Vitamin B₆ requirements of women using oral contraceptives¹, ²

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ABSTRACT Fifteen women who used combined estrogen-progestogen oral contraceptives and nine control women were given a vitamin B₆-deficient diet for 4 weeks and the same diet supplemented with 0.8, 2.0, or 20.0 mg of pyridoxine hydrochloride for an additional 4 weeks. At weekly intervals a variety of indices of vitamin B₆ nutrition were measured to determine rates of depletion and repletion. The tryptophan load test (2.0 g) was significantly different in the contraceptive users. However, other indices, including urinary cystathionine (3.0 g L-methionine load), urinary 4-pyridoxic acid, plasma phosphate, and erythrocyte alanine and aspartate aminotransferases, were not significantly different. Since altered tryptophan metabolism persisted in contraceptive users even when other indices of vitamin B₆ nutrition were normal, we suggest that the use of oral contraceptives specifically affects tryptophan metabolism by some means other than through a vitamin B₆ deficiency. Am. J. Clin. Nutr. 28: 535–541, 1975.

Women using estrogen-containing oral contraceptives excrete elevated amounts of tryptophan metabolites after a tryptophan load test compared with women not taking oral contraceptives (1–3). Elevated levels of 3-hydroxyanthranilic acid were also observed in basal urines from such subjects (4). When pyridoxine was administered to such subjects the excretion of tryptophan metabolites was decreased toward normal levels, but in some subjects large amounts of the vitamin may be required (3). These data have been widely interpreted as indicating that the use of oral contraceptive drugs causes an increased requirement for vitamin B₆, although other explanations for the altered tryptophan metabolism may be possible. To evaluate the effect of oral contraceptive usage on the requirement for vitamin B₆, a variety of indices of vitamin B₆ nutrition were measured to determine the rate at which control and oral contraceptive using women became depleted of this vitamin while ingesting a diet low in vitamin B₆. The same indices were used to measure the rate at which these subjects became repleted when supplemented with physiological levels of pyridoxine.

Methods and materials

The experimental details of these studies concerning the selection of subjects, diets used, and indices of vitamin B₆ nutrition measured were reported previously (5). In brief, 9 healthy control women (average age 22.3 ± 1.9 years), and 15 women (23.2 ± 3.11 years) who had used oral contraceptive agents for at least 6 months were given a diet that contained only 0.19 mg of pyridoxine equivalents per day (6). Oral contraceptive users started the diet on day 11 of a 21-day pill sequence. Control subjects started 14 days after onset of the previous menses. For the first 4 days, while baseline studies were made, the diet was supplemented daily with 0.8 mg of pyridoxine hydrochloride (PN-HCl). This supplement was then withdrawn and both groups of subjects consumed only the deficient diet for 28 days. After this depletion period, subjects were supplemented with either 0.8, 2.0, or 20 mg/day of PN-HCl for another 28 days. Before release from the study, subjects were supplemented for a final 4 days with 100 mg PN-HCl/day. During the baseline period, and at weekly intervals throughout the study, several indices of vitamin B₆ nutrition were measured in each subject. The indices measured included urinary tryptophan metabolites before and after a 2.0 g oral load of L-tryptophan (7, 8), urinary cystathionine after a load

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of 3.0 g of L-methionine (9, 10), urinary 4-pyridoxic acid (5), plasma pyridoxal phosphate (5), and erythrocyte activities of alanine aminotransferase and aspartate aminotransferase (5). In order to study metabolism along the kynurenine pathway and to circumvent any variations in activity of tryptophan oxygenase, loading doses of L-kynurenine sulfate (200 mg/dose) were given initially, at the time of maximum deficiency, and after repletion with pyridoxine for 4 weeks.

**Results**

Measurement of urinary tryptophan metabolites after tryptophan loading prior to starting the deficient diet showed that the oral contraceptive users excreted elevated levels of kynurenine, acetylkynurenine, 3-hydroxykynurenine and xanthurenic acid compared with the control subjects (8). These differences persisted and increased during the period of vitamin B6 depletion. This is summarized in Fig. 1, which shows the yield of tryptophan metabolites excreted. During depletion, both groups of subjects excreted increasingly large amounts of metabolites, with the oral contraceptive users excreting consistently larger amounts than the controls. Supplementation of control subjects with 0.8 mg of PN-HCl/day dramatically reduced the excretion within 1 week, and by the third week of repletion the excretion level had stabilized at essentially the predepletion values. The oral contraceptive users supplemented with 0.8 mg of PN-HCl/day also exhibited a marked reduction in excretion of tryptophan metabolites. However, even after 4 weeks of supplementation, the excretion was still slightly higher than the starting (predepletion) values for these subjects and was markedly higher than the control subjects at the same time. The low value observed in oral contraceptive users at day 47 and the inflection at day 19 were a consistent finding which coincided with the 7-day period during which oral contraceptive use was interrupted.

Supplementation of the control subjects with 2.0 mg of PN-HCl daily restored tryptophan metabolism to normal ranges within 1 to 2 weeks. Excretion by oral contraceptive users was also promptly decreased by daily supplements of 2.0 mg of PN-HCl, with excretion values considerably lower than the predepletion values for these subjects, but still not at control levels. Supplementation of oral contraceptive users with 20 mg/day promptly restored excretion to control levels.

At comparable times of depletion and at equal levels of PN-HCl supplementation, the mean excretion of tryptophan metabolites was consistently greater than that of controls, and suggests that the requirement for vitamin B6 may be slightly greater in oral contraceptive users than in controls. However, it should be pointed out that because of large individual variations and limited numbers of subjects, many of these differences do not achieve statistical significance. Details of the excretion of individual tryptophan metabolites are published elsewhere (8).

The urinary excretion of cystathionine after a 3.0 g oral load of L-methionine is shown in Fig. 2. The pattern is different from that of the tryptophan metabolite yield in that predepletion and week 1 levels of cystathionine were similar in both groups. However, apparent differences occurred at later times, and during

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**FIG. 1.** Yield of metabolites excreted after a 2.0 g tryptophan load by control subjects and oral contraceptive users (O.C.). During the first 4 days the deficient diet (-B6) was supplemented with 0.8 mg pyridoxine hydrochloride. During the second +B6 period the diets were supplemented with the number of milligrams of pyridoxine hydrochloride indicated in the figure. The shaded bars below the figure designate the 7-day period during which oral contraceptive use was interrupted.
FIG. 2. Urinary excretion of cystathionine after a 3.0 g oral load of L-methionine in oral contraceptive users (O.C.) and control women. During the −B6 period the diet contained the equivalent of 0.19 mg of pyridoxine. During the +B6 period the diet was supplemented daily with 0.8, 2.0, or 20 mg of pyridoxine hydrochloride. Cystathionine was measured by an amino acid analyzer (modified Beckman model 120) using a modified buffer gradient system (9).

The first 2 weeks of repletion with PN-HCl the excretion by oral contraceptive users remained above that of controls at comparable times. Again, because of wide variations between individuals and because of the small number of subjects, these differences were not of significance statistically.

Excretion of urinary 4-pyridoxic acid decreased at similar rates in both groups of subjects (Fig. 3) and repletion rates during supplementation with PN-HCl were also quite similar. These data suggest that use of oral contraceptives has no measurable effect on absorption, utilization or conversion of pyridoxine to 4-pyridoxic acid (5).

Plasma pyridoxal phosphate (PLP) levels of oral contraceptive users were slightly lower than control values initially and remained slightly lower throughout the depletion period (Fig. 4). During repletion with PN-HCl no differences between groups were found. Supplements of 0.8 mg PN-HCl/day were not enough to restore PLP values to predepletion levels in either group. However, supplements of 2.0 mg/day for 2 and 3 weeks resulted in PLP levels appreciably higher than starting values in both groups (5).

To evaluate metabolic effects of oral contraceptive use on the tryptophan metabolic pathway independent of any effects on the activity of tryptophan oxygenase, 200 mg loads of L-kynurenine sulfate monohydrate were given before deficiency, at peak deficiency, and after pyridoxine supplementation. The excretions of kynurenine, 3-hydroxykynurenine, acetyl-kynurenine, and xanthurenic acid are shown in Fig. 5. Excretions of kynurenine and 3-hydroxykynurenine were slightly higher in the oral contraceptive group than in controls at all three times studied and were highest in both groups when at the peak of deficiency. Excretions of acetyl-kynurenine and xanthurenic acid were essentially unchanged. Because of sizable individual variations, none of these differences were significant but they suggest that the use of oral contraceptives has an effect on tryptophan metabolism at one or more steps beyond kynurenine in addition to any effects they may have on tryptophan oxygenase activity.

Activity of erythrocyte alanine aminotrans-
and controls were found initially and activity of both groups decreased similarly during the depletion period. Daily supplements of 0.8 mg of PN-HCl slowly increased the activity but not to predepletion levels. With 2.0-mg supplements, the activity of both groups had risen to approximately predepletion levels. The final points shown were after 3 or 4 days of supplementation with 100 mg PN-HCl/day. This level of supplement resulted in normal or supernormal levels.

The above erythrocyte preparations were also assayed after fortification in vitro with saturating levels of PLP (Fig. 6). The percent stimulation by PLP increased as the deficiency progressed, and did so to the same extent in controls and oral contraceptive users. During deficiency, in vitro stimulation by PLP did not restore activity to predepletion levels, suggesting that the decreased activity was due, in part, to loss of apoenzyme. Dietary supplementation with PN-HCl again decreased the percent stimulation with no consistent differences between comparable groups.

Similar unstimulated and PLP-stimulated assays were done for erythrocyte aspartate aminotransferase (not fortified with PLP in vitro) at weekly intervals throughout the study is shown in Fig. 6. Details were reported elsewhere (5). No differences between oral contraceptive users

FIG. 4. Concentration of plasma pyridoxal phosphate (PLP) in controls and oral contraceptive users. Footnotes are the same as in Fig. 3. PLP was assayed with tyrosine apodecarboxylase using L-tyrosine-1-14C as substrate.

FIG. 5. Excretion of kynurenine (KYN), acetyl-kynurenine (AK), 3-hydroxykynurenine (HK), and xanthurenic acid (XA) by control subjects and oral contraceptive users (O.C.) (given an oral load of 200 mg of L-kynurenine sulfate) prior to vitamin B6 depletion (period 1), at the peak of deficiency (period 2) and after repletion with pyridoxine (period 3). The abbreviated metabolic pathway serves to identify the metabolites in each group of bars. Metabolites were measured as previously described (7).

FIG. 6. The upper figure shows changes in erythrocyte alanine aminotransferase basal activity (without addition of PLP in vitro) in controls (CONT.) and oral contraceptive users (O.C.) during vitamin B6 depletion and repletion. The daily supplements of PN-HCl (in mg) are shown on each curve. The lower figure shows the percent stimulation observed in the activity of this enzyme when saturated in vitro by added PLP.
aminotransferase, and the findings paralleled those of the alanine enzyme although the relative decreases induced by vitamin B₆ deficiency were not as great. Details of these studies have been presented elsewhere (5).

Discussion

The excretion of tryptophan metabolites by oral contraceptive users, after the tryptophan load test, was significantly different from similarly loaded controls prior to induction of vitamin B₆ deficiency. This confirmed previous observations of altered tryptophan metabolism in oral contraceptive users (1–3). Other indices of vitamin B₆ nutrition measured before vitamin B₆ depletion were not clearly different in oral contraceptive users. Thus, urinary cystathionine and urinary 4-pyridoxic acid were not different from controls; and plasma PLP and the erythrocyte aminotransferases, while slightly lower in oral contraceptive users, were not statistically different. When the rates of change of these indices were compared during dietary depletion of vitamin B₆ in both groups, the excretion of tryptophan metabolites and cystathionine suggested that the oral contraceptive group might be depleting at a slightly faster rate than the controls. Other indices, including 4-pyridoxic acid excretion, plasma PLP, and erythrocyte aminotransferases changed at virtually the same rate in both groups during depletion and reached the same nadir at the time of maximum deficiency.

Supplementation with pyridoxine (0.8 mg/day) resulted in very similar restoration rates of plasma PLP, erythrocyte alanine aminotransferase and urinary 4-pyridoxic acid. However, correction of urinary excretion of cystathionine and tryptophan metabolites was slightly slower in the oral contraceptive group, although not significantly so. Similarly, repletion rates between groups supplemented with 2.0 mg of PN-HCl were not significantly different. The data clearly indicate that a daily supplement of 0.8 mg of PN-HCl for 28 days is not enough to replete either controls or oral contraceptive users. However, 2.0 mg/day for 4 weeks restored all indices in both groups to their respective predepletion levels and, in some cases, to ultranormal levels.

The detailed data (8) show that the excretion of tryptophan metabolites by the oral contraceptive group was still elevated above control values after supplementation with 2.0 mg, at which time all other indices were within normal control ranges. This suggests that the oral contraceptives may have some relatively specific effect on the metabolism of tryptophan which is independent of vitamin B₆ levels. This effect may be primarily on the activity of tryptophan oxygenase, since studies in rats have shown direct as well as adrenal-mediated effects of estrogens on the activity of this enzyme (11). However, the present observations in subjects given small kynurenine loads, which bypass any tryptophan oxygenase effects, suggest that the usage of contraceptive hormones also may have an effect elsewhere in the pathway of kynurenine metabolism. Previous studies (12, 13) indicate that steroids or steroid conjugates can affect the activity of the PLP-dependent enzymes of the kynurenine pathway, i.e., kynureninase and kynurenine aminotransferase. Thus, it seems most likely that the altered tryptophan metabolism of oral contraceptive users is the summation of hormonalmal effects on tryptophan oxygenase and kynureninase rather than the production of a general vitamin B₆ deficiency. This interpretation is in agreement with the recent report by Lumeng et al. (14) in which urinary xanthurenic acid and plasma PLP levels were compared in oral contraceptive users and controls. They found that xanthurenic acid excretion was elevated in most contraceptive users even though plasma PLP levels were, in most cases, not correspondingly low. However, in contrast to the present study, they reported that about 20% of oral contraceptive users in the 20- to 34-year-age range had subnormal levels of plasma PLP. Further, plasma PLP levels decreased in subjects during the first months of oral contraceptive use but tended toward normal with continued usage. Since no dietary information was presented, the possibility of contraceptive-induced changes in diet must be considered. Salkeld et al. (15) found the erythrocyte aspartate aminotransferase stimulation test to be abnormal in almost 50% of oral contraceptive users, but observed no effect of duration of contraceptive usage on this index. Rose et al. (16) found low urinary 4-pyridoxic acid levels and increased hydroxykynurenine—hydroxyxanthanilate ratios in about 19% of oral contraceptive users although the mean 4-pyridoxic acid value for the whole group was not significantly different from controls.
The activities of erythrocyte aminotransferases, particularly the PLP activation thereof, are considered reliable indices of vitamin B₆ nutrition (17). In the present study these indices changed as expected with the induction of a vitamin B₆ deficiency, but there were no consistent or significant differences between control subjects and oral contraceptive users. Rose et al. (18) found an increased in vitro PLP stimulation of erythrocyte alanine aminotransferase in about 15% of contraceptive users.

The brief review of the literature presented above indicates that ample evidence exists to suggest that the use of estrogen-containing oral contraceptives may produce a vitamin B₆ deficiency in certain subjects. In the present study in which precise dietary control was maintained, it was not possible (with the exception of the tryptophan load test) to demonstrate clear-cut differences in vitamin B₆ requirements between control subjects and oral contraceptive users when a variety of indices of vitamin B₆ nutrition were measured. Perhaps because of the limited number of subjects studied, we might not have obtained experimental subjects susceptible to contraceptive-induced vitamin B₆ deficiency. Perhaps with a much larger number of subjects some of the minor differences would have reached statistical significance. However, it is possible to say that the use of oral contraceptives per se does not significantly or consistently increase the requirement for vitamin B₆ in the majority of women using these agents. However, a small subgroup of women may well exist who are particularly susceptible to steroid-induced vitamin B₆ deficiency, and in these women other metabolic abnormalities may result (19).

**Summary**

Fifteen women who used estrogen-containing oral contraceptives and nine control women who had never used these agents were given a diet deficient in vitamin B₆ (containing the equivalent of 0.19 mg of pyridoxine/day). After 4 weeks, this diet was supplemented daily with 0.8, 2.0 or 20.0 mg of pyridoxine hydrochloride for an additional 4 weeks. Initially, and at weekly intervals, measurements were made of several indices of vitamin B₆ nutrition, including urinary tryptophan metabolites (before and after a 2.0 g load of L-tryptophan), urinary cystathionine (after a 3.0 g load of L-methionine), urinary 4-pyridoxic acid, plasma pyridoxal phosphate, and erythrocyte alanine and aspartate aminotransferases. In addition, 200 mg oral loads of L-kynurenine sulfate were given initially, at the peak of deficiency, and after pyridoxine repletion.

No significant differences were observed between oral contraceptive users and controls in the above measured indices with the exception of the tryptophan load test, although very minor differences occurred in several of the indices. The data suggest that the use of oral contraceptives may specifically affect tryptophan metabolism by some means other than through a vitamin B₆ deficiency, since altered tryptophan metabolism persisted even when other indices of vitamin B₆ nutrition were normal. The amount of vitamin B₆ (as pyridoxine) needed to maintain normal levels of these indices (except for tryptophan metabolism) was between 0.8 and 2.0 mg/day. The data suggest that if the use of oral contraceptives of the combined estrogen-progestogen type does alter the requirement for vitamin B₆, the effect is a minor one and of doubtful clinical significance to the majority of women taking these steroid preparations.

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