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ORIGINAL ARTICLE *Reproductive endocrinology*

Oral, transdermal and vaginal combined contraceptives induce an increase in markers of chronic inflammation and impair insulin sensitivity in young healthy normal-weight women: a randomized study

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STUDY QUESTION: What is the effect of alternative administration routes of combined contraceptives (CCs) on androgen secretion, chronic inflammation, glucose tolerance and lipid profile?

SUMMARY ANSWER: The use of oral, transdermal and vaginal CCs impairs glucose tolerance and induces chronic inflammation.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: Oral CCs worsen insulin sensitivity and are associated with increased levels of circulating inflammatory markers, whereas the metabolic effects of transdermal and vaginal CCs have been reported to be minimal. This is the first study comparing three different administration routes of CCs on metabolic variables.

STUDY DESIGN, SIZE AND DURATION: This randomized (computer-generated) open-label 9-week follow-up study was conducted at the Oulu University Hospital, Finland. Fasting blood samples were collected at baseline and thereafter at 5 and 9 weeks of treatment, and serum levels of 17-hydroxyprogesterone, androstenedione, testosterone, C-reactive protein (CRP), sex hormone-binding globulin (SHBG), glucose, insulin, C-peptide, total, low-density lipoprotein and high-density lipoprotein cholesterol and triglycerides were measured. Oral glucose tolerance tests were performed and plasma levels of pentraxin 3 (PTX-3) were measured at 0 and 9 weeks. The randomization list, with an allocation ratio of 1:1:1 and block size of six, was computer generated and constructed by a pharmacist at the Oulu University Hospital. The research nurse controlled the randomization list and assigned participants to their groups at the first visit.

PARTICIPANTS AND SETTING: Forty-two of 54 healthy women who entered the study used oral contraceptive pills ($n = 13$), transdermal contraceptive patches ($n = 15$) or contraceptive vaginal rings ($n = 14$) continuously for 9 weeks. Inclusion criteria were regular menstrual cycles, at least a 2-month washout as regards hormonal contraceptives and no medication.

[†] These authors contributed equally to this work and they both should be considered as first authors.

MAIN RESULTS AND THE ROLE OF CHANCE: Serum levels of SHBG increased and consequently the free androgen index (FAI) decreased in all study groups from baseline to 9 weeks of treatment [FAI, oral: 1.3 (95% confidence interval, CI: 0.94; 1.62) to 0.40 (0.25; 0.54); transdermal: 1.2 (0.96; 1.4) to 0.36 (0.30; 0.43); vaginal: 1.6 (1.1; 2.1) to 0.43 (0.29; 0.58), $P < 0.001$ in all groups]. Insulin sensitivity was reduced at 9 weeks in all three groups according to the Matsuda index [oral: 7.3 (5.5; 9.0) to 5.6 (3.9; 7.3); transdermal: 9.1 (6.7; 11.4) to 6.6 (4.5; 8.8); vaginal: 7.7 (5.9; 9.5) to 5.4 (3.9; 7.0), $P = 0.004–0.024$]. Levels of HDL cholesterol, triglycerides and CRP rose in all three groups [CRP, oral: 0.70 (0.38; 1.0) to 5.4 (1.0; 9.9) mg/l; transdermal: 0.77 (0.45; 1.1) to 2.9 (1.4;4.4) mg/l; vaginal: 0.98 (0.52; 1.4) to 3.7 (−0.25; 7.7, a negative value due to skewed distribution to right) mg/l, $P \leq 0.002$ in all groups] and PTX-3 levels increased in the oral and transdermal study groups ($P = 0.007$ and $P = 0.002$).

WIDER IMPLICATIONS OF THE FINDINGS: Although the long-term consequences of the present results remain undetermined, these findings emphasize the importance of monitoring glucose metabolism during the use of CCs, especially in women with known risks of type 2 diabetes or cardiovascular diseases.

BIAS, LIMITATIONS, GENERALIZABILITY: The number of subjects was relatively low. Moreover, the 9-week exposure to CCs is too short to draw conclusions about the long-term health consequences. However, as the subjects were healthy, normal-weight young women, the possible alterations in the glucose and inflammatory profiles among women with known metabolic risks might be even greater.

STUDY FUNDING/COMPETING INTERESTS: This work was supported by grants from the Academy of Finland, the Sigrid Jusélius Foundation, the Finnish Medical Foundation, the Research Foundation of Obstetrics and Gynecology, Oulu University Scholarship Foundation, the North Ostrobothnia Regional Fund of the Finnish Cultural Foundation, the Tyyni Tani Foundation of the University of Oulu and the Finnish-Norwegian Medical Foundation. No competing interests.

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Key words: combined contraception / administration route / insulin sensitivity / lipid profile / chronic inflammation

Introduction

Over 100 million women of fertile age use oral contraceptives worldwide (United Nations, Department of Economic and Social Affairs, Population Division (2011), 2010). Besides birth control, combined contraceptives (CCs) are also used in the treatment of several gynaecological conditions, such as menstrual disorders, dysmenorrhea and endometriosis. Furthermore, the use of CCs often continues for years, occasionally even until menopause. Today, CCs can be administered through three different routes, namely oral, transdermal and vaginal, with similar contraceptive effectiveness (Lopez *et al.*, 2010).

CCs are also often prescribed to manage acne and hirsutism as they effectively reduce androgen action on sebaceous glands and hair follicles (Paparodis and Dunaif, 2011). Ethinyl estradiol (EE) in CCs has a marked effect on hepatic metabolism, as it increases sex hormone-binding protein (SHBG) synthesis (Sitruk-Ware and Nath, 2011), resulting in a decrease in the free androgen index (FAI; Elkind-Hirsch *et al.*, 2007).

The results of some (Wynn *et al.*, 1979; Godsland *et al.*, 1990; Godsland *et al.*, 1992; Watanabe *et al.*, 1994; Elkind-Hirsch *et al.*, 2007; Morin-Papunen *et al.*, 2008; Cagnacci *et al.*, 2009b) but not all (Gaspard *et al.*, 2003) previous studies have suggested that oral CCs worsen glucose metabolism, while transdermal and vaginal contraceptives have not been found to have any effect (Duijkers *et al.*, 2004; Elkind-Hirsch *et al.*, 2007; Cagnacci *et al.*, 2009b; Grodnitskaya *et al.*, 2010; Kiriwat and Petyim, 2010). Although no clear connection between CC use and type 2 diabetes (T2DM) has been demonstrated so far among the general population (Rimm *et al.*, 1992; Chasan-Taber *et al.*, 1997), a recent prospective population-based study revealed that ongoing use of oral contraceptives results in a 7-fold greater risk of having impaired glucose tolerance (IGT; Deleskog *et al.*, 2011). The contradictory results concerning glucose metabolism in CC studies may be related to different study settings, doses of EE, types of progestin and routes of administration.

In addition to glucose metabolism, the results regarding the effects of CCs on lipid metabolism have not been consistent either. Oral and transdermal contraceptives have been suggested to change the lipid profile, in most cases by increasing serum levels of triglycerides and high-density lipoprotein cholesterol (HDL-C) (Wynn *et al.*, 1979; Godsland *et al.*, 1990; Creasy *et al.*, 2003; Frempong *et al.*, 2008; Kiriwat and Petyim, 2010), whereas the use of a vaginal ring has been reported to increase both triglycerides and HDL-C or have no effect at all (Tuppurainen *et al.*, 2004; Elkind-Hirsch *et al.*, 2007; Cagnacci *et al.*, 2009b; Barreiros *et al.*, 2011).

According to the results of previous studies, current oral CC use is associated with an increased risk of cardiovascular disease (CVD), although the risk seems to become normalized when CC use is stopped (Baillargeon *et al.*, 2005; Merz *et al.*, 2006). Furthermore, the induction of chronic inflammation is considered to be an independent risk factor of CVD, as the development of atherosclerosis is regarded as an inflammatory process (Ross, 1999; Ridker *et al.*, 2000). The level of chronic inflammation can be measured by assay of C-reactive protein (CRP), which is mainly produced by the liver. Another recently discovered marker of inflammation, pentraxin 3 (PTX-3), is produced by peripheral tissues (such as vascular endothelial cells, smooth muscle cells and leukocytes) in response to proinflammatory signals. Therefore, unlike CRP synthesis, PTX-3 is not influenced by drug-induced hepatic protein synthesis (Mantovani *et al.*, 2008), which makes it a useful and possibly even better marker of inflammatory changes. Orally administered contraceptives have been shown to increase serum levels of CRP (van Rooijen *et al.*, 2006; Krintus *et al.*, 2010). There is only one study available on the effects of transdermal contraception on inflammation, and the results suggest that the use of a transdermal contraceptive patch increases serum CRP levels (White

et al., 2006). To our knowledge, the relationship between the use of a vaginal ring and chronic inflammation has not been studied so far.

As the route of administration and serum steroid concentrations during CC use may affect the extent of metabolic side effects, the present study was designed to compare the effects of 9 weeks continuous use of oral, transdermal and vaginal CC on androgen synthesis, insulin sensitivity, lipid metabolism and chronic inflammation in young healthy normal-weight women.

Materials and Methods

This randomized, prospective, open-label, single-centre study was conducted at the Oulu University Hospital, Finland, between September 2008 and December 2010. The study protocol was approved by the Ethics Committee of the Oulu University Hospital and the Finnish Medicines Agency. All the subjects signed a written informed consent document. The study was registered at the Clinical Trials web-pages (identifier code NCT01087879; http://clinicaltrials.gov) and EU Clinical Trials Register (identifier code 2007-004984-23; https://www.clinicaltrialsregister.eu).

Subjects

A total of 54 healthy Caucasian women (aged 20–33 years; body mass index (BMI): 17.9–26.4 kg/m^2) participated in the study. All the subjects had regular menstrual cycles and used no medication. Twenty-eight of these women had used CCs during the previous year and were required to have a wash-out period for a minimum of 2 months before entering the study. The pituitary–ovarian axis recovers fast and the suppressive effect of the oral pill disappears very shortly after cessation of CCs, and after 2 months the hormonal parameters are indistinguishable from controls (Klein and Mishell, 1977). Exclusion criteria were impaired fasting glucose (IFG), IGT, type 1 diabetes, T2DM, cigarette smoking, abuse of alcohol, contraindications as regards the use of CCs and lactation.

The subjects were randomized to use one of the following preparations for three continuous cycles (9 weeks): a combined oral contraceptive pill (EE, 20 μg and desogestrel, 150 μg; Mercilon®; Organon Ltd., Dublin, Ireland; $n = 18$), a transdermal contraceptive patch (EE, 20 μg/day and norelgestromin, 150 μg/day; Ortho Evra®; Janssen Pharmaceutica N.V., Beerse, Belgium; $n = 18$) or a contraceptive vaginal ring (EE, 15 μg/day and etonogestrel, active metabolite of desogestrel, 120 μg/day; NuvaRing®; N.V. Organon, Oss, the Netherlands]; $n = 18$). The randomization list, with an allocation ratio of 1:1:1 and block size of six, was computer generated and constructed by a pharmacist at the pharmacy of the Oulu University Hospital. The research nurse controlled the randomization list and assigned participants to their groups at the first visit. T.P. and J.P. enrolled the subjects. Before randomization, in all subjects systolic and diastolic blood pressure (BP) was measured and a normal ovarian morphology was confirmed with transvaginal ultrasonography. The subjects started treatment after the baseline measurements on cycle days 2–4 and they were advised to use barrier contraception during the first week of CC use and not to change their physical activities or dietary habits during the study.

After randomization, two women (one from the oral pill group and one from the vaginal ring group) were excluded because of IFG and two women (one from the transdermal patch group and one from the vaginal ring group) because of IGT diagnosed after the oral glucose tolerance tests (OGTTs) at baseline. None of these subjects had a known history of abnormal glucose metabolism before entering the study. Furthermore, during the study two women were excluded as a result of non-adherence to the study protocol (one from the oral pill group and one from the vaginal ring group). During the first month of the study, one subject withdrew because of difficulties using transdermal patch (when swimming), one subject from the oral pill group was lost to follow-up, one subject from the transdermal patch group withdrew for personal reasons and two subjects from the oral pill group and one from the vaginal ring group dropped out because of mood changes. Thus, analyses were performed for the 42 normoglycaemic women who completed the study (oral pill group: $n = 13$; transdermal patch group: $n = 15$; vaginal ring group: $n = 14$; Fig. 1).

Measurements for androgens, lipid profile and inflammatory markers

Fasting blood samples were collected at baseline and thereafter at 5 and 9 weeks of treatment for analyses of serum 17-hydroxyprogesterone (17-OHP), androstenedione, testosterone, SHBG, total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides and high sensitivity CRP (hs-CRP). For measurement of PTX-3, samples of EDTA plasma were collected at baseline and at 9 weeks of treatment. All samples were frozen at −80°C for later analyses. The FAI and the LDL-C to HDL-C ratio were also calculated.

Oral glucose tolerance tests

OGTTs (involving a 75 g load of glucose in 300 ml of water) were performed after an overnight fast of 12 h at the beginning of the study and at 9 weeks of treatment. Blood samples for assay of glucose, insulin and C-peptide, a cleavage product of proinsulin, were drawn before the OGTT and at 30, 60 and 120 min thereafter. Serum glucose measurements were performed immediately after blood sampling and the rest of the serum was frozen at −80°C for later analyses.

Glucose tolerance was defined according to World Health Organization criteria: IFG was diagnosed when in OGTTs fasting glucose concentrations were 6.1–6.9 mmol/l, and IGT was diagnosed when glucose levels at 2 h were 7.8–11.0 mmol/l (Alberti and Zimmet, 1998).

Incremental insulin and glucose areas under the curve ($AUC_{insulin/glucose}$) were calculated by the trapezoidal method. In order to evaluate the

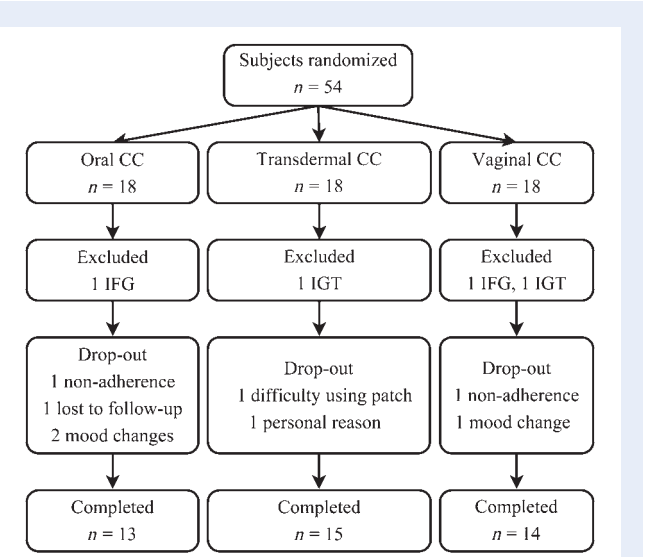

Figure 1 Flow chart of the study of metabolic effects of CC administered by oral, transdermal and vaginal routes. IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

degree of glucose tolerance and β-cell function, several indexes derived from either fasting or OGTT-stimulated measurements were used. The insulin sensitivity index (ISI) was calculated as follows: fasting or 2 h glucose (mmol/l)/fasting or 2 h insulin (mIU/l). The whole body ISI, i.e. the Matsuda index was calculated according to the following equation: $\{10\,000/\sqrt{[(\text{fasting glucose (mg/dl)} \times \text{fasting insulin (mIU/l)}) \times (\text{glucose}_{\text{meanOGTT}} \text{ (mg/dl)} \times \text{insulin}_{\text{meanOGTT}} \text{ (mIU/l)})]}\}$ (Matsuda and DeFronzo, 1999).

Assays

Serum levels of 17-OHP were measured by radioimmunoassay (Siemens Healthcare Diagnostics, Los Angeles, CA, USA), with a sensitivity of 0.2 nmol/l. Serum samples for testosterone were analyzed by using Agilent triple quadrupole 6410 LC/MS equipment with an electrospray ionization source operating in a positive ion mode (Agilent Technologies, Willington, DE, USA), with a sensitivity of 0.03 nmol/l. SHBG and androstenedione were analyzed by chemiluminometric immunoassays (Immulite 2000; Siemens Healthcare Diagnostics, Los Angeles, CA, USA), with a sensitivity of 0.02 nmol/l for SHBG and 1.0 nmol/l for androstenedione. Serum glucose, total cholesterol, LDL-C, HDL-C and triglycerides were assayed by using an automatic chemical analyzer (Advia, 1800; Siemens Healthcare Diagnostics, Tarrytown, NY, USA), insulin and C-peptide by using an automated chemiluminescence system (Advia Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY, USA), hs-CRP by immunonephelometry (BN ProSpec; Siemens Healthcare Diagnostics, Marburg, Germany) and plasma PTX-3 by enzyme-linked immunosorbent assay, following the instructions of the manufacturer (Quantikine DPTX 30; R&D Systems, Inc., Minneapolis, MN, USA). The mean detection limit for PTX-3 is 0.025 ng/ml and the assay exhibits no cross-reactivity with CRP. Intra- and inter-assay coefficients of variation were 7.1 and 7.3% for 17-OHP (at 4.2 nmol/l), 6.3 and 7.0% for androstenedione (at 4.5 nmol/l), 5.3 and 5.3% for testosterone (at 0.6 nmol/l), 2.7 and 5.2% for SHBG (at 63 nmol/l), 2.9 and 3.6% for insulin (at 23.1 and 25.0 mU/l), 3.2 and 7.1% for C-peptide (at 0.56 and 0.4 nmol/l), 0.9 and 3.5% for hs-CRP (at 1.75 and 1.63 mg/l) and 3.8 and 6.1% for PTX-3 (at 2.6 and 2.8 ng/ml), respectively. All samples (i.e. baseline, and 5 and 9 weeks) from the same subject were analysed in the same assay. 17-OHP and PTX-3 were assayed in duplicate.

Statistics

The primary outcomes were androgen secretion and protein secretion from the liver (CRP, SHBG) and the secondary outcomes were glucose tolerance and lipid profile. Power analysis was based on the assumption that serum levels of hs-CRP increase from 0.8 to 1.6 mg/l (with SD 0.9 mg/l) during the use of oral CCs and no change occurs during the use of transdermal CCs (with SD 0.5 mg/l), as demonstrated in postmenopausal women using hormone-replacement therapy (Ropponen *et al*., 2005). The power analysis revealed that 13 subjects would be needed in each group for the study to have 80% power, when an α error was set at a significant level of 0.05. A total of 51 subjects needed to be recruited after adjusting for a possible 20% dropout rate.

All variables with a skewed distribution went through logarithmic transformation prior to statistical analysis. All statistical analyses were conducted by using the Statistical Package for the Social Sciences (SPSS) software (version 15.0 for Windows, SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P \leq 0.05$.

To explore changes in the levels of serum androgens, SHBG, glucose, insulin, C-peptide, lipids, CRP and ISIs within the same study group at 5 and 9 weeks of treatment versus baseline, repeated measures analysis of variance (ANOVA) was performed for normally distributed variables and Friedman's test was used for variables with a skewed distribution. To compare serum levels of the above-mentioned parameters at baseline versus 5 or 9 weeks of treatment within the same study group, the paired samples *t*-test was used as a *post hoc* test for normally distributed variables and Wilcoxon's non-parametric test for variables with a skewed distribution. The paired samples *t*-test and Wilcoxon's non-parametric test were also used to compare $AUC_{insulin/glucose}$, the Matsuda index and PTX-3 at baseline and 9 weeks of treatment. The change in BMI was used as a covariate to adjust for changes in the $AUC_{insulin/glucose}$ in repeated measures ANOVA. Differences between the study groups at baseline were compared by using one-way ANOVA and differences between the study groups during treatment by using independent samples *t*-test for normally distributed variables and the Mann–Whitney *U*-test for variables with a skewed distribution.

Results

The mean age, BMI, waist-to-hip ratio (WHR) and systolic and diastolic BP at baseline were comparable in the three study groups (Table I). BMI and WHR did not change during the study (data not shown) except in the transdermal patch group, in which BMI increased from 21.6 (SD: 2.3) kg/m^2 to 22.0 (SD: 2.3) kg/m^2 at 9 weeks ($P = 0.004$). Baseline measurements of glucose tolerance and chronic inflammation (data not shown) were similar in women with ($n = 28$) and without ($n = 14$) prior use of CCs.

Serum levels of androgens and SHBG

Serum levels of 17-OHP and androstenedione decreased significantly over time in all study groups, whereas testosterone levels remained

Table I Baseline demographics of women in the study population in different CC treatment groups.

	Oral	Transdermal	Vaginal	*P* (ANOVA)*
No. subjects	13	15	14	
Age (years)	23.5 (3.1)	24.3 (3.5)	24.1 (3.7)	0.807
BMI (kg/m^2)	22.0 (2.2)	21.6 (2.3)	22.4 (2.2)	0.637
WHR	0.80 (0.04)	0.78 (0.04)	0.79 (0.04)	0.587
Systolic BP (mmHg)	109.9 (10.2)	112.6 (9.0)	114.6 (8.5)	0.419
Diastolic BP (mmHg)	69.5 (6.6)	66.5 (7.9)	69.1 (8.2)	0.533

Data are shown as mean (SD). WHR, waist to hip ratio; BP, blood pressure.
*Comparison between study groups by one-way analysis of variance (ANOVA).

Table II Parameters related to androgen secretion in different CC treatment groups.

	Weeks	Oral	P*	Transdermal	P*	Vaginal	P*
17-OHP (nmol/l)	0	5.4 (2.3)	0.001	5.5 (2.5)	<0.001	4.8 (1.5)	<0.001
	5	3.8 (2.2)[b]		3.3 (2.2)[a]		3.0 (0.94)[a]	
	9	3.2 (2.1)[a]		3.1 (1.8)[a]		3.0 (1.2)[a]	
Androstenedione (nmol/l)	0	10.1 (2.8)	0.009	10.3 (3.1)	<0.001	10.0 (3.4)	<0.001
	5	8.2 (3.7)[a]		7.8 (2.3)[a]		8.2 (4.2)[a]	
	9	8.2 (4.5)[b]		7.6 (2.2)[a]		7.1 (2.7)[a]	
Testosterone (nmol/l)	0	0.89 (0.43)	0.243	0.76 (0.35)	0.044	0.88 (0.39)	0.473
	5	1.1 (0.50)		0.86 (0.34)		1.0 (0.60)	
	9	1.0 (0.50)		0.97 (0.38)[b]		1.0 (0.62)	
SHBG (nmol/l)	0	76.2 (26.9)	<0.001	63.8 (18.4)	<0.001	61.2 (19.1)	<0.001
	5	280.6 (89.5)		248.6 (65.6)		217.4 (65.7)	
	9	285.0 (81.2)		264.9 (52.3)		219.2 (63.0)	
FAI	0	1.3 (0.57)	<0.001	1.2 (0.44)	<0.001	1.6 (0.93)	<0.001
	5	0.42 (0.22)[a]		0.35 (0.12)[a]		0.45 (0.25)[a]	
	9	0.40 (0.24)[a]		0.36 (0.12)[a]		0.43 (0.25)[a]	

Data are shown as mean (SD).
[a,b]Analysed by the paired samples *t*-test or Wilcoxon's test.
[a]Compared with baseline, $P \leq 0.009$.
[b]Compared with baseline, $P = 0.012–0.036$.
*Analysed by repeated measures analysis of variance or Friedman's test.

Table III Parameters related to glucose metabolism in different CC treatment groups.

	Weeks	Oral	P*	Transdermal	P*	Vaginal	P*
Glucose (mmol/l)	0	5.2 (0.3)	0.102	5.0 (0.3)	0.828	5.0 (0.2)	0.059
	5	5.1 (0.4)		5.1 (0.5)		5.1 (0.1)	
	9	5.0 (0.4)		5.1 (0.5)		5.3 (0.5)	
Insulin (mU/l)	0	7.0 (4.7)	0.008	7.6 (4.0)	0.315	6.7 (1.9)	0.008
	5	11.2 (9.3)[a]		9.2 (4.6)		9.5 (3.6)[a]	
	9	9.4 (5.2)[b]		10.0 (7.0)		10.0 (4.0)[a]	
C-peptide (nmol/l)	0	0.26 (0.15)	0.292	0.21 (0.10)	0.034	0.26 (0.11)	0.259
	5	0.35 (0.27)		0.24 (0.12)		0.29 (0.08)	
	9	0.28 (0.11)		0.27 (0.15)		0.27 (0.13)	
ISI_{0h}	0	0.95 (0.39)	0.008	0.83 (0.37)	0.421	0.81 (0.25)	0.021
	5	0.65 (0.32)[b]		0.68 (0.37)		0.62 (0.25)[b]	
	9	0.68 (0.30)[a]		0.68 (0.32)		0.62 (0.28)[b]	
ISI_{2h}	0	0.16 (0.07)		0.19 (0.12)		0.17 (0.10)	
	9	0.13 (0.07)[b]		0.16 (0.06)[b]		0.13 (0.05)[b]	
Matsuda index	0	7.3 (2.9)		9.1 (4.0)		7.7 (3.1)	
	9	5.6 (2.8)[a]		6.6 (3.7)[b]		5.4 (2.6)[b]	

Data are shown as mean (SD). ISI_{0h}, fasting ISI.
[a,b]Analysed by the paired samples *t*-test or Wilcoxon's test.
[a]Compared with baseline, $P \leq 0.008$.
[b]Compared with baseline, $P = 0.014–0.04$.
*Analysed by repeated measures analysis of variance or Friedman's test

unchanged in the oral pill and vaginal ring groups but increased during the use of transdermal patch. Serum levels of SHBG increased over time, and consequently the FAI decreased, in all study groups (Table II).

Oral glucose tolerance tests

Fasting serum levels of glucose remained unchanged during 9 weeks of treatment but the AUC values of glucose in OGTTs rose significantly in all three study groups (Table III, Fig. 2). Fasting serum levels of insulin increased significantly from baseline during the use of oral and vaginal contraceptives, and a similar trend was seen in the transdermal patch group (Table III). The AUC of insulin rose significantly during the use of oral and transdermal CCs, and there was a tendency to increase in the vaginal ring group (Fig. 2). The increase in BMI in the transdermal patch group did not explain the changes in AUC of glucose or insulin. Serum levels of C-peptide rose significantly from

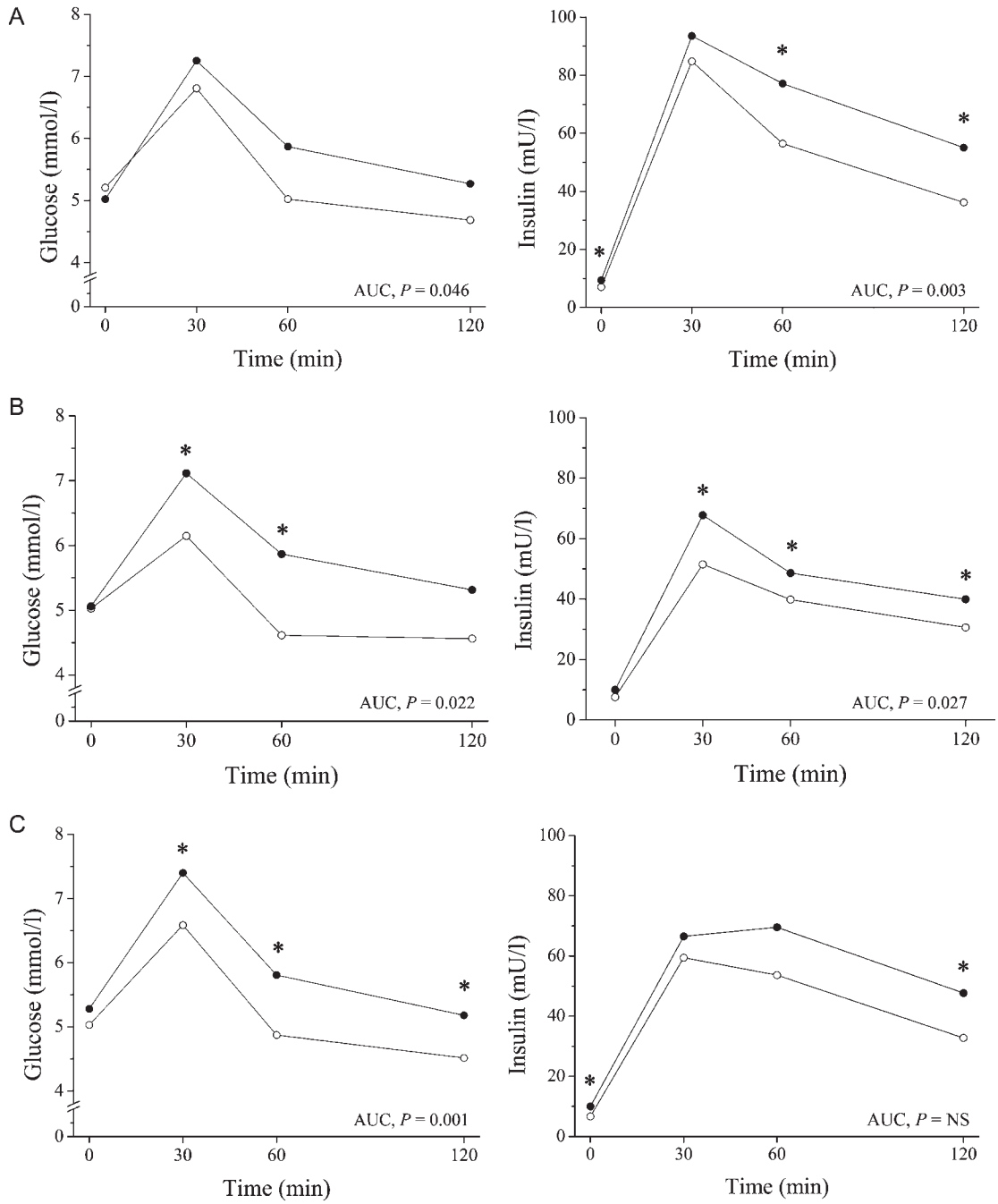

Figure 2 Glucose and insulin responses during OGTTs in the (**A**) oral pill group; (**B**) transdermal patch group and (**C**) vaginal ring group. *Specific time point during OGTT where concentration is significantly higher after 9 weeks use of oral ($P \leq 0.030$), transdermal ($P \leq 0.041$) and vaginal ($P \leq 0.013$) contraception. Open circle, baseline; closed circle, 9 weeks of treatment. AUC, area under the curve.

baseline to 9 weeks in the transdermal patch group but remained unchanged in the oral pill and vaginal ring groups (Table III). The fasting ISI, shown as ISI_{0h}, decreased significantly in the oral pill and vaginal ring groups, and the same trend was seen in the transdermal patch group (Table III). The ISI at 2 h (ISI_{2h}) and the Matsuda index, reflecting whole-body insulin sensitivity, had decreased at 9 weeks in all three study groups (Table III).

Serum levels of lipids, CRP and PTX-3

Serum total cholesterol levels remained unchanged and triglyceride and HDL-C concentrations increased significantly over time in all the study groups. Levels of LDL-C increased significantly among the users of oral CC (Table IV). The concentrations of CRP rose significantly in all the three study groups at 9 weeks (Fig. 3). There were three subjects in the oral pill group, one in the transdermal patch

Table IV Parameters related to the lipid profile in different CC treatment groups.

	Weeks	Oral	P*	Transdermal	P*	Vaginal	P*
Cholesterol (mmol/l)	0	4.5 (0.6)	0.063	4.4 (0.7)	0.280	4.3 (0.9)	0.149
	5	4.9 (0.9)		4.5 (0.6)		4.5 (0.7)	
	9	4.7 (0.8)		4.4 (0.5)		4.4 (1.0)	
LDL-C (mmol/l)	0	2.3 (0.7)	0.028	2.1 (0.6)	0.842	2.1 (0.7)	0.182
	5	2.8 (1.1)[b]		2.1 (0.5)		2.3 (0.7)	
	9	2.6 (0.9)		2.1 (0.5)		2.2 (0.7)	
HDL-C (mmol/l)	0	1.5 (0.2)	0.037	1.5 (0.2)	<0.001	1.4 (0.3)	0.002
	5	1.6 (0.3)[b]		1.7 (0.3)[a]		1.6 (0.3)[a]	
	9	1.5 (0.3)		1.7 (0.2)[a]		1.5 (0.3)[b]	
LDL/HDL ratio	0	1.6 (0.4)	0.520	1.4 (0.4)	0.075	1.5 (0.5)	0.359
	5	1.7 (0.6)		1.2 (0.3)[b]		1.5 (0.4)	
	9	1.7 (0.5)		1.3 (0.4)		1.4 (0.4)	
Triglycerides (mmol/l)	0	0.73 (0.2)	<0.001	0.75 (0.3)	<0.001	0.78 (0.1)	<0.001
	5	1.1 (0.4)[a]		1.1 (0.3)[a]		1.2 (0.2)[a]	
	9	1.3 (0.5)[a]		1.2 (0.4)[a]		1.2 (0.4)[a]	

Data are shown as mean (SD). LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.
[a,b]Analysed by the paired samples *t*-test or Wilcoxon's test.
[a]Compared with baseline, $P \leq 0.005$.
[b]Compared with baseline, $P = 0.01–0.05$.
*Analysed by repeated measures analysis of variance or Friedman's test.

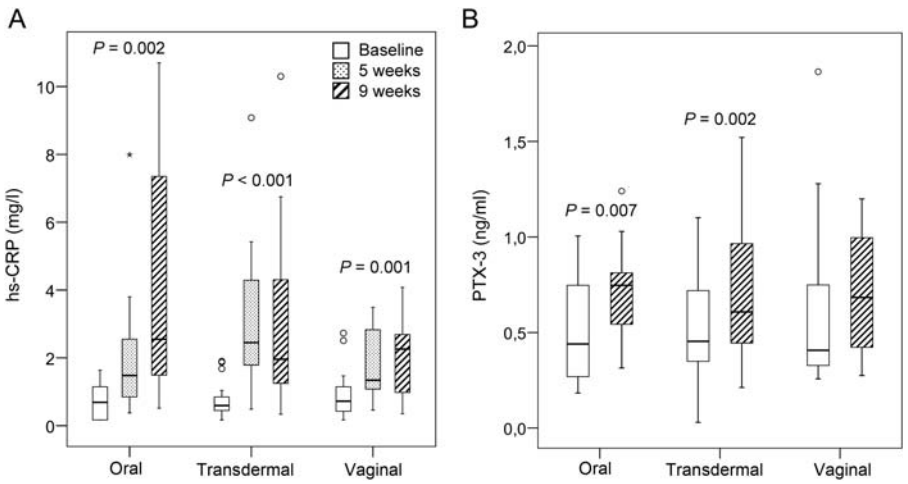

Figure 3 Changes in the markers of inflammation, CRP and pentraxin 3 (PTX-3), during the study. Open circle, outlier; asterisk, extreme outlier. CRP: one reading in the oral pill group and one in the vaginal ring group were extreme outliers, with CRP levels of 26.7 and 27.5 mg/l at 9 weeks of treatment. They were included in the analyses but are not shown in the figure.

group and one in the vaginal ring group whose serum CRP levels were over 10 mg/l at 9 weeks, which may reflect occult infection. Yet, the overall increase in CRP levels persisted after excluding these five women from the analyses. Plasma concentrations of PTX-3 rose significantly during the use of oral and transdermal contraceptives, and a similar trend was seen in the vaginal ring group (Fig. 3).

Comparison of changes in hormonal and metabolic parameters between study groups

The increase in serum SHBG levels during CC treatment was significantly smaller in the vaginal ring group than in the oral pill and transdermal patch groups, and the change in fasting glucose was higher in the vaginal ring group than that in the oral pill group. The changes

Table V Comparison of the changes in hormonal and metabolic parameters between groups during the use of CCs.

	Oral change	Transdermal change	Vaginal change	*P*		
				O-Tr	O-V	Tr-V
17-OHP (nmol/l)	−2.2 (−3.5; −1.0)	−2.4 (−3.5; −1.3)	−1.8 (−2.7; −0.88)	0.447	0.530	0.369
Androstenedione (nmol/l)	−1.9 (−4.2;0.28)	−2.7 (−3.8; −1.5)	−2.9 (−4.3; −1.6)	0.518	0.332	0.348
Testosterone (nmol/l)	0.14 (−0.16;0.44)	0.21 (0.011;0.40)	0.12 (−0.18;0.42)	0.533	0.789	0.484
SHBG (nmol/l)	208.8 (171.2;246.5)	201.0 (176.1;225.9)	158.0 (123.9;192.1)	0.534	0.039	0.035
FAI	−0.88 (−1.2; −0.59)	−0.84 (−1.1; −0.61)	−1.2 (−1.6; −0.73)	0.765	0.257	0.169
Glucose (mmol/l)	−0.18 (−0.47; 0.10)	0.033 (−0.23; 0.30)	0.25 (−0.035; 0.54)	0.233	0.027	0.120
Insulin (mU/l)	2.4 (−0.4; 5.2)	2.4 (−1.6; 6.3)	3.3 (0.67; 6.0)	0.629	0.604	0.337
C-peptide (nmol/l)	0.02 (−0.09; 0.12)	0.060 (−0.0094; 0.13)	0.0071 (−0.076; 0.090)	0.449	0.698	0.300
ISI_{0h}	−0.27 (−0.45; −0.09)	−0.15 (−0.38; 0.074)	−0.19 (−0.42; 0.031)	0.377	0.550	0.780
ISI_{2h}	−0.033 (−0.069; 0.0019)	−0.033 (−0.083; 0.017)	−0.037 (−0.082; 0.0090)	0.945	0.905	0.694
Matsuda index	−1.6 (−2.7; −0.64)	−2.4 (−4.5; −0.37)	−2.3 (−4.5; −0.10)	0.469	0.578	0.921
$AUC_{glucose}$	66.2 (−8.1; 141)	105 (10.4; 200)	90.2 (35.7; 145)	0.495	0.437	0.771
$AUC_{insulin}$	1795 (630; 2961)	1251 (−56.1; 2557)	1427 (100; 2753)	0.409	0.627	0.818
Cholesterol (mmol/l)	0.25 (−0.064; 0.6)	0.081 (−0.23; 0.40)	0.12 (−0.16; 0.41)	0.407	0.437	0.793
LDL-C (mmol/l)	0.22 (−0.071; 0.5)	0.026 (−0.23; 0.28)	0.093 (−0.15; 0.33)	0.285	0.465	0.685
HDL-C (mmol/l)	0.10 (−0.025; 0.22)	0.16 (0.058; 0.27)	0.16 (0.034; 0.29)	0.333	0.433	0.710
LDL/HDL ratio	0.05 (−0.15; 0.25)	−0.11 (−0.29; 0.076)	−0.12 (−0.34; 0.10)	0.225	0.230	0.920
Triglycerides (mmol/l)	0.53 (0.29; 0.78)	0.46 (0.29; 0.62)	0.43 (0.22; 0.64)	0.569	0.467	0.662
CRP (mg/l)	4.8 (0.2; 9.3)	2.2 (0.79; 3.6)	2.8 (−1.2; 6.8)	0.253	0.286	0.407
PTX-3 (ng/ml)	0.19 (0.05; 0.32)	0.21 (0.090; 0.32)	0.075 (−0.12; 0.27)	0.790	0.318	0.209

Data are presented as mean change with 95% confidence intervals in parentheses. Comparisons between the study groups were performed using the independent samples *t*-test or the Mann–Whitney *U*-test. O, oral CC; Tr, transdermal CC; V, vaginal CC. AUC, area under the curve; CRP, C-reactive protein; PTX-3, pentraxin 3.

in all other measurements were comparable in the three study groups (Table V).

Discussion

This is the first study in which the metabolic effects of three different administration routes of CCs have been compared. The results demonstrate that the use of oral, transdermal and vaginal CCs equally decreases androgenicity, worsens insulin sensitivity and increases the level of markers of chronic inflammation.

During the use of oral, transdermal and vaginal CCs, decreases in serum levels of 17-OHP and androstenedione were observed. Moreover, as shown in several studies (Tuppurainen *et al.*, 2004; White *et al.*, 2005; White *et al.*, 2006; Elkind-Hirsch *et al.*, 2007), serum levels of SHBG increased and consequently the FAI decreased in all the study groups. However, the increase in SHBG was more pronounced during the use of oral and transdermal CCs when compared with vaginal administration probably reflecting the lowest exposure to EE in the vaginal ring group (van den Heuvel *et al.*, 2005). On the other hand, in two earlier studies a greater increase in SHBG levels during the use of vaginal ring compared with oral CC has been reported (Tuppurainen *et al.*, 2004; Elkind-Hirsch *et al.*, 2007). The contradiction between these results and the present ones may be a result of a different progestin component used in the oral pills, as the androgenicity of the progestins has been shown to modify the effect of EE on the synthesis of SHBG (Odlind *et al.*, 2002; Sitruk-Ware, 2006).

An interesting observation was that although fasting serum glucose levels remained unchanged over 9 weeks of treatment with oral, transdermal and vaginal CCs, insulin sensitivity decreased in all the three study groups, i.e. independent of the route of administration. This was reflected by increased fasting and glucose-stimulated insulin levels and by worsened indexes that reflect glucose tolerance. In line with the present results, several previous investigators have also reported a decrease in insulin sensitivity during oral CC use (Wynn *et al.*, 1979; Godsland *et al.*, 1990; Godsland *et al.*, 1992; Watanabe *et al.*, 1994; Elkind-Hirsch *et al.*, 2007; Morin-Papunen *et al.*, 2008; Cagnacci *et al.*, 2009b). It is noteworthy that, as for the oral pill group, glucose metabolism worsened during the use of transdermal and vaginal CCs, even though these routes avoid first-pass liver metabolism. Besides the present study, the effects of transdermal CCs on glucose metabolism have been investigated in only one (non-controlled) study, in which a decrease in fasting glucose levels during treatment was reported (Kiriwat and Petyim, 2010). However, in contrast to our results, four previous studies on the vaginal ring CC observed no changes in glucose metabolism (Duijkers *et al.*, 2004; Elkind-Hirsch *et al.*, 2007; Cagnacci *et al.*, 2009b; Grodnitskaya *et al.*, 2010).

Some of the discrepancies between the present results and those of previous studies concerning glucose metabolism during the use of transdermal and vaginal CCs may be explained by different dosing patterns than the continuous 9 weeks of administration used in the present study. Our study population included only young non-obese

women, who were able to compensate for the worsening insulin sensitivity, as reflected by increased fasting insulin levels in all three study groups. In line with this, a recent cohort study in young non-obese CC users reported raised fasting serum insulin levels, i.e. lowered insulin sensitivity but no elevation in fasting glucose levels (Morin-Papunen et al., 2008). Our finding of stable serum C-peptide levels despite increased insulin concentrations in the oral pill and vaginal ring groups could be explained by the hepatic effects of CCs, as insulin is mainly cleared by the liver, whereas C-peptide is primarily cleared by the kidneys (Lebowitz and Blumenthal, 1993). Thus, the present observation suggests that increased serum insulin levels might partly reflect altered liver clearance of insulin caused by CCs. The results of earlier studies support this interpretation (Godsland et al., 1992; Kojima et al., 1993), although enhanced insulin secretion from β-cells seems to be involved, as reflected by moderately increased serum C-peptide levels in the transdermal patch group.

As a long-term concern it is also important to note that worsening of insulin resistance and the concomitant increase in insulin levels not only increases the risk of developing T2DM but also CVD (Isomaa et al., 2001). The present observations emphasize the role of OGTTs and measurement of serum insulin in assessing alterations in glucose metabolism in clinical practice, as fasting glucose levels failed to reveal a worsening of glucose tolerance during the use of CCs, although the levels of insulin rose significantly at the same time and ISIs worsened.

The most noteworthy changes in the lipid profile during the use of oral, transdermal and vaginal CCs were the increases in serum HDL-C and triglyceride levels. Serum total cholesterol levels did not change over time and were comparable between the study groups, which is in line with the results of a recent cross-sectional study in which oral, transdermal and vaginal CCs were compared (Palan et al., 2010). The small increase in serum HDL-C may be of significance, as HDL-C is considered to be a strong, independent inverse predictor of CVD, and even in subjects with low LDL-C levels a significant protective role of HDL-C on cardiovascular events has been reported (Barter et al., 2007). Several investigators have reported an increase in serum HDL-C during the use of oral and transdermal CCs (Creasy et al., 2003; Frempong et al., 2008; Cagnacci et al., 2009a; Kiriwat and Petyim, 2010), whereas the results of the majority of studies on the vaginal ring suggest no effect on HDL-C levels (Tuppurainen et al., 2004; Elkind-Hirsch et al., 2007; Cagnacci et al., 2009b). The increase in triglyceride concentrations may also be of significance, as triglycerides are considered to represent an independent risk factor of CVD (Harchaoui et al., 2009). Increased levels of triglycerides during the use of oral and transdermal CCs have already been commonly reported (Wynn et al., 1979; Creasy et al., 2003; Morin-Papunen et al., 2008; Kiriwat and Petyim, 2010; Krintus et al., 2010), whereas results concerning vaginal rings and triglycerides have been conflicting (Tuppurainen et al., 2004; Elkind-Hirsch et al., 2007; Cagnacci et al., 2009b; Barreiros et al., 2011). All in all, the changes in lipid profiles in our study were moderate and the absolute long-term clinical significance is difficult to assess.

Elevated levels of serum CRP, a marker of systemic inflammation, are of prognostic importance as regards the development of CVD (Ridker et al., 2000). Serum levels of CRP increased in all study groups, which is in line with the results of previous studies on oral and transdermal CCs (van Rooijen et al., 2006; White et al., 2006; Morin-Papunen et al., 2008; Krintus et al., 2010). The present results show for the first time that levels of CRP also increased during the use of vaginal CCs. In addition, of all study subjects, serum levels of CRP increased from less than 1 to 1–3 mg/l in 12 subjects and to >3 mg/l in 7 subjects. This finding may be of significance, as a CRP serum level of >3 mg/l is estimated to double the risk of CVD when compared with that of <1 mg/l (Pearson et al., 2003). As CRP is mainly produced by the liver, plasma levels of PTX-3, another inflammatory marker, were also analysed. PTX-3 is produced by peripheral tissues (Mantovani et al., 2008). Plasma levels of PTX-3 increased significantly during the use of oral and transdermal CCs, supporting the idea that the increase in CRP concentrations was not only a consequence of liver induction but also reflected a true increase in inflammation rate caused by CCs. The pronounced inflammatory milieu in CC users may play a role in long term through activation of endothelial dysfunction and promoting the accumulation of asymptomatic atherosclerotic changes in these women, although the clinical importance of this remains to be investigated in long-term follow-up studies.

Several metabolic alterations during use of the CCs studied can be explained by the dosing pattern and the effects of EE, which progestin is used and the balance between EE and progestin action. Continuous administration of transdermal and vaginal CCs results in steady serum steroid concentrations when compared with oral CCs, and exposure to EE seems to be lowest with vaginal and highest with transdermal administration (van den Heuvel et al., 2005). Interestingly, the effects on glucose metabolism, the lipid profile and chronic inflammation were similar in all study groups, probably as a result of the fact that a threshold stimulus for metabolic alterations was reached with all CC regimens, independent of route of administration. This is supported by previous studies showing that EE has a significant hepatic effect, independent of whether it is administered orally or vaginally (Goebelsmann et al., 1985). Thus, routes of delivery other than oral administration may not offer metabolic benefits, as a result of the potency of EE. The smaller increase in serum SHBG levels which we observed in the vaginal ring group, however, may reflect less pronounced liver induction. The development of CCs that contain natural estrogens may provide preparations with fewer metabolic effects, as the hepatic effects of natural estrogens are significantly weaker than those of EE (Sitruk-Ware and Nath, 2011).

Progestins may influence various metabolic parameters and modulate estrogen-induced metabolic alterations (Sitruk-Ware, 2006). It has been shown that EE decreases insulin sensitivity, whereas progestins, depending on their androgenicity, extend the half-life of insulin and increase the insulin response to glucose when combined with EE (Godsland et al., 1992; Kojima et al., 1993). The androgenicity of the progestins used in the present study was somewhat comparable, as the androgenic potency of the oral CC was calculated to be 0.51, that of the transdermal CC 0.285 and that of the vaginal CC 0.408, when compared with a pill containing 1 mg of norethindrone (Greer et al., 2005). Changes in the lipid profile depend on the balance between EE and progestins because EE increases levels of HDL-C and decreases those of LDL-C, whereas progestins (again depending on their androgenicity, dose and regimen) have opposite effects (Sitruk-Ware and Nath, 2011).

The weaknesses of the present study are the short follow-up period, the non-blinded study protocol and the limited number of

subjects. As the follow-up period was restricted to 9 weeks, conclusions concerning any long-term consequences of the present findings cannot be drawn and the long-term effects of CCs on metabolic parameters have to be investigated, especially in women with metabolic risks. The non-blinded study protocol was necessary for the researchers to be able to advise the participants and ensure appropriate use of different CCs. The drop-out rate of 20% may raise some concern regarding the validity of the results. However, based on the power analysis, 39 subjects were needed to have 80% power and we believe that drop-out of 12 subjects (oral pill 5, transdermal patch 4, vaginal ring 3) did not skew the data. Finally, the fact that all treatment groups experienced changes in the same direction may have been related to extrinsic (e.g. seasonal) factors but this is unlikely as the subjects were recruited steadily throughout the year and the follow-up period was relatively short.

In conclusion, the present results demonstrate that commonly used CCs have unfavourable effects on glucose metabolism, and their use may lead to an induction of chronic inflammation, independent of the administration route. In recent decades the indications for CC use have broadened and the duration of CC use has become prolonged, which may influence the extent of adverse metabolic effects related to these preparations. However, the present observations have to be treated with caution, as the long-term consequences of these metabolic alterations remain unclear and they have to be investigated in long-term follow-up studies, especially in women at an increased risk of T2DM or CVD. Although the use of reliable contraception is a priority, the present findings emphasize the importance of monitoring glucose metabolism during CC use and the possibility of considering alternative contraception methods in women with known risk factors.

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Authors' roles

T.P. and J.S.T. designed the study. T.P., J.P. and L.M.-P. contributed to data collection. J.P., P.H., A.R., S.J.M., KH.H. and A.N. performed laboratory analyses. T.P., J.P. and J.S.T. analysed the results. J.P. wrote the first draft of the manuscript. J.S.T. supervised the overall project. All authors took part in revising the manuscript and approved the final version. L.M.-P. was an Associate Editor of Human Reproduction until 2012. The corresponding author is chairman of publication subcommittee and Chairman-Elect of ESHRE.

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Conflict of interest

None declared.

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