

Serum Folate and Cobalamin Levels in Women Using Combined Contraceptive Vaginal Ring

Folsäure- und Vitamin-B₁₂-Serumkonzentrationen bei Anwenderinnen eines vaginalen Rings zur hormonellen Kontrazeption

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Key words

- folate
- combined contraceptive vaginal ring
- hormonal contraceptives
- vitamin B₁₂

Schlüsselwörter

- Folsäure
- hormonelle Kontrazeption
- Verhütungsring
- Vitamin B₁₂

Abstract

Purpose: The aim of our study was to evaluate the effects of combined contraceptive vaginal rings on serum concentrations of folate and cobalamin in healthy users.

Material and Methods: Case-control study on cobalamin and folate status of 45 healthy female nulligravidae using a combined contraceptive vaginal ring for >3 months and 45 healthy controls. Factors interfering with vitamin metabolism were thoroughly controlled.

Results: Cobalamin and folate levels did not differ between the groups. Vegetarian diet, smoking or obesity did not have a significant influence.

Conclusions: The use of a combined contraceptive vaginal ring provides an appropriate hormonal contraception in women with pre-existing cobalamin deficiency or restrictive diet habit in order to avoid interferences between vitamin B₁₂ metabolism and exogenously applied estrogens.

Zusammenfassung

Fragestellung: Ziel dieser Studie war es, den Einfluss der Anwendung eines Verhütungsrings zur hormonellen Kontrazeption auf die Folsäure- und Vitamin-B₁₂-Serumkonzentrationen bei gesunden Anwenderinnen zu untersuchen.

Material und Methodik: Die Folat- und Vitamin-B₁₂-Serumspiegel von 45 gesunden Ringanwenderinnen wurden nach einer Anwendungsdauer von 3 Monaten mit denen eines altersentsprechenden Kontrollkollektivs unter sorgfältiger Berücksichtigung möglicher Einflussfaktoren verglichen.

Ergebnisse: Die Folat- und Vitamin-B₁₂-Serumkonzentrationen unterschieden sich zwischen Studien- und Kontrollkollektiv nicht signifikant. Es konnte kein Einfluss von vegetarischer Ernährung, Nikotinabusus oder Übergewicht nachgewiesen werden.

Schlussfolgerung: Bei fehlendem Nachweis eines negativen Einflusses auf den Folsäure- und Vitamin-B₁₂-Stoffwechsel müssen bei der Anwendung des vaginalen Rings zur hormonellen Kontrazeption keine besonderen Ernährungs- oder Supplementierungsempfehlungen berücksichtigt werden.

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Introduction

The use of hormonal contraceptives is widespread throughout the fertile period from adolescence to perimenopause [1–8]. A wide variety of formulas [9–11], application methods [12–15] and therapeutic regimes [16–22] have been developed and assessed.

The information that is available in the literature concerning the serum levels of folate (folic acid) in women using oral contraceptive pills is still somewhat conflicting. Some studies have shown diminished serum or plasma concentrations of fo-

late in users of oral contraceptives when compared to controls [23,24], whereas others have found similar levels of folate in oral contraceptive users and non-pregnant female volunteers [25–29]. These divergent observations are hard to reconcile. It should be mentioned, however, that the folate concentration in serum and, to a lesser extent, in the erythrocyte is dependent on fasting and nutritional status, eating habits and recent vitamin supplementation, and that previous studies may not have been able to control all these confounding factors. In addition, the process of handling and storage of blood samples has to be

considered. As the folate concentration within the erythrocyte exceeds the corresponding serum level, hemolysis during the pre-analytic phase may lead to elevated levels. On the other hand, it has been well documented that women using oral contraceptives show decreased intestinal resorption of folate [29], supporting the assumption that there should be a tendency towards decreased serum folate levels in women taking oral birth control pills. Differences between studies may also have been attributable to the type of progestin and the dosage of ethinyl estradiol of the oral contraceptives. Previous studies mainly referred to oral contraceptives containing 35–50 µg ethinyl estradiol per pill but during the past several years there has been a shift towards the preferential use of low dose oral contraceptives containing 20 µg [14, 17, 21].

A significant decrease in serum or plasma levels of cobalamin in women using oral contraceptives has been shown by several authors [24–26, 30, 31]. A similar effect has been observed in men treated with high doses of estrogens in order to treat prostatic cancer [32]. The suppression of serum cobalamin levels is presumably dependent on the dose of ethinyl estradiol contained in the oral contraceptives [25].

While the low dose oral contraceptives contain at least a daily dosage of 20 µg ethinyl estradiol, the combined contraceptive vaginal ring releases only 15 µg ethinyl estradiol per day [33–36]. Little information is available on the effect of combined contraceptive vaginal rings on the vitamin B₁₂ and folic metabolism. Therefore, we considered it appropriate to investigate the effects of a combined contraceptive vaginal ring on folate and cobalamin concentrations in a case-control setting and to determine the prevalence of folate and cobalamin deficiency among combined contraceptive vaginal ring users.

Material and Methods

Subjects and controls

The study included 45 volunteers who were healthy nulligravid females of reproductive age (mean ± standard deviation: 23.2 ± 4.5 years, range: 18–38 years). None of them had a history of gastrointestinal, hepatobiliary, vascular or renal disease, thyroid dysfunction, epilepsy or eating disorder, none was currently taking any medication that could possibly interfere with the folate or cobalamin metabolism or had been on vitamin supplementation during the last 3 months. The mean body mass index (± standard deviation) was 22.5 ± 2.6 kg/m² (range: 19.0–27.1 kg/m²). Obese women (BMI > 30 kg/m²) were not included. Smoking and vegetarian eating habits were permitted. All volunteers were continuously using a combined contraceptive vaginal ring (Nuva-ring®, Essex Pharma, München, Germany). This device is a flexible ring, made of ethylene vinyl acetate copolymer, 54 mm in diameter releasing a daily dosage of 120 µg etonogestrel and 15 µg ethinyl estradiol. The ring was placed intravaginally and left for three weeks followed by a ring-free week. The combined contraceptive vaginal ring was used for at least 3 months before the folate and cobalamin studies were performed. The mean duration of usage (± standard deviation) was 28.1 ± 25.2 months (range: 22–153 months). Informed consent was obtained from all women before recruitment.

45 nulligravid female volunteers of similar age served as controls (mean ± standard deviation: 24.3 ± 4.2 years, range 18–39 years) and body mass index (mean ± standard deviation: 21.2 ± 2.6 kg/m² range: 18.8–27.3 kg/m²); had not been using hormonal con-

traceptives during the last 3 months and met the above-mentioned criteria. Fasting venous blood samples were routinely drawn between 8:00 and 10:00 a.m. without visible hemolysis using dry 7.5 ml-vacutainer tubes with beads and clot activator for serum separation (Sarstedt Monovette®, Nürnberg, Germany). Samples were protected from light and kept at +4 °C up to 24 hours until the folate and cobalamin measurements were performed. If the samples were not analyzed on the day of blood collection, they were centrifuged and the supernatants were frozen at –20 °C until analysis was performed as recommended by the manufacturer.

Folate and cobalamin analysis

Serum concentrations of folate and cobalamin were determined by commercially available assays. For the measurement of folic acid, an ion-capture assay (IMx Folsäure®, Abbott, Wiesbaden, Germany) was used according to the manufacturer's instructions. The normal range for the serum concentration of folic acid was 3.1–12.4 ng/ml. Values < 2.8 ng/ml were defined as folate deficiency and those between 2.8 and 3.1 ng/ml as "subnormal folate status". The lowest level of detection of folate was 0.8 ng/ml. The intra- and interassay coefficients of variation were < 8.3 and < 9.3% respectively.

For the measurement of vitamin B₁₂, an automated intrinsic-factor coated micro-particle enzyme immunoassay (MEIA; IMx B₁₂, Abbott, Wiesbaden, Germany) was used according to the manufacturer's instructions. The normal range for the serum concentration of vitamin B₁₂ was 223–1132 pg/ml, vitamin B₁₂ deficiency was defined as values < 179 pg/ml, and those between 197 and 223 pg/ml as "subnormal vitamin B₁₂ status". The lowest level of detection of vitamin B₁₂ was 60 pg/ml. The intra- and interassay coefficients of variation were < 4.5 and 8.5%, respectively. All assays allow the storage of sera at +2–8 °C for up to 24 h or at –20 °C up to 1 month before the actual measurement.

Statistical analysis

Results for age, body mass index and duration of ring use are given in mean ± standard deviation (SD). Values for cobalamin and folate serum concentrations are expressed as median and range. The distribution of serum folate and cobalamin levels in study and control subjects was compared using Mann-Whitney U-Test, as both vitamins did not show a Gaussian normal distribution in the test of van der Waerden. Correlation between the serum concentrations of folate and cobalamin and between the levels of both vitamins and age, body mass index and duration of combined contraceptive vaginal ring use were calculated using Spearman's rank coefficient of correlation (ρ). P < 0.05 was considered to be significant.

Results

Cobalamin serum levels of women using a combined contraceptive vaginal ring (median: 571 range: 191–1140 pg/ml) were similar to those of the controls (median: 653 pg/ml, range: 201–1665 pg/ml, p = 0.53) (● Fig. 1). As shown in ● Table 1, the percentages of reduced, normal and elevated cobalamin levels did not differ significantly (p = 0.82) between the study and the control group. There was no significant difference (p = 0.72) between the serum folate concentrations in the study group (median: 8.9 ng/ml, range: 3.0–15.5 ng/ml) and in controls (median: 9.2 ng/ml, range: 2.9–16.2 ng/ml) (● Fig. 2). Furthermore, the percentages of re-

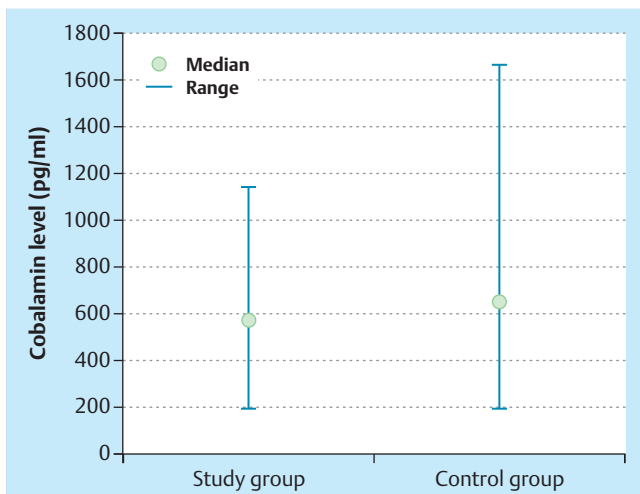


Fig. 1 Distribution of serum cobalamin concentrations in users of a combined contraceptive vaginal ring (n = 45) and controls (n = 45) showing the mean value and standard deviations.

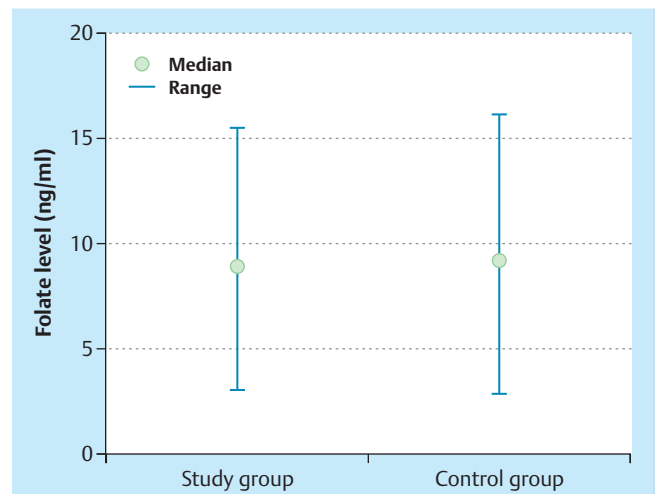


Fig. 2 Distribution of serum folate concentrations in users of a combined contraceptive vaginal ring (n = 45) and controls (n = 45) showing the mean value and standard deviations.

duced, normal and elevated folate levels did not differ significantly ($p=0.76$) between the two groups (Table 2). Seventy percent of women (71% of the study and 77% of the control group) had concentrations of both vitamins within the normal range. However, 5 women (4 study and 1 control subjects) had subnormal vitamin B₁₂ levels and 4 women (1 study and 3 control subjects) had subnormal folate levels as defined previously. Marked cobalamin or folate deficiency was identified in none of the study or control subjects.

No significant correlation was found between age and vitamin concentrations in either study group ($p=0.28$ for cobalamin, $p=0.32$ for folate, n.s.), in controls ($p=0.29$ and $p=0.22$, n.s.) or in the entire population ($p=0.19$ and $p=0.28$, n.s.). The body mass index did not correlate with the cobalamin or folate status in the two groups ($p=0.31$). A significant correlation between the two vitamins was observed neither in the study group ($p=0.38$), nor in the controls ($p=0.29$) or in the entire population ($p=0.35$). In ring users, there was no significant correlation between the duration of combined contraceptive vaginal ring use and cobalamin ($p=0.67$) or folate levels ($p=0.55$). Vegetarian eating habits (9%) and smoking (29%) had no significant impact on the serum levels of vitamin B₁₂ ($p=0.60$ and 0.16 , n.s.) and folate ($p=0.72$ and 0.57 , n.s.).

Discussion

Several previous case-control studies demonstrated decreased cobalamin and/or folate serum concentrations in users of orally administered combined contraceptives [24,26,28,30,31]. There was evidence that the influence of oral contraceptives on the metabolism of those two vitamins is dose-dependent since the impact was more obvious in formulations with 35–50 µg than in low dose pills containing 20 µg ethinyl estradiol [25]. It is presumed that the influence on vitamin B₁₂ or folic acid metabolism is associated with the intake of ethinyl estradiol contained in the pill because cobalamin concentrations were unchanged in elderly women using hormonal replacement therapy with either estradiol or conjugated equine estrogens but similar progestins as contained in modern oral contraceptives [35]. As the effect of the use of oral contraceptives on cobalamin concentration is presumably dose-dependent, it could be assumed that this depression should be either diminished or absent with a contraceptive vaginal ring releasing only a daily dosage of 15 µg ethinyl estradiol. It should be noted that we were not able to identify ring users with particularly low concentrations, fulfilling the criteria for marked cobalamin deficiency. We feel confident that our results are reliable because we were aiming at a careful and

Table 1 Percentages of normal, elevated, subnormal and deficient cobalamin status in users of combined contraceptive vaginal ring and controls.

Cobalamin levels	Deficient (< 179)	Subnormal (179–223)	Normal (223–1132)	Elevated (> 1132)
Ring users (n = 45)	0	4 (9%)	36 (80%)	5 (11%)
Controls (n = 45)	0	1 (2%)	38 (84%)	6 (13%)

All cobalamin values are in pg/ml.

Table 2 Percentages of normal, elevated, subnormal and deficient folate status in users of combined contraceptive vaginal ring and controls.

Folate levels	Deficient (< 2.8)	Subnormal (2.8–3.1)	Normal (3.1–12.4)	Elevated (> 12.4)
Ring users (n = 45)	0	1 (2%)	40 (89%)	4 (9%)
Controls (n = 45)	0	3 (7%)	37 (82%)	5 (11%)

All folate values are in ng/ml.

thorough control of variables that are known to have an influence on the cobalamin levels and metabolism. Main variation factors for vitamin B₁₂ concentrations include vegetarian eating habits [36], smoking and alcohol consumption, previous medical or surgical history, use of drugs and the ratio of weight and height [37–39]. Subjects with a cobalamin deficiency may be asymptomatic or may have hematologic, neuropsychiatric, or gastrointestinal problems [40–42]. The exact biological significance of cobalamin deficiency without clinical symptoms remains unclear [43]. There is now general consensus that periconceptional supplementation with 400 µg folate per day is effective in the prevention of neural tube defects [44–46].

Conclusion

From the observations in this study, it is unlikely that previous use of a combined contraceptive vaginal ring should interfere with folate metabolism. Women who plan to conceive should receive a daily supplementation with 400 µg folate 3 months prior to discontinuing use of oral contraceptives.

Conflict of Interest

None.

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