

The effect of oral contraceptives on the apparent vitamin B₆ status in some Sudanese women

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1. In vitro activation of erythrocyte aspartate aminotransferase (EC 2.6.1.1) activity by pyridoxal phosphate was used to assess vitamin B₆ nutritional status in forty Sudanese women taking combined, low-dose oral contraceptives (oestrogen-progestogen; OC) and in thirty healthy, non-pregnant women not taking OC.
2. Fourteen (35%) out of forty OC users showed apparent vitamin B₆ deficiency.
3. Side-effects associated with OC were more common among the apparently vitamin-B₆-deficient OC users than among OC users and non-OC users not deficient in vitamin B₆.

Rose (1966) reported that oral contraceptive (OC) users frequently show signs of abnormal tryptophan metabolism which are similar to those observed in vitamin B₆ deficiency. Subsequently many workers have attempted to show vitamin B₆ deficiency among OC users (Rose *et al.* 1972, 1973*a, b*; Salkeld *et al.* 1973; Adams *et al.* 1974; Miller *et al.* 1974; Prasad *et al.* 1975; Shane & Contractor, 1975; Pitkin, 1976; Rose, 1978). Some workers have even suggested supplementing the diet of women using OC with vitamin B₆ (Applegate *et al.* 1979). However, Smith *et al.* (1975) reported no difference in vitamin B₆ status between OC users and non-users.

Previous studies have shown that the tryptophan load test may not be a valid index of vitamin B₆ nutritional status in women receiving oestrogens because oestrogen-conjugates inhibit kynureninase (EC 3.7.1.3) independently of vitamin B₆ status (Rose, 1966; Bender & Wynick, 1981).

The present study was undertaken to determine if erythrocyte aspartate aminotransferase (EC 2.6.1.1; AsT) activation by pyridoxal phosphate (PLP) could be used to assess the vitamin B₆ nutritional status in OC users in Sudan.

SUBJECTS

A total of seventy Sudanese women attending family planning clinics of Khartoum Civil Hospital and Buluk Hospital of Omdurman were selected for this study. Forty were taking a combined, low-dose OC pill (oestrogen 50 µg and less: Noriday, Eugynon 30, Neogynon 30, Minovlar or Nordette) for at least 6 months. The control group of thirty women was not using OC. The age range of both groups was between 18 and 45 years. Women taking OC were also matched for parity with the control group. The last pregnancy of women in the control group was completed at least 6 months before the study. A complete medical history and physical examination was conducted with special attention to personal and family history of hypertension, obesity, and method of contraception and use of steroids within the previous 6 months. Complications of OC were monitored by an experienced physician using subjective medical history reports with rating scales as well as objective clinical assessment of changes in indices such as body-weight and blood pressure. Symptoms of depression, such as feelings of misery, irritability, disturbance of sleep, loss of appetite and loss of interest, were rated in all women interviewed at the end of the study period. Depression was diagnosed among sixteen out of the forty women using OC and

three out of the thirty control women. An increase of 10% or more in body-weight, 10 mmHg in diastolic blood pressure or 20 mmHg in systolic blood pressure over the 6-month study period was considered significant. The dietary history revealed that both groups belonged to the middle socio-economic class with an adequate intake of foods that contained vitamin B₆.

METHODS

Blood (5 ml) was collected from each subject in the study, into a heparinized tube, transferred to the laboratory in packed ice and the erythrocytes separated. The packed cells were sampled and deep-frozen (-16°) until assayed for AsT activity within 2 weeks. Vitamin B₆ status was assessed as described by Bayoumi & Rosalki (1976) where the activity of AsT is increased on in vitro addition of PLP. The enzyme activity with and without PLP is expressed in units (U)/g haemoglobin (Hb). The percentage in vitro activation has been suggested as an indirect measure of vitamin B₆ content of erythrocytes. The higher the activation, the greater the vitamin B₆ deficiency. The percentage activation was calculated as

$$\text{Percentage activation} = \frac{\text{activity with PLP} - \text{activity without PLP}}{\text{activity without PLP}} \times 100$$

RESULTS

The upper limit of erythrocyte AsT activation by PLP for the normal healthy adult Sudanese population was found to be 120% (mean and 2 SD) (Zein, 1984). This was used as the reference and values above 120% were considered as indicative of apparent biochemical vitamin B₆ deficiency (Fig. 1).

The results of erythrocyte AsT activation by PLP in forty Sudanese women using OC and thirty control women not using OC are shown in Table 1. The mean percentage activation in OC users, as evaluated by Student's *t* test, was significantly higher than that for the control group ($P < 0.01$). Fourteen (35%) of the forty women using OC were apparently vitamin-B₆-deficient, with percentage AsT activation ranging between 120 and 438%. In the control group only one had 138% activation, otherwise all had lower than 101% activation (range 25–138%). Among the OC users, the fourteen women who were apparently vitamin-B₆-deficient had higher incidences of depression, fatigue, headache and nausea compared with the remaining OC users and non-OC users who were not vitamin-B₆-deficient (Table 2).

DISCUSSION

Women using OC are known to develop functional and apparent biochemical manifestations similar to those observed with vitamin B₆ deficiency and our observations are in agreement with those earlier findings (Rose *et al.* 1973*a, b*; Adams *et al.* 1973; Nobbs, 1974), even though a low dose of OC was used by women in this study. The depression and other symptoms and signs usually associated with the use of OC were also observed in Sudanese women and the incidence of such symptoms and signs was more frequent in the OC users who had biochemical evidence of vitamin B₆ deficiency.

No satisfactory explanation has been offered as to the mechanism by which vitamin B₆ deficiency is induced in women using OC. Adams *et al.* (1973) suggested that an increased metabolism of tryptophan through the nicotinic acid ribonucleotide pathway increases the requirement for pyridoxal phosphate. However, there is little evidence of increased metabolism of tryptophan in response to the administration of oestrogens. Bender *et al.* (1983) have shown that under a variety of conditions tryptophan oxygenase (tryptophan

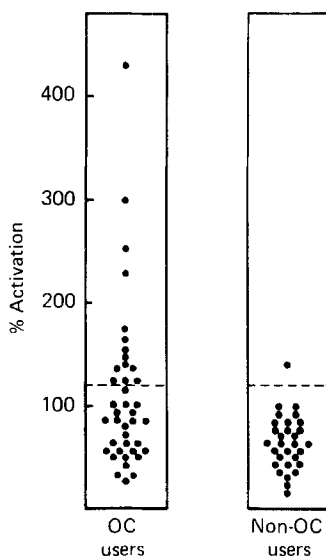


Fig. 1. Vitamin B₆ status in a group of Sudanese women using oral contraceptives (OC) and a group of healthy, non-pregnant non-OC users indirectly assessed by the pyridoxal phosphate activation of erythrocyte aspartate aminotransferase (EC 2.6.1.1). Dotted lines indicate upper limit of normal (mean and 2SD) for the healthy adult Sudanese population.

Table 1. Activation with pyridoxal phosphate of erythrocyte aspartate aminotransferase (EC 2.6.1.1) in a group of forty Sudanese women using oral contraceptives (OC) and a group of thirty healthy non-pregnant non-OC users

(Mean values and standard deviations)

Group	Mean activity of erythrocyte aspartate aminotransferase (U/g haemoglobin)				Mean percentage activation	
	Without coenzyme		With coenzyme		Mean	SD†
	Mean	SD	Mean	SD		
OC users	3.09	1.29	6.01	2.08	94*	80
Healthy non-OC users	3.47	1.19	5.65	2.01	63	55

* Significantly different from healthy non-OC users ($P < 0.01$).

† SD for mean percentage activation was calculated from individual percentage activation values.

2, 3-dioxygenase; EC 1.13.11.11) is not induced by oestrogens, and even the acute administration of oestradiol does not result in increased oxidative metabolism of tryptophan (Bender, 1983). Indeed, Bender & Wynick (1981), Bender *et al.* (1982) and Bender (1983) have suggested that oestrogens do not deplete vitamin B₆ but rather that the effects on tryptophan metabolism are due to competitive inhibition of kynureninase by oestrogen conjugates, and hence an artefact of the tryptophan load test used to assess vitamin B₆ status. Mason *et al.* (1969) and Prasad *et al.* (1975) have previously suggested that oestrogen conjugates may competitively inhibit PLP binding to certain enzymes, and recently Bender & Wynick (1981) have shown uncompetitive inhibition of PLP binding to kynureninase by

Table 2. *Some of the symptoms and signs associated with the use of oral contraceptives (OC) observed in a group of forty Sudanese women using OC and in a group of thirty healthy, non-pregnant, non-OC users**

Symptoms and signs	Number of OC users with symptoms or signs						Number of non-OC users with symptoms or signs (n 30)	
	Showing apparent vitamin B ₆ deficiency (n 14)		Not vitamin-B ₆ -deficient (n 26)		Total (n 40)			
	No.	%	No.	%	No.	%	No.	%
Symptoms†:								
Symptoms of depression	11	79	5	19	16	40	3	10
Headache	8	57	5	19	13	33	2	7
Migraine	1	7	1	4	2	5	0	0
Fatigue	7	50	4	15	11	28	3	10
Nausea	11	79	11	42	22	55	1	3
Vomiting	2	14	1	4	3	8	0	0
Itching	3	21	3	12	6	15	0	0
Signs†:								
Increased body-wt	5	36	12	46	17	43	4	13
Increased blood pressure	5	36	8	31	13	33	0	0

* The number of women with specific symptoms and signs was often too small for statistical analysis.

† More than one symptom or sign was often observed per individual.

physiological oestrogen conjugate concentrations. However, Bender *et al.* (1982) showed that although tryptophan metabolism was inhibited by the administration of oestrogens, other indices of vitamin B₆ status were not affected. In a recent study, Bior & Bender (1986) have established that AsT activity and binding to PLP are not inhibited by oestrogen conjugates, suggesting thereby that evaluation of vitamin B₆ deficiency by PLP activation of erythrocyte AsT is a valid method, though indirect, for assessing the nutritional status of vitamin B₆ among women using OC.

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