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C-reactive protein and homocysteine as predictors of cardiovascular risk associated to hormonal contraceptives use

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Abstract

Hormonal contraceptives (HC) are currently the most commonly prescribed method to prevent pregnancy. Although the composition of oral contraceptives has markedly changed over time, venous thromboembolism is the main determinant of the risk-benefit profile in HC use. High concentrations of homocysteine and C reactive protein (CRP) are linked as independent predictor factors of cardiovascular events risk and might be sensitive to hormonal changes in HC users. The aim of present study was to synthesise the available data on the relationship between CRP and homocysteine levels in HC use. We found considerable amount of evidence to support the association between increased CRP level and HC use, while the relationship with the homocysteine level is not well established. However, given the evidence linking inflammation and homocysteine to cardiovascular risk, homocysteine-folate to hormonal changes, as well as HC use to thromboembolic risk, elucidating these aspects by long term prospective studies for various types of combined HC is needed. Filling the gap of knowledge on the subject might allow the development of preventive strategies for thromboembolic risk. Demonstrating possible additional benefits of adding folic acid to HC on reducing the cardiovascular risk demands further investigation.
Keywords: *hormonal contraceptives, C-reactive protein, homocysteine, folic acid, venous thromboembolism, cardiovascular risk factors*

Introduction

Firstly introduced about 50 years ago, hormonal contraceptives (HC) currently represent the most commonly prescribed method to prevent pregnancy and are used by millions of women worldwide. While natural estrogens may have a protective effect against cardiovascular disease in young women[1], HC use was associated with increased risk of weight gain, cardiovascular disease, dyslipidemia, stroke, and venous thromboembolism (VTE)[2]. HC use explains an important part of thrombotic phenomena in premenopausal women and VTE is the main determinant of the risk-benefit profile in HC use[3].

Looking for lowering the risk associated with HC chronic use, especially VTE, composition of oral contraceptives (OC) has markedly changed over time and new technologies of delivery have emerged: lower doses of estrogens, different types of estrogens, newer selective or partial antiandrogenic progestins, and alternative routes of delivery, such as vaginal ring or transdermal patch. The safety profile of so called third generation HC compared to second generation of HC is a matter of debate. Prospective, active studies found no difference between the two classes, while observational, generally retrospective studies found a higher thrombotic risk associated with newer combinations[4]. There is insufficient data from long term, large studies to investigate thromboembolic risk for the new contraceptive combinations, and information on their effects on metabolic pathways is limited, as well[4].

Different combinations of oestrogens and progestogens in HC modify various metabolic factors such as factors involved in haemostasis, lipids and carbohydrate metabolism, and inflammation. Observational studies have revealed an association between markers of inflammation, such as C reactive protein (CRP) and HC use in healthy women, while CRP is a well known risk predictor for cardiovascular disease[5]. Low grade inflammation has been suggested to be associated with VTE[6]. Mechanisms of HC-induced CRP increase, whether this increase has pathological consequences, or the impact of higher CRP levels on VTE risk for different types of HC have not been elucidated.

Similar to CRP, homocysteine has been associated with cardiovascular risk in observational studies[7]. There are few studies investigating the hormonal modulation of homocysteine levels before menopause, and studies in women post-menopause have conflicting results[8]. Data on HC effects on homocysteine levels are also insufficient.

The aim of present study was to synthesise the available data on the relationship between CRP and homocysteine levels and HC use, which might help in understanding the mechanisms of these interactions and in finding strategies to lower the VTE risk.

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Methods

For this narrative review, we performed a PubMed literature search to identify the publications which had evaluated and observed the effect of combined oral contraceptive (COC) on CRP and homocysteine levels among healthy women. The search terms C reactive protein, homocysteine, combined oral contraceptive for title, abstract and keywords were used. The references of all the studies were manually searched for additional eligible studies. We also inspected review articles and references of other pertinent articles to identify related articles. There was no date limitation for the included studies.

CRP and hormonal contraceptives

CRP is synthesised mainly in hepatic cells under the control of interleukin-6 cytokine. CRP production is stimulated via an inflammatory pathway that begins with local injury and reaction at cellular level with activation of macrophages, monocytes, leucocytes, fibroblasts and endothelial cells in the immediate area surrounding the disturbance[9]. An increased level of CRP is widely recognised as a predictor factor for cardiovascular risk and was found as the strongest independent predictor for myocardial infarction and stroke in women[5,10]. CRP was found to be related to atherothrombosis, but the mechanism involved in the pathogenesis (proatherogenic, prothrombotic, or both), is still uncertain, although it seems more likely to be involved in plaque vulnerability, thrombus formation and vascular occlusion[11]. A prothrombotic rather than a protoatherogenic mechanism might be involved in the effects of HC as well, an idea supported by the studies showing that users of HC do not have an increased risk of myocardial injury and that the abnormalities of coagulation return to basic normal level after stopping the treatment with HC[12].

Many epidemiological studies have found an association between increased CRP levels and HC use. In a study on 12 684 healthy blood donors, OC use was found as a strong predictor for low grade inflammation (LGI), defined in the study by a CRP serum level of 3-10 mg/L, and the strongest predictor in premenopausal woman (odds ratio OR: 8.98). LGI was found in 29.9% of women using OC compared to 7.9% of women not using OC, and 6.1% in men(13). Similar proportions of LGI were found in a study on 277 young (mean age 23 years), fertile, non-obese women: LGI was found in 27.3% of third generation OC users (n=77), compared to 8.5% of non-users (n=200), OR: 4.04 (95% Confidence Interval CI: 1.99-8.18)[8]. Body mass index and waist circumference were additionally associated with OC use(13). Buchbinder S. et al. studied the association between OC, endogenous oestrogens, age, gender, smoking, BMI and serum cholesterol levels and CRP concentrations in 850 blood donors (438 males, 412 females), including 227 women using OC. CRP value was found to be influenced by overweight and OC use, the combination of the two resulting in a 6-fold increase of median CRP level in women[14]. Similar results were found in a population-based study on 2,120 subjects aged 24-39 from Finland, aimed to investigate the distribution and determinants of CRP levels: the combination of BMI + OC use was found to have the greatest influence on the variation of CRP levels. CRP serum levels higher than 3 mg/L were found in 35% of women using OC, compared to 10.3% in non-user women and 8.8% in men[15]. In a study on 1,761 men and 2,248 women aged 25 to 84 from Australia, OC use was identified by logistic regression analysis as the second strongest independent predictor of increased CRP in women (OR: 4.6, 95% CI: 3.3-6.5), after obesity (OR: 7.8, 95% CI: 5.8 to 10.6)[16]. Association between OC use and increased serum levels of CRP have been found in many other observational studies[17-23]. In most of these studies both second and third generation OC were used, but no analyses were made according to OC type. In one of the studies, involving 1,257 women (aged 24-39) the correlations between serum triglyceride level and CRP were tested separately in different COC users in accordance with progestogen content and dosage, the analysis revealing significant association only in women using a high dosage of progestogen or cyproterone[22]. The haplotypes of CRP gene had no significant association with CRP concentration in COC users, while independent effects on CRP were found in non-users. In another study higher mean values of CRP in healthy young women using OC were found for third generation OC, compared to second generation, but the results should be interpreted with caution, as the study had a low number of subjects (34 OC users, in total) and it was not a prospective study[23].

There are few studies investigating the relationship between non-oral hormonal contraceptives and CRP levels and the results are inconclusive. In a prospective study on 45 premenopausal women randomly assigned to either contraceptive vaginal ring (CVR) (n=23) containing levonorgestrel and ethinyl estradiol (EE), or an OC with levonorgestrel and EE, CRP increased in both groups after three menstrual cycles[24-26]. A significant larger increase was found in the subjects with CVR, compared with OC users, in cases with low baseline CRP, while no difference in the CRP increase was found for the highest tertile of pre-treatment CRP levels[26]. The authors interpreted their results as most likely explained by a specific effect of nestorone alone, or in combination with EE26. In another randomized study on 23 women, comparing transdermal patch delivering EE and norelgestromin with OC containing EE and norgestimate combination, after three menstrual cycles CRP levels significantly increased in the transdermal patch group and the increase approached significance in the OC treated group[24]. In the only study comparing the effect on CRP levels of oral (EE-desogestrel combination) (n=13), transdermal (EE-norelgestromin combination) (n=15) and vaginal (EE- etonogestrel) contraceptives (n= 14), no difference among the groups was found in the significant CRP increase after 9 weeks

of treatment[25]. Moreover, serum level of pentraxin 3 (PTX-3), a newer marker of inflammation produced in peripheral tissues as response to inflammatory stimuli, increased significantly in oral and transdermal contraceptives groups and registered a similar trend in vaginal ring group[25]. As PTX-3 production is not influenced by hepatic protein synthesis induction, the results sustain the hypotheses that the CRP increase following HC use is the consequence of true inflammation and not of the hepatic protein induction. On the contrary, the results of a cross over-study on 35 women randomized to either second or third generation OC suggested the increase of serum CRP to be related rather to an effect on hepatic synthesis than to IL-6 mediated inflammation, or endothelial activation[27]. Hepatic protein synthesis induced by HC might be weaker in case of vaginal ring combination[25].

Homocysteine, folic acid and hormonal contraceptives

Similar to CRP, high homocysteine level has been associated with thrombotic and other cardiovascular events[28]. It has been involved in oxidative stress, vascular inflammation, endothelial dysfunction and inhibition of endothelial dependent vascular relaxation[20]. Additionally, increased serum level of homocysteine was associated with neurodegenerative disorders, pregnancy pathology and central nervous system diseases[8].

The results of the studies investigating the association of HC use with homocysteine levels are contradictory. A prospective study on 100 healthy women with normal menstrual cycles aimed to assess the effect of a low dose OC pills on homocysteine and nitric oxide (NO) levels, in the group treated with OC (n=50) homocysteine levels significantly increased and NO concentration significantly decreased after three months of treatment[29]. In an observational cross-sectional study of 90 healthy, non-obese women, homocysteine levels were significantly higher in women treated with OC, compared to non-users (13.268±3.475 vs. 7.288±2.621 μmol/L)[20]. This association was also suggested in a study investigating the effects of OC on metabolites of one-carbon metabolism, tryptophan catabolism, and inflammation[30]. On the contrary, the association between OC use and homocysteine level could not be proved in some cross-sectional observational studies[31-34]. In a study aimed to investigate the effect of 2 types of low dose combined OC (EE/desogestrel vs. EE/levonorgestrel) on serum homocysteine levels, lower homocysteine concentrations were observed in EE/desogestrel group, compared to EE/levonorgestrel and control groups[35]. The small number of studies, as well as differences in study design and types of OC used do not allow for definitive conclusions.

Homocysteine, an amino acid derived from methionine by demethylation is inversely correlated with the folate level. Folic acid, a group B vitamin, is a cofactor in methylation processes involved in DNA synthesis, gene expression, synthesis of neurotransmitters or proteins essential in cellular growth and multiplication[36]. After being metabolized to methylene-tetrahydrofolate during its passage across intestinal mucosa, folic acid is involved as a methyl group donor in the methylation of homocysteine to methionine. Folic acid deficit can cause neural tube defects in developing embryos, macrocytic anaemia, peripheral neuropathy, and also leads to increased level of serum homocysteine. Hyperhomocysteinemia has been associated with increased cardiovascular risk, late pregnancy complications and preeclampsia[37-39]. Even since the introduction of OC some studies showed that their use could lead to folate deficiency. Later on, this hypothesis was confirmed in some, but not all studies[40].

Palmery et al. have found that serum levels of folate decreased during the administration of the OC and returned to baseline levels in 3 months after women stopped using pills40. It was shown that acid folic supplementation as enriched diet, folic acid, or levomefolate calcium is efficient in decreasing the homocysteine level, but a number of months are needed to attain a steady serum and red blood cells (RBC) level of folic acid[41,42]. This depends on baseline values, but the needed time seems to be of at least 8 weeks in population were national programmes of folate supplementation through diet are implemented[43]. In other populations, the steady state is reached after 12 weeks for serum concentration and after 24 weeks for RBC[44]. In order to obtain a maximal protection against neural tube defects, Lamers et al. recommended a minimum 12 weeks of folic acid supplementation before conception[42]. Considering the high number of unplanned pregnancies, an alternative strategy would be adding folic acid compounds to OC. Folate fortified HC have been recently introduced in USA as a method to attain a sufficient folate level for sexually active women until the moment of pregnancy, when acid supplementation would be initiated[45].

The impact of folic acid supplementation on reducing the risk of neural tube defects has been demonstrated by multiple studies[46]. Considering the relationship between inflammation, cardiovascular risk, homocysteine and folate, possible additional benefits of adding folic acid to HC on reducing the cardiovascular risk should be investigated by long-term prospective studies.

Conclusion

This review summarizes the available evidence on the relationship between CRP and homocysteine levels and HC use. While there is considerable evidence to support the association between increased CRP level and OC use, the relationship with the homocysteine level is not well established. There are not enough longitudinal studies to evaluate the level of homocysteine and folate before and after HC use. However, given the evidence linking inflammation and homocysteine to cardiovascular risk, homocysteine-folate to hormonal changes, as well as HC use to thromboembolic risk, elucidating these aspects by long term prospective studies for various types of

combined HC is needed. Filling the gap of knowledge on the subject might allow the development of preventive strategies for thromboembolic risk. Demonstrating possible additional benefits of adding folic acid to HC on reducing the cardiovascular risk demands further investigation. ■

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