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ORAL CONTRACEPTIVE USE AND VITAMIN NUTRITION STATUS OF MALNOURISHED WOMEN—EFFECTS OF CONTINUOUS AND INTERMITTENT VITAMIN SUPPLEMENTS

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SUMMARY

Indian women from a low income group who had used oral contraceptives containing either 30 or 50 μg ethynyl oestradiol and 0.15 mg D-norgestrel for 12 cycles, had significantly elevated urinary excretion of xanthurenic acid after a tryptophan load. A third of the women had pyridoxine deficiency as judged by the erythrocyte asparate aminotransferase (EAspAT) test and the majority of women showed riboflavin deficiency as judged by the erythrocyte glutathione reductase (EGR) test. Administration of multivitamins containing 2 mg riboflavin and 10 mg pyridoxine daily, or twice the dose for only the 7 "non hormone" days in the cycle, corrected the abnormality in tryptophan metabolism. Enzymatic tests showed an improvement with regard to coenzyme saturation, but a number of women continued to be in the deficient range even after 6 months of supplements. This was partly due to increase in apoenzymes EGR and EAspAT. Several women developed glossitis despite the vitamin supplements. The usefulness of routine schedules of vitamin supplements to women using oral contraceptives remains equivocal.

INTRODUCTION

Reports from both developed and developing countries show that the use of combination type of oral contraceptives (OC) containing 50 μg or more of oestrogen and 0.25 mg or more of progestogen leads to biochemical changes suggestive of deterioration in vitamin status. Serum levels of vitamin A rise but experiments in animals suggest that the increase may be due to mobilisation of the vitamin from the liver with consequent depletion of liver reserves. These observations raise a number of questions relating to the molecular mechanisms involved, the clinical implications in terms of established and non-established effects of vitamin deficiency, the need for supplementation and dose and schedule required, and the possibility of mitigating these changes by reducing the dose of the hormones or altering the oestrogen to progestogen ratio.

Experiments in women and female rats have suggested that OC alter the vitamin economy by increasing the concentration of specific apo proteins such as carrier proteins (retinol binding protein) [1, 2], tissue binders (folate binders) [3], and apo enzymes (flavo proteins, pyridoxal phosphate dependent enzymes) [4, 5]. This leads to an increased requirement for the vitamins, and their redistribution between tissues and between functions within a tissue. For instance, increase in retinol binding protein results in a shift of vitamin A from liver into blood [1] whereas an increase in certain flavoproteins in the liver leads to a rise in liver riboflavin and a fall in blood riboflavin [4]. In the case of folate, OC appear to reduce the half-life of the labile pool of folate which is in equilibrium with dietary folate, but increase the half-life of the stable pool of folate which is in equilibrium with the bound folate in liver. The former increases urinary excretion of folate, whereas the latter raises liver folate and decreases serum folate [6].

Steroids can also activate or inhibit enzyme activities selectively. Thus the increase in urinary excretion of xanthurenic acid and other tryptophan metabolites following a tryptophan load, seen in OC treated women is due to an increase in the activity of tryptophan oxygenase, the first enzyme of the tryptophan niacin pathway, and inhibition of the pyridoxal phosphate dependent enzyme-kynureninase by oestrogen metabolites [5]. It appears therefore that OC steroids alter vitamin economy through their effects on protein metabolism and enzymes. Mechanisms underlying alterations in vitamin C and vitamin B_{12} status have yet to be investigated.

To obtain answers to questions concerning the biochemical and clinical implications of OC use particularly in malnourished women, the World Health Organization's Special Programme for Research in Human Reproduction recently supported a study in India (Hyderabad and Bombay) and Thailand (Chiangmai) in which the effects of OC were examined in women of low income groups known to be of poor nutritional status. In the same study, the effect of pills containing either 50 or 30 μg oestrogen and

Table 1. Effect of oral contraceptives containing 30 or 50 μg ethynyl oestradiol (EE) and 0.15 mg d-norgestrel on urinary xanthurenic acid (XA) excretion after 2 g tryptophan load

	Pill preparation		
	Control	30 μg EE	50 μg EE
XA μmol/8 h	22.6 ± 8.6* (14)	103.4 ± 9.9† (22)	103.4 ± 18.6† (15)

* Values are mean ± S.E.
† $P < 0.001$ compared to control.
Figures in parentheses indicate number of subjects.

0.15 mg laevo-norgestrel were compared. The details of this study design have been previously [10] described.

Preliminary data generated in this study suggest that amongst the vitamin deficiences, Hyderabad women show a very high prevalence of riboflavin deficiency as judged by the erythrocyte glutathione reductase (EGR) test. Their serum vitamin A and RBC folate levels also tended to be low. Pyridoxine deficiency judged by erythrocyte aspartate aminotransferase test (EAspAT) was seen in 25–30% women, but thiamin deficiency as judged by erythrocyte transketolase test (ETK) was not common. This may be due to the fact that thiamin requirement is related to calorie intake and the calorie intake of these women was low.

Both 30 and 50 μg ethynyl oestradiol, pills produced deterioration in the riboflavin status of women whose initial status was adequate or low (EGR activity coefficient (AC) less than 1.4), but in more severely deficient women (EGR-AC greater than or equal to 1.4) OC effects were not clear. Perhaps they were masked by the already existing severe deficiency. A similar trend was seen with regard to pyridoxine status as judged by the EAspAT test. However, a majority of women showed a marked increase in the post tryptophan load urinary excretion of xanthurenic acid, within 1–3 months of OC use, suggesting a relative B_6 deficiency. The magnitude of increase was similar with 30 μg and 50 μg oestrogen pills (Table 1).

While the analysis of all these data generated in this study is underway, other investigations have been initiated by WHO and the Indian Council of Medical Research to assess the clinical implications of OC use in relation to established and non-established effects of vitamin deficiencies. We have also initiated studies on vitamin supplements to OC treated women, and I will now present some of our preliminary data on two different schedules of vitamin supplements given to OC treated women.

EXPERIMENTAL

Twenty four healthy young women who had been using the combination type OC for one year, were randomly allocated to "complete vitamin supplementation group" or "intermittent vitamin supplementation group". The former received a multi-vitamin tablet containing thiamin 3 mg, riboflavin 2 mg, nicotinamide 20 mg, vitamin C 30 mg, vitamin A 5000 i.u., plus 10 mg pyridoxine daily. The latter group received twice this dose of vitamins but only for the 7 days in the cycle when the pill was not taken. The women continued to use the OC along with the vitamin supplements.

Blood samples were analysed before giving the vitamin supplements and at convenient intervals thereafter (2–3 months, 4–5 months, and 6–8 months). Blood was always collected between days 15–25 of the menstrual cycle, and analysed for erythrocyte glutathione reductase activity (EGR) and its *in vitro* activation with FAD (EGR-AC) for riboflavin status, and erythrocyte aspartate aminotransferase activity (EAspAT) and its *in vitro* activation with pyridoxal phosphate (EAspAT-AC) for pyridoxine status. In a few women, pyridoxine status was also assessed by measuring urinary excretion of xanthurenic acid after a tryptophan load. Impaired tryptophan metabolism is one of the earliest and most sensitive metabolic effects of OC use.

Data were analysed by paired *t*-test at each time point, and values expressed as changes in relation to values before the vitamin supplements were taken. Cut-off points for judging vitamin status were as follows.

Riboflavin—EGR–AC	< 1.25	acceptable
	1.25–1.4	marginal–low
	> 1.4	deficient
Pyridoxine—EAspAT–AC	< 1.7	acceptable
	≥ 1.7	deficient

RESULTS

Data on the effects of vitamin supplements on riboflavin status of OC users as judged by the EGR-test are presented in Tables 2 and 3. Intermittent as well as complete supplementation regimes produced significant increases in both the basal and stimulated EGR activities. The EGR-AC tended to decrease. However data on distribution of deficient low and normal subjects presented in Tables 2B and 3B, show that 50–70% of the subjects continued to have an ERG-AC greater than 1.4, even after 6 months of supplementation indicating a persistence of riboflavin deficiency. In the continuous supplementation group, 3 subjects had an AC less than 1.25 before vitamin supplements (Table 2B). In these subjects the AC showed a slight increase after supplementation, because of a marked increase in total enzyme activity.

In Tables 4 and 5 are presented data on pyridoxine nutrition status as judged by the EAspAT test. Continuous as well as intermittent pyridoxine supplements produced an increase in both basal and stimulated enzyme activities. The AC tended to fall. However data presented in Tables 4B and 5B show that 30–50% of subjects continued to have an AC greater

Table 2. Effect of continuous vitamin supplementation on riboflavin mutrition status of OC users

A. EGR test

Duration of supplementation	Changes from values prior to supplements (mean ± S.E.)		
Months ⟶	2	4–5	6–8
EGR activity − FAD	+21.0 ± 7.0*	+32.2 ± 19.0	±40.7 ± 12.0†
EGR activity + FAD	+18.7 ± 15.6	+17.7 ± 17.1	+31.7 ± 9.4†
EGR-AC	−0.22 ± 0.16	−0.36 ± 0.19	−0.15 ± 0.16
Number of subjects	9	6	10

* $P < 0.01$.
† $P < 0.001$ compared to values prior to supplements.

B. Distribution of normal low and deficient subjects

	Duration of supplementation (months)			
EGR-AC	Initial	2	4–5	6–8
	Number of subjects			
<1.25 (normal)	3	0	2	5
1.25–1.4 (low)	1	1	2	1
>1.4 (deficient)	8	8	2	4
Total	12	9	6	10

than 1.7, even 6 months after supplementation indicating vitamin B_6 deficiency.

Before taking the vitamin supplements, all women had high post tryptophan load urinary excretion of xanthurenic acid. After continuous as well as intermittent supplementation with vitamins, there was a significant and similar improvement, which continued beyond 6 months of treatment (Table 6).

Table 3. Effect of intermittent vitamin supplementation on riboflavin status

A. EGR test

Duration	Change from values prior to supplements (mean ± S.E.)		
Months ⟶	2	4–5	6–8
EGR activity − FAD	+29.9 ± 7.8‡	+36.5 ± 9.9‡	+34.4 ± 7.2‡
EGR activity + FAD	+23.2 ± 11.5	+36.1 ± 12.1†	+24.7 ± 9.9*
EGR-AC	−0.38 ± 0.09‡	−0.32 ± 0.13*	−0.38 ± 0.13†
Number of subjects	11	11	9

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$ compared to values prior to supplements.

B. Distribution of normal low and deficient subjects

	Duration of supplements (months)			
EGR-AC	Initial	2	4–5	6–8
	Number of subjects			
<1.25 (normal)	—	0	3	2
1.25–1.4 (low)	—	4	0	1
>1.4 (deficient)	12	7	8	6
Total	12	11	11	9

Table 4. Effect of continuous vitamin supplements on pyridoxine nutrition status

A. EAsp-AT test

Duration	Change from values prior to supplements (mean ± S.E.)		
Months ⟶	2	4–5	6–8
EAspAT activity − PLP	+22.3 ± 5.1†	+37.5 ± 14.0*	+11.6 ± 11.9
EAspAT activity + PLP	+24.7 ± 7.6*	+32.3 ± 16.3	+5.1 ± 15.7
EAspAT-AC	−0.12 ± 0.08	−0.23 ± 0.09	−0.07 ± 0.12
Number of subjects	10	7	11

* $P < 0.01$.
† $P < 0.001$, compared to values prior to supplements.

B. Distribution of normal and deficient subjects

EAspAT-AC	Duration of supplements (months) Initial	2	4–5	6–8
	Number of subjects			
<1.70 (normal)	8	6	5	6
⩾1.70 (deficient)	4	4	2	5
Total	12	10	7	11

DISCUSSION

In the present study the effects of two types of vitamin supplementation schedules, i.e. 21 days hormones, 28 days vitamins (continuous) and 21 days hormones, 7 days vitamins at twice the dose (intermittent), were tested. The practical advantages of the latter are obvious. Both treatments produced significant and sustained improvement in the altered tryptophan metabolism induced by OC.

Some of the metabolic and clinical side effects such as altered glucose tolerance and mental depression associated with OC use have been attributed to changes in tryptophan metabolism. The administration of 10 mg pyridoxine daily or 20 mg for 7 non-hormone days may therefore be considered as being beneficial.

Vitamin supplements also produced significant increases in EGR and EAspAT-activities, but due to

Table 5. Effect of intermittent vitamin supplements on pyridoxine nutrition status

A. EAspAT test

Duration	Change from values prior to supplement (Mean ± S.E.)		
Months ⟶	2	4–5	6–8
EAspAT activity − PLP	+32.2 ± 11.9*	+26.2 ± 7.7‡	+18.8 ± 5.8†
EAspAT activity + PLP	+29.1 ± 16.9	+32.9 ± 11.3†	+22.4 ± 5.9‡
EAspAT-AC	−0.24 ± 0.11*	−0.08 ± 0.11	−0.10 ± 0.13

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$ compared to values prior to supplements.

B. Distribution of normal and deficient subjects

EAspAT-AC	Duration of supplements (months) Initial	2	4–5	6–8
	Number of subjects			
<1.7 (normal)	8	9	7	6
⩾1.7 (deficient)	4	2	4	3
Total	12	11	11	9

Table 6. Effect of intermittent and continuous vitamin supplements on urinary xanthurenic acid excretion (μmol/8 h) in women using oral contraceptives

Duration	After vitamin supplements			
Months ⟶	Initial	2–3	4–5	6
Intermittent supplements	107.2 ± 10.4* (23)	58.4 ± 6.1§ (21)	50.2 ± 8.3† (5)	42.3 ± 5.4‡ (5)
Continuous supplements	106.4 ± 19.7 (10)	33.8 ± 7.7§ (10)	—	45.6 (2)

* Mean ± S.E.
† $P < 0.02$.
‡ $P < 0.01$.
§ $P < 0.001$, compared to initial.
Figures in parenthesis indicate number of subjects.

a concomitant increase in the total (stimulated) enzyme activity as well, the effects on EGR-AC and EAspAT-AC (which indicate enzyme unsaturation with coenzyme) were not marked and a number of subjects continued to have AC values in the deficient range even after supplementation. This observation is in line with our earlier findings, that the administration of 2 mg riboflavin and 10 mg pyridoxine daily to women treated with OC can prevent the deterioration in vitamins B_2 and B_6 nutritional status due to pill use, but that it cannot correct their initial state of deficiency [7].

Most nutritionists use AC values rather than basal enzyme activity values for evaluating vitamin nutrition status. Some doubts however, have been expressed about relying entirely on AC values, and it has been suggested that enzyme activity should also be considered.

The ultimate consideration should be the effects on health of the women, and the decision regarding vitamin supplements to women using OC will therefore have to depend on feed back from clinical trials with adequate sample size. In the present study quite a few women developed glossitis while receiving the vitamin supplements, and there was no substantial improvement in their sense of well being, raising doubts about the usefulness of routine administration of such supplements.

Briggs and co-workers[8] however, have found considerable improvement in clinical signs and symptoms of nutritional deficiencies, (judged by self-rating scales) in women who received vitamins along with OC compared with those who did not receive vitamins. They used higher doses of all the vitamins compared with the doses employed in the present study. Prasad *et al.*[9] have reported that OC produced significant increases in the incidence of nutritional deficiency signs in high income group women but not in the low income group women, in whom prevalence of nutritional deficiency signs was high even without OC use. Benefits of vitamin supplements were more clearly apparent in women belonging to the high income group, than in those belonging to the low income group.

Thus the advantages of vitamin supplements to women using OC seem equivocal at the present time. Clearly more studies are needed. However, data presented here suggest that if future clinical trials do indicate the need for giving such supplements, an intermittent 21-day hormones, 7-day vitamins schedule can be adopted since it is as effective and more convenient than a 21-day hormone, 28-day vitamins schedule.

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