AN ABSTRACT OF THE THESIS OF

Judith Ann Hoaglund (Name) For the degree MASTER OF SCIENCE (Degree)

in FOODS AND NUTRITION (Major Department) presented on June 6, 1980 (Date)

Title: THE RELATIONSHIP OF DIETARY INTAKE TO BLOOD VITAMIN B_6 IN ORAL CONTRACEPTIVE USERS

Abstract approved: [redacted]

Oral contraceptive (OC) users frequently have lower vitamin B_6 status than non-oral contraceptive (NOC) users. However, normal dietary intake, a possible factor, has not been adequately studied. Therefore, 26 OC users and 25 NOC users, of college age, were compared with respect to dietary intake of vitamin B_6 and blood vitamin B_6 levels. OC users had been taking "the pill" for at least five months and NOC users had not taken any estrogen-progestin hormones for at least five months. A 72-hour continuous dietary intake record, kept by each subject, was used to calculate intakes of vitamin B_6 and nine other nutrients. Subjects consumed self-selected diets and none had used vitamin B_6 supplements within two weeks of this study.

Intakes of all nutrients studied were comparable between the two groups. The mean intakes exceeded the recommended dietary allowance (RDA) for all nutrients except iron, calories and vitamin B_6. The mean intake of vitamin B_6 (1.4 ± 0.5 mg/day for OC and 1.6 ± 0.5 mg/day for NOC) did not differ significantly between the two groups. The RDA for this age group is 2.0 mg/day of vitamin B_6. The mean protein intakes

were not significantly different for OC versus NOC users (72.6 ± 19.4 g/day for OC and 66.9 ± 13.6 g/day for NOC). The ratio of vitamin B_6 to protein was calculated for each subject. Mean ratios were 0.020 ± 0.004 for OC and 0.025 ± 0.01 for NOC users. This difference was significant at $p \leq 0.05$. The mean ratio for both groups exceeded 0.019, which is considered to be adequate.

Fasting blood samples were collected during the luteal phase (NOC) or after seven days of the pill cycle for OC users. These samples were analyzed for whole blood and plasma (by Lind, 1980) vitamin B_6, using a microbiological assay (S. uvarum). These values were used to calculate vitamin B_6 levels in the red blood cell (RBC). A significant difference ($p \leq 0.05$) was found between the mean level of RBC vitamin B_6 in the OC users versus the NOC (12.4 ± 5.4 ng/ml for OC and 16.8 ± 8.5 ng/ml for NOC). Plasma vitamin B_6 concentrations were also significantly different between the two groups. The mean ratio of plasma vitamin B_6 to RBC vitamin B_6 was not statistically different between OC and NOC users.

A questionnaire was used to compare the subject groups with respect to exercise, alcohol intake, general health, general vitamin B_6 intake and other indices. With the exception of alcohol intake, the mean scores for both groups, from this questionnaire, were similar. OC users had a significantly higher intake of alcohol than NOC users, as measured by the questionnaire. However, the actual alcohol intake from the dietary record did not differ statistically between the two groups.

The lack of a significant difference in vitamin B_6 intake, coupled with significantly different blood vitamin B_6 levels for OC versus NOC users, tends to indicate that the OC may be altering vitamin B_6 metabolism. Estrogens may cause a redistribution of vitamin B_6 in various body pools, with the vitamin leaving the blood and entering other tissues. Blood levels are generally used to determine vitamin status. By this assessment, OC users have a lower vitamin B_6 status than controls. It is recommended that OC users be encouraged to consume at least 2.0 mg/day of vitamin B_6 in their normal diets.

The Relationship of Dietary Intake to Blood Vitamin B_6 in Oral Contraceptive Users

by

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A Thesis submitted to

Oregon State University

In Partial Fulfillment of
The Requirements for
the Degree of

Master of Science

Completed June 1980

Commencement June 1981

APPROVED:

Professor of Foods and Nutrition in charge of Major

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Date thesis is presented June 6, 1980

ACKNOWLEDGEMENTS

Every form I see is Thine own form, my Lord,
And every sound I hear is Thine own voice;
In the perfume of flowers I perceive the fragrance of Thy spirit;
In every word spoken to me I hear Thy voice, my Lord.
All that touches me is Thine own touch;
In everything I taste I enjoy the savor of Thy delicious spirit.
In every place I feel Thy presence, Beloved;
In every word that falls on my ears I hear Thy message.
Everything that touches me thrills me with the joy of Thy kiss;
Wherever I roam, I meet Thee; wherever I reach I find Thee, my Lord;
Wherever I look, I see Thy glorious vision; whatever I touch, I touch Thy beloved hand.
Whomsoever I see, I see Thee in his soul;
Whoever aught gives to me, I take it from Thee.
To whomsoever I give I humbly offer it to Thee, Lord.
Whoever comes to me, it is Thou who comest;
On whomsoever I call, I call on Thee.

- Hazrat Inayat Khan

I am grateful for the contributions, participation, guidance and friendship of everyone who made this thesis a reality. Thank you all!

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INTRODUCTION

Oral contraceptives have been used by millions of women since they were first approved in 1960 (Hodges, 1971). In the past 15 years considerable literature has been published on the subject of oral contraceptives (OC) and their effects on metabolism and nutrient status both in animals and humans. Vitamin B_6 is one such nutrient that has been studied extensively in this respect.

Women taking OC agents were reported by Rose (1966) to have abnormal tryptophan metabolism. This abnormal pattern resembled that of a vitamin B_6 deficiency. Furthermore, it could be corrected by supplementation with vitamin B_6. Rose, therefore, suggested that OC agents may be altering the user's requirement for this vitamin. Other indices of vitamin B_6 status have since been studied. These studies have included blood levels of vitamin B_6 or its major coenzyme form, activities of certain vitamin B_6 requiring enzymes, urinary excretion of vitamin B_6 or its major metabolite, and metabolites of the tryptophan-kynurenine pathway. Many of these investigators have reported lower vitamin B_6 status in OC users than in controls.

Generally, these studies have attempted to relate changes in nutrient levels to the use of OC drugs, but have ignored the dietary intakes of their subjects. The recommended dietary allowance (RDA) for vitamin B_6 in young women is 2.0 mg/day. Several studies indicate that the average young women in the U.S. may not be ingesting this amount. Cinnamon and Beaton (1970) found vitamin B_6 intake in young men on self-selected diets to be only about 1.5 mg/day. In a study by Donald *et al.*, (1971) young women had an average intake of 1.6 mg/day of vitamin B_6. These authors suggested, however, that this was adequate based on the protein

intake. Other studies have shown similar low intakes for vitamin B_6 (Driskell et al., 1976; Kirsey et al., 1978).

It therefore appeared desirable to investigate the dietary intake of vitamin B_6 in similar populations of OC users and non-users (NOC) to determine the actual consumption of vitamin B_6 and to compare these intakes with the RDA and blood vitamin B_6 levels.

REVIEW OF LITERATURE

Metabolic Effects of Oral Contraceptives

In the U.S. alone there are estimates of between ten and 15 million women using oral contraceptives (Massey and Davidson, 1979). The great majority of these women are taking "the pill" for contraception, and are otherwise healthy individuals. A great deal of research has been done toward a better understanding of the metabolic effects of these compounds.

The most common types of OC agents used in the U.S. today are the combination types that contain both estrogens and progestins in each tablet. The lesser-used sequential types contain 21 tablets of estrogen and five tablets of progestin taken in sequence. Combination type OC's are much more effective in preventing pregnancy than the sequential forms. Currently most physicians favor the lower dose, combination types where both estrogens and progestins are in reduced amounts (Hodges, 1971). The lower doses are still effective contraceptives, yet produce fewer and less noticeable side effects. The mechanism of action was originally assumed to be the suppression of ovulation. It appears now that the low-dose "pills" frequently do not suppress ovulation. Instead their effects are probably due to changes in the chemical and physical characteristics of the cervical mucus and endometrium which are less favorable for fertilization and implantation (Rudel, 1970).

Since many of the side effects of OC agents are dose related, it is important to keep the levels as low as possible, particularly for estrogens. The lowest level which continues to be effective as a contraceptive is 30 μg ethinyl estradiol per day (Briggs, 1979). Some of the side effects seen with OC use are nausea, vomiting, depression, headache, weight gain and breast discomfort. These are generally explained to and accepted by women taking "the pill". Major problems implicated by the use of OC agents are increased risk of thromboembolism and some types of cancer (Goodman and Gilman, 1975); hypertension (Weinberger *et al.*, 1970); stroke and diabetes mellitus (Hodges, 1971).

There are also nutritional effects of oral contraceptives. As early as 1960 it was noted that estrogens might alter the activity of vitamin B_6-dependent enzymes (Rose, 1978). Estrogens are known to induce several metabolic enzymes such as tryptophan oxygenase (pyrrolase), tyrosine and alanine amino transferases (Rose and Braidman, 1971; Brin, 1971). Ornithine decarboxylase and phosphorylase phosphatase are also induced by steroid hormones (Litwack, 1979). Other enzymes, such as kynureninase, appear to be inhibited by OC use (Mason et al., 1969). As one might expect from this list of enzymes, amino acid metabolism is also affected by OC agents. Hormones, including estrogen, affect the operation of certain amino acid transport systems (Guidotti et al., 1978). Total plasma α-amino nitrogen is lowered by OC use (Rose et al., 1976). This is due mainly to decreases in arginine, glycine, proline and tyrosine (Aly et al., 1971; Craft and Peters, 1971). Rose et al. (1975) reported that alanine and phenylalanine levels were depressed in OC users. Fasting levels of valine, methionine, isoleucine, histidine and glutamic acid were significantly lower in OC users than in controls (Potera and Rose, 1978). Tryptophan metabolism has been studied extensively since 1966 when estrogens were reported to influence its metabolism (Rose, 1966). Changes in protein levels and synthesis also occur with OC use.

Carbohydrate metabolism is influenced by the use of OC agents (Beck, 1973; Rose et al., 1972). Impaired glucose tolerance in OC users has been studied (Wynn and Doar, 1966; Rose et al., 1975). Wynn and Doar (1966) also reported abnormal increases in serum insulin levels after glucose loads. Fasting insulin levels are also elevated in OC users (Beck et al., 1975). Rose et al. (1972) have suggested that the decreased glucose tolerance may be caused by elevated xanthurenic acid, produced during altered tryptophan metabolism, which binds to insulin making it inactive. This abnormal glucose tolerance is reversed by vitamin B_6 supplementation (Rose et al., 1975). These authors also suggest that the OC may stimulate gluconeogenesis by increasing the activity of hepatic phosphoenolpyruvate carboxykinase.

Alterations due to OC therapy have been noted in lipid metabolism as well (Beck et al. 1973). Rose et al. (1977) reported a highly significant increase in serum triglyceride levels among OC users. Fasting triglycerides were more than 70% higher in OC subjects. These authors suggest this is due to increased metabolism of alanine to pyruvate (via estrogen-induced alanine aminotrasferase) and its subsequent incorporation into lipids. Hazzard et al. (1969) state that serum triglyceride levels may be elevated partly in response to impaired clearance of triglycerides by OC agents. Increased triglyceride levels appear to be related to estrogen dose (Stokes and Wynn, 1971; Beck et al., 1973). Cholesterol levels are also elevated by the combination type OC. Stokes and Wynn (1971) found significantly increased fasting cholesterol levels to be related to progestin dose in the OC. Beck et al. (1973) noted a similar relationship. Progestin when given alone, however, decreased levels of both triglycerides and cholesterol (Beck et al., 1975).

Blood vitamin levels appear to be affected by OC therapy. Ascorbic acid levels in blood have been reported to decrease during OC use (Rivers and Devine, 1972), although not all investigators have observed this (Prasad et al., 1975). Possibly there is an increased need for vitamin C due to estrogen-elevated levels of ceruloplasmin in the blood of OC users (Theuer, 1972). This author suggests that this is due to increased degradation of vitamin C by the increased levels of ceruloplasmin.

Shojania et al. (1971) noted that folate levels were lower in OC users. Others have reported similar findings (Ahmed et al., 1975; Prasad et al., 1975). It appears that lower folate levels may be due to reduced absorption of food folate by the use of OC agents (Theuer, 1972). However, Shojania et al. (1971) indicated that absorption is not impaired, but folate metabolism is significantly altered.

Plasma vitamin A levels have been reported to increase with OC therapy (Gal et al., 1971; Ahmed et al., 1975; Prasad et al., 1975; Ahmed and Bamji, 1976). Prasad et al. (1975) noted lower plasma

carotene levels in the OC users. Liver vitamin A appears to decrease in response to OC therapy suggesting a redistribution of vitamin A from the liver to the blood, possibly due to the rise in retinol binding protein (Ahmed and Bamji, 1976).

OC users appear to have decreased serum vitamin B_{12} levels (Wertalik et al., 1972), and an increased serum vitamin B_{12} binding capacity (Theuer, 1972). Massey and Davidson (1979) propose that abnormalities in vitamin B_{12} metabolism are possibly due to abnormalities in the metabolism of vitamin B_6.

The need for riboflavin may also be increased as the metabolism of vitamin B_6 is altered during OC use (Theuer, 1972). Ahmed et al., (1975) reported decreases in red cell riboflavin and glutathione reductase activity, and increased in vitro stimulation with FAD in OC users. Others have noted similar decreases in riboflavin status (Prasad et al., 1975; Wynn, 1975).

Vitamin B_6 status has been studied extensively with respect to OC use, and does appear to be altered by these drugs. This topic will be discussed later.

Mineral metabolism affected by the use of OC includes copper, zinc, calcium and iron. Serum copper has been found to be elevated in OC users (O'Leary and Spellacy, 1968). These authors noted that the ceruloplasmin protein which carries copper in the blood is increased by the use of estrogens. Halsted et al., (1968) reported that women using OC agents had significantly higher plasma copper levels than controls. Plasma zinc levels were significantly lower in these same OC users. Estrogen appears to decrease plasma zinc but increase zinc levels in certain tissues such as liver, spleen, adrenals, and uterus (Theuer, 1972). Massey and Davidson (1979) have stated that erythrocyte zinc levels also increase with OC use. Calcium is affected by estrogens. Bone resorption is inhibited by this hormone (Theuer, 1972). Calcium absorption from the intestine was improved by the use of the OC according to Caniggia et al. (1970). A decrease in urinary calcium has also been noted during OC use (Massey and Davidson, 1979). Serum

iron and total iron binding capacity are increased in OC users (Burton, 1967; Mardell and Zilva, 1967). Theuer (1972) states that OC agents have no significant effect on hemoglobin concentrations. Other researchers have reported similar findings for hemoglobin and hematocrit values (Rose et al., 1973; Miller et al., 1975; Bosse and Donald, 1979).

Functions of Vitamin B_6

Vitamin B_6 occurs in three forms: pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM), each of which can be phosphorylated. PN is the form usually found in plants, while PL and PM are the major forms seen in animal tissues (Sauberlich and Canham, 1973). Pyridoxal-5-phosphate (PLP) is the major physiologically active form of the vitamin (Sauberlich et al., 1972) although pyridoxamine phosphate (PMP) may also activate some transaminases (Sauberlich and Canham, 1973). All three free forms are approximately equal in supporting animal growth, and can be readily interconverted and phosphorylated (see Figure 1). PLP functions as a coenzyme for well over 60 enzymes in the body. Vitamin B_6 is involved in the metabolism of protein, carbohydrates and lipids, but functions mainly in amino acid metabolism. Examples of reactions which involve PLP are transamination, decarboxylation, racemization, dehydration, desulfhydration, cleavage and synthesis. Transaminases are a major enzyme group involved in the removal and transfer of the α-amino group from amino acids such as alanine, arginine, asparagine, aspartic acid, cysteine, isoleucine, lysine, phenylalanine, tyrosine, valine and tryptophan. Decarboxylases act on the amino acids tyrosine, histidine and tryptophan, for example, and are involved in the production of serotonin. Racemization enzymes convert L-forms of amino acids to D-forms. Dehydratases participate in interconversions of amino acids. Cysteine is converted to pyruvic acid via desulfhydration. Kynureninase is an example of a cleavage enzyme. The PLP dependent enzyme δ-aminolevulinic acid synthetase is involved in the synthesis of heme. PLP is also important in the conformation or structure of the phosphorylase enzyme. This enzyme catalyzes the breakdown of glycogen to glucose-1-phosphate.

The liver is the site of further metabolism of pyridoxal to 4-pyridoxic acid (4-PA) via aldehyde oxidase. This compound is no longer biologically active and cannot be reconverted to active form (Brin, 1978), therefore, it is excreted in the urine.

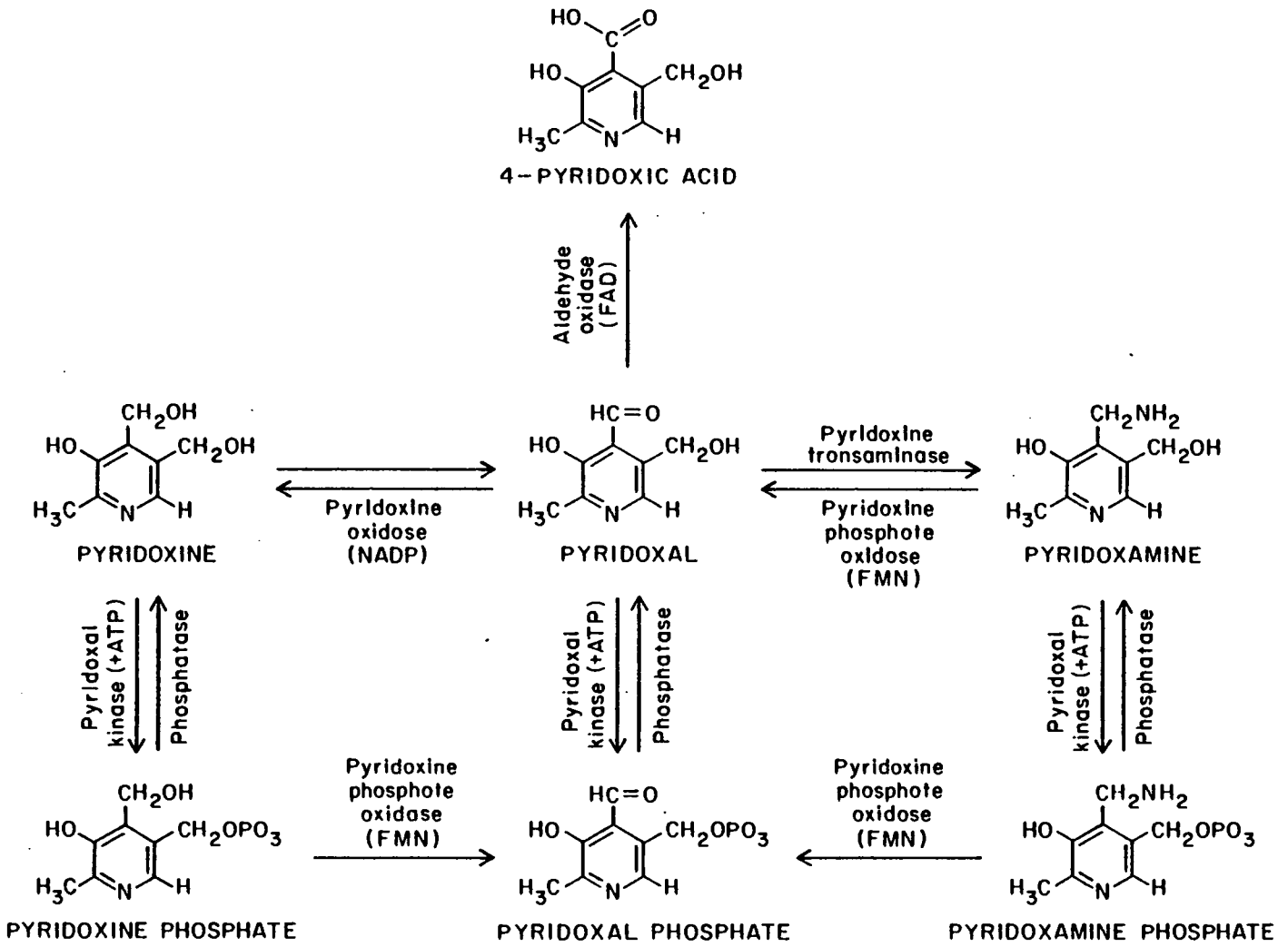

Figure 1: Interconversions and structures of vitamin B_6.

Altered Vitamin B_6 Metabolism with Drug Use

The activity of vitamin B_6 is altered by a number of compounds (Bauernfeind and Miller, 1978). Four-deoxypyridoxine is an antagonist to vitamin B_6, and has been used to induce vitamin B_6 deficiencies in animals and humans (Sauberlich, 1968). Another common antagonist is isonized (INH), a drug used in the treatment of tuberculosis. This compound also increases the urinary excretion of vitamin B_6 (Coursin, 1961). Penicillamine, a specific antidote to copper poisoning, complexes with PL rendering it inactive (Bauernfeind and Miller, 1978). Certain antibiotics may also interfere with vitamin B_6. Cycloserine, for example, inactivates the vitamin (Sauberlich and Canham, 1973).

Oral contraceptives have been found to produce a number of effects on vitamin B_6 metabolism. Investigations of these effects have covered a variety of metabolic pathways. Basically,there are two major methods for the assessment of vitamin B_6 status in addition to the measurement of urinary vitamin B6 and 4-PA. Direct methods measure the levels of vitamin B_6 or PLP in body tissues, usually the blood. Indirect methods are used to measure products formed via vitamin B_6 dependent metabolic pathways. Transaminase activity and *in vitro* stimulation, and urinary tryptophan-kynurenine pathway metabolites are most commonly used.

Pyridoxal phosphate

PLP, the active form of vitamin B_6, is the major form found in blood (Sauberlich *et al.*, 1972). It is most easily measured in plasma, and has been found to correlate with other indices of vitamin B_6 status (Lumeng *et al.*, 1974b). Shane and Contractor (1968) have reported that plasma PLP does not fluctuate with changes in the menstrual cycle. PLP is considered to be a sensitive indicator of vitamin B_6 status (Baysal *et al.*, 1966; Kelsay *et al.*, 1968; Donald *et al.*, 1971). In a longitudinal study involving 55 OC and 77 NOC users Lumeng *et al.*(1974b) observed that the OC users did have significantly lower plasma PLP than NOC users. When the subjects were

divided according to age there was no significant difference between the two groups in the 20-24 year range, but there was a significant difference in the 25-30 age group. Lumeng et al. (1974b) also determined plasma PLP from two weeks prior to OC therapy through six months of continuous OC use. During the first three months plasma PLP decreased, but by six months it had returned to the pretreatment level for most subjects. These authors suggest that the increased level of estrogens caused an increase in the PLP binding proteins which then produced a redistribution of PLP in the body tissues and fluids. Further, in most women this temporary "deficiency" tends to correct itself over time.

Brown et al. (1975) using a depletion-repletion design, reported predepletion PLP levels of 11.70 ng/ml for nine NOC and 9.15 ng/ml for 15 OC. After 28 days of depletion of vitamin B_6 their PLP levels were 3.18 ng/ml, NOC, and 2.99 ng/ml for OC users. These subjects were then repleted for 28 days with 0.8 mg/day of PN·HCl. Plasma PLP levels increased to 5.66 ng/ml and 4.50 ng/ml, respectively. At a repletion level of 2.0 mg/day the mean plasma PLP level was 15.00 ng/ml for NOC and 12.90 ng/ml for OC users. These differences were not statistically significant. These authors suggest the lack of significance is due to the small number of subjects and the different steroid preparations the subjects were using. Miller et al. (1975) reported finding no significant difference between the levels of plasma PLP in ten OC and 11 NOC users, either before or after two days of supplementation with 50 mg/day of PN·HCl. In a study involving OC users and controls from two different socioeconomic levels in India, plasma PLP values were significantly lower in the OC versus NOC users from the high socioeconomic level (Prasad et al., 1975).

In the only study of whole blood PLP (Shane and Contractor, 1975), levels were significantly lower among OC users than controls. Following a dose of 20 mg/day PN·HCl for four weeks, no significant differences in blood PLP were noted between the two groups of subjects.

Blood vitamin B_6

Blood levels of vitamin B_6 and vitamin B_6 activity are usually considered to be a reflection of vitamin B_6 intake (Baysal et al., 1966; Kelsay et al., 1968). Vitamin levels can be measured in serum, plasma, whole blood or the red cell. Bosse and Donald (1979) measured red cell PL in OC and NOC subjects. They reported PL levels were lower in the OC group, but this was not statistically significant. These subjects were depleted for 30 days then repleted. During repletion OC users required larger doses of PN to reach the same degree of increase seen in the controls; i.e. the erythrocyte vitamin B_6 levels responded to repletion with less sensitivity in the OC users. Donald et al. (1971) reported that in young women the vitamin B_6 level in the red cell declined during depletion and rose during repletion with vitamin B_6. This suggests that the red cell can be used to indicate long term vitamin B_6 status (Bosse and Donald, 1979). A summary of blood vitamin B_6 levels is given in Table 1.

Whole blood vitamin B_6 has been studied by Miller et al. (1974). These authors observed lower vitamin B_6 levels in OC users versus controls (6.0 ng/ml and 7.7 ng/ml, respectively), but this difference was not statistically significant. The mean concentration of vitamin B_6 in whole blood was inversely related to the length of time subjects had been taking the OC. In 1975, Miller et al. reported the response of ten OC and 11 NOC users to vitamin B_6 supplements. Whole blood vitamin B_6 was assayed before and after two days of 50 mg PN·HCl doses. The vitamin B_6 levels were not significantly different between the two groups either before or after the supplements. OC users were slightly higher than controls before and controls slightly higher than OC users after the supplement. There was also no relationship between whole blood vitamin B_6 before and after supplementation.

Serum vitamin B_6 levels were studied by Haspels et al. (1975). These authors reported that the OC had no effect on serum levels of the vitamin. Most recently Miller et al. (1978) reported plasma vitamin B_6 levels were lower in OC users (5.9 ng/ml versus 9.9 ng/ml for NOC users.)

Table 1. Report levels of blood vitamin B_6 and PLP in OC users and NOC users.

Reference	No. of Subjects	Measurement	Assay Method	Vitamin B_6 ng/ml	Dietary Intake mg/day
Lumeng et al., 1974	55 OC users	Plasma PLP	Tyrosine decarboxylase	7.8 ± 3.7	Not available
	77 NOC users			9.4 ± 4.2	
Miller et al., 1974	3 OC users	Whole blood Vitamin B_6	Microbiological Assay S. uvarum	6.0 ± 0.4	1.9 mg/day
	2 NOC users			7.7 ± 1.1	1.9 mg/day
Brown et al., 1975	15 OC users	Plasma PLP	Tyrosine decarboxylase	9.15 ± 2.57	Pre-depletion
	10 NOC users			11.7 ± 3.2	
	15 OC users			12.9 ± 2.95	Post depletion & repletion of 2.0 mg/day
	10 NOC users			15.0 ± 5.0	
Miller et al., 1975	10 OC users	Plasma PLP	Tyrosine decarboxylase	5.9 ± 3.7	Not avail.
	11 NOC users			6.4 ± 3.8	Not avail.
		Whole blood Vitamin B_6	Microbiological Assay S. uvarum	8.3 ± 4.3	Not avail.
				7.0 ± 1.5	Not avail.
Shane and Contractor, 1975	9 OC users	Whole blood PLP	Fluorometric	7.6 ± 1.1	Not avail.
	12 NOC users			9.6 ± 1.7	
Miller et al., 1978	5 OC users	Plasma vitamin B_6	Microbiological Assay	5.9 ± 3.4	1.5 ± .05
	4 NOC users			9.9 ± 1.9	1.6 ± .4
	11 Lab control women		S. uvarum	10.0 ± 3.6	
Bosse and Donald, 1979	8 OC users	RBC PL	Microbiological Assay S. uvarum	11.6 ± 1.6	2.06 mg/day
	8 NOC users			13.5 ± 3.1	2.06 mg/day

These authors suggested the marginal intake of vitamin B_6 by OC users in this study may have been responsible for the lower blood values. The assessment of dietary intake of vitamin B_6 has been lacking in most of the studies of blood vitamin B_6.

Erythrocyte transaminase activity

As previously mentioned, RBC transaminase activity is an indirect method for assessing vitamin B_6 status. PLP serves as cofactor for these enzymes, and vitamin B_6 status can be estimated by measuring enzyme activity and percent stimulation by PLP. Erythrocyte transaminases most accurately reflect vitamin B_6 status (Linkswiler, 1967; Sauberlich et al., 1972). Erythrocyte alanine aminotransferase (glutamic-pyruvic transaminase EC 2.6.1.2 or EGPT) and erythrocyte aspartate aminotransferase (glutamic-oxaloacetic transaminase EC 2.6.1.1 or EGOT) are most commonly studied. The activity of these enzymes and their percent stimulation in vitro by PLP are measured and expressed as an activation factor or ratio of total enzyme activity to apoenzyme (Shane, 1978).

Raica and Sauberlich (1964) determined that the enzyme levels in the red cells themselves were not an accurate assessment of vitamin B_6 status, but that in vitro stimulation was useful. Cinnamon and Beaton (1970) also found that in vitro stimulation correlated well with vitamin B_6 depletion. A decrease in the activity of both enzymes plus a rise in the in vitro stimulation were reported by Ahmed and Bamji (1976) to be associated with a vitamin B_6 deficiency. Others have noted an increased enzyme activation in pregnancy (Heller et al., 1973). All researchers, however, do not agree on which, if either, enzyme is a more sensitive indicator of vitamin B_6 status in OC users, and results are frequently conflicting. Aly et al. (1971) found EGOT activity higher in OC users. Rose et al. (1972) and Miller et al. (1975) concur with these findings. Miller et al. (1978) reported no difference in EGOT activity for OC users and controls. Lower EGOT activity was noted with OC use by Salkeld et al. (1973) and Kishi et al. (1977). Driskell et al. (1976) found greater in vitro stimulation for EGPT

among OC users. This finding agrees with Doberenz et al. (1971) who found both lower EGPT activity and greater in vitro stimulation in OC users. Aly et al. (1971), however, concluded that OC use did not affect EGPT activity. Still other investigators could find no significant difference between OC users and controls for either EGOT or EGPT (Rose et al., 1975; Brown et al. 1975). Variability between laboratories, a wide range in subject's values, small numbers of subjects and lack of correlation to dietary intake of vitamin B_6 has led to the conclusion by some that transaminases are not reliable indicators for vitamin B_6 status, especially during pregnancy or OC therapy (Shane and Contractor,1975; Driskell et al., 1976; Bosse and Donald, 1979). Lack of standardization of experimental methodology is a possible explanation for these results (Shane, 1978). Other factors may alter the sensitivity of transaminases to vitamin B_6. During vitamin B_6 deficiency there may be a destabilization of the apoenzyme (Brin et al. 1960; Jacobs et al., 1968). Certain proteases may act on the apoenzyme (Kominami et al., 1972). Hormonal induction and altered cofactor affinity may occur (Brin, 1971). Estrogen conjugates may inhibit enzymes by competing with PLP for binding sites on the apoenzyme (Rose and Braidman, 1971). There may also be cofactor induction of apoenzyme synthesis resulting from administration of supplemental vitamin B_6 (Rose et al. 1972).

Tryptophan metabolism

A second indirect method for determining vitamin B_6 status is the tryptophan load test (TLT). Tryptophan can be converted to niacin and other metabolites through a series of reactions, some of which are PLP dependent (see Figure 2). When a person deficient in vitamin B_6 is given a loading dose of tryptophan, an increased excretion of kynurenine (K), 3-hydroxykynurine (HK) and xanthurenic acid (XA) results along with an elevated HK/3-hydroxyanthranilic acid (HA) ratio (Rose et al. 1972). This abnormal tryptophan metabolism may result from a shortage of PLP cofactor or competition for binding sites.

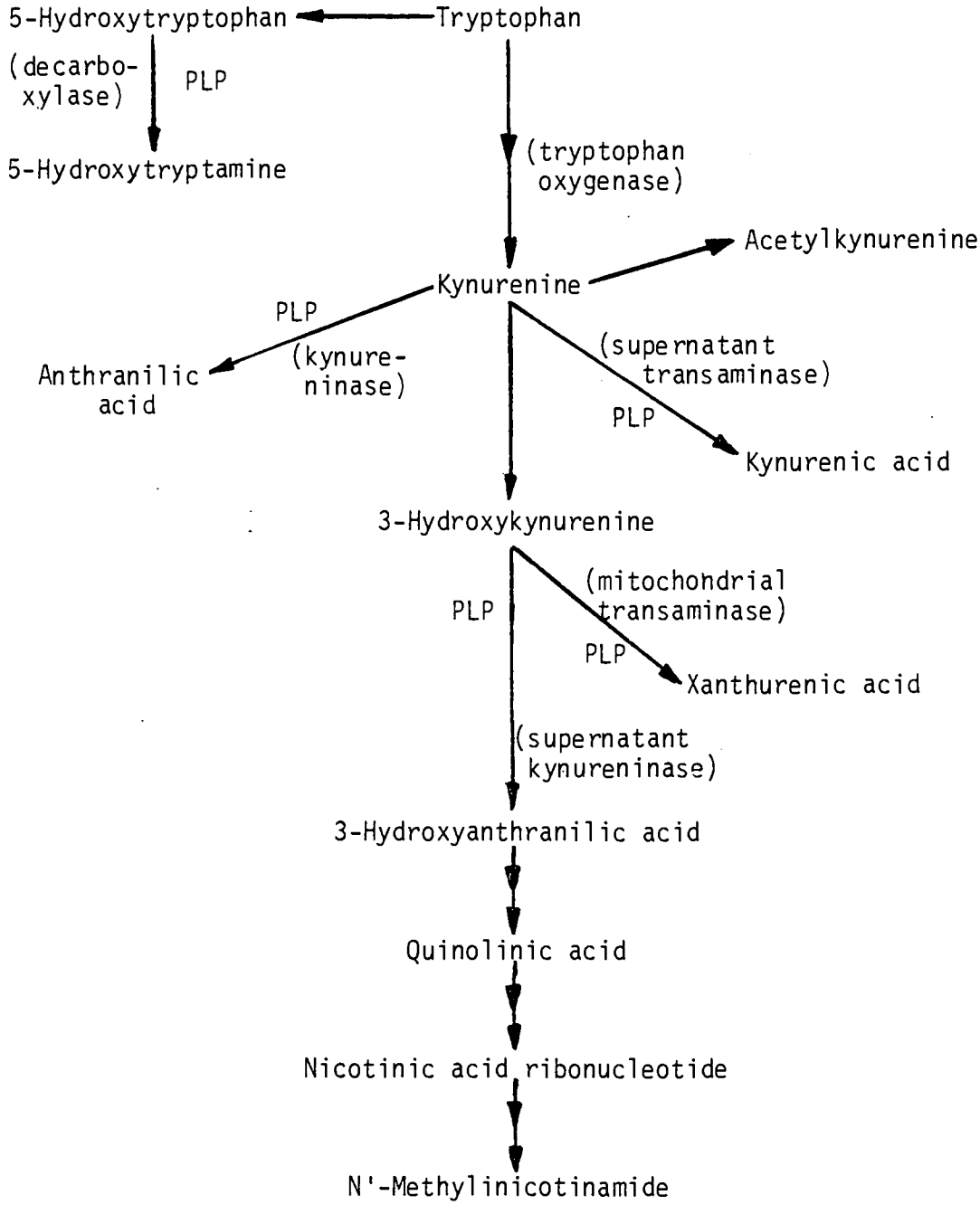

Figure 2. Tryptophan-kynurenine pathway (adapted from Rose et al., 1972; and Leklem et al., 1975). PLP indicates the known pyridoxal phosphate-dependent enzyme reactions.

Abnormal tryptophan metabolism is also seen in women taking OC agents. This was first noted by Rose (1966) and has since been documented by many other studies. When subjects taking OC drugs were given loading doses of 5g, L-trytophan excretion of HK and XA increased. When three of these subjects were given PN supplements, their excretion of HK and XA returned to normal.

Price et al. (1967) reported increased excretion of XA, HK, K and kynurenic acid (KA) following a 2g dose of tryptophan among OC users. Similar results were noted by Aly et al. (1971) for XA, KA and K following a TLT and by Luhby et al. (1971); Lumeng et al. (1974b) and Haspels et al. (1975) for XA in OC users. Leklem et al. (1973) administered 2g of L-tryptophan and 200 mg of L-kynurenine sulfate to OC users and observed increased excretion of K, HK, HA and XA, plus an elevated HK/HA ratio. In a study by Miller et al. (1974) differences in excretion of tryptophan metabolites were found (in one OC user) when the tryptophan dose was given at different times during the day, suggesting a circadian variable may be present in tryptophan metabolism. These authors also noted altered tryptophan metabolism when OC pills were interrupted for the menses interval. Luhby et al. (1971) had also observed a decreased excretion of XA in OC users following a TLT during the menses interval. Leklem et al. (1975b) noted this as well.

Investigators have also studied the effects of vitamin B_6 depletion and repletion on tryptophan metabolism in OC users. Leklem et al. (1975b) noted tryptophan metabolism deteriorated more rapidly in OC users than in controls. Upon repletion with vitamin B_6, the OC group showed a slower improvement in tryptophan metabolism. This was also reported by Donald and Bosse (1979).

The exact causes of abnormal tryptophan metabolism in OC users has not yet been fully explained. Several hypotheses have been suggested. Estrogen conjugates, as mentioned before, may be competing with PLP for binding sites and thereby inhibiting these enzymes (Mason et al., 1969; Rose and Braidman, 1971). Rose et al. (1972) has further suggested that the supernatant kynureninase enzyme may be inhibited by estrogen esters while the mitochondrial kynureninase transaminase

is protected, leading to an increased excretion of XA. Again, induction of transaminases and altered affinity for PLP may result from estrogen use. This effect is believed to be in response to elevated glucocorticoid levels caused by estrogen use (Rose and Braidman, 1971). An increased transaminase level would create increased competition of the binding sites for PLP, possibly leaving more sensitive enzymes, like kynureninase, with less cofactor (Brin, 1971). In addition to these effects, estrogen may have a direct effect on tryptophan oxygenase. Brin (1971) found estrogen induction of this enzyme in rats and proposed that increased tryptophan metabolites would stress the capacity of the tryptophan-niacin pathway. Recently, however, it has been suggested that tryptophan oxygenase activity is similar in women using and not using OC compounds (Green, 1978). Possibly this finding is due to a modification of the estrogenic effect on tryptophan by the type and dose of progestin in the combination type OC (Leklem et al. 1973).

Urinary Vitamin B_6 and 4-Pyridoxic Acid

Vitamin B_6 status may be estimated by the amount of vitamin B_6 or 4-PA (the major metabolite) in the urine. These two indices generally reflect dietary vitamin B_6 intake. Both have been found to decrease with depletion and increase with repletion of vitamin B_6 (Baysal et al., 1966; Linkswiler, 1967; Kelsay et al., 1968; Donald et al., 1971; Rose et al., 1972 and Donald and Bosse, 1979). Urinary 4-PA comprises between 40 and 50% of the vitamin B_6 ingested in adult males (Sauberlich et al., 1972). Donald et al. (1971) observed a 22-35% conversion of dietary B_6 to 4-PA in adult women. The balance consists of various forms of vitamin B_6, mainly PL, and a number of other metabolites. Wozenski et al. (1980) stated that urinary vitamin B_6 comprised about 8% of a vitamin B_6 dose, and 4-PA excretion was between 35 and 63% of this vitamin dose in males. Aly et al. (1971) found that OC use

did not affect the excretion of 4-PA or vitamin B_6. Further studies have confirmed this (Brown et al., 1975; Leklem et al., 1975a; Donald and Bosse, 1979). Miller et al. (1974) reported that OC users excreted about 30% less total vitamin B_6 than controls when a diet containing 1.9 mg of vitamin B_6 was consumed. Excretion of 4-PA was not significantly different between these two groups. It appears that urinary vitamin B_6 and 4-PA are most useful in assessing recent vitamin B_6 intakes. According to Wozenski et al. (1980) these are the "most sensitive indicators of vitamin B_6 absorption and metabolism", with a dose of at least 1 mg of vitamin B_6 producing measurable changes.

Tissue Distribution of Vitamin B_6

Vitamin B_6 is found in all tissues of the body. Various studies have been used to determine relative amounts of this vitamin in different body compartments. The liver appears to be a major site for vitamin B_6 on a ng/g tissue basis. Thiele and Brin (1966), using rats, reported the highest total vitamin B_6 level was in the liver, then kidney, brain, skeletal muscle and finally heart. Lyon et al. (1962) ranked vitamin B_6 tissue levels in mice as liver, then brain and then muscle. In monkeys, liver vitamin B_6 levels were higher than blood vitamin B_6 levels (Benson et al., 1976). Rats studied by Bhagavan et al. (1976) showed the highest levels in kidney followed by heart, adrenals and then lungs on a ng/g fresh weight basis.

The form of vitamin B_6 present in greatest concentration in animal tissues is PM for all tissues except muscle (Thiele and Brin, 1966). PLP is the major form in muscle and may be stored there attached to phosphorylase enzymes. Muscle thus represents the largest total body vitamin B_6 pool. Lyon et al. (1962) reported that the phosphorylated forms PMP and PLP were highest in all tissues. They also found vitamin B_6 in the brain to be mostly PMP, with PLP the main form in muscle. Levels of PN are routinely found to be very low in animal tissues (Thiele and Brin, 1966; Benson et al., 1976).

The predominant form of vitamin B_6 in human plasma is PLP (Sauberlich et al., 1972; Lumeng and Li, 1974; Parker et al., 1979). Anderson et al. (1971) consider the main form in plasma to be PL. These differences of opinion may be due to the large variety of assay methods used (Shane, 1978). PLP is formed almost entirely in the liver and then released into the blood (Lumeng et al., 1974a). PLP is transported in the plasma mainly bound to the protein albumin according to these authors.

Red cell vitamin B_6 appears to be mainly PL (Anderson et al., 1971). Erythrocytes also contain all the enzymes necessary for interconversions of vitamin B_6 to its various forms (Shane, 1978; Anderson, 1980). See Figure 3 for these interconversions. The red cell cannot take up protein bound PLP from the plasma, nor is PLP normally released into plasma (Lumeng et al. 1974a; Anderson et al., 1974). The red cell does take up the three free forms of vitamin B_6. PL is maintained in an approximately 2:1 ratio between erythrocytes and plasma (Anderson et al., 1971). This distribution is believed to be governed by competing protein binders in the plasma (albumin) and RBC (hemoglobin) for vitamin B_6 (Anderson et al., 1974). Erythrocytes are unable to phosphorylate PL directly (Anderson et al., 1971). PN and PM are converted in the red cell to PNP and PMP via pyridoxine kinase, and then oxidized to PLP. This oxidase enzyme is flavin mononucleotide (FMN) dependent. PLP formed in the RBC may remain bound to hemoglobin (Suzue et al., 1970) or may be split by phosphatase to PL. Thus, riboflavin metabolism plays a role in the interconversions of vitamin B_6 in erythrocytes, and the mature red cell can synthesize FMN and FAD from riboflavin (Anderson, 1980). Levels of PLP in the red cell are also controlled by the membrane-bound phosphatase enzyme (Lumeng and Li, 1974, Anderson et al., 1971). PN has been reported to induce RBC pyridoxine kinase and EGOT (Solomon and Hillman, 1977). These authors also noted that PLP induced EGOT enzymes.

Anderson (1980) postulates that a significant portion of blood vitamin B_6 enters the red cell, and that most PL in the blood is the result of RBC conversion. Further, the red cell may serve as a reservoir

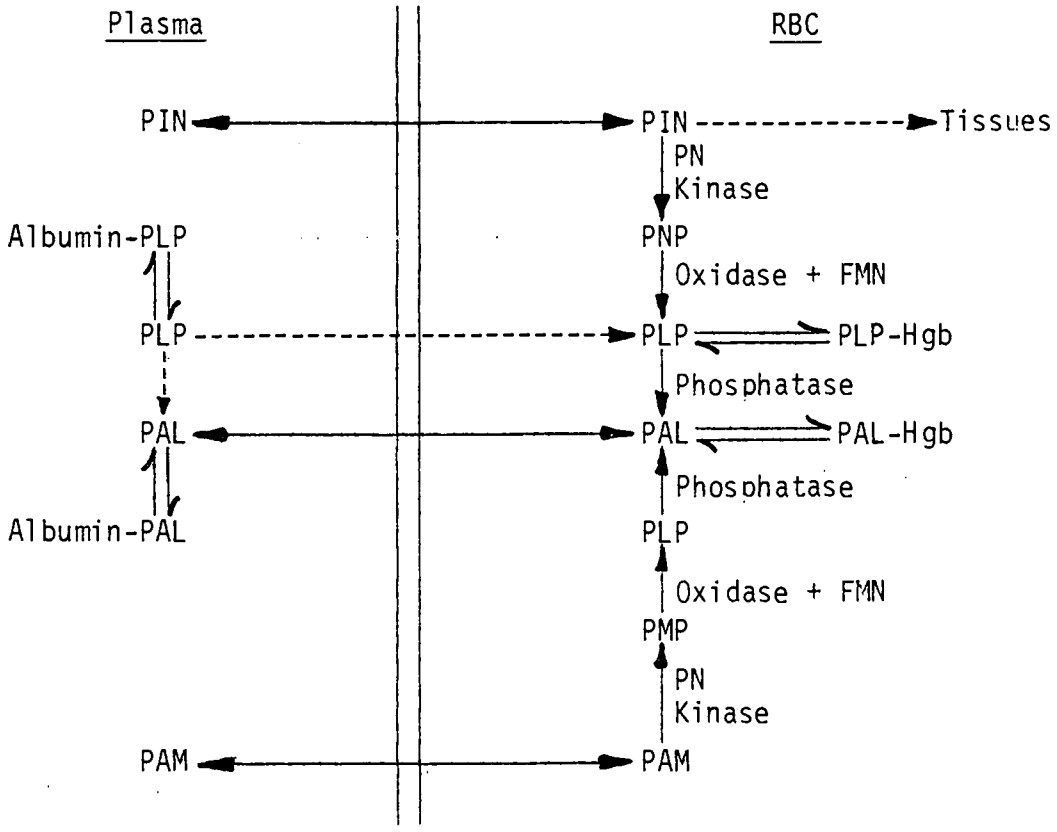

Figure 3. Interconversions of vitamin B_6 in the RBC.

Adapted from Shane, 1978 and Anderson, 1980.

for PL, and a transport mechanism for uptake of vitamin B_6 by other tissues. PN may be able to pass directly from the RBC to body tissues, without entering the plasma, in the same manner suggested for amino acids (Anderson, 1980). Thus, the red cell appears to be active in the metabolism, transport and storage of vitamin B_6 in the body.

At present leukocytes are thought to have a minor role in vitamin B_6 metabolism (Shane, 1978).

Vitamin B_6 Requirements and Intakes

The requirement for vitamin B_6 is in part determined by the level of protein in the diet. In a study of young men, Kelsay et al. (1968) determined that the demand for vitamin B_6 was increased in subjects who consumed a high, as opposed to low, protein diet. It was due to this and similar studies (Donald, 1978) that the Canadian dietary requirement for vitamin B_6 was related to protein intake. Consumption of 0.02 mg. of vitamin B_6 per gram of protein is suggested (Bureau of Nutritional Sciences, 1975).

Vitamin B_6 requirements in young women have been studied by Donald et al. (1971) and Shin and Linkswiler (1974). Data from control groups in studies of vitamin B_6 requirements of OC users (Brown et al., 1975; Leklem et al., 1975a and 1977; Donald and Bosse, 1979) may be considered as well. These studies were conducted using depletion-repletion designs. Different levels of vitamin B_6 were supplied in the diets and various time periods were used in each study. A summary of these studies is given in Table 2. Various biochemical parameters were used to determine vitamin B_6 requirements, with restoration of pre-depletion levels being the usual criterion. These researchers generally agree that 1.0 mg/day of vitamin B_6 is not sufficient and 2.2 mg/day more than restored most parameters to normal. Thus an intake of between 1.5 mg and 2.0 mg/day is generally considered adequate. The requirement for OC uses, although controversial, may be slightly more (Donald and Bosse, 1979). At pre-

Table 2. Summary of studies determining the vitamin B_6 requirement of young women, including OC users.

	Donald et al., 1971	Shin and Linkswiler, 1974	Brown et al., 1975 NOC Users	Brown et al., 1975 OC Users	Bosse and Donald 1979 and Donald and Bosse, 1979 NOC Users	Bosse and Donald 1979 and Donald and Bosse, 1979 OC Users
Subjects						
Number	8	5	10	15	8	8
Mean age, years	24.7	23.2	22.3	23.2	21.0	20.0
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	
Assessment Criteria	RBC vitamin B_6 EGOT and EGPT Urinary vitamin B_6 and 4-PA	Tryptophan & Methionine Metabolites	Plasma PLP EGOT and EGPT Urinary 4-PA		RBC pyridoxal EGOT and EGPT Urinary vitamin B_6 and 4-PA Tryptophan metabolites	
"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		NOC users: 1.5 mg/day OC users: 1.5 to 5.0 mg/day, probably closer to 1.5 mg/day.	

Adapted from Donald, 1978.

sent the RDA for vitamin B_6 is 2.0 mg/day for adult women, including OC users (NRC, 1980).

The average woman in the U.S. may only be consuming between 1.17 and 1.6 mg/day of vitamin B_6 (Donald et al., 1971; Driskell et al., 1976; Miller et al., 1978). In a study by Kirksey et al. (1978) female adolescents were ingesting 1.24 mg of vitamin B_6 per day or about 79% of the RDA for the 11-14 age group. Chrisley et al. (1979) analyzing dietary vitamin B_6 in 83 women, reported that less than 100% of the RDA was consumed by virtually all subjects. The average vitamin B_6 intake in this study was about 70% of the RDA.

Without a doubt, oral contraceptive use results in changes in many of the nutritional aspects studied to date. However, vitamin B_6 intake, an important variable, has largely been neglected. It therefore seemed appropriate to assess the intakes of vitamin B_6 in OC users and controls, and to determine what, if any,relationship this intake would have to the blood vitamin B_6 levels in these women.

METHODS AND MATERIALS

The Subjects

The subjects consisted of 51 women (49 caucasian and 2 oriental) most of whom were students at Oregon State University who freely consented to participate in this study. All were between the ages of 18 and 26. Each subject was in apparent good health as judged by the health questionnaire, hematocrit and the subject herself. Twenty-six were OC users, and had been taking a combination type OC for longer than five months (See Table 3 for the types of OC used). The 25 NOC users had not taken any estrogen-progestin hormones for at least five months. Blood was drawn during the second half of the menstrual cycle for the NOC users, and after day seven of the pill pack for the OC users in an attempt to narrow the fluctuating hormone levels in these subjects. None of the subjects was taking vitamin B_6 supplements within two weeks of the study. Those who declared medical or metabolic problems were eliminated, as were those receiving medication that might have intereferred with the growth of the assay microorganism or the availability of vitamin B_6 to it. The number of subjects rejected was 20. Vitamin B_6 supplements were being taken by 15, one had exercised heavily before the blood drawing, one plasma assay was not obtained, one had congenital anemia, one did not yield any blood and one subject was taking antibiotics (Ampicillin).

Procedure

Each subject was given verbal instructions, using examples and food models, on how to keep a 3-day diet record. The diet record forms also contained examples and instructions for recording all foods eaten (see appendix for the diet record forms). A 3-day diet has been found to be a reliable index for intake of nutrients when it is used to assess a group (Whiting, 1960; Young et al., 1953) The subjects

Table 3. Oral Contraceptive user data.

Subject No.	O.C. Brand Name	Dose Score[a]	Duration/Mos.	Composition of O.C. Estrogen	Progestin
301	Ovral	3	5	50 ug ethinyl estradiol	0.5 mg norgestrel
302	Lo/Ovral	1	16	30 ug ethinyl estradiol	0.3 mg norgestrel
303	Ortho-Novum 1/50	3	7	50 ug mestranol	1.0 mg norethindrone
305	Ortho-Novum 1/50	3	36	50 ug mestranol	1.0 mg norethindrone
306	Norinyl 1 + 50	3	12	50 ug mestranol	1.0 mg norethindrone
307	Lo/Ovral	1	12	30 ug ethinyl estradiol	0.3 mg norgestrel
308	Norlestrin 2.5	3	19	50 ug ethinyl estradiol	2.5 mg norethindrone acetate
309	Modicon	2	18	35 ug ethinyl estradiol	0.5 mg norethidrone
310	Lo/Ovral	1	16	30 ug ethinyl estradiol	0.3 mg norgestrel
311	Norinyl 1 + 50	3	28	50 ug mestranol	1.0 mg norethindrone
312	Ovral	3	9	50 ug ethinyl estradiol	0.5 mg norgestrel
313	Lo/Ovral	1	20	30 ug ethinyl estradiol	0.3 mg norgestrel
314	Ovral	3	23	50 ug ethinyl estradiol	0.5 mg norgestrel
317	Lo/Ovral	1	9	30 ug ethinyl estradiol	0.3 mg norgestrel
318	Norinyl 1 + 50	3	14	50 ug mestranol	1.0 mg norethindrone
319	Modicon	2	8	35 ug ethinyl estradiol	0.5 mg norethindrone
320	Lo/Ovral	1	15	30 ug ethinyl estradiol	0.3 mg norethindrone
322	Lo/Ovral	1	22	30 ug ethinyl estradiol	0.3 mg norethindrone
324	Demulen	3	10	50 ug ethinyl estradiol	1.0 mg ethynodiol diacetate
325	Demulen	3	36	50 ug ethinyl estradiol	1.0 mg ethynodiol diacetate
326	Demulen	3	34	50 ug ethinyl estradiol	1.0 mg ethynodiol diacetate
327	Norlestrin	3	38	50 ug ethinyl estradiol	1.0 mg norethindrone acetate
328	Ortho-Novum 1/50	3	48	50 ug mestranol	1.0 mg norethindrone
330	Brevicon	2	24	35 ug ethinyl estradiol	0.5 mg norethindrone
334	Norinyl 1 + 50	3	18	50 ug mestranol	1.0 mg norethindrone
335	Lo/Ovral	1	11	30 ug ethinyl estradiol	0.3 mg norgestrel
Mean		2.3	20		
± SD			11		

[a]See appendix for scoring procedure

were instructed to record all food and drink consumed during a 72 hour period. A diet history form designed to estimate each subject's average vitamin B_6 intake per week was included in the diet record and questionnaire forms. This question, No. 18, made up the vitamin B_6 foods/week score and will be discussed later.

The health questionnaire was completed by each subject. This instrument was developed with assistance from the O.S.U. Survey Research Center (see the appendix for the questionnaire). A general health score was computed from the survey as an index of health status. Several questions from this questionnaire were also used in conjunction with question No. 18 to determine a general vitamin B_6 score (see the appendix for all questionnaire scoring procedures). The questionnaire and its scoring were designed to evaluate and compare OC users to NOC users rather than evaluate any one individual per se. The scores given for each possible answer were arranged so that the higher the score the better the health, or vitamin B_6, status. The questions were not weighted in a particular way to emphasize any one parameter, but were essentially scored equally. The remaining questions on the survey were used to screen subjects for possible problems, vitamin B_6 supplementation, type of OC and length of time on OC therapy. Each questionnaire and 3-day food record was cross-checked with the subject at the time it was received by us. The subjects were also asked if their 3-day diet was representative of their usual dietary pattern (Plough and Bridgforth 1960). All subjects responded that these three days were fairly representative of their normal diets.

Fasting blood samples were drawn on the morning of the day following the 3-day diet record. Prior to any strenuous exercise, 20 ml of blood were drawn from the left antecubital vein using heparinized vacutainer tubes. All samples were protected from light and refrigerated immediately. Hematocrit was determined in duplicate on the fresh blood using the microhematocrit method. Hematocrit was used as an index of health status for the two groups of subjects, and also to calculate the vitamin B_6 levels in the RBC from the plasma and whole blood vitamin B_6 values. The formula used for calculating RBC vitamin B_6 was:

$$\text{RBC vitamin } B_6 \text{ (ng/ml whole blood)} = \frac{\text{Whole blood vitamin } B_6 - \text{Plasma vitamin } B_6(1\text{-Hct.})}{\text{Hct.}}$$

Microbiological Assay of Whole Blood Vitamin B_6

Microbiological assay as detailed by Storvick et al. (1964) was adapted for use with whole blood. Saccharomyces uvarum 4228; ATCC No. 9080 was used to determine total vitamin B_6 values. S. uvarum responds to PL, PN and PM. Yeast cultures were grown on lactobacilli agar and transferred every two weeks (at least) to retain vigor (see the appendix for culture preparation). The inherent error for any microbiological assay is 10-15%, and replicates which do not differ by more than this are considered identical (Freed, 1966).

Since vitamin B_6 is not stable to light, samples and assays were protected at all times. Blood samples were run in duplicate. The blood was gently mixed by hand, and two ml were pipetted slowly while stirring into ten ml of ten percent trichloroacetic acid (TCA) in centrifuge tubes. Round bottom tubes were found to be the most suitable. The whole blood was immediately coagulated by this procedure. The samples were then placed in the dark, at room temperature, for 30 minutes and stirred three times during this period. Next the tubes were centrifuged, at room temperature, for five minutes at 5,000 rpm. The supernatant was decanted into 50 ml glass beakers. Five ml of 10% TCA were added to the precipitate which was then resuspended by stirring. The samples were centrifuged as above and the supernatant collected. Again five ml of the TCA were added, stirred well and centrifuged again. The supernatant was collected and the precipitate discarded. It should be noted that the frozen samples used for the interassay control values were noticeably easier to work with, at this stage of the assay, than was the fresh blood. The accumulated supernatant for each sample was covered with a watch glass and autoclaved for 30 minutes at 15 psi. This step hydrolyzed all the phosphorylated forms of vitamin B_6 to the free forms (Wozenski, 1980). Most of the TCA was driven off during this treatment. When cool, the pH of each sample was adjusted to 4.5

with potassium hydroxide. The samples were transferred quantitatively to a glass-stoppered cylinder and diluted with redistilled water to a total volume of 40 ml (1:20 dilution). The cylinder was gently shaken and the samples transferred to brown plastic bottles. These were refrigerated overnight.

In the morning, a standard curve using PL·HCl was prepared along with the samples. The standard solution was prepared from stock PL·HCl (10 μg/ml) which was diluted to give a concentration of 1.0 ng/ml of PL. Standards were run in triplicate at concentrations of 0.5, 1, 2, 3, 4 and 5 ng/ml PL. The samples were prepared in volumes of 1, 2, 3, 4 and 5 ml sample/tube. Lipped Evelyn tubes were used. Standards and samples were covered and autoclaved for ten minutes at 15 psi. Double strength basal medium (see the appendix for reagents and preparation) was steamed for ten minutes and cooled. Five ml of this basal medium were added aseptically to each tube. Two negative blanks were prepared as above with five ml of redistilled H_2O and five ml of double strength basal medium, and two positive blanks which contained, in addition, one drop each of the yeast innoculum. All sample and standard tubes were aseptically innoculated with one drop (0.01 ml) of prepared yeast innoculum (see the appendix for yeast preparation).

All tubes were covered with a sterile cloth and incubated with constant shaking for 22 hours at 30° C. Immediately after incubation all tubes were steamed for five minutes to arrest yeast growth. When cool, the tubes were mixed on a vortex mixer and read in the Evelyn colorimeter. Transmittance was set at 100% with the negative blank; transmittance was then read and reset at 100% using the positive blank. All standard and sample tubes were read against the positive blank, and percent transmittance was recorded. The readings from the standard tubes were used to prepare a standard curve. These data were also fed into a Hewlett-Packard programable calculator to determine nanogram values on the standard curve. The program, interpolation of unequally spaced data, was supplied by Dr. J. E. Leklem. Nanogram values were divided by the volume of sample per tube and multiplied by the dilution factor to give ng vitamin B_6/ml of whole blood.

An inter-assay control, using a single pooled blood sample frozen in aliquots, was run with each assay. This control sample averaged 9.2 ± 1.1 ng/ml, with a coefficient of variability (CV) of 11.5. Recoveries were also run as a check on assay technique. Standard PL (10.0 ng) was added for each ml of whole blood. This was done to randomly selected duplicates. The PL was added to the TCA simultaneously with the whole blood. The recoveries averaged 9.9 ± 1.1 ng/ml, with a CV of 10.9. As a further check on the interassay variability, standard curves were compared for each assay throughout the study. All standard curves obtained were virtually the same (see appendix for standard curve values).

Three-day Diet Records

The 3-day diet records were coded using Handbook No. 8 (Watt and Merrill, 1975), the A.H.E.A. Handbook of Food Preparation and the Ohio State Diet Survey (Schaum et al., 1973). The subjects were asked to include lists of ingredients or recipes for food combinations; most subjects complied. For food combinations where ingredients were not listed, the recipe was assumed to be typical for that combination, and a standard cookbook was consulted (Charley, 1971.; Betty Crocker's Cookbook, 1969). Additional assumptions included foods that were not fully named such as "milk" or "bread." These foods were assumed to be the most commonly consumed type for that food. For example "milk" was coded as whole milk and "bread" as white enriched bread. To determine what foods are most commonly consumed, food consumption surveys for the U.S. were consulted (Kinder, 1979). Substitutions were made for foods which have no vitamin B_6 value listed on the Ohio State Diet Survey Computer tape. In these cases, the food closest in composition for which there was a vitamin B_6 value listed was substituted. Examples of substitutions made are: 2% milk was changed to whole milk, jack cheese was coded as brick, red snapper was replaced with cod. These substitutions were made using Handbook No. 8 and Orr, (1969). If there was a value listed for a cooked item, but not raw, the cooked value was

coded taking into consideration the changes in weight or volume. In general, substitutions were made to the next closest state of preparation for that same food item. In cases where a good substitute for a food combination was not available, a standard recipe was used to code the basic ingredients singly. If the "amount" column was not filled in on the diet record, the amount eaten was assumed to be a standard serving for that food item (Hand. Food Prep., 1975; Kinder, 1979). This occurred in fewer than four percent of the diet records.

The diet records were randomly divided into two groups, one group was coded by Lind (1980) and the other by myself. We then exchanged these groups to do the final coding and hand calculations of vitamin B_6 and protein. This served as a check on each other's accuracy and overall consistancy. The vitamin B_6, in micrograms, and protein, in grams, were calculated for each subject, manually, on a per day basis; and as a 3-day average intake. These were used to check the computer values for vitamin B_6 and protein for each subject. Hand calculations were nearly identical to the computer values; no value differed by more than five percent. The vitamin B_6/protein ratio was then calc manually from the computer values.

These data were keypunched directly from the calculation sheets to eliminate errors in transcribing the data to standard keypunch forms. The following dietary data was then generated: 3-day average intake for each nutrient listed on the Ohio State Diet Survey tape, vitamin B_6/protein ratio based on the 3-day averages and the percent of the RDA ingested, as a 3-day average, for those nutrients having an RDA.

Statistical Analysis

All data from the questionnaire and 3-day diet records were keypunched and verified by the O.S.U. Computer Center. Programming was arranged through a grant from the Computer Center and through the services of the O.S.U. Statistics Department. As mentioned previously, the dietary data were generated by the Ohio State Diet Survey Computer

Program. The statistical analyses were performed via the S.I.P.S. (Statistical Interactive Programing System) computer banks.

Descriptive statistics were generated from the questionnaire, dietary data and blood values for the OC and NOC users. Student's t-test (McClave and Dietrich, 1979) was used to evaluate differences between the two subject groups. Regression analysis was run (using S.I.P.S.) on all variables within each group. Pearson's Coefficient of Correlation (McClave and Dietrich, 1979) was used to determine if linear relationships existed between any of the variables.

RESULTS

Demographic Data

The descriptive data for the two groups of subjects are given in Tables 4 and 5. The OC and NOC groups were similar in age, height, weight, body mass, hematocrit and general health score (GHS). The hematocrit and GHS were used to assess the health status of the two groups. Mean hematocrit values were 40.5 $\pm$ 2.7% for OC and 40.2 $\pm$ 1.6% for NOC. The normal range for women in this age group is 37.0 - 47.0%. Most subjects were within this range. Four subjects were below 37% (3 OC and 1 NOC) and one subject was above 47% (1 OC). These five subjects were close enough to the normal range to be included. The mean GHS was 20.1 $\pm$ 1.8 for OC and 21.0 $\pm$ 2.0 for the NOC. The maximum possible score was 25 with a score of 15 considered acceptable for this study. All subjects had scores greater than 15.

Results from the questionnaire are included in Tables 4 and 5. The mean vitamin B_6 general score was 15.8 $\pm$ 1.3 for OC and 16.4 $\pm$ 0.8 for NOC. The mean vitamin B_6 foods/week score was 4.0 $\pm$ 0.6 and 4.1 $\pm$ 0.6, OC and NOC, respectively. Neither of these scores was statistically different between OC and NOC subjects. Alcohol intake (Question No. 5) was significantly different between the two groups ($P \leq 0.05$). The mean score was 3.9 $\pm$ 0.7 for OC and 4.4 $\pm$ 0.6 for NOC users. These scores indicate that the OC users were generally consuming more alcohol than the NOC group. Question No. 16, the exercise score, means were 2.5 $\pm$ 1.0 for OC and NOC: 2.3 $\pm$ 1.4. This difference was not significant.

Specific OC user data is given in Table 3. The average length of time on OC therapy was 20 $\pm$ 11 months. The range was five to 48 months. There was no correlation between the length of time on OC agents and vitamin B_6 levels in the blood, nor between estrogen dose level and blood vitamin B_6. Blood vitamin B_6 results will follow.

Table 4. Descriptive Statistics for OC Users.

Subject No.	Age Yrs.	Height cm.	Weight kg.	Body Mass kg/m^2	Hematocrit Percent	Vitamin B_6 general[a]	B_6/week score[a]	Alcohol score[a]	Exercise score[a]	General Health[a]
301	19	158	49.1	19.8	42.5	16	3.7	4	4	23
302	21	163	65.9	25.0	42.5	16	4.2	4	3	19
303	20	163	63.6	24.1	43.5	16	4.1	4	3	21
305	22	153	51.4	22.1	38.0	17	4.8	4	1	18
306	25	160	54.5	21.3	41.5	16	4.4	4	1	18
307	23	170	60.9	21.2	38.5	15	3.3	4	4	20
308	19	163	59.1	22.4	39.0	18	4.6	5	3	23
309	19	170	63.9	22.0	36.5	15	3.9	3	3	20
310	20	165	51.4	18.9	48.5	16	4.3	4	2	19
311	19	163	55.0	20.8	42.5	16	3.7	4	3	19
312	21	178	75.9	24.1	41.0	14	3.9	3	4	22
313	21	163	50.9	19.3	42.0	13	3.5	3	3	16
314	23	163	57.7	21.9	44.0	16	3.1	5	1	20
317	21	160	54.5	21.3	39.0	15	2.9	4	3	22
318	25	163	56.8	21.5	40.0	18	4.6	5	3	22
319	19	168	59.1	21.1	42.5	16	4.0	4	4	23
320	21	155	58.2	24.2	40.0	15	4.6	2	3	20
322	24	155	52.3	21.8	41.0	17	4.6	4	2	20
324	23	165	55.9	20.5	37.5	17	4.7	4	2	20
325	22	175	65.9	21.5	36.0	15	4.1	3	2	19
326	22	155	55.0	22.9	38.5	16	3.8	4	3	21
327	21	160	58.0	23.1	40.5	14	3.3	3	1	18
328	20	155	49.1	20.4	36.5	18	4.6	5	3	21
330	21	173	63.6	21.4	40.0	17	4.8	4	3	21
334	18	160	54.5	21.3	39.0	15	3.4	4	1	19
335	22	158	54.5	22.0	41.5	15	4.3	4	1	18
Mean	21	163	57.6	21.8	40.5	15.8	4.0	3.9	2.5	20.1
± SD	1.9	6.5	6.2	1.5	2.7	1.3	.6	.7	1.0	1.8

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

Table 5. Descriptive Statistics for NOC Users.

Subject No.	Age Yrs.	Height cm.	Weight kg.	Body Mass kg/m^2	Hematocrit Percent	Vitamin B_6 general[a]	B_6/week score[a]	Alcohol score[a]	Exercise score[a]	General Health[a]
102	24	170	75.9	26.3	40.5	17	5.2	4	3	20
103	25	165	65.9	24.2	41.5	15	4.4	4	2	21
106	24	168	61.4	21.9	40.5	17	4.7	4	1	17
107	21	155	54.5	22.7	41.0	17	3.8	5	3	23
108	18	163	61.8	23.4	40.0	16	4.0	4	4	22
109	24	173	56.8	19.1	39.5	17	4.7	4	4	20
111	20	165	63.6	23.4	39.5	17	4.1	5	1	21
112	25	160	54.5	21.3	40.5	15	3.4	5	2	22
115	19	170	66.4	23.0	41.0	18	5.4	5	4	24
116	24	148	48.2	22.2	43.5	16	4.2	4	2	21
117	21	173	62.3	20.9	41.5	17	3.9	5	4	24
121	21	163	64.5	24.4	41.0	16	4.4	4	1	20
122	23	175	69.1	22.6	39.5	16	3.9	4	5	24
123	22	160	56.8	22.2	40.0	17	3.6	5	1	21
124	19	160	59.1	23.1	36.0	17	4.7	4	3	22
125	25	163	61.4	23.2	38.5	17	3.7	5	1	21
126	21	170	65.9	22.8	37.5	16	3.8	4	1	20
127	21	158	55.5	22.4	39.5	15	3.9	4	2	20
131	24	158	49.1	19.8	42.5	16	3.1	5	1	21
133	21	168	72.7	25.9	41.0	15	3.7	3	2	17
134	26	173	60.0	17.3	40.5	17	4.2	5	5	25
135	25	168	48.6	20.0	40.0	16	3.1	5	1	19
136	20	165	58.5	20.1	42.5	17	4.7	4	1	20
137	20	160	51.4	21.4	39.0	16	3.6	4	1	20
138	20	173	63.6	20.2	39.0	16	3.9	4	2	21
Mean	22	165	60.1	22.1	40.2	16.4	4.1	4.4	2.3	21
± SD	2.3	6.8	7.2	2.1	1.6	1.3	.6	.6	1.4	2.0

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

Dietary Intake

The data for dietary intake are included in Tables 6 and 7. The 3-day mean vitamin B_6 intake was 1.4 $\pm$ 0.5 mg/day for OC and 1.6 $\pm$ 0.5 mg/day for NOC (difference not significant). The mean protein intake was 72.6 $\pm$ 19.4 g/day for OC and 66.9 $\pm$ 13.6 g/day for NOC this difference was also not significant. The vitamin B_6/protein ratio, computed from the average B_6 intake (in mg) divided by the average protein intake (in g) was 0.020 $\pm$ 0.004 and 0.025 $\pm$ 0.01 for the OC and NOC groups. This difference in ratios is significant ($P \leq 0.05$). The vitamin B_6/protein ratio, vitamin B_6 and protein intakes are summarized in Figure 4.

Actual alcohol consumption as indicated by the 3-day diet record averaged 5.5 $\pm$ 6.5 g/day for OC and 3.5 $\pm$ 7.4 g/day for NOC subjects. This difference was not statistically significant, but does support the higher alcohol consumption reported by OC on the questionnaire.

The percent of the RDA consumed by the subjects is shown for 11 nutrients in Tables 8 and 9. There were no significant differences in percent RDA between the two groups except for vitamin C ($P \leq 0.05$) and vitamin A ($P \leq 0.05$). The OC group had a lower intake of both vitamin C and A than the NOC users, but both groups consumed over 100% of the RDA for both vitamins. One subject (#107) consumed 1115% of the RDA for vitamin A. If this woman is deleted from the calculation of the mean and SD for vitamin A the significance between OC and NOC is increased ($P \leq 0.02$). The average intake exceeded the RDA for all nutrients except calories, iron and vitamin B_6. These three nutrient intakes were below the RDA in both OC and NOC users. The mean calorie intake, as a percentage of the RDA, was 94 $\pm$ 25% for OC and 86 $\pm$ 23% for controls. Average iron levels, in percent of the RDA, were found to be 71 $\pm$ 23% for OC and 67 $\pm$ 20% for NOC. Vitamin B_6 intakes averaged 71 $\pm$ 24% and 78 $\pm$ 24% for OC and NOC users, respectively. Almost all subjects recorded diets containing more than 70% of the RDA for the remaining eight nutrients.

Table 6. Selected diet intake and blood vitamin B_6 levels for OC users.

Subject No.	B_6 Intake[a] mg/day	Protein[a] g/day	B_6/Protein mg/g	Alcohol[a] g/day	Plasma B_6[b] ng/ml	Whole Blood B_6[c] ng/ml	RBC B_6[d] ng/ml	Log RBC B_6	Plasma B_6 / RBC B_6
301	1.1	60.4	.018	3.3	8.6	11.6	15.6	1.19	0.55
302	1.4	103.5	.014	14.9	7.8	9.3	11.3	1.05	0.69
303	1.6	96.6	.016	14.6	14.8	20.3	27.4	1.44	0.54
305	1.7	81.0	.021	7.9	6.3	12.5	21.8	1.34	0.29
306	1.0	58.8	.017	7.9	7.5	10.3	13.8	1.14	0.54
307	0.8	47.3	.018	3.3	7.6	7.0	6.1	0.79	1.24
308	1.2	85.2	.014	4.3	6.6	6.7	5.6	0.75	1.18
309	1.3	77.7	.017	0.0	8.4	9.8	12.2	1.09	0.69
310	1.4	58.2	.024	0.0	6.3	6.7	7.1	0.85	0.89
311	1.8	88.6	.020	8.6	5.1	9.1	14.5	1.16	0.35
312	2.4	102.1	.024	18.8	6.0	7.6	9.9	1.00	0.61
313	1.0	42.5	.025	13.6	4.2	5.9	8.2	0.91	0.51
314	0.6	42.9	.015	0.0	6.5	7.0	7.6	0.88	0.86
317	1.0	44.9	.022	0.0	6.9	9.6	13.8	1.14	0.50
318	1.3	59.4	.022	0.0	9.2	12.8	18.2	1.26	0.51
319	1.5	76.9	.019	0.0	6.7	9.5	13.3	1.12	0.50
320	1.8	65.5	.027	13.7	4.3	6.1	8.8	0.95	0.49
322	0.9	51.1	.018	15.8	5.1	6.4	8.3	0.92	0.61
324	2.1	91.2	.023	13.0	5.7	10.4	18.5	1.27	0.31
325	1.1	74.2	.015	0.0	6.2	8.3	12.0	1.08	0.52
326	1.0	77.6	.013	0.0	6.0	6.5	7.3	0.86	0.82
327	1.5	90.9	.017	0.0	13.8	12.6	10.8	1.03	1.28
328	1.2	71.0	.018	0.0	8.0	8.2	8.6	0.94	0.94
330	2.4	107.2	.023	0.0	4.8	5.6	6.8	0.83	0.71
334	1.6	59.3	.026	2.1	9.0	11.8	12.2	1.09	0.56
335	2.1	74.6	.028	0.0	9.8	13.2	18.0	1.26	0.55
Mean	1.4	72.6	.020	5.5	7.4	9.3	12.3	1.05	0.66
± SD	0.5	19.4	.004	6.5	2.5	3.2	5.3	0.18	0.27

[a]From Schaum et al., 1973.
[b]Mary Beth Lind, 1980.
[c]Microbiological assay (S. uvarum).
[d]Calculation described in appendix.

Table 7. Selected diet intake and blood vitamin B_6 levels for NOC users.

Subject No.	B_6 Intake[a] mg/day	Protein[a] g/day	B_6/Protein mg/day	Alcohol[a] g/day	Plasma B_6[b] ng/ml	Whole Blood B_6[c] ng/ml	RBC B_6[d] ng/ml	Log RBC B_6	Plasma B_6 / RBC B_6
102	2.3	44.1	.053	2.9	8.8	15.2	24.6	1.39	0.36
103	2.1	68.9	.031	0.5	14.8	18.0	22.5	1.35	0.66
106	1.9	68.1	.022	31.7	11.8	19.8	31.5	1.50	0.37
107	2.2	40.4	.054	0.0	7.5	9.3	12.9	1.11	0.58
108	1.3	74.3	.018	17.3	6.4	8.6	11.9	1.08	0.54
109	2.1	72.8	.028	0.0	5.7	7.3	9.7	0.97	0.58
111	1.3	94.7	.014	0.0	8.3	11.7	16.9	1.23	0.49
112	1.2	83.8	.014	0.0	11.0	14.3	19.1	1.28	0.58
115	1.8	68.4	.027	0.0	8.9	13.8	20.9	1.32	0.42
116	1.4	74.2	.019	3.6	12.6	9.6	5.8	0.76	2.20
117	1.4	69.7	.020	0.0	24.5	24.4	24.0	1.38	1.10
121	1.0	51.9	.020	0.0	8.9	10.0	5.8	0.76	0.77
122	1.3	74.5	.017	13.0	12.1	16.6	23.5	1.37	0.51
123	1.5	92.8	.016	0.0	9.7	8.1	5.7	0.76	1.71
124	1.6	70.6	.023	0.0	4.7	5.2	6.1	0.79	0.77
125	1.5	64.2	.023	0.0	8.7	8.2	7.4	0.87	1.22
126	0.9	50.8	.018	0.0	8.4	7.8	6.8	0.83	1.20
127	1.4	67.8	.020	9.2	10.5	10.3	9.8	0.99	1.10
131	1.2	51.3	.024	0.0	11.6	8.9	5.3	0.72	0.30
133	1.4	57.1	.024	0.0	7.3	12.2	19.3	1.29	0.39
134	1.3	55.5	.023	0.0	11.6	18.4	29.3	1.47	0.38
135	1.2	57.7	.021	0.0	8.8	14.8	23.8	1.38	0.37
136	1.0	70.4	.014	2.9	16.6	21.9	29.1	1.46	0.57
137	1.7	79.8	.021	0.0	4.8	9.0	15.6	1.19	0.31
138	2.9	68.3	.043	5.8	8.0	15.5	27.2	1.44	0.29
Mean	1.6	66.9	.025	3.5	10.1	12.9	16.8	1.14	0.71
± SD	0.5	13.6	.010	7.4	4.2	5.1	8.5	0.27	0.47

[a]From Schaum et al., 1973.
[b]Mary Beth Lind, 1980.
[c]Microbiological assay (S. uvarum).
[d]Calculation described in appendix.

Table 8. Nutrient intake of OC users as percent RDA (NRC, 1974).

Subject No.	Energy	Thiamin	Protein	Phosphorus	Iron	Riboflavin	Vit. A	Calcium	Niacin	Vit. B_6	Vit. C
301	91	116	131	126	51	83	72	76	79	54	267
302	122	108	225	260	76	160	124	193	102	72	137
303	109	143	210	200	82	121	126	92	116	79	274
305	128	166	176	159	102	140	210	97	181	87	104
306	75	138	128	123	66	106	142	75	109	50	406
307	66	96	103	79	61	93	37	43	101	42	70
308	72	69	185	183	35	127	61	172	88	58	118
309	96	89	169	164	65	116	128	97	97	67	185
310	78	113	127	140	75	96	260	94	125	71	65
311	124	150	193	201	93	191	91	168	165	88	370
312	143	131	222	258	74	241	126	197	133	121	184
313	68	91	92	87	58	84	132	51	156	52	50
314	74	62	93	81	46	69	44	43	75	32	30
317	57	82	98	97	49	69	57	53	81	50	248
318	67	105	129	129	45	111	162	94	93	64	397
319	75	119	167	133	73	97	30	57	119	73	248
320	93	190	142	178	94	215	84	128	188	90	103
322	94	121	111	128	54	101	86	79	102	46	98
324	92	106	198	158	73	117	175	47	223	107	175
325	82	113	161	187	70	108	357	140	143	57	331
326	74	72	169	153	50	103	71	105	103	50	64
327	106	109	198	206	67	188	143	168	117	77	133
328	140	179	154	180	95	164	101	121	143	62	102
330	142	343	233	285	145	183	96	189	245	121	368
334	80	88	124	92	53	121	55	66	100	78	192
335	95	117	162	166	81	102	92	112	135	103	100
Mean	94	124	158	160	71	127	118	106	128	71	185
± SD	25	55	42	55	23	45	73	49	43	24	116

Table 9. Nutrient intake of NOC users as percent RDA (NRC, 1974).

Subject No.	Energy	Thiamin	Protein	Phosphorus	Iron	Riboflavin	Vit. A	Calcium	Niacin	Vit. B_6	Vit. C
102	75	148	96	115	59	90	382	70	106	117	512
103	61	122	150	138	54	137	81	87	209	107	321
106	137	151	148	144	70	146	64	70	192	94	360
107	58	129	88	113	75	90	1115	85	82	108	317
108	117	132	155	122	67	150	73	77	89	66	259
109	78	123	158	161	75	117	697	121	133	104	622
111	151	106	206	253	67	120	125	144	172	65	191
112	77	184	182	163	71	163	108	99	102	59	308
115	70	180	149	180	99	145	175	77	137	92	403
116	84	128	161	172	58	126	296	159	111	71	580
117	79	80	152	142	53	100	108	89	106	71	342
121	66	98	113	169	53	159	381	190	94	52	98
122	113	188	162	150	77	173	82	83	134	64	171
123	91	127	202	200	73	166	97	160	120	76	227
124	83	127	154	185	85	118	297	94	145	80	266
125	82	130	140	158	70	153	187	123	105	73	314
126	73	86	111	105	47	66	36	51	114	47	333
127	71	81	148	152	45	100	204	95	126	70	144
131	74	103	112	106	42	107	59	70	106	61	315
133	84	89	124	131	61	97	98	72	111	69	262
134	94	119	121	134	72	99	308	110	90	63	219
135	53	101	125	101	59	117	171	65	95	60	235
136	82	78	153	182	40	104	150	113	93	50	79
137	93	145	174	173	70	127	317	110	159	84	150
Mean	86	127	145	153	67	126	233	102	127	78	295
± SD	23	37	29	35	20	31	235	34	40	24	134

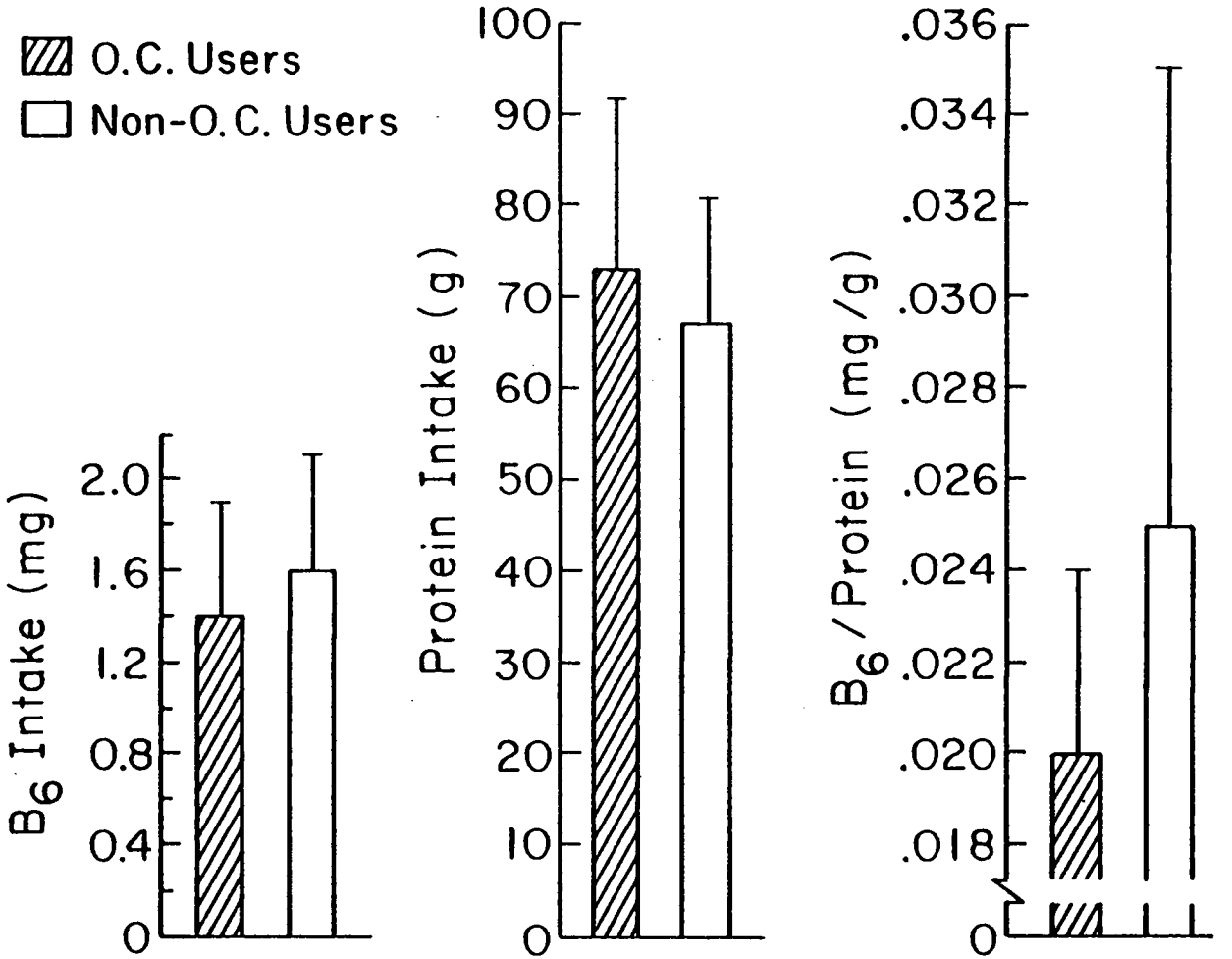

Figure 4. Summary of dietary intakes.

Daily variation of vitamin B_6 intake was calculated manually for each subject by subtracting the lowest daily vitamin B_6 intake from the highest. These differences in vitamin B_6 intake between days are shown in Figure 5. Approximately 68% of the OC users and 78% of the non-users had more than a 0.5 mg/day daily variation in vitamin B_6 intake. This variation exceeded the 1.4 mg/day mean intake for OC users in nearly 20% of these subjects. More than 20% of the NOC users reported intake values above the 1.6 mg/day mean for this group.

Blood Vitamin B_6 Levels

The values for blood vitamin B_6 are given in Tables 6 and 7. Average vitamin B_6 levels in the red cell differed by 4.4 ng/ml between the two subject groups. OC red cell levels averaged 12.4 $\pm$ 5.4 ng/ml blood, while the mean for NOC was 16.8 $\pm$ 8.5 ng/ml blood. This difference was significant at $P \leq 0.05$. The mean whole blood vitamin B_6 levels were also significantly different ($P \leq 0.05$), averaging 9.3 $\pm$ 3.2 ng/ml blood for OC and 12.9 $\pm$ 5.1 ng/ml blood for NOC users. The mean plasma levels differed by 2.7 ng/ml between the two groups ($P \leq 0.05$). The OC group had a mean of 7.4 $\pm$ 2.5 ng/ml and NOC mean was 10.1 $\pm$ 4.2 ng/ml (see Lind, 1980, for a discussion of the plasma values). The ratio of plasma vitamin B_6 to RBC B_6 was also computed (see Tables 6 and 7). The OC users had a mean ratio of 0.66 $\pm$ 0.27 and NOC mean ratio was 0.71 $\pm$ 0.47. This difference was not statistically significant (see Figure 6 for the distribution). This distribution was skewed to the right with nearly 70% of the subjects in both groups clustered between 0.25 and 0.75.

Since the vitamin B_6 levels in the RBC were skewed, especially for OC users (see Figure 7), a log transformation was performed on the RBC vitamin B_6 values. The resulting histogram for these data is given in Figure 8. The values for the OC users were normalized by this transformation. The values for the control group appeared to be slightly bimodal both for the RBC vitamin B_6 levels and the log transformation. The difference between the log RBC vitamin B_6 values for the two groups

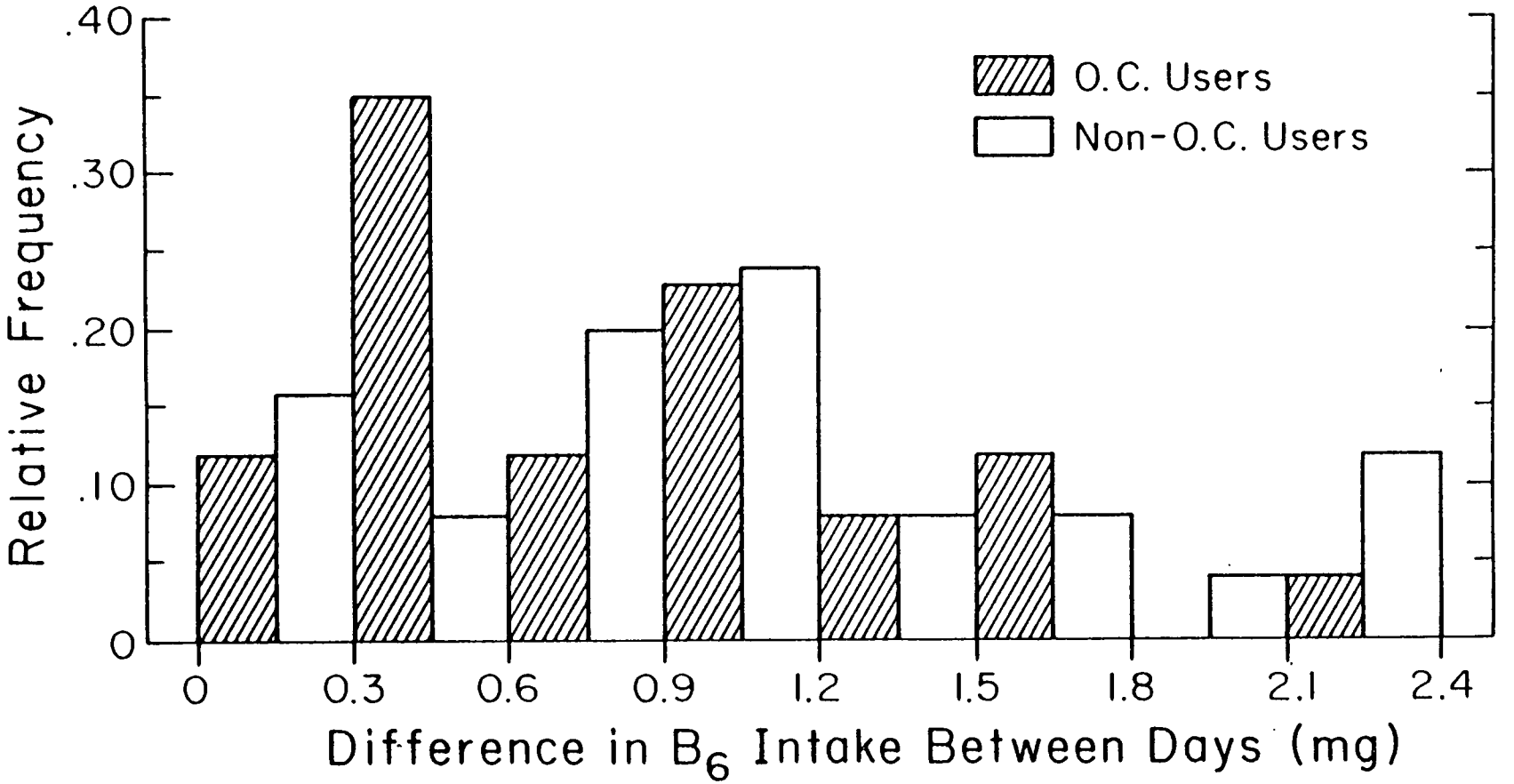

Figure 5. The difference in vitamin B_6 intake between days.

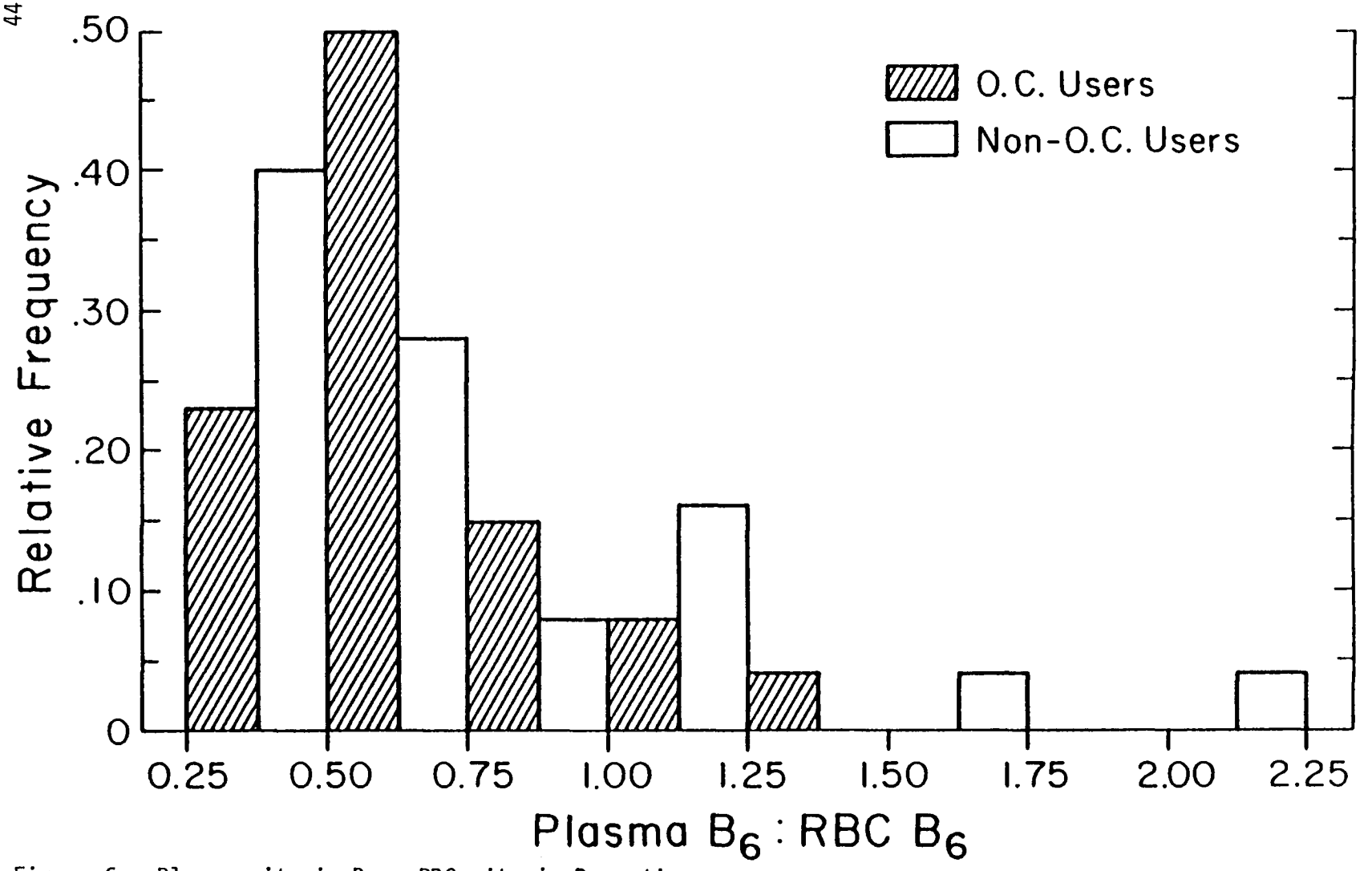

Figure 6. Plasma vitamin B_6 : RBC vitamin B_6 ratio.

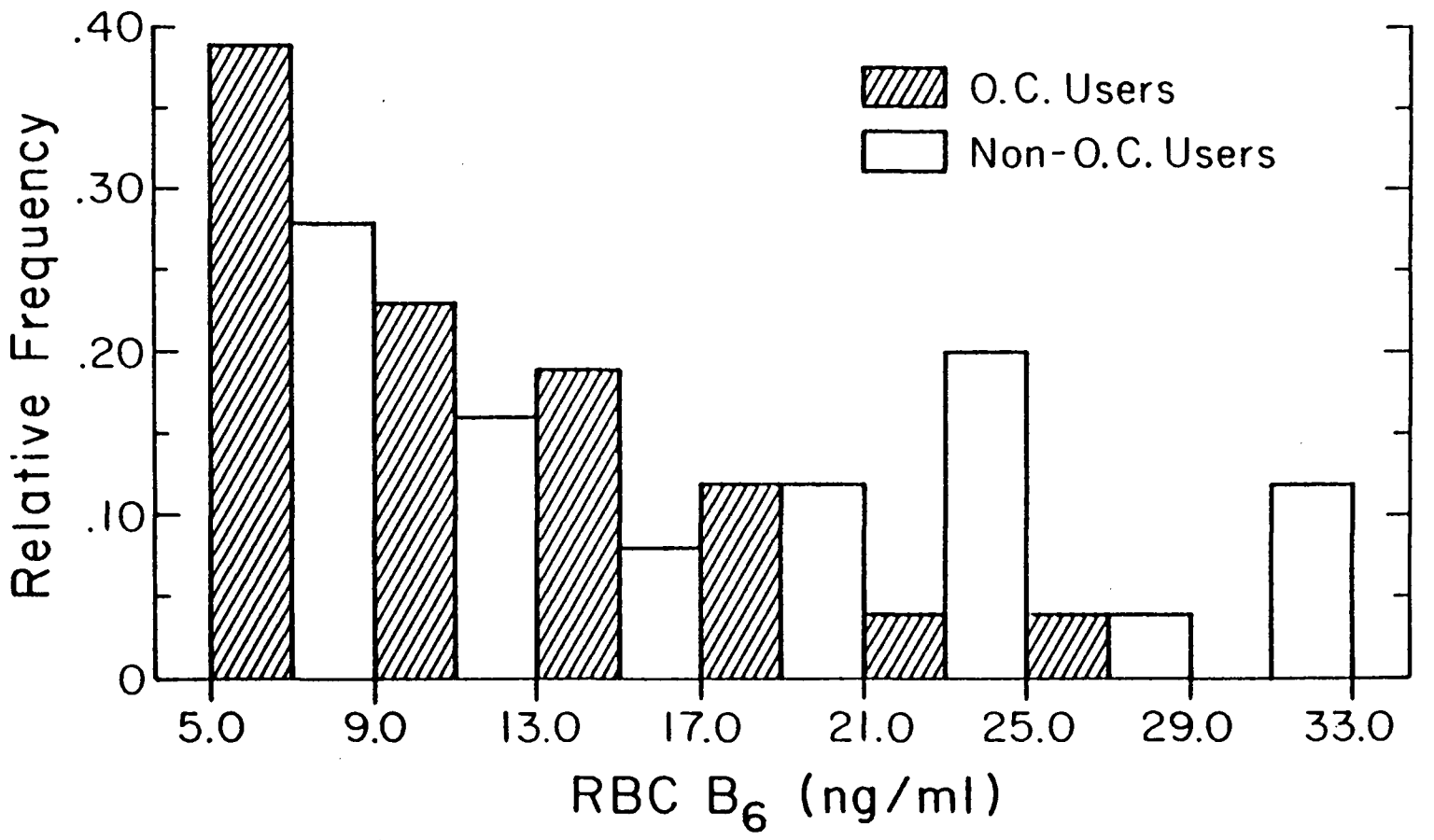

Figure 7. RBC vitamin B_6 levels.

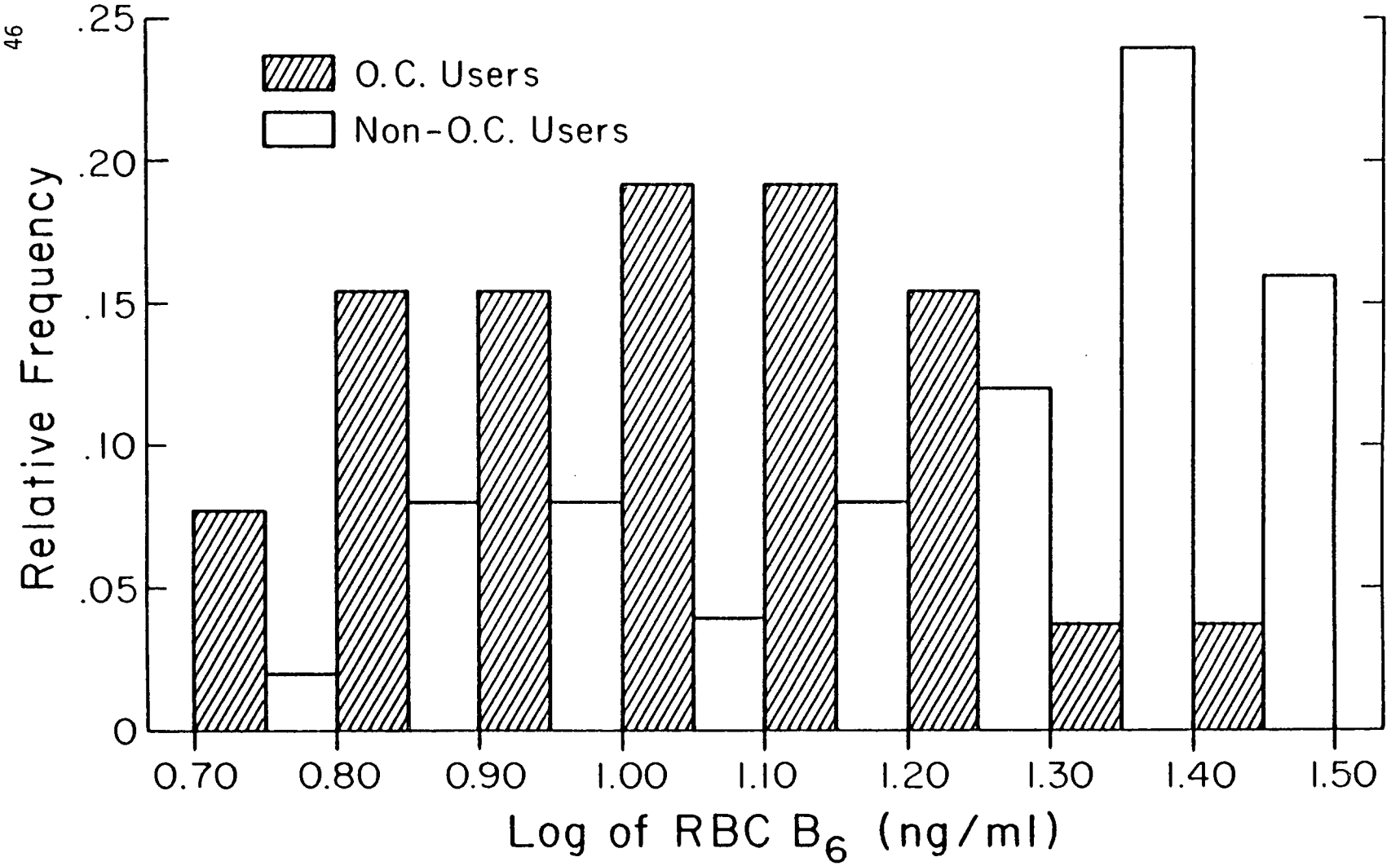

Figure 8. Log of RBC vitamin B_6.

was considerably less significant ($P \leq 0.15$) than for the RBC vitamin B_6 values. Histograms summarizing the plasma, RBC and log RBC vitamin B_6 levels plus the plasma vitamin B_6/RBC vitamin B_6 ratio are given in Figure 9.

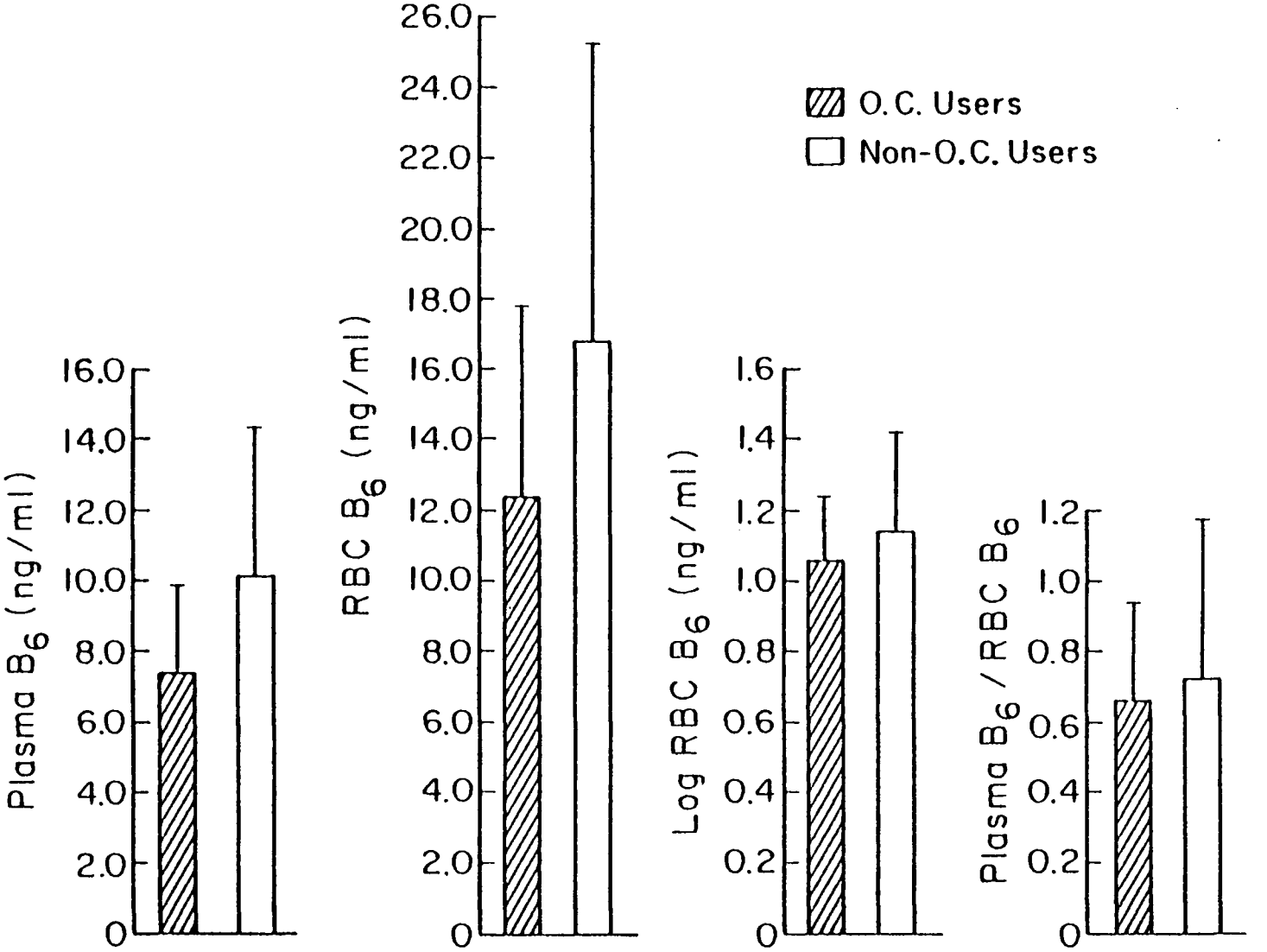

Figure 9. Summary of blood vitamin B_6 levels.

DISCUSSION

As described in the section on results, the OC and NOC subjects were very similar in all aspects as measured by the questionnaire, except alcohol consumption. Thus, for the purpose of this study they can be considered comparable subject groups. Although the OC users in this study had been on OC therapy for varying lengths of time, they were combined into a single group. Driskell et al. (1976) found no signficiant difference in data from subjects using OC agents for less than six months and those using the OC for longer than six months. The current results agree with these authors' findings.

The alcohol intake, as assessed by the questionnaire, was significantly higher for OC users. Actual alcohol intake from the 3-day diet record, although not significantly different, tended to support this. It is generally accepted that the process of alcohol oxidation can alter the metabolism of vitamin B_6 and contribute to lower B_6 tissue levels (Li, 1978). Acetaldehyde, the oxidation product, appears to accelerate the degradation of PLP (Lumeng, 1978). Parker et al. (1979) reported that, in dogs, low alcohol dose did not significantly alter plasma PLP levels. Although it is improbable that large changes in vitamin B_6 status would result from the levels of alcohol reported here, alcohol intake may be a possible factor in the vitamin B_6 status of the OC users.

Dietary Intake

The mean vitamin B_6 intake was 1.4 $\pm$ 0.5 mg/day for the OC users and 1.6 $\pm$ 0.5 mg/day for the controls. Using the level suggested by Donald et al. (1971) of 1.5 mg/day of vitamin B_6, 58% of the OC users and 56% of the NOC users consumed less than this amount of the vitamin. If the 2.0 mg/day vitamin B_6 intake, suggested by Shin and Linkswiler (1974), Brown et al. (1975) and NRC (1980), is used, the majority of subjects in both groups of this study appear to have marginal vitamin

B_6 intakes (see Tables 6 and 7). Driskell et al. (1976) also found that both OC and NOC subjects were consuming less than the RDA for vitamin B_6. These authors reported mean intakes, as percent of the RDA, of 62.9 ± 26.3% for OC and 61.8 ± 24.9% for NOC users. Chrisley and Driskell (1979) noted a mean intake of 69.0 ± 30.0% of the RDA for vitamin B_6 in young women. Although the RDA was not attained by these subjects, the authors of both studies concluded that their subjects were not deficient. The values reported in the present study, (71 ± 24% of the RDA for OC and 78 ± 24% for NOC) are slightly higher than the above studies found, but are still well below the RDA for vitamin B_6 for young women.

The percent of the RDA for protein (158 ± 42% for OC and 145 ± 29% for NOC) are similar to those reported by Driskell et al. (1976) of 158.7 ± 68.0% for OC and 165.5 ± 76.5% of the RDA for protein in controls. Chrisley and Driskell (1979) noted the mean intake of protein to be 167 ± 66% of the RDA for young women. In the current study, protein intake was slightly higher in the OC than the NOC users (OC intake of protein, 72.6 ± 19.4 g/day versus the NOC intake of 66.9 ± 13.6 g/day). As discussed previously, this higher protein intake would tend to raise the requirement of these subjects for vitamin B_6 (Donald, 1978). At the same time, the vitamin B_6 intake was slightly lower in the OC group. Neither of these differences in intakes was significant by itself. However, the vitamin B_6/protein ratio was significantly different ($P \leq 0.05$). This indicates that the OC group, with its higher protein intake, may have had an increased requirement for vitamin B_6, at the same time they were ingesting less of this vitamin. Excess protein consumed causes an increase in the catabolism of amino acids as assessed by the excretion of urinary vitamin B_6 and 4-PA, plasma PLP and blood vitamin B_6 (Baysal et al., 1966). These authors found decreased vitamin B_6 status in subjects consuming high protein diets. Donald (1978) has suggested that a vitamin B_6/protein ratio of 0.019 may be considered adequate in terms of vitamin B_6 status, while a ratio

of 0.017 is considered inadequate. Using the above guideline, the vitamin B_6/protein ratios reported here (0.020 for OC and 0.025 for NOC) are both adequate. Although both these ratios are adequate, they are still significantly different ($P \leq 0.05$).

Caloric intake was below the RDA for both groups, with 94 $\pm$ 25% of RDA reported by OC users and 86 $\pm$ 23% of the RDA for NOC subjects. Driskell et al. (1976) reported intakes of 85.0 $\pm$ 29.9% of the RDA for calories in OC users and 87.5 $\pm$ 33.4% for controls. Chrisley and Driskell (1979) found the mean percentage of RDA for calories ingested by young women to be 94 $\pm$ 31%. Thus, the calorie intakes for subjects in this study seem to agree fairly well with literature values.

The low iron intake of the women in this study is not unexpected since the RDA for this age group (18 mg/day) is difficult to meet on the standard American diet. As judged by the hematocrit, however, the iron status of these subjects appears normal.

The significant differences in the intakes of vitamins A and C are probably not important parameters in the vitamin B_6 status of these women. Shultz and Leklem (1980) found that large doses of vitamin C did not significantly affect vitamin B_6 metabolism.

There are a number of factors affecting the assessment of dietary intake. The National Research Council has commented on the lack of information on the vitamin B_6 content of foods and its bioavailability (NRC, 1980). This problem has been acknowledged by other researchers as well (Wozenski, 1980). Vitamin B_6 is distributed widely in foods (Passmore et al., 1974) but is usually present in small amounts. It is also subject to destruction before being consumed and, as suggested above, may not be total absorbable. Vitamin B_6, being water soluble, may be drained off during cooking procedures. It is quickly destroyed by ultraviolet light in both neutral and alkaline solutions. It is also less stable to heat at these pH levels. Natural, raw foods may supply adequate amounts of vitamin B_6 to the diet, while refined, processed and canned foods may have lost significant amounts of this vitamin

(Schroeder, 1971). Canning, freezing and drying of meats and produce can result in significant losses of vitamin B_6 (De Ritter, 1976). Additional sources of error in the use of diet records come from limitations of food composition tables, inaccurate food records and errors in interpretation and calculation of the records (Chrisley and Driskell, 1979).

In this study, another source of variability in the assessment of vitamin B_6 intake can be seen within each subject. Intrapersonal variation can be considerable (review Figure 5). The differences in vitamin B_6 intake between any two days of the 3-day diet record have been discussed. The OC users were not significantly different from NOC users in terms of variability of daily vitamin B_6 intake. More than 70% of all subjects showed a greater variability than the standard deviation (0.5 mg/day) for both groups. Over 20% of all subjects had daily variations exceeding the mean intakes (1.4 mg/day for OC and 1.6 mg/day NOC) for these subjects. As stated previously, these subjects responded that the diet records were representative of their normal eating habits. Thus, there appears to be considerable variation of vitamin B_6 intake within normal populations of OC users and non-users consuming self-selected diets. Studies done on dietary intakes of vitamin B_6 to date have not spoken to this daily variability of vitamin B_6 intake among their subjects. Subsequent studies should include an assessment of this variability with those of mean nutrient intakes. This variability, and the fact that mean vitamin intakes are used should be considered when relating dietary intake to nutrient levels in the blood.

Blood Levels of Vitamin B_6

Baker et al. (1966) have suggested that 20 ng/ml is a normal vitamin B_6 level for the RBC in adults. The eight women in their study averaged 19.1 ng/ml, with a range of 13 to 24 ng/ml. The value for NOC users reported here (16.8 ng/ml) is fairly close to this normal value. Unfortunately Baker et al. (1966) did not record the vitamin B_6 intake of

their subjects. Red cell PL was determined by Bosse and Donald (1979) to be 11.6 ± 1.6 ng/ml blood (28.7 ng/ml packed RBC) for OC users and 13.5 ± 3.1 ng/ml blood (32.2 ng/ml packed RBC) for controls. These values are slightly lower than the levels reported here. This is expected since the above authors measured only PL. Had all three forms of vitamin B_6 been measured, these values would most likely be slightly higher. The lack of a significant difference between OC users and controls reported by Bosse and Donald (1979) is perhaps due to the higher vitamin B_6 intake (2.06 mg/day) in their subjects and/or the fact that all subjects received this same amount of the vitamin. This suggests that if there is a difference in blood vitamin B_6 levels between OC users and controls, it may be related to differences in dietary intake. Also these difference may be more marked at lower as opposed to higher intakes of vitamin B_6. Vitamin B_6 levels in the RBC were also studied by Donald et al (1971). These authors used the same protozoological assay as was used by Baker et al. (1966 and 1969), but they reported levels of vitamin B_6 that were much higher than those reported by Baker et al. The eight women studied had pre-depletion RBC vitamin B_6 levels averaging 143 ng/ml blood (336 ng/ml packed RBC). No information was given on the vitamin B_6 intake of these women before the study. After depletion and repletion with an intake of 1.54 mg/day of vitamin B_6, the RBC vitamin B_6 level was 112 ng/ml blood (263 ng/ml packed RBC). These authors could not explain the large discrepancy between their results and those of Baker et al. (1966).

Whole blood vitamin B_6 levels in adults were determined by Baker et al. (1966) to be 37 ± 6 ng/ml with a range of 24 to 45 ng/ml. Whole blood values for women in this study did not differ significantly from those for men. This level is considerably higher than the 9.3 ± 3.2 ng/ml for OC and 12.9 ± 5.1 ng/ml for NOC whole blood vitamin B_6 presented here. Results from Miller et al. (1974) for whole blood vitamin B_6 (OC: 6.0 ± 0.4 ng/ml and NOC: 7.7 ± 1.1 ng/ml) were much closer to present values. Vitamin B_6 levels from Miller et al. (1975) for

whole blood averaged 8.3 ± 4.3 ng/ml for OC and 7.0 ± 1.5 ng/ml for NOC users, using S. uvarum; and 8.0 ± 1.1 ng/ml for OC and 10.2 ± 1.1 ng/ml (NOC) using S. faecium as the assay organism.

Shane and Contractor (1975), using a fluorometric assay, reported whole blood PLP levels of 7.6 ± 1.1 ng/ml (OC) and 9.6 ± 1.7 ng/ml (NOC). Again, these levels are reasonably close to those presented here if adjustment is made for the presence of all three vitamin B_6 forms.

In the studies discussed here it can be seen that there is a general trend of OC users to have consistently lower vitamin B_6 status than controls whether vitamin B_6 is assessed in plasma, whole blood or the red cell. This is further illustrated by the plasma vitamin B_6/RBC vitamin B_6 ratio. As reported previously this ratio was 0.66 for OC users and 0.71 for controls. These values approximate the normal ratio of 0.50 suggested by Anderson (1980). Baker et al. (1966) determined this ratio in women to be 0.80 with a range of 0.11 to 3.0. This range includes all the ratios that are reported in the present study (see Figure 6). These ratios ranged from 0.29 to 1.28 for OC users and in NOC: 0.29 to 2.20. While the difference in vitamin B_6 levels in both plasma and the red cell was significant, the difference between the plasma viatmin B_6/RBC vitamin B_6 ratios was not. This indicates that lower RBC vitamin B_6 levels were generally associated with lower plasma vitamin B_6 levels, and vice versa, in both OC users and controls. No comparable studies were found in the literature that attempted to relate the total vitamin B_6 content of the RBC to total vitamin B_6 levels in plasma for OC users and NOC users.

In view of the variety of factors that may affect blood vitamin B_6 levels, a series of possible relationships between these factors and blood vitamin B_6 levels were examined.

Correlations

Regression analysis was performed on all indices measured. These included age, height, weight, hematocrit, body mass, exercise, general health score, vitamin B_6 foods/week score, OC dose and length of time on OC therapy, 3-day average vitamin B_6 intake, vitamin B_6/protein ratio, protein intake, plasma vitamin B_6, whole blood vitamin B_6, RBC vitamin B_6 and the log of RBC vitamin B_6.

There was no correlation between blood vitamin B_6 levels and dose of estrogen or the length of time the subject had been on OC therapy. This lack of correlation may be due to the relatively low dose of estrogens in all OC agents being taken in this study. Bosse and Donald (1979) also found no correlation between blood vitamin B_6 levels and the type of OC used or the duration of use.

Plasma vitamin B_6 levels were only weakly correlated to RBC vitamin B_6 levels, $r = 0.51$ ($P \leq 0.05$) for OC users and $r = 0.39$ ($P \leq 0.1$) for NOC users. The fact that this correlation was weak suggests that the distribution of vitamin B_6 between the plasma and the RBC is not a simple one.

The 3-day average vitamin B_6 intake and the vitamin B_6 foods/week score were significantly related among the OC users, $r = 0.42$ ($P \leq 0.05$); but less significnatly for the NOC group, $r = 0.34$ ($P \leq 0.1$). This tends to indicate that these subjects were consuming their normal diet, with respect to vitamin B_6, during their 3-day diet record. It also suggests that the vitamin B_6 foods/week score sheet may be useful as a quick approximation of the general vitamin B_6 intake.

No correlation was obtained between dietary intake of vitamin B_6 and levels of this vitamin in the blood, in spite of a wide range of vitamin B_6 intakes. Recall that the dietary intakes of OC and NOC subjects did not differ significantly, yet the blood vitamin B_6 levels were consistently and significantly lower in the OC users when measured

in the RBC, in plasma and in whole blood. This seems to indicate that dietary vitamin B_6 intake, at least at the levels consumed in this study, is not a major factor influencing blood vitamin B_6 levels.

What are the other possible factors that may contribute to the lower vitamin B_6 status in the OC users? The effects of alcohol intake and the vitamin B_6/protein ratio have been discussed previously. No correlation was seen between blood levels of vitamin B_6 and alcohol intake from the 3-day diet record or alcohol intake as reported on the questionnaire. There was also no correlation between the vitamin B_6/protein ratio or protein intake and blood vitamin B_6 levels. Exercise has been suggested as a factor for vitamin B_6 levels in the blood (Leklem et al., 1979). No correlation of exercise and blood vitamin B_6 was found in the present study. Although not statistically significant, the OC users were slightly lower in their vitamin B_6 general score, vitamin B_6 foods/week score, 3-day vitamin B_6 intake and body mass index. In an attempt to assess a variety of parameters multiple regression was performed using age, body mass, alcohol intake, exercise, vitamin B_6/protein ratio and the OC dose level versus blood vitamin B_6 status. No significant correlation was found.

The only other variables that were significantly correlated were height with RBC vitamin B_6 and height with the log of RBC vitamin B_6. It is not clear what relationship might exist between height and vitamin B_6 levels in the blood. These correlations may be incidental.

Although actual vitamin B_6 metabolism was not studied, there is an indication of altered vitamin B_6 status with OC use. Lower vitamin levels may be caused by decreased absorption, increased excretion or altered metabolism. As discussed in the review of literature, excretion of vitamin B_6 and 4-PA do not appear to be altered by OC agents. The question of absorption of vitamin B_6 in OC users has not been studied in any of the literature surveyed. Many of the above studies have implied altered vitamin B_6 metabolism. It is possible that the use of OC agents results in increased tissue requirements for vitamin B_6

via estrogen induced enzyme synthesis and altered kinetics. Johansson et al. (1966) have demonstrated that there are two body compartments for vitamin B_6: (1) a rapid turnover pool from which excretion into the urine occurs, and (2) a slow turnover or storage compartment in which the vitamin is bound within the tissues. Shane and Contractor (1975) estimated the total body store of a normal woman to be 60 mg of vitamin B_6. These authors suggest that with a total blood content of less than 0.5 mg of vitamin B_6, an increased tissue requirement could have a substantial effect on the levels of vitamin B_6 in the blood. Skeletal muscle represents the largest tissue compartment for vitamin B_6 (Li, 1978). It is possible that estrogen induced increases in enzyme levels in such a large tissue pool as muscle could significantly lower the levels of vitamin B_6 in the blood. The blood represents the major pool from which vitamin B_6 could be drawn into the tissues.

CONCLUSION

Some attempt must be made to explain what these data mean in real terms. In considering the various aspects, it is clear that the blood levels of vitamin B_6 in OC users are significantly lower than in controls. Several possible explanations present themselves.

First, these data may be artifactual due to the large variation in intakes of vitamin B_6 and the errors in dietary assessment discussed previously. Blood levels tend to be a reflection of dietary intakes in general. Shane and Contractor (1975) consider the direct estimation of vitamin B_6 in the blood to be "more reflective of true vitamin status than the indirect tests." One can reasonably assume that the errors associated with the assessment of dietary intake and intrapersonal variation were similar in both subject groups. There is also the evidence of the many other studies discussed herein. Thus, these data are probably not artifactual.

Second, there are other factors present which may possibly contribute to the lower vitamin B_6 status of the OC users. Although there were no significant correlations, including multiple regression, it cannot be said conclusively that these factors do not contribute to the blood values presented. The lack of statistical significance means that this is unlikely. There may be some synergistic effect of these variables. This possibility remains to be clearly evaluated.

A third possibility is altered vitamin B_6 metabolism in the OC users. This remains a viable consideration for the lower vitamin B_6 levels seen in the OC users. It is important to note, however, that deficiency does not necessarily follow from lowered blood vitamin levels. Redistribution of vitamin B_6 between the body compartments may be occurring, while the total body pool of this vitamin remains unchanged. Nutritional recommendations for OC users thus appear to be a matter of value judgment as to what constitutes true vitamin B_6 status.

SUMMARY

Two apparently normal populations of young women, those taking OC agents and those not taking these drugs, were compared with respect to general health, vitamin B_6 intake, blood vitamin B_6 levels and a variety of other factors thought to influence vitamin B_6 status.

(1) The blood levels of vitamin B_6 were found to be significantly lower in OC users when measured as RBC, whole blood or plasma vitamin B_6 (plasma vitamin B_6 levels are presented by Lind, 1980). The ratio of plasma vitamin B_6 to RBC vitamin B_6 was not significantly different between the OC users and controls.

(2) Alcohol intake, as indicated by questionnaire, was significantly higher in the OC users. This was generally supported by the actual 3-day diet intake.

(3) Differences in mean vitamin B_6 intake from the 3-day diet records were not significant between OC users and controls. Wide variation of intake between the three days was observed.

(4) Intakes of other nutrients studied were comparable between the two subject groups. Nearly half of the subjects from both groups recorded intakes below 67% of the RDA for vitamin B_6, iron and calories. Intakes of other nutrients met the RDA in nearly all subjects.

(5) The vitamin B_6/protein ratio was significantly different between OC and NOC subjects, although both ratios were deemed adequate.

(6) Vitamin B_6 blood levels did not correlate with dietary vitamin B_6 intake. OC agents and other factors may be influencing vitamin B_6 status in the OC users.

(7) A possible explanation for the altered blood vitamin B_6 levels in the OC users was suggested via altered tissue distribution of vitamin B_6.

Further research in this area will most likely answer the questions raised here. Studies of tissue levels and vitamin B_6 distribution in the body would be helpful. The question of altered absorption and bioavailability of vitamin B_6 in OC users and controls should be addressed. Longer term studies where the dietary vitamin B_6, alcohol, protein and other nutrient intakes are controlled might prove useful in assessing the influence of these variables on vitamin B_6 status.

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APPENDIX

REAGENTS AND PREPARATION

Vitamin solution I:

Dissolve 10 mg thiamin[9] and 1 gm inositol[4] in about 200 ml water, and make to 1 liter. Store in refrigerator. (1 ml = 10 µg thiamin, and 1 mg inositol)

Vitamin solution II:

Dissolve 10 mg biotin[5] in 100 ml 50% alcohol-water. Store in refrigerator. (1 ml = 100 ug biotin)

Dissolve 200 mg calcium pantothenate[6] and 200 mg niacin[9] in about 200 ml water; add 8 ml of the biotin solution; make to 1 liter with water. Store in refrigerator. (1 ml = 200 ug each of calcium pantothenate and niacin, 0.8 ug biotin)

Salt solution I:

Dissolve 17 g KCl, 10.3 g $MgSO_4 \cdot 7H_2O$, 100 mg $FeCl_3 \cdot 6H_2O$, and 100 mg $MnSO_4 \cdot H_2O$ in about 800 ml water. Add 2 ml conc. HCl.[2] Dissolve 5 g $CaCl_2 \cdot 2H_2O$ in about 100 ml water; add to the first solution and make to 1 liter with water. Stores in refrigerator. (1 ml = 17 mg KCl, 10.3 mg $MgSO_4 \cdot 7H_2O$, 100 ug each $FeCl_3 \cdot 6H_2O$, and $MnSO_4 \cdot H_2O$, and 5 mg of $CaCl_2 \cdot 2H_2O$.)

Salt solution II:

Dissolve 22 g KH_2PO_4[7] and 40 g $(NH_4)_2HPO_4$[7] in water and make to 1 liter. Store in refrigerator. (1 ml = 22 mg KH_2PO_4 and 40 mg $(NH_4)_2HPO_4$.

"Tween 80" solution:

Weigh 2.5 g of Tween 80[1] in a small beaker. Transfer with warm water (45°C) and make to 500 ml volume. Store in refrigerator. (1 ml = 5 mg Tween 80)

Citric acid solution:

Dissolve 50 g of citric acid[7] in 50 ml of water. Store at room temperature in a bottle with a plastic stopper.

Amonium phosphate solution:

Dissolve 25 g $(NH_4)_2HPO_4$ in 50 ml water. Store at room temperature in a bottle with a plastic stopper.

Citrate buffer solution:

Dissolve 100 g potassium citrate[7] and 20 g citric acid and make to 1 liter with water. Store in refrigerator. (1 ml = 100 mg potassium citrate and 20 mg citric acid.)

Basal medium stock solution (for 200 tubes):

To make a liter of medium add to about 400 ml water: 100 ml citrate buffer solution, 10 g Casamino acid powder[3], 50 ml vitamin solution I, 25 ml vitamin solution II, 50 ml salt solution I, and 50 ml salt solution II. Dissolve 100 g dextrose[7] in the liquid. Dissolve 22 mg dl-tryptophan[8], 27 mg l-histidine HCl[8], 100 mg dl-methionine[2], 216 mg dl-isoleucine[10], and 256 mg dl-valine[10] in 10 ml of 10% HCl in a small beaker and add to the above. Add 20 ml "Tween 80" solution. Make to 1 liter with water and store in a pyrex bottle plugged with cotton in refrigerator. Prepare not longer than 24 hours before use. When ready, steam for 10 minutes and cool.

Test organism:

Saccharomyces uvarum (carlsbergensis) American Type Culture Collection No. 9080.

Agar culture medium:

Suspend 25 g of bacto-wort agar in about 400 ml water in a marked 500 ml wide mouth erlenmeyer flask. Plug with cotton, steam for about 10 minutes to dissolve the agar, adjust the volume to 500 ml. Pipet the hot agar in about 10 ml amounts into 20 x 150 mm test tubes, plug with absorbent cotton and autoclave for 15 minutes at 15 pounds pressure. Avoid overheating. Tilt the hot agar tubes to form slants and cool in this position.

Liquid culture medium:

Pipet 5 ml of mixed solution (2 ml each of 1.0 ug/ml pyridoxine[10], pyridoxal[10] and pyridoxamine[10]) into 16 x 150 mm test tubes, containing two glass beads (4 mm), plug with absorbent cotton, autoclave for 10 minutes at 15 pounds pressure. Add 5 ml of the steamed vitamin B_6-free basal medium stock solution, under aseptic conditions. Store in the refrigerator.

Inoculum Rinse:

Pipet 5 ml of water into test tubes, plug with absorbent cotton, and autoclave for 10 minutes at 15 pounds pressure. Add 5 ml of steamed vitamin B_6-free basal medium under aseptic conditions. Store tubes in refrigerator.

Culture Care:

Maintain the S. uvarum culture by weekly transfers on wort agar slants. Incubate these freshly seeded agar slants at 30°C. for 24 hours and then refrigerate.

Assay Inoculum:

Incubate cells for inoculum on agar at 30°C. for 24 hours just before use. Transfer these cells under aseptic conditions to liquid broth couture tubes. Plug with absorbent cotton held on with masking tape, and place the tubes on the shaker at 30°C. for 20 hours. Centrifuge at 2500 rpm for 1.5 minutes. Decant the liquid and resuspend in 10 ml of inoculum rinse. Separate by centrifugation at 2500 rpm for 1.5 min. Decant the liquid, resuspend in a second 10 ml of sterile inoculum rinse, centrifuge for 1.5 min. and decant. The cells suspended in the third 10 ml of inoculum rinse are the assay inoculum.

REAGENT SOURCES

1. J.T. Baker Chemical Co., Phillipsburg, N.J.: "Tween 80" and trichloroacetic acid.

2. Calbiochem Co., Los Angeles, CA.: dl-methionine

3. Difco Labs., Detroit, Mich.: vitamin-free casamino acids (#565735)

4. Fisher Scientific Co., Fair Lawn, N.J.: inositol, KCl, $CaCl_2 \cdot 2H_2O$.

5. General Biochemicals, Inc., Chagrin Falls, Ohio: biotin

6. INC Pharmaceuticals, Inc., Cleveland, Ohio: calcium pantothenate

7. Mallinckrodt, Inc., St. Louis, MO.: $MgSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, $MnSO_4 \cdot H_2O$, KH_2PO_4, potassium citrate, $(NH_4)_2HPO_4$, dextrose, and citric acid.

8. Nutritional Biochemicals Corp., Cleveland, Ohio: dl-tryptophan and l-histidine

9. Merck and Co., Inc., Rahway, N.J.: thiamin and niacin

10. Sigma Chemical Co., St. Louis, MO.: pryidoxine HCl, pyridoxal HCl and pyridoxamine HCl dl-isoleucine and dl-valine

Appendix Table 1. Percent Transmittance for the Standard Curves.

	Concentration of Standard ng/ml PAL					
Date of Assay	0.5	1.0	2.0	3.0	4.0	5.0
5/18/79	93.2	86.7	76.2	68.0	62.2	57.2
5/25/79	92.0	85.5	75.7	67.8	61.8	56.2
6/1/79	93.0	86.5	75.5	68.0	61.0	56.0
10/5/79	-	84.2	75.0	68.0	62.0	56.5
10/12/79	92.7	84.5	74.5	68.0	61.0	55.1
10/19/79	93.2	88.3	78.7	71.3	66.2	61.3
10/26/79	93.2	85.0	74.5	67.2	59.7	53.7
11/2/79	91.3	83.3	71.3	65.3	60.0	55.3
11/9/79	92.5	85.2	74.7	67.8	61.5	56.7
11/22/79	92.3	84.5	74.5	66.5	60.3	55.0

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.

Instructions for the Diet Record

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	____Dried fruit	____Banana (1/3 med.)
____apples	____raisins (1/3 c.)	____avocado (1/4 med.)
____berries		
____melon		
____Other____		
_____		

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

____carrots	____greens	____dried beans
____green beans	____broccoli/cauliflower	____lentils
____tomatoes	____sweet potato	____soybeans
____potatoes	____corn	
____Other____	____cabbage	
_____		

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

____white bread	____whole wheat bread	____Brewers yeast (3 Tbs.)
____white rice	____whole wheat pasta	____wheat germ (1/4 c.)
____saltine/soda crackers (4-6)	____brown rice	____soy flour (1/4 c.)
____Other____	____rye bread	____wheat bran (1/2 c.)
_____	____cornbread	

MEATS (3 ounces is one serving)

____shellfish	____fish	____organ meats (liver)
____shrimp	____red meats	____fresh tuna or salmon
____Other____	____eggs (2 is 1 serv.)	
_____	____poultry	

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.)	____peanut butter (1 & 1/2 Tbs.)	____sunflower seeds (1/4 c.)
____honey (2 Tbs.)		____walnuts (14 halves)
____Other____	____almonds (10 nuts)	____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 ¼ pack

3 points -- Less than ½ pack

2 points -- Less than 1 pack

1 point -- More than or equal to 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 or equal to 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 1 yes answer
3 points -- For 2 yes answers
2 points -- For 3 yes answers
1 point -- For 4 yes answer

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 basis, 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.

General Vitamin B_6 Score

1) Question 5: How much, if any, alcoholic beverage do you drink?

Scored in the same manner as the General Health Score.

2) Question 11: Pregnancy in the last 5 years.

5 points -- None
4 points -- Not full term
3 points -- 3 or more years ago
2 points -- 1-3 years ago
1 point -- in the last year

3) Question 18: Vitamin B_6 foods/week.

Vitamin B_6 foods/week score was used vide infra.

Vitamin B_6 Foods/Week Score

The vitamin B_6 foods/week 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. Foods listed in "other" categories were placed in the appropriate column based on data from Orr (1969).