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Research Article

THE EFFECT OF VITAMIN SUPPLEMENTATION ON THE URINARY EXCRETION OF TRYPTOPHAN METABOLITES BY PREGNANT WOMEN *

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The first demonstration by Lepkovsky, Roboz and Haagen-Smit (1) of the occurrence of xanthurenic acid in urine of vitamin B_6-deficient rats has since been extended and confirmed in a number of species (2–4) including man (5, 6), and the measurement of xanthurenic acid in urine following a loading dose of tryptophan has been proposed as an index of vitamin B_6 nutrition (7). The role of this vitamin in tryptophan metabolism has been studied in intact rats by Dalgliesh (8), and enzyme studies by a number of investigators (9–12) provide a reasonable explanation for the elevated excretion of tryptophan metabolites found in the urine of pyridoxine-deficient animals. Although xanthurenic acid sometimes occurs in elevated amounts in urine, it is not an abnormal tryptophan metabolite, since it can be detected in normal human urine (13).

The observations that pregnant women excrete elevated amounts of xanthurenic acid (14–16) and other metabolites of tryptophan (17) have been interpreted as signifying a vitamin B_6 deficiency in pregnancy, inasmuch as supplementation of the diet with pyridoxine restored the excretion of xanthurenic acid to control levels. Xanthurenic acid excretion was not elevated in pregnant swine (18), rats, guinea pigs, rabbits or dogs (19). However, dietary pyridoxine deficiency caused elevated xanthurenic acid excretion in most of these species.

It has been reported that pre-eclamptic and eclamptic patients excreted more xanthurenic acid than normal pregnant or nonpregnant women (14). However, since there are differing reports on the efficacy of pyridoxine in reducing the incidence of pre-eclampsia (20, 21), factors in addition to vitamin B_6 nutrition are probably involved.

Since quantitative studies of urinary tryptophan metabolites have been chiefly concerned with xanthurenic acid, the present study was undertaken to evaluate the quantity of this and other tryptophan metabolites excreted during pregnancy, and to determine the effect, if any, of other vitamins in addition to vitamin B_6 on the excretion of tryptophan metabolites by normal pregnant women. The results indicate that the elevated levels of tryptophan metabolites found in the urine of normal pregnant subjects were lowered by pyridoxine administration to near the levels found in nonpregnant subjects, with the exception of N-methyl-2-pyridone-5-carboxamide excretion, which remained two or three times higher in pregnancy. Administration of other vitamins had no effect on the excretion of tryptophan metabolites. The pattern of urinary metabolites found suggests that other factors in addition to vitamin B_6 nutrition may be involved in regulating the metabolism of tryptophan in pregnant subjects.

METHODS

The effect of vitamin supplementation on the metabolism of tryptophan was studied in 14 pregnant subjects (ages 21 to 37) seen by one of us (M. T.) as outpatients in the Department of Gynecology and Obstetrics. In stage of pregnancy, 4 subjects were in the third trimester, 9 were in the second trimester, and one was late in the first trimester. Prior to the first study, the subjects received no vitamin supplement for at least 2 weeks. After collection of a 24 hour basal urine, each subject was given an oral test dose of 2 g of L-tryptophan and two more complete 24-hour urine collections were obtained. Thus, all urine was collected for 1 day before and for 2 days after tryptophan administration (Group II, pregnant, no vitamins). The urine was preserved

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under toluene and refrigerated until analyzed. Following this first study, each subject received a multivitamin preparation lacking only pyridoxine of the common vitamins.[1] After ingestion of this vitamin preparation for 11 days, the 3-day tryptophan study was repeated (Group III, pregnant, vitamins, no B_6). The subjects were then given daily the same multivitamin preparation, containing in addition 6 mg of pyridoxine hydrochloride,[2] for another 11 days when a third tryptophan study was obtained (Group IV, pregnant, vitamins with B_6). The subjects then continued to receive the complete multi-vitamin supplement until term. Of the 14 subjects on whom complete studies were obtained during pregnancy, 9 were available for postpartum study (Group V). This 3-day study was done 1 to 16 months post partum when the subjects had stopped lactating, and had received no vitamin supplement for at least 2 weeks prior to study.

Mild anemia was the chief prenatal complication. All infants (7 males and 7 females) were delivered at term, two by Cesarean section, two by forceps and the remainder spontaneously. Postpartum bleeding and a subsequent transfusion reaction in one subject were the only postpartum complications encountered.

For comparison, the tryptophan metabolism of a group of 10 normal, nonpregnant women (ages 19 to 45) was measured (Group I). Two of the controls were postmenopausal, the others were premenopausal. Four of the controls had had one or more pregnancies, 6 were nulliparous; no detectable difference in tryptophan metabolism was observed between these subgroups.

In addition, 7 normal nonpregnant women were given the tryptophan load test just before menstruation, just after menstruation, and at the estimated time of ovulation, in an attempt to evaluate the influence on tryptophan metabolism of changes in hormonal levels during the menstrual cycle. Subjects with regular cycles were chosen in order that it might be possible to accurately predict the time of ovulation and the start of menstruation.

The tryptophan metabolites measured were kynurenine, acetylkynurenine, *o*-aminohippuric acid (22), 3-hydroxykynurenine (23), kynurenic and xanthurenic acids (24), and N-methyl-2-pyridone-5-carboxamide (pyridone) (25). Only the natural L-tryptophan was used in these studies since previous reports (26) demonstrated that use of DL-tryptophan leads to the excretion of metabolites of D-tryptophan.

[1] Each daily dose contained 1.5 mg thiamine mononitrate, 3 mg riboflavin, 7.5 μg vitamin B_{12}, 100 mg ascorbic acid, 15 mg nicotinamide, 0.4 mg folic acid, 6,000 USP units vitamin A, 75 mg ferrous sulfate, 6 mg racemic calcium pantothenate and 1.87 g calcium carbonate. We are indebted to Dr. J. M. Maas and Mr. R. Glenn Weiss of Eli Lilly and Co., Indianapolis, Ind., for supplying us with this preparation.

[2] Compren Pulvules no. 25, Eli Lilly and Co., Indianapolis, Ind., kindly supplied by Dr. J. M. Maas and Mr. R. Glenn Weiss.

RESULTS

Table I outlines the metabolic relationships of the compounds measured, and shows the average quantities excreted by the various groups of subjects before and after a loading dose of 2 g of L-tryptophan. Statistical evaluation by means of a *t* test was made of the basal (pre-tryptophan) levels of excretion between groups and also of the yields of metabolites due to tryptophan loading (post-tryptophan values minus basal values) between groups (Table II).

The basal excretions of kynurenine, hydroxykynurenine, and xanthurenic acid were significantly higher ($p \leq 0.05$) in pregnancy (Group II) than in the control subjects (Group I), whereas basal kynurenic acid excretion was significantly lower ($p \leq 0.02$) in the pregnant subjects. In comparing the basal levels of excretion by pregnant subjects (Group II) with the same subjects post partum (Group V), it was found that pregnant subjects excreted significantly elevated levels of kynurenine, hydroxykynurenine

TABLE I

The average urinary excretion of metabolites of tryptophan by various groups of women †

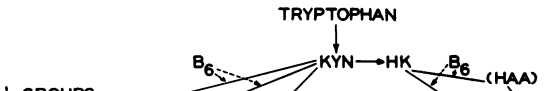
GROUP NO.	SUBJECTS		TRYPTOPHAN	oAH	KA	ACK			XA	PYR
				TRYPTOPHAN → KYN → HK → (HAA)						
				B_6				B_6		
I	10	CONTROL	0	22	11	10	11	25	7	87
			2	42	61	16	29	51	30	120
II	14	PREGNANT, NO VITAMINS	0	21	9	13	25	56	10	98
			2	34	82	29	183	306	254	207
III	14	PREGNANT, VITAMINS NO B_6	0	21	8	13	28	63	11	172*
			2	31	74	23	172	315	412	293*
IV	14	PREGNANT, VITAMINS WITH B_6	0	23	9	16	25	56	12	163*
			2	32	42	20	60	116	94	319*
V	9	POST-PARTUM	0	19	12	13	13	18	11	65
			2	45	132	46	133	135	95	102

† The units are in micromoles of metabolite per 24 hours except for the dose of L-tryptophan which is 2.0 g (9,800 μmoles). The abbreviations are: oAH, *o*-aminohippuric acid; KA, kynurenic acid; ACK, acetylkynurenine; KYN, kynurenine; HK, 3-hydroxykynurenine; HAA, 3-hydroxyanthranilic acid; XA, xanthurenic acid; PYR, N-methyl-2-pyridone-5-carboxamide; B_6, pyridoxal phosphate or pyridoxine hydrochloride.

* Pyridone values are elevated because of 15 mg (123 μmoles) of niacinamide present in the multivitamin supplement. The average increase in pyridone excretion after multivitamin supplementation was 69.5 μmoles, which accounted for 56.5 per cent of the niacin given.

TABLE II

Results of statistical comparison (t test) of the yield of metabolites excreted after tryptophan (post-tryptophan value minus basal value) by various experimental groups *



GROUPS COMPARED	o-AH	KA	ACK			XA	PYR
I-II	NS	NS	II>I	II>I	II>I	II>I	II>I
II-III	NS	NS	NS	NS	NS	NS	NS
III-IV	NS	III>IV	NS	III>IV	III>IV	III>IV	NS
I-IV	I>IV	I>IV	NS	IV>I	IV>I	NS	IV>I
I-V	NS	V>I	V>I	NS	NS	V>I	NS
II-V	V>II	NS	NS	NS	NS	II>V	II>V
IV-V	V>IV	V>IV	V>IV	NS	NS	NS	IV>V

* The group numbers and abbreviations are those used in Table I. Where a difference is indicated, the p value is less than 0.05, indicating that such a difference would be expected to occur by chance fewer than 5 times in 100.

and pyridone and significantly lower levels of kynurenic acid. No significant differences occurred in basal excretion levels between control subjects (Group I) and the postpartum subjects (Group V). Similarly, administration of vitamins to Groups III and IV did not alter the basal level of excretion significantly, except for increases in pyridone levels due to the additional niacin in the vitamin preparation.

In comparing the yield of metabolites after tryptophan (post-tryptophan value minus basal value), a number of significant differences were observed between groups (Table II). The pregnant subjects of Group II excreted significantly more kynurenine, acetylkynurenine, hydroxykynurenine, xanthurenic acid, and pyridone than did the control subjects (Group I). Kynurenic acid and *o*-aminohippuric acid levels were unchanged. The multivitamin supplement lacking pyridoxine had no effect on the excretion of tryptophan metabolites by pregnant subjects, as shown by a comparison of Groups II and III. Addition of pyridoxine to the supplement (Group IV) resulted in a significant decrease in the yield of kynurenine, hydroxykynurenine, kynurenic acid and xanthurenic acid as compared with levels excreted by Group III. However, kynurenine, hydroxykynurenine and pyridone levels in Group IV still remained significantly higher than those of the controls (Group I), xanthurenic acid was no different from controls, while kynurenic acid and *o*-aminohippuric acid levels were significantly lower than those of the controls.

Comparison of Group II with postpartum subjects (Group V) showed that yields of xanthurenic acid and pyridone were significantly higher in Group II than in Group V, whereas *o*-aminohippuric acid was significantly lower in Group II. Although there were no significant differences in basal excretion levels between Groups I and V before tryptophan, after the test dose, the postpartum subjects excreted significantly more acetylkynurenine, kynurenic acid and xanthurenic acid than did the control subjects. The data suggest that the metabolism of the postpartum subjects had not returned to normal at the time they were studied. That the differences observed between postpartum subjects (Group V) and nonpregnant controls (Group I) were not due to biased sampling of these groups during specific phases of their menstrual cycles was shown by separate studies of seven subjects at specific times during their cycles (Table III). With the exception of significantly decreased ($p \leq 0.05$) excretion of hydroxykynurenine postmenstrually, no other significant variations could be associated with the stage of the menstrual cycle. Thus, the differences in tryptophan metabolism observed in the postpartum subjects were probably related to their postpartum metabolic and nutritional condition.

It is of interest that the yield of pyridone after tryptophan in all the pregnant groups was significantly higher than in either of the nonpregnant groups. Expressed as a percentage of the administered tryptophan, the conversion to pyridone was 0.46 and 0.79 for nonpregnant Groups I and V and was 1.72, 1.57 and 2.20 for pregnant Groups II, III and IV, respectively. Thus, even in the apparent vitamin B_6 deficiency in Groups II and III, the yield of pyridone from tryptophan was about three times that observed in Groups I and V and administration of pyridoxine in Group IV did not significantly alter the pyridone excretion.

Table I shows that although kynurenine was elevated in Groups II, III and IV, the excretion of kynurenic acid was not significantly elevated and was actually lower in Group IV. This is in contrast to the high levels of xanthurenic acid associated with high levels of hydroxykynurenine.

TABLE III

The average urinary excretion of tryptophan metabolites by 7 nonpregnant women at various stages of their menstrual cycles *

Stage of cycle	Tryptophan	oAH	KA	ACK	KYN	HK	XA	PYR
Postmenstruation	0	19	14	9	9	11†	7	97
	2	38	48	12	19	20‡	24	154
Ovulation	0	20	14	9	9	20	7	82
	2	40	47	14	18	37	27	122
Premenstruation	0	22	16	9	10	20	9	111
	2	35	55	13	34	45	29	144

* The abbreviations used are the same as in Table I. Zero and 2 refer, respectively, to pre- and post-tryptophan loading with 2.0 g of L-tryptophan.

† Significantly lower ($p < 0.05$) than the corresponding value in ovulation or premenstruation periods.

‡ Significantly lower ($p < 0.05$) than the value found in the ovulation period. The average post-tryptophan value of HK is quite different between the post- and premenstruation periods, but a large variance in the latter values precluded statistical significance.

The ratio of hydroxykynurenine to kynurenine changed only slightly throughout Groups I to V, whereas the ratio of xanthurenic acid to kynurenic acid changed 12-fold.

In addition to the 14 subjects described above, the tryptophan metabolism of 14 other normal pregnant women was studied under conditions identical with those used for Group II (no vitamin supplementation). Although the results were entirely comparable with those obtained from Group II, they were not included in Table II since these subjects were not studied subsequently in the manner described for Group II.

In Figure 1 is shown graphically the average post-tryptophan excretion levels of nonpregnant and pregnant women receiving no vitamin supplements, and for comparison, the excretion patterns of a male subject who received deoxypyridoxine and isoniazid at different periods (27). The excretion pattern of the pregnant group is noticeably different from that of the controls and differs from the patterns observed with deoxypyridoxine and isoniazid chiefly in that pregnant subjects excreted larger amounts of pyridone than did subjects with either of the drug-induced abnormalities.

DISCUSSION

The quantitative results presented above confirm the findings of elevated excretion not only of xanthurenic acid (14–16), but of other tryptophan metabolites by pregnant subjects given loading doses of tryptophan (17). The present quantitative results show that pregnant women not supplemented with pyridoxine excrete significantly elevated amounts of kynurenine, acetylkynurenine, hydroxykynurenine, xanthurenic acid and pyridone after tryptophan loading. In addition, elevated excretion of kynurenine, hydroxykynurenine and xanthurenic acid occurred even in the basal urine before tryptophan supplementation. It was reported that administration of 5 to 10 mg of pyridoxine was sufficient to restore xanthurenic acid excretion to nonpregnancy levels (16). In the present study, administration of 6 mg per day for 12 days before tryptophan loading lowered the xanthurenic acid excretion level to that of controls, but did not completely restore kynurenine, hydroxykynurenine or pyridone to normal nonpregnancy levels. Administration of a vitamin mixture lacking pyridoxine had no detectable effect on urinary levels of tryptophan metabolites. Similarly, Zartman, Barnes and Hicks (28) reported that aspirin or niacin did not alter the elevated xanthurenic acid excretion of pregnant subjects.

Since *o*-aminohippuric acid is the chief urinary metabolite of anthranilic acid in humans (29), its level of excretion should provide a guide to the kynureninase activity *in vivo*. In livers from pyridoxine-deficient animals, a decrease in kynureninase activity was observed (9, 10). In humans with a functional pyridoxine deficiency induced by isoniazid or deoxypyridoxine (Figure 1), the excretion of *o*-aminohippuric acid was unchanged or reduced even when high levels of

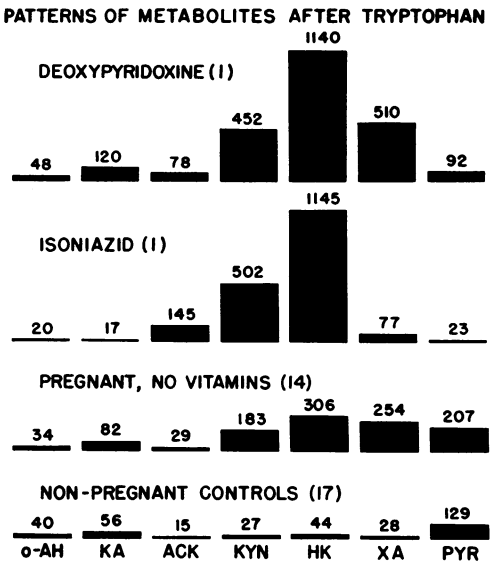


FIG. 1. LEVELS OF URINARY EXCRETION OF TRYPTOPHAN METABOLITES IN THE 24 HOURS FOLLOWING AN ORAL DOSE OF 2.0 G OF L-TRYPTOPHAN. The abbreviations are the same as those used in Table I. The number above each bar represents micromoles of metabolite excreted; the number of subjects in each group is given in parentheses.

kynurenine were excreted, but excretion was normal when the diet was supplemented with pyridoxine (27). In contrast, the low excretion of *o*-aminohippuric acid by the pregnant subjects in this study was not increased by pyridoxine supplements (Table I), suggesting that perhaps hormonal factors were in some way inhibiting the kynureninase activity. The excretion of *o*-aminohippuric acid post partum was restored to normal levels even though there was some evidence that these subjects were not normal with respect to excretion of other metabolites. These data suggest the possibility that the kynureninase activity may be more directly affected by the hormonal changes of pregnancy than by the state of pyridoxine nutrition in these subjects.

Similarly, the increased excretion of kynurenine, hydroxykynurenine and xanthurenic acid by pregnant subjects without a corresponding increase in excretion of kynurenic acid suggests that kynurenine transaminase activity is regulated by the hormonal status of pregnancy in such a way that production of xanthurenic acid is favored over kynurenic acid even though high levels of both precursors are present. This suggests the possibility that kynurenine transaminase and hydroxykynurenine transaminase might be different enzymes under different hormonal regulation. Mason and Gullekson (30) reported that certain estrogen conjugates inhibited the reactivation of resolved kynurenine transaminase and phosphorylase. Endocrine regulation of other transaminases was demonstrated by Rosen, Roberts and Nichol (31), who showed that treatment of rats with glucocorticoids resulted in marked increases in glutamic-pyruvic transaminase activity in tissues with negligible changes in glutamic-oxaloacetic transaminase activity. It was suggested that these enzyme changes were primarily responsible for gluconeogenesis initiated by these steroids.

In previous studies (27) abnormal tryptophan metabolism was induced by administration of isoniazid or deoxypyridoxine and these abnormalities were completely reversed by pyridoxine supplements, thus suggesting a functional pyridoxine deficiency. In such subjects, pyridone yields were usually below normal. Similarly, the effect of dietary pyridoxine deficiency in rats was to lower the extent of conversion of tryptophan to niacin (32, 33). This is in contrast to the relatively high efficiency of the pregnant subjects in converting tryptophan to niacin even though they were apparently deficient in vitamin B_6 as indicated by elevated excretion of tryptophan metabolites. Supplementation of these subjects with pyridoxine, while decreasing the excretion of other tryptophan metabolites, had no detectable effect on the efficiency of the tryptophan to niacin conversion. This suggests that the increased efficiency of conversion found in pregnancy is not due to pyridoxine deficiency as such, but is probably related to the endocrine changes associated with pregnancy. Similar results were reported by Wertz, Lojkin, Bouchard and Derby (34), who found that pregnant subjects excreted increased levels of niacin metabolites. Various reports in the literature have indicated the influence of endocrine organs on tryptophan metabolism. Civen and Knox (35) have studied the effect of cortisone on the activity of tryptophan pyrrolase, and Chiancone, Ginoulhiac, Mainardi and Tenconi (36) reported that ovariectomy or hypophysectomy of rats caused increased excretion of xanthurenic acid and that adrenalectomy caused a decrease. The report by

Mehler, McDaniel and Hundley (37) suggests that an adrenal mechanism exists for the regulation of hydroxyanthranilic acid conversion to nicotinic and picolinic acids. The increased efficiency of niacin synthesis observed in pregnancy could very well be mediated through hormonally regulated changes in the relative amounts of nicotonic, picolinic and quinolinic acids produced.

While ample evidence exists demonstrating altered tryptophan metabolism in normal pregnancies, the present data suggest that the pattern of excretion of tryptophan metabolites in pregnancy is different in several respects from the pattern observed in vitamin B_6 deficiency. Those differences are probably a result of the endocrine changes associated with pregnancy, and the overall excretion pattern of tryptophan metabolites observed is probably the result of both a vitamin B_6 deficiency and endocrine changes.

SUMMARY

The excretion of the tryptophan metabolites—kynurenine, kynurenic acid, acetylkynurenine, hydroxykynurenine, xanthurenic acid, *o*-aminohippuric acid and N-methyl-2-pyridone-5-carboxamide—was measured quantitatively in the urine of nonpregnant women, pregnant women without and with vitamin supplementation, and in the same subjects post partum.

The urinary excretion patterns of metabolites suggested a vitamin B_6 deficiency in pregnancy, but the patterns were modified probably by the normal hormonal changes associated with pregnancy. During pregnancy there was an increase in excretion of xanthurenic acid with no change or a decrease in the excretion of kynurenic acid, even though large amounts of kynurenine and hydroxykynurenine were excreted. The pyridone metabolite of niacin (N-methyl-2-pyridone-5-carboxamide) was also excreted in larger amounts by pregnant subjects, suggesting increased efficiency of niacin synthesis from tryptophan during pregnancy. Pyridone excretion remained high even after pyridoxine supplementation had significantly lowered the excretion of xanthurenic acid, hydroxykynurenine and kynurenine. Administration of vitamins other than pyridoxine had no detectable effect on excretion of metabolites. Subjects studied at various intervals post partum still excreted somewhat elevated levels of several metabolites, suggesting that their metabolism had not completely returned to normal.

Normal nonpregnant women, when tested just before or just after menstruation, or during ovulation, had normal urinary levels of tryptophan metabolites, with the exception of hydroxykynurenine, which was significantly decreased after menstruation.

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