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

Folsäure- und Vitamin-B$_{12}$-Serumkonzentrationen bei Anwenderinnen eines Vaginalrings zur hormonellen Kontrazeption

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Key words

- folic acid
- combined contraceptive vaginal ring
- hormonal contraceptives
- vitamin B$_{12}$

Schlüsselwörter

- Folsäure
- hormonelle Kontrazeption
- Verhütungsring
- Vitamin B$_{12}$

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 B$_{12}$ 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-B$_{12}$-Serumkonzentrationen bei gesunden Anwenderinnen zu untersuchen.

**Material und Methodik:** Die Folat- und Vitamin-B$_{12}$-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-B$_{12}$-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-B$_{12}$-Stoffwechsel müssen bei der Anwendung des Vaginalrings zur hormonellen Kontrazeption keine besonderen Ernährungs- oder Supplemementierungsempfehlungen berücksichtigt werden.

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 folate 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 theses 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 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].

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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 consistent approach.

#### Table 1

<table>
<thead>
<tr>
<th>Cobalamin levels</th>
<th>Deficient (&lt; 179)</th>
<th>Subnormal (179–223)</th>
<th>Normal (223–1132)</th>
<th>Elevated (&gt; 1132)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring users (n = 45)</td>
<td>0</td>
<td>4 (9%)</td>
<td>36 (80%)</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>Controls (n = 45)</td>
<td>0</td>
<td>1 (2%)</td>
<td>38 (84%)</td>
<td>6 (13%)</td>
</tr>
</tbody>
</table>

All cobalamin values are in pg/ml.

#### Table 2

<table>
<thead>
<tr>
<th>Folate levels</th>
<th>Deficient (&lt; 2.8)</th>
<th>Subnormal (2.8–3.1)</th>
<th>Normal (3.1–12.4)</th>
<th>Elevated (&gt; 12.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring users (n = 45)</td>
<td>0</td>
<td>1 (2%)</td>
<td>40 (89%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Controls (n = 45)</td>
<td>0</td>
<td>3 (7%)</td>
<td>37 (82%)</td>
<td>5 (11%)</td>
</tr>
</tbody>
</table>

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 $B_12$ 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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