	158.8	83.5	149.5
Mean	85.5	127.0	145.2	152.9	67.1	126.4	233.0	101.6	126.8	77.9	294.7
± SD	23.0	37.0	29.3	34.8	19.7	30.8	234.9	34.2	39.6	23.9	134.2

No statistically significant differences in percent RDA were noted between the two groups except for vitamin C. Non-OCA users ingested significantly more vitamin C than OCA users, however, both groups were consuming well over 100 percent of their RDA for the vitamin. This difference is probably not important to vitamin B_6 metabolism (Shultz and Leklem, 1980).

Most of the subjects were ingesting diets that contained above 70 percent of the RDA for most nutrients, except for iron, calories and vitamin B_6. The mean intake of the remaining nutrients was above 100 percent of the RDA for both the OCA and non-OCA users.

The subjects in this study consumed diets with a slightly higher percentage of the RDA for vitamin B_6 than subjects studied by Driskell et al. (1976) or Chrisley and Driskell (1979). Driskell et al. reported that OCA users and non-OCA users consumed diets containing 62.9 ± 26.3 percent and 61.8 ± 24.9 percent of the RDA for vitamin B_6, respectively. Chrisley and Driskell (1979) reported that the mean intake of vitamin B_6 of young women was 69.0 ± 30.0 percent of the RDA. In the present study, values were 77.9 ± 23.9 percent and 71.2 ± 23.5 percent of the RDA for this vitamin, respectively, for non-OCA users and OCA users.

In the present study, the mean vitamin B_6 intakes by the OCA users was 1.4 ± 0.5 mg/day and by the non-OCA users was 1.6 ± 0.5 mg/day, which is not significantly different. Donald et al. (1971) suggested 1.5 mg/day might be an adequate intake of vitamin B_6 for young women. In light of this, 14 of the non-OCA users and 15 of the OCA users in the present study were consuming diets containing less than 1.5 mg of vitamin B_6. The reader should recall (Table 1) that Donald and Bosse (1979) recommended that OCA users need an intake of 1.5 to 5.0 mg/day. Leklem et al. (1975) suggested 2.0 mg/day, which is adequate for non-OCA users, is also adequate for OCA users with perhaps some exceptions. This suggests that the vitamin B_6 intake of the majority of the subjects in the present study may be marginal.

Since the vitamin B_6 requirement is related to protein intake (NRC, 1980), it is important to note the protein intake of the sub-

jects. The mean protein intake of the OCA users and non-OCA users was, respectively, 72.6 ± 19.4 g/day and 66.9 ± 13.6 g/day which represents 157.7 ± 42.4 percent and 145.2 ± 29.3 percent of the RDA. This difference was not statistically significant. In the study reported by Chrisley and Driskell (1979) the mean protein intake by young women was 167 ± 66 percent of the RDA. Driskell et al. (1976) noted that the mean protein intake of OCA users and non-OCA users was 158.7 ± 68.0 percent and 165.5 ± 76.5 percent of RDA, respectively.

A direct comparison of the vitamin B_6 and protein intake of the subjects in the present study was made by a ratio of the milligrams of vitamin B_6 to the grams of protein (B_6/protein ratio). The resulting ratios were 0.020 ± 0.004 and 0.025 ± 0.01, respectively, for OCA users and non-OCA users. Donald (1978) suggests that a ratio of 0.019 is "adequate" whereas an intake of 0.017 is "inadequate." Although this indicates that both groups had adequate B_6/protein ratios, the difference between the two groups is significant ($p<0.05$).

The lower percent intake for iron is expected since women of childbearing age have a high RDA for iron, 18 mg/day (NRC, 1980). The caloric intake is comparable to the research of Driskell et al. (1976) and Chrisley and Driskell (1979). Driskell et al. (1976) reported the mean percent RDA of caloric intake for OCA users as 85.0 ± 29.9 and that for non-OCA users as 87.5 ± 33.4 percent. The mean percent RDA of caloric intake for women 19-22 years old was 94 ± 31 percent in the study conducted by Chrisley and Driskell (1979). This compares with the values from the present study of a mean percent RDA for caloric intake of 85.5 ± 23.0 percent and 94.0 ± 25.4 percent for non-OCA users and OCA users, respectively.

Plasma Vitamin B_6

The mean plasma vitamin B_6 values were significantly lower ($p<0.05$) in the OCA users than in the non-OCA users (7.4 ± 2.5 vs 10.1 ± 4.2 ng/ml). Data for the individual subjects are given in Table 6 for the

OCA users and Table 7 for the non-OCA users. Hoaglund (1980), who measured whole blood vitamin B_6 in these subjects, also noticed lower levels of vitamin B_6 in OCA users.

Because the histogram of the plasma values was skewed, a logarithmic transformation of the values was obtained. The geometric means from these transformations when re-transformed as antilogarithms, were 7.0 and 9.4 ng/ml for the OCA users and non-OCA users, respectively. This difference was still statistically significant ($p < 0.05$).

The range of plasma vitamin B_6 values was greater among the non-OCA users (4.7 to 24.5 ng/ml) than among the OCA users (4.2 to 14.8 ng/ml). The median value for the OCA users was 6.6 ng/ml, which indicates that half of the subjects had values below this level. However, only four of the non-OCA users had values below this level. On the other hand, the median value for non-OCA users was 8.9 ng/ml. Only five OCA users had values above this level.

Table 8 gives the normal plasma and serum values for vitamin B_6 and PLP according to a survey of the literature. All of these studies, like the present study, show a lower vitamin B_6 concentration in the plasma or serum of OCA users than of non-OCA users. Both Miller *et al.* (1975, 1978) and Brown *et al.* (1975) reported consistently lower values for OCA users than non-OCA users. However, in none of these studies was the difference statistically significant. Since the number of subjects used in these studies was smaller than the number used in this one, their lack of statistical difference may be more the result of a small sample size than a lack of significant difference between the two in data.

Lumeng *et al.* (1974), measuring the plasma PLP in a larger number of women, noted that OCA users had significantly lower value than non-OCA users. In a study on women in their fifth month of pregnancy, Roepke and Kirksey (1979) observed that women who prior to the pregnancy had been taking OCAs for more than 30 months had lower serum vitamin B_6 levels than women who had not used OCAs prior to pregnancy.

Table 6. Vitamin B_6 status of OCA users.

Subject No.	Vitamin B_6 Diet History Score[a]	Vitamin B_6[b] mg/day	Protein[b] g/day	B_6/Protein Ratio mg/g	Plasma B_6[c] ng/ml
301	3.68	1.1	60.4	.018	8.6
302	4.17	1.4	103.5	.014	7.8
303	4.13	1.6	96.6	.016	14.8
305	4.79	1.7	81.0	.021	6.3
306	4.38	1.0	58.8	.017	7.5
307	3.29	0.8	47.3	.018	7.6
308	4.60	1.2	85.2	.014	6.6
309	3.87	1.3	77.7	.017	8.4
310	4.32	1.4	58.2	.024	6.3
311	3.69	1.8	88.6	.020	5.1
312	3.90	2.4	102.1	.024	6.0
313	3.45	1.0	42.5	.025	4.2
314	3.09	.6	42.9	.015	6.5
317	2.88	1.0	44.9	.022	6.9
318	4.63	1.3	59.4	.022	9.2
319	4.00	1.5	76.9	.019	6.7
320	4.64	1.8	65.5	.027	4.3
322	4.63	.9	51.1	.018	5.1
324	4.71	2.1	91.2	.023	5.7
325	4.13	1.1	74.2	.015	6.2
326	3.82	1.0	77.6	.013	6.0
327	3.25	1.5	90.9	.017	13.8
328	4.55	1.2	71.0	.018	8.0
330	4.82	2.4	107.2	.023	4.8
334	3.40	1.6	59.3	.026	9.0
335	4.29	2.1	74.6	.028	9.8
Mean	4.04	1.4	72.6	.020	7.4
± SD	.57	0.5	19.4	.004	2.5

[a]See Appendix for questionnaire and calculation of scores.
[b]From computer print out using the nutrient data bank compiled at Ohio State University (Schaun *et al.*, 1973).
[c]Microbiological assay (*S. uvarum*) of plasma from fasting subjects.

Table 7. Vitamin B_6 status of non-OCA users.

Subject No.	Vitamin B_6 Diet History Score[a]	Vitamin B_6[b] mg/day	Protein[b] g/day	B_6/Protein Ratio mg/g	Plasma B_6[c] ng/ml
102	5.21	2.3	44.1	.053	8.8
103	4.38	2.1	68.9	.031	14.8
106	4.66	1.9	68.1	.022	11.8
107	3.77	2.2	40.4	.054	7.5
108	4.02	1.3	74.3	.018	6.4
109	4.74	2.1	72.8	.028	5.7
111	4.05	1.3	94.7	.014	8.3
112	3.41	1.2	83.8	.014	11.0
115	5.35	1.8	68.4	.027	8.9
116	4.21	1.4	74.2	.019	12.6
117	3.90	1.4	69.7	.020	24.5
121	4.43	1.0	51.9	.020	8.9
122	3.90	1.3	74.5	.017	12.1
123	3.60	1.5	92.8	.016	9.7
124	4.71	1.6	70.6	.023	4.7
125	3.70	1.5	64.2	.023	8.7
126	3.79	0.9	50.8	.018	8.4
127	3.85	1.4	67.8	.020	10.5
131	3.09	1.2	51.3	.024	11.6
133	3.66	1.4	57.1	.024	7.3
134	4.21	1.3	55.5	.023	11.6
135	3.12	1.2	57.7	.021	8.8
136	4.72	1.0	70.4	.014	16.6
137	3.64	1.7	79.8	.021	4.8
138	3.94	2.9	68.3	.043	8.0
Mean	4.08	1.6	66.9	.025	10.1
± SD	.58	0.5	13.6	.010	4.2

[a]See Appendix for questionnaire and calculation of scores.

[b]From computer print out using the nutrient data bank compiled at Ohio State University (Schaun *et al.*, 1973).

[c]Microbiological assay (*S. uvarum*) of plasma from fasting subjects.

Table 8. Reported values of plasma and serum vitamin B_6 and PLP in OCA users and non-OCA users.

Reference	No. of Subjects	Measurement of[a]	Assay Method	Vitamin B_6	Dietary Intake
Miller et al., 1975	10 OCA users 11 non-OCA users	Plasma PLP	Tyrosine decarboxylase	5.9 ± 3.7 6.4 ± 3.8	Not available
Miller et al., 1978	5 OCA users 4 Non-OCA users 11 Lab control women	Plasma vitamin B_6	Microbiological Assay *S. uvarum*	5.9 ± 3.4 9.9 ± 1.9 10.0 ± 3.6	1.5 ± .05 1.6 ± .4
Brown et al., 1975	15 OCA users 10 Non-OCA users 15 OCA users 10 Non-OCA users	Plasma PLP	Tyrosine decarboxylase	9.15 ± 2.57 11.7 ± 3.2 12.9 ± 2.95 15.0 ± 5.0	Prior to depletion; Following depletion & repletion of 2.0 mg/day
Roepke & Kirksey, 1979 (Pregnant women at 5 months gestation who had previously used OCAs)	11 used OCA (30 mo.) 31 used OCA (1-30 mo.) 38 no OCA previously	Serum vitamin B_6	Microbiological Assay *S. uvarum*	8.1 ± 2.0 13.7 ± 2.0 16.6 ± 1.9	6.7 ± 1.3 4.6 ± 0.6 6.6 ± 1.0
Lumeng et al., 1974	55 OCA users 77 Non-OCA users	Plasma PLP	Tyrosine decarboxylase	7.8 ± 3.7 9.4 ± 4.2	Not available

[a]Measurement of vitamin B_6 and PLP is not equivalent. Vitamin B_6 assay measures dephosphorylated and free forms of the vitamin, while the PLP assay measures only the active form.

The results obtained in this study are in agreement with the control values from this laboratory. Miller et al. (1978) reported the laboratory control women had plasma vitamin B_6 levels of 10.0 ± 3.6 ng/ml. The non-OCA users in the present study had plasma vitamin B_6 levels of 10.1 ± 4.2 ng/ml.

Correlations

Table 9 gives the correlations of ten (for non-OCA users) or twelve (for OCA users) independent variables with the log of the vitamin B_6 concentration in plasma. The correlation of hematocrit with the log of the plasma vitamin B_6 for non-OCA users is the only significant correlation (p 0.05), besides the correlation of vitamin B_6 concentration in plasma and whole blood. This correlation between hematocrit and the log of the plasma vitamin B_6 for the non-OCA users is probably spurious.

The weak negative correlation between the log of plasma and the variables related to dietary intake of vitamin B_6 (vitamin B_6 diet history score, three-day dietary record, and the B_6/protein ratio) was unexpected. The correlations are, however, not significant, possibly because of the relatively small variation in dietary intake. Since all the subjects were of normal health, of similar nutritional status, of a limited age group (18-26 years old) and from a population of students at Oregon State University, the data did not contain large variations. Possibly for these reasons significant correlations were not obtained.

Attempting to discern what factors may influence plasma vitamin B_6, we performed multiple regression analysis using age, body mass, alcohol intake, exercise, B_6/protein ratio of dietary intake, and dose level (although all subjects were using OCAs with a low estrogen content--see Appendix) and length of time of OCA therapy as the independent variables. These are variables that have been suggested as possibly explaining some of the variation in blood levels of vitamin B_6. No significant correlation was found. In fact, with all seven variables in

Table 9. Correlations of the log of plasma vitamin B_6 with other variables.

Variable	Correlation Coefficient OCA Users	Non-OCA Users
Hematocrit	.04	.63[a]
General Health Score[b]	.08	.22
B_6 Diet History Score[b]	-.22	-.03
B_6 Three-Day Dietary Record	-.06	-.23
B_6/Protein Ratio	-.24	-.18
Age	-.10	.34
Alcohol Intake	.21	.25
Exercise	-.19	.04
Whole Blood B_6[c]	.81[a]	.75[a]
Body Mass Index	.24	-.11
Dose Level of Therapy	.29	
Time on Therapy	-.09	

[a]Statistically significant at $p < 0.05$.
[b]See Appendix for calculation of scores.
[c]See thesis by Hoaglund, 1980.

the model, less than 30 percent of the variation could be explained. No t value or partial correlation allowed the variable to be kept in the model. The statistical conclusion is that the addition of these seven variables did not significantly reduce the variability of vitamin B_6 in plasma.

What then are the reasons for the lower plasma levels of vitamin B_6 in the OCA users than non-OCA users? Possibly, redistribution of vitamin B_6 is effected by the estrogens as suggested by Lumeng et al. (1974), resulting in a higher level of vitamin in the liver and/or muscle, and a lower plasma level. This could be a result of the increased enzyme activity, e.g., transaminases (Brin 1971) resulting from the estrogenic stimulation of glucocorticoids. Comparison of erythrocyte, leukocyte and plasma levels of total vitamin B_6 and/or PLP might be enlightening. Leukocytes may give an indication of tissue distribution of vitamin B_6.

What is the significance or consequence of the lower plasma levels of vitamin B_6 in OCA users than non-OCA users? No "acceptable" levels of vitamin B_6 in plasma have been set nor have exact clinical conditions as a result of "low" vitamin B_6 been identified in young women. Winston (1973) suggested that depressive mood changes in OCA users who are predisposed to depression may be alleviated by pyridoxine. However, until some criteria are developed to identify what is a "low" level of vitamin B_6, the significance of the lower levels in OCA users than non-OCA users is debatable. Meanwhile the difference does exist suggesting the vitamin B_6 status of OCA users should be monitored. Of special concern are the women who become pregnant after having used OCAs.

This research seems to add more questions than answers. Further studies using a larger number of subjects of similar age and physical condition, who are receiving controlled amounts of vitamin B_6, protein, and alcohol, would add more light to the present subject. Also of interest is the possible effect of the bioavailability of dietary vitamin B_6. Perhaps the determination of fecal and/or urinary 4-pyridoxic

acid and vitamin B_6 would be appropriate. Since the dietary vitamin B_6 to protein ratios were different between the two groups, it might be interesting to look at the ratio of urinary 4-pyridoxic acid to urea nitrogen.

SUMMARY AND CONCLUSIONS

The vitamin B_6 status of young women using OCAs was determined by evaluating a three-day dietary record and determining the level of vitamin B_6 in plasma. Women between 18 and 26 years of age served as subjects: 26 had been using an OCA for five months or longer (OCA users) and 25 had never used or had not taken these drugs for five months or longer (non-OCA users). A questionnaire served as a check. From these data, the following conclusions can be drawn:

1) The mean plasma vitamin B_6 level of OCA users (7.4 ± 2.5 ng/ml), as assessed by microbiological assay with S. uvarum, was significantly lower ($p < 0.05$) than that of the non-OCA users (10.1 ± 4.2 ng/ml).

2) The difference in the mean dietary intake of vitamin B_6 between the OCA users (1.4 ± 0.5 mg/day) and that of the non-OCA users (1.6 ± 0.5 mg/day was not statistically significant. Almost half of the subjects from each group had intakes of less than 2/3 the RDA for this vitamin.

3) The dietary intake of vitamin B_6 expressed as a B_6/protein ratio was adequate (0.019) (Donald 1978) for both groups. Non-OCA users (0.025 ± 0.01) had a significantly higher ($p < 0.05$) ratio of vitamin B_6 to protein than did OCA users (0.020 ± 0.004).

4) According to the questionnaire, the OCA users had a higher average consumption of alcohol than the non-OCA users. There was, however, no significant correlation betwen the alcohol consumption and the vitamin B_6 in the plasma.

5) Multiple regression was performed using age, body mass (kg/m^2), alcohol consumption, exercise, B_6/protein ratio, estrogen content of the OCA and length of OCA therapy as the independent variables. No significant correlation was found. In fact, with all seven variables in the model, less than 30 percent of the variation could be explained, perhaps because there was relatively little variation in most of the variables.

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APPENDIX

Appendix Table 1. Composition of OCAs used by subjects.

Product	Estrogen	Progestin
Brevicon	35 μg ethinyl estradiol	0.5 mg norethindrone
Demulen	50 μg ethinyl estradiol	1 mg ethynodiol diacetate
Lo/Ovral	30 μg ethinyl estradiol	0.3 mg norgestrel
Modicon	35 μg ethinyl estradiol	0.5 mg norethindrone
Norinyl 1 + 50	50 μg mestranol	1 mg norethindrone
Norlestrin	50 μg ethinyl estradiol	1 mg norethindrone acetate
Norlestrin 2.5	50 μg ethinyl estradiol	2.5 mg norethindrone acetate
Ortho-Novum 1/50	50 μg mestranol	1 mg norethindrone
Ovral	50 μg ethinyl estradiol	0.5 mg norgestrel

Informed Consent Form

Nutritional Survey of Young Women

The purpose of this study is to examine the normal intake by young women aged 18-25, of certain nutrients and also to assess the blood levels of these nutrients. We are especially interested in oral contraceptive users (those who have taken oral contraceptives for six months or longer) and in non-users (those who have not taken oral contraceptives in the past six months).

As a participant you are asked to:

1. Record all the food and beverages you consume for three consecutive days.

2. Answer some questions concerning health and diet. All information will be kept strictly confidential.

3. On the morning following the diet record before eating breakfast and before any strenuous physical exercise, allow a licensed medical technician at the Department of Foods and Nutrition to draw 20 ml of blood (about 2 Tbs.) from your arm.

Important scheduling information: Three day diet records should be on Sun., Mon. and Tues. because blood will be drawn only on Wed. Please schedule your blood sampling during the last half of your menstrual cycle, that is between 15-28 days after the first day of flow. This is because the cyclic hormone pattern affects the levels of some vitamins in the blood.

To receive the questionnaire and diet record sheets, and to schedule your diet and blood drawing please call Mary Beth Lind at 753-1416 or Judy Hoaglund at 752-6890, or leave a message with Lori Bates at the Department of Foods and Nutrition at 754-3561, between 8:00-12:30 or 1:00-4:30.

This research has been approved by the Oregon State University Committee for the Protection of Human Subjects. In accordance with their regulations, you must be informed of the rationale, procedure, and safety of this study; you must be informed of your right to withdraw at any time; and you must sign an informed consent statement in the presence of a witness in order to participate. Furthermore, the confidentiality of any information provided must be strictly maintained by the principal investigators with the use of a number-code system. Do not write your name on the questionnaire.

INFORMED CONSENT STATEMENT

I have been informed of the rationale, procedure, and safety of this study, and of my right to withdraw at any time. I freely consent to participate as a subject.

Witness: Signature:

Date: Date:

Please print name:

TELEPHONE (to clarify any information on diet record)

ADDRESS

CODE NO.

Dietary Record Instructions

INSTRUCTIONS FOR RECORDING FOOD

1. Please record each food and beverage you consume (except water) on a separate line. Be sure to indicate all snacks.

2. Record them in reasonably exact amounts: liquids in cups, fluid ounces or milliliters; vegetables and fruits in cups or inches using the ruler on the record sheets; beans, grains and pasta in cups dry or cups cooked; bread in slices, indicate what kind of bread; meats, fish and cheeses in ounces (an average meat portion is 3 oz., a slice of American cheese is about 1 oz.) or measure your servings with the ruler.

   If it is impractical to measure foods at certain meals, measure a comparable food at least once to establish in your mind the measure of certain quantities. Remember: the more accurate your record the more accurate the analysis will be.

3. Please specify if a food is consumed raw. Also indicate if it was prepared from fresh, canned or frozen products.

4. Indicate how the food was prepared, such as fried, boiled, baked etc.

5. If a food is a mixture (sandwich, soup, stew) list the major ingredients separately in their proportions or amounts as eaten.

6. Use brand names wherever possible, or mention comparable brand name products.

7. Specify if a food is fortified with vitamins and minerals, or if it is a diet product. Please include brand names.

8. For fruits and vegetables indicate if skin was removed.

9. Provide any other information you feel might be helpful.

10. Indicate if milk is whole, skim, 2% or dry non-fat milk.

11. Be sure to include sauces, gravies, milk in coffee etc. Everything you eat or drink.

If there are any questions on your diet record, please call Mary Beth Lind at 753-1416 or Judy Hoaglund at 752-6890, or leave a message with Lori Bates at the Dept. of Foods and Nutrition 754-3561, from 8:00-12:30 and 1:00-4:30.

DIET RECORD SHEET

CODE NO. _____ DATE CONSUMED: _____

LEAVE A BLANK SPACE BETWEEN EACH MEAL
USE A SEPARATE SHEET FOR EACH DAY

FOOD specify each food or beverage on a separate line	SOURCE canned, dried fresh etc.	BRAND be specific	PREPARATION fried, baked, raw etc.	AMOUNT measure in cups inches etc.	FOR OFFICE USE AMT. code	 WT. code

Questionnaire

Code No. _____

Please answer the following questions as completely and as honestly as possible. The accuracy of this survey depends upon you. Do NOT write your name on this questionnaire. All information is confidential. If you do NOT wish to answer a question, draw a line thru it.

If you have any conditions such as tuberculosis, malabsorption problem or heavy drug use, please do not take part in this study.

1. Age _____ years

2. Height _____inches

3. Weight _____ pounds

---

4. How many, if any, cigarettes do you smoke per day?

_____ None

_____ Less than $\frac{1}{4}$ pack

_____ Less than $\frac{1}{2}$ pack

_____ Less than 1 pack

_____ More than 1 pack

---

5. How much, if any, alcoholic beverages do you drink?

_____None

_____ Less than 10 oz. beer, or less than 5 oz. wine or less than 1 oz. liquor

_____ Less than 20 oz. beer, or less than 10 oz. wine or less than 2 oz. liquor

_____ Less than 30 oz. beer, or less than 15 oz. wine or less than 3 oz. liquor

_____ More than 30 oz. beer, or more than 15 oz. wine or more than 3 oz. liquor

---

Yes No

___ ___ 6. Have you been taking oral contraceptives regularly in the last six months? If yes, taking contraceptives, answer the following. If no, go to question 7.

How long have you been taking them continuously? _____ months.

What is the complete brand name of your pills? _____

(include a label from pack if possible; be sure to remove your name from label)

___ ___ Have you switched brands in the past six months? If so, what brand did you take before? _____

For how many months did you take this brand? _____ months.

Code No. _____ Page 2

Yes No

___ ___ Are you taking the pill for reasons other than contraception?
If so, please explain _____

___ ___ Has your weight changed since you started taking the pill?
Amount of increase _____ lbs. or decrease _____ lbs.

___ ___ 7. Are you taking any other medications now?

DRUG	AMOUNT USED PER WEEK	HOW LONG YOU HAVE USED
_____	_____	_____
_____	_____	_____
_____	_____	_____

Use back of page if more space is needed.

___ ___ 8. Are you under a doctor's care now?
If yes, please explain _____

___ ___ 9. Do you have a history of any medical problems?
If yes, please explain _____

___ ___ 10. Do you have a history of any gynecological problems?
If yes, please explain _____

---

11. In the last five years have you

_____ Had no pregnancy

_____ Been pregnant but not full term.

_____ Had a full term pregnancy 3 or more years ago.

_____ Had a full term pregnancy 1-3 years ago.

_____ Had a full term pregnancy in the last year.

Yes No

___ ___ 12. Is your menstrual cycle usually regular?
How many days between periods usually? _____ days
What day of your menstrual cycle will you be on the day your blood is drawn? _____ day

---

Code No. _____ Page 3

Yes No

___ ___ 13. Are you on any kind of special diet now?
If yes, please explain _____

___ ___ Are you on a weight reducing diet?

___ ___ Do you have any food allergies?
If yes, please explain _____

___ ___ 14. Have you changed your eating habits in the past six months?
If yes, please explain _____

---

15. Where do you usually eat:

	DON'T EAT	AT RESIDENCE	AWAY FROM RESIDENCE	FIX AT RESIDENCE BUT EAT AWAY
BREAKFAST:	_____	_____	_____	_____
LUNCH:	_____	_____	_____	_____
DINNER:	_____	_____	_____	_____
SNACKS:	_____	_____	_____	_____

___ ___ 16. Do you regularly (4 or more times/week) engage in physical activity:
___ Running How many miles per day? _____ miles
___ Walking How many miles per day? _____ miles
___ Riding bike How many miles per day? _____ miles
___ Sport What kind _____ Hours per week: _____
___ Swimming How much time per day? _____ hours
___ Other Explain what and give time per day _____

___ ___ 17. Do you take any supplements such as vitamins, minerals, protein, etc.?
Give brand name _____ include label if possible.
Amount (number of tablets, etc.) per week _____
How many months have you taken the supplement? _____ months
Please, give this information for each supplement.
(If more space is needed use back of page.)

Code No. _____ Page 4

18. Please put a number indicating approximately how many servings of each of the following you eat per week:

FRUITS (one small fruit or ½ cup is one serving)

____Citrus
____apples
____berries
____melon
____Other____
_____

____Dried fruit
____raisins (1/3 c.)

____Banana (1/3 med.)
____avocado (1/4 med.)

VEGETABLES (1 small veg. or ½ cup is one serving)

____carrots
____green beans
____tomatoes
____potatoes
____Other____
_____

____greens
____broccoli/cauliflower
____sweet potato
____corn
____cabbage

____dried beans
____lentils
____soybeans

BREADS AND CEREALS (1 slice or ½ cup is one serving)

____white bread
____white rice
____saltine/soda crackers (4-6)
____Other____
_____

____whole wheat bread
____whole wheat pasta
____brown rice
____rye bread
____cornbread

____Brewers yeast (3 Tbs.)
____wheat germ (1/4 c.)
____soy flour (1/4 c.)
____wheat bran (1/2 c.)

MEATS (3 ounces is one serving)

____shellfish
____shrimp
____Other____
_____

____fish
____red meats
____eggs (2 is 1 serv.)
____poultry

____organ meats (liver)
____fresh tuna or salmon

MILK AND MILK PRODUCTS

____Milk, all kinds (1 fluid cup is one serving)
____yogurt (1 cup is one serving)
____cheeses, all kinds (1 ounce is one serving)

MISC.

____jam/jelly (2 Tbs.)
____honey (2 Tbs.)
____Other____
_____

____peanut butter (1 & 1/2 Tbs.)
____almonds (10 nuts)

____sunflower seeds (1/4 c.)
____walnuts (14 halves)
____filberts (1/4 c.)
____peanuts (10 nuts)

Calculation of Scores

CALCULATIONS OF SCORES

General Health Score

From the questionnaire, questions 2-10 and 16 were used to determine the general health score of each subject. The scores for each subject are given in Tables 2 and 3. Calculations are as follows:

1) The subjects were evaluated on their height and weight relationship (questions 2 and 3) using the nomograph provided by Thomas et al. (1976).

5 points -- Within ± 10 percent of desirable weight

3 points -- Within ± 10-20 percent of desirable weight

1 point -- Not within ± 20 percent of desirable weight

2) Question 4: How many, if any, cigarettes do you smoke per day?

5 points -- None

4 points -- Less than $\frac{1}{4}$ pack

3 points -- Less than $\frac{1}{2}$ pack

2 points -- Less than 1 pack

1 point -- More than 1 pack

3) Question 5: How much, if any, alcoholic beverages do you drink?

5 points -- None

4 points -- Less than 10 oz. beer, or less than 5 oz. wine or less than 1 oz. liquor

3 points -- Less than 20 oz. beer, or less than 10 oz. wine or less than 2 oz. liquor

2 points -- Less than 30 oz. beer, or less than 15 oz. wine or less than 3 oz. liquor

1 point -- More than 30 oz. beer, or more than 15 oz. wine or more than 3 oz. liquor

4) Questions 7 through 10 dealt with the medical history of the subjects, such as the type and amount of medications taken, or the medical and/or gynecological problems of the subject.

5 points -- If no checked on all questions

4 points -- For one yes answer

3 points -- For 2 yes answers

2 points -- For 3 yes answers

1 point -- For 4 yes answers

5) The question relating to physical activity (no. 16) was difficult to rate because of the many variations and possibilities. In general, the following standard was used. If the subject engaged in no regular physical activity other than required for her normal life, e.g., walking to class, one point was given. If the subject engaged in a competitive sport or intensive exercise, e.g., running 5 miles/day on a regular bases, five points were given. Points of two through four were given for differing degrees of exercise between these two described limits.

Thus a total of 25 points was possible and a minimum of five points was insured.

Vitamin B_6 Dietary History Score

The vitamin B_6 score was derived from page 4 of the questionnaire. All foods in the first column (generally low in vitamin B_6 content) were given two points per serving. Foods in the second and third columns (indicating food generally moderate and high in vitamin B_6 content), respectively, were scored six and ten points. The total score was then divided by the number of servings to get an average value. A high score would indicate a tendency for the subject to eat more foods high in vitamin B_6.

Human Subjects Committee Approval

OREGON STATE UNIVERSITY

Committee for Protection of Human Subjects

Chairman's Summary of Review

Title: A Survey of Vitamin B6 Status in Young Women: Oral Contraceptive Users and Non-users

Program Director: James E. Leklem; Lorraine T. Miller

Recommendation:

____ Approval

_X_ Provisional Approval

____ Disapproval

____ No Action

Remarks: 1. Question 6 - "Include a label from pack if possible". This is not compatible with confidentiality since the label could contain the patients name. Can add the statement "If patients name on label please remove".
2. Question 7 - If respondant includes illegal use of drugs would the principle investigator be bound to report the authorities? Please have this revalued by O.S.U. Lawyers or remove and use as base for being excluded from the study.
3. Question 7, 8, 9, 10 and 11 - should be omitted since they do not seem to be related to the project proposed and they infringe on right not to give unnecessary information. If certain diseases, post pregnancies, abortions, street drugs, etc. interfere or are not compatible with your methods or objectives state those as conditions whereby individuals will be excluded from the study.

Date: May 4, 1979 Signature: [signature]

If the recommendation of the committee is for provisional approval or disapproval, the program director should resubmit the application with the necessary corrections within one month.