

Pyridoxal, Vitamin B₁₂ and Folate Metabolism in Women Taking Oral Contraceptive Agents

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SUMMARY

Serum pyridoxal, vitamin B₁₂, serum and red cell folate and haemoglobin levels were measured in a large group of women taking part in the Busselton population survey, to determine the effect of oral contraceptive agents on these measurements.

Serum pyridoxal levels were measured in 107 women taking oral contraceptive agents, and compared with the results obtained from 107 age-matched non-pregnant women who were not taking these agents. The mean serum levels for the two groups were not significantly different. Nine per cent of women taking oral contraceptive agents had a serum pyridoxal below the lower limit of the reference range, compared with 13% in the controls.

Blood was available from 1 067 non-pregnant women for the measurement of serum vitamin B₁₂, serum and red cell folate and haemoglobin levels; 166 of them were taking oral contraceptive agents. The group taking oral contraceptive agents had significantly lower mean concentrations of vitamin B₁₂ and serum and red cell folate. The mean haemoglobin level was the same in both groups (13.3 g/100 ml).

The low mean red cell folate suggests that the available folate pool in women taking oral contraceptive agents is significantly lowered. There were fewer women with serum folate and pyridoxal levels below the lower limit of the reference range in the group taking oral contraceptive agents than in the controls. This was no doubt due to the inclusion of a greater number of women of relatively low economic status in the controls, since oral contraceptive agents are not subsidised by the Health Service in Australia.

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Changes in pyridoxal metabolism have been reported in women taking oral contraceptive agents (OCA) and the administration of pyridoxine has given relief in some women from mental depression associated with the pill.^{1,2} Rose *et al.*³ studied the pyridoxal metabolism in 80 women taking OCA and concluded that the results in some reflected a subclinical vitamin B₆ deficiency.

Wertalik and colleagues⁴ observed a significant reduction in vitamin B₁₂ levels in 20 women taking OCA compared

with matched controls. They found that this reduction may occur within 5 months of commencing treatment with anti-ovulatory agents, and serum levels may fall to values indistinguishable from other forms of vitamin B₁₂ deficiency, although this is not accompanied by any evidence of anaemia.

Shojania *et al.*⁵ found that the mean serum folate levels for 62 women taking OCA were significantly less than those found in 24 controls. Streiff⁶ reported treating 7 young women with folate deficiency and anaemia who were taking OCA. Five of these patients responded to 250 µg folic acid daily while continuing to take OCA, and 2 responded after discontinuing OCA alone. Streiff also found that naturally-occurring folate polyglutamates were poorly absorbed in volunteers taking OCA, although the monoglutamate was utilised normally. Castrén and Rossi⁷ measured the serum folate levels in 30 women both before and during treatment with OCA, and concluded that OCA did not affect serum folic acid levels. In a similar study McLean *et al.*⁸ also concluded that treatment with OCA did not affect serum folate levels.

The measurement of serum vitamin B₁₂ and folate levels is possible by direct assay. However, the assessment of vitamin B₆ status has generally been based on indirect tests such as the measurement of xanthurenic acid and other metabolites of tryptophan excreted in the urine after an oral loading dose of tryptophan. Alternatively, pyridoxal phosphate-dependent enzymes such as aspartate aminotransferase (E.C.2.6.1.2.) can be measured.^{9,10} Since 4-pyridoxic acid is the end product of pyridoxal phosphate metabolism, the concentration of this metabolite in the urine has also been used as an index of pyridoxal status.

The relationship between these vitamins and the changes which occur in women taking OCA is a problem of considerable interest, but so far there has been no direct evidence of an absolute vitamin B₆ deficiency in women taking OCA. The recent development of a fully automated method for the measurement of serum pyridoxal¹¹ has made it possible to determine pyridoxal status as a routine. This article describes a study of pyridoxal, vitamin B₁₂ and folate levels in a large group of women taking OCA.

Vitamin B₁₂ was measured using *Euglena gracilis* as the test organism, with reference range 160 - 875 ng/litre.¹²

Serum and red cell folate was measured microbiologically using *Lactobacillus casei* as the test organism, the reference range being 2.5 - 18.5 µg/litre for serum and 115 - 600 µg/litre for red cells.¹³ Haemoglobin was measured as cyanmethaemoglobin, reference range 11.3 - 14.9 g/100 ml.

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MATERIALS AND METHODS

Blood samples for this study were obtained from women who had volunteered to take part in a population survey in the town of Busselton.

Because of the large numbers involved it was not possible to bleed the volunteers at a given time of the day, which would have been necessary to avoid diurnal variations and changes in plasma vitamin levels owing to recent meals. Volunteers were bled at 2-minute intervals throughout the day. Blood was collected into vials containing EDTA for the haemoglobin and red cell folate measurements, and into a sterile tube for serum vitamin measurements. Serum was separated for the clotted samples within 4 hours of collection and stored at -20°C until required for assay.

A total of 1126 women between the ages of 20 and 59 years were available for study; 59 of them were found to be pregnant, and were excluded, leaving 1067. Of these, 166 admitted to taking OCA for periods ranging from one month to 9 years (mean 36 months, SD 27). The type of OCA used was not recorded.

Serum pyridoxal was estimated using *Lactobacillus casei* as the test organism. The reference range of this vitamin is related to age, thus volunteers were divided into groups representing age by decades, and the results from women taking OCA were then compared with results from those in a similar age group not taking these agents.¹¹

RESULTS

Serum for the assay of pyridoxal was available from 107 women taking OCA and these were age-matched with 107 women not taking OCA. No significant difference was found between the two groups nor was there any

TABLE I. COMPARISON OF MEAN SERUM PYRIDOXAL PHOSPHATE LEVELS IN 107 WOMEN TAKING OCA AND AGE-MATCHED CONTROLS

Age (yrs)	No. in each group	Women taking		Significance
		OCA	Controls	
20 - 29	46	48,5	51,8	NS
30 - 39	28	43,2	36,5	NS
40 - 49	31	36,3	35,1	NS
50 +	2	31,5	37,0	Insufficient number

TABLE II. MEAN RESULTS OF WOMEN TAKING OCA COMPARED WITH THOSE OF WOMEN WHO WERE NOT TAKING OCA

	No.	Serum folate ($\mu\text{g/L}$)	Red cell folate ($\mu\text{g/L}$)	Serum vitamin B ₁₂ (ng/L)	Hb (g/100 ml)
Women taking OCA	166	4,6	260,9	310,0	13,3
Women not using OCA	901	5,1	292,9	363,4	13,3
Significance		$P < 0,001$	$P < 0,001$	$P < 0,001$	NS

difference between the groups at any age level (Table I).

The results of the serum vitamin B₁₂, serum and red cell folate assays and haemoglobin measurements are shown in Table II.

There was a significant difference between the mean serum vitamin B₁₂ ($P < 0,001$), mean serum ($P < 0,001$) and red cell folate levels ($P < 0,001$) of women taking OCA and those of women not taking these agents. The mean haemoglobin levels were identical in both groups.

The number of women with serum pyridoxal, vitamin B₁₂ and serum or red cell folate levels below the lower limit of the reference range is shown in Table III.

TABLE III. FREQUENCY OF LOW VITAMIN B₁₂, FOLATE AND PYRIDOXAL LEVELS IN WOMEN TAKING OCA AND IN CONTROLS

	Women taking OCA	Controls
Serum folate	9 (5,4%)	73 (8,1%)
Red cell folate	9 (5,4%)	45 (5,0%)
Vitamin B ₁₂	0	7 (0,8%)
Pyridoxal	10 (9,3%)	14 (13,1%)

Ten women taking OCA had a serum pyridoxal below the lower limit of the reference range; in 9 this was the only abnormal finding, and in the tenth it was associated with low serum and red cell folate. Fourteen women in the control group had a low serum pyridoxal; in 12 this was the only abnormal finding, and in 2 it was associated with a reduced red cell folate level.

None of the women taking OCA had a reduced serum vitamin B₁₂ concentration, compared with 7 in the control group. However, only 1 had a value below 100 ng/litre (95 ng/litre).

Eight women taking OCA had a low serum folate, and 1 had low levels of both serum and red cell folate. Sixty-five women in the control series had a low serum folate, 37 had a low red cell folate and 8 had both low serum and red cell folates.

A low serum folate (1,9 $\mu\text{g/litre}$) in 1 woman taking OCA was accompanied by evidence of a mild iron deficiency (haemoglobin 11,2 g/100 ml, MCHC 29,4%, MCV 69 fl). Three women in the control group with a low serum folate (1,6, 1,8 and 2,3 $\mu\text{g/litre}$) also had a slight reduction in haemoglobin (11,1, 11,1 and 11,2 g/100 ml). One showed evidence of iron deficiency with an MCV of 65 fl and an MCHC of 28,7%. Two women in

the control group had both low serum and low red cell folates (1,8 and 111, 2,1 and 114 $\mu\text{g}/\text{litre}$) and this was associated with a reduced level of haemoglobin (11,0 and 10,9 g/100 ml).

DISCUSSION

Because of difficulty in the direct measurement of serum pyridoxal phosphate levels, it has been usual to rely on indirect methods. Of the many tests available, the ratio of 3-hydroxykynurenine to 3-hydroxyanthranilic acid excreted in the urine after an oral loading dose of tryptophan, is thought to be a reliable indicator of an absolute vitamin B₆ deficiency.^{1,14,15} Using this criterion, Adams *et al.*¹ found that 11 of 22 women taking OCA had evidence of an absolute deficiency of vitamin B₆. It has been shown previously that tryptophan metabolism is frequently disturbed in women taking OCA, and this is characterised by elevation of the urinary excretion of xanthurenic acid and other metabolites of the tryptophan-nicotinic acid pathway.^{16,17} Luhby *et al.*¹⁸ calculated that a daily dose of 25 mg of pyridoxine would be required to correct the tryptophan metabolism in all women using OCA. This is more than 12 times the recommended dietary allowance.¹⁹ In animals the administration of oestrogenic steroids reduces the affinity for pyridoxal phosphate of two of the vitamin B₆-dependent enzymes of tryptophan metabolism, kynureninase and kynurenine transaminase.²⁰

Depression is a problem in some women taking OCA and it has been suggested that the cause of this may be a reduction of brain 5-hydroxytryptophan decarboxylase. Adams *et al.*¹ found that women with depression associated with OCA, and showing biochemical characteristics which suggested an absolute deficiency of vitamin B₆, responded to treatment with pyridoxine. In contrast, women with other urinary tryptophan metabolite abnormalities, which did not relate to an absolute deficiency of vitamin B₆, did not respond to treatment with pyridoxine. This work indicated that there were two types of depression associated with the taking of OCA, one of which was due to an absolute deficiency of vitamin B₆. It has been claimed that the symptoms of OCA-induced depression differ from those found in reactive and endogenous depression, although these may also be associated with changes in pyridoxal or tryptophan metabolism.²¹

The frequency of depression among the women seen in this series was not investigated, but Herzberg *et al.*²² found that 6,6% of women taking OCA were more severely depressed than women not taking these agents. If depression in women taking OCA is caused by an absolute deficiency of pyridoxal, then the frequency of pyridoxal deficiency in this series should have been higher in those taking OCA. This was not the case. Only 9,3% of women had an absolute deficiency of pyridoxal phosphate, compared with 13,1% in the age-matched controls. These findings indicate that pyridoxine-responsive depression in women taking OCA is not due to an absolute deficiency of pyridoxine, but rather to interference with the binding of pyridoxal phosphate to one or more

apo-enzymes. There is some evidence that depression may be the result of a decrease in brain serotonin.^{17,28} 5-Hydroxytryptophan is the immediate precursor of serotonin, and changes in tryptophan metabolism may reduce the tryptophan available for 5-hydroxytryptophan production.²⁴ This, coupled with the pyridoxal phosphate requiring 5-hydroxytryptophan decarboxylase, may account for the success reported with pyridoxine treatment in women with depression caused by OCA.^{1,2,8}

Pyridoxal phosphate plays an important role in iron metabolism and a deficiency of the vitamin has been associated with hypochromic anaemia in man²⁵ and animals.²⁶ In this study none of the women with low serum levels of pyridoxal showed any evidence of hypochromic anaemia.

Women taking OCA were found to have lower mean serum vitamin B₁₂ and serum and red cell folate levels than women not taking these agents and this difference was highly significant, $P < 0,001$.

It was interesting to find identical mean haemoglobin levels in the two groups. Women taking OCA could be expected to have a smaller menstrual blood loss and therefore an increased level of haemoglobin. However, it has been reported that fluid retention frequently occurs during treatment with oestrogens²⁷ and this would tend to reduce the haemoglobin level.

The number of women with a serum pyridoxal, vitamin B₁₂ or folate level below the lower limit of the reference range was greater among the controls than among those taking OCA. The frequency of low red cell folate levels was similar in both groups.

Decreased serum vitamin B₁₂ levels have previously been reported in women using OCA. Wertalik *et al.*⁴ found a mean level of 221 ng/litre in women taking OCA, which was 40% lower than their control mean of 372 ng/litre, compared with means of 310 and 363 ng/litre respectively in the present study. These authors found that 3 women had a vitamin B₁₂ level which was clearly in the deficient range, but this was not accompanied by anaemia, an increased polymorph lobe count or other evidence of tissue depletion.

In the present series, although there was a reduction in the mean vitamin B₁₂ level of women taking OCA, no level was found to be below the lower level of the reference range of 160 ng/litre. Serum vitamin B₁₂ levels have been found to fall progressively in pregnancy,^{28,29} but the reason for this is not clear. Hansen and Klewesahl-Palm³⁰ also found that while serum vitamin B₁₂ levels were lower in pregnant women, they were restored to the range for normal, healthy, non-pregnant women as early as one week postpartum.

The fall in the mean serum folate level in women taking OCA is very similar to that seen in pregnancy. Davis *et al.*³¹ measured the serum folate concentration in 100 pregnant women and found a mean value of 4,7 $\mu\text{g}/\text{litre}$ compared with a mean of 4,59 $\mu\text{g}/\text{litre}$ in the present series. It is known that oral contraceptives containing oestrogen analogues induce metabolic changes which simulate pregnancy.³² The reduced serum folate level in pregnancy may be due to the increased demand

made by the fetus, but this is not likely to be the only reason. In women taking OCA other causes must be sought. Streiff⁹ found that the absorption of naturally occurring folate was reduced in women taking OCA, but if this is so, the change is not sufficient to cause overt folate deficiency. In this study no attempt was made to relate serum vitamin levels to the different types and strengths of OCA used, nor to the length of time for which they had been used.

Although the reduction of vitamin B₁₂ and folate levels in women taking OCA is similar to that found in pregnancy, there is no evidence of a common mode of action to account for this. The suggestion that malabsorption of folate may be responsible for the reduced serum levels is not a wholly satisfactory answer since a deficiency of folate is seldom severe enough to cause clinical signs in women taking OCA.

The lower mean levels of serum and red cell folate in women taking OCA indicate not only a negative vitamin balance but a reduction in the body folate pool compared with the controls.

Although the mean vitamin B₁₂ and folate levels were significantly lower in the group taking OCA, the number of women with levels below the lower limit of the reference range was greater among the controls. In Australia OCA are not subsidised by the Health Service, and the group not taking OCA would be biased towards the lower end of the socio-economic scale, resulting in an increased number of subnormal results. The excess of low results in the controls has tended to reduce the difference between the mean levels in the two groups.

Reduced levels of some vitamins may have an undesirable effect on pregnancy. Teratogenicity has been reported in vitamin B₆-deficient pregnant rats,³² and the maintenance of a normal serum pyridoxal level during pregnancy may be of importance in humans. If, as suggested by Rose *et al.*,³ a number of women taking OCA develop a pyridoxal deficiency, then pregnancy following treatment with OCA may carry an increased risk. This study has shown that the frequency of pyridoxal deficiency is not increased in women taking OCA.

Although Martin and Davis³³ treated pregnant patients with reduced serum folate activity as early as the 8th week of gestation, they were unable to restore normal growth to the fetus or placenta. This was not surprising because it was already known that ovarian hormones cannot benefit a deficiency of folic acid in the same way as they can deficiencies of some other vitamins. To be effective in restoring proper growth, folic acid would have to be given very early in pregnancy or, preferably, before conception. Additional folic acid may have a beneficial effect in women wishing to conceive after treatment with OCA.

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