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ORIGINAL ARTICLE

Combined oral contraceptives may activate the contact system in healthy women

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Abstract

Background: The contact system (CAS) is part of the coagulation system, consisting of a group of plasma proteins stimulating inflammation, coagulation, and fibrinolysis when activated. CAS can be triggered by several activating surfaces, and CAS may play a potential role in thrombus formation. Combined oral contraceptives (COCs) are known to increase the risk of venous thromboembolism, and COCs induce various pro-thrombotic changes in the coagulation system, whereas the effect of COC on CAS has not been thoroughly investigated.

Objectives: To investigate CAS in COC users compared with nonusers.

Methods: Blood samples from 62 study subjects, 30 COC users, and 32 nonusers, were analyzed. Coagulation factor XII (FXII), prekallikrein (PK), H-Kininogen (HK), cleaved HK (cHK), C1-esterase inhibitor (C1-inh), and the endogenous kallikrein potential (EKP) were measured.

Results: COC users had significantly higher FXII (median, 38.4 vs 28.9 mg/L) and lower C1-inh levels (0.20 vs 0.23 g/L) than nonusers. The levels of PK and HK were not significantly different. Measurement of EKP indicated an increased capacity of CAS in COC users (1860 vs 1500 nmol/L × min), and increased plasma levels of cHK (2.02 vs 1.07 μg/L) indicated an increased activity *in vivo*.

Conclusion: This study demonstrates an increased CAS capacity in women using COC compared with nonusers and also an increased activity *in vivo*. The results indicate that increased contact activation may contribute to the increased thrombotic risk caused by COC.

KEYWORDS
combined oral contraceptives, contact system, complement C1 inhibitor protein, endogenous kallikrein potential, factor XII

Essentials

- The contact system (CAS) may play a potential role in thrombus formation
- Methods have been developed to measure the capacity and activity of CAS
- CAS capacity is increased in women using combined oral contraceptives (COCs) (birth-control pills)
- Increased CAS capacity may contribute to the increased thrombotic risk caused by COC

1 | INTRODUCTION

The contact system (CAS) consists of a group of plasma proteins, coagulation factor XII (FXII), prekallikrein (PK), and H-kininogen (HK), promoting inflammation, coagulation, and fibrinolysis when activated by an activating surface [1–4]. Both FXII and PK have trace amounts of endogenous enzymatic activity. Activating surfaces constitute cofactor properties and enhance reciprocal activation of FXII and PK causing a generation of significant amounts of FXIIa and plasma kallikrein (PKa) [4]. These reactions lead to the activation of coagulation through the formation of activated coagulation FXIa or to activation of the kallikrein-kinin pathway causing the release of bradykinin and formation of cleaved HK (cHK) [2,4–7]. Substances, such as inorganic polyphosphates (polyP), secreted by platelets, extracellular RNA, collagen exposed on injured endothelium, misfolded proteins, or mast cell heparin, serve reportedly as physiological activating surfaces; similarly, artificial surfaces, such as glass, silica, and kaolin, can activate CAS *in vitro* [8–13]. C1-esterase inhibitor (C1Inh), which targets FXIIa and kallikrein, is the main endogenous inhibitor of CAS [1–3]. Contact activation has been shown to be a potential and important factor in thrombus growth [1,8].

Combined oral contraceptives (COCs) are significantly associated with an increased risk of venous thromboembolism (VTE); the VTE risk in women treated with COC is 2 to 6 times higher than in nonusers, higher for third-generation COC than second-generation COC. The risk of VTE is highest within the first 6 to 12 months of use [14–19]. The estrogen component of COC causes prothrombotic alterations in the coagulation system, such as reduced plasma concentrations of antithrombin, protein S, and tissue factor pathway inhibitor [20–23]. The thrombin generation capacity is increased by COC, both in healthy women and women with polycystic ovary syndrome [23,24]. Most recently, a study in healthy women [25] demonstrated increased thrombin generation in women taking COC, which was suggested to be a possible effect of activation of CAS. However, the effect of COC on the complex interactions characterizing the initiation and propagation of CAS has not been systematically investigated.

Thus, the aim of the present study was to investigate in detail, the effect of COC on CAS with new methods focusing on the dynamic interactions of the CAS proteins and the total capacity of CAS in blood.

2 | METHODS

2.1 | Study population

Sixty-nine healthy women were recruited at the blood bank at Aalborg University Hospital after informed consent. Inclusion criteria were women aged between 18 and 55 years, using COC or not, and not taking any other medicine. According to Danish legislation and agreement with the organization of blood donors, it is allowed to perform an investigation similar to this when the samples are anonymized without formal approval from an ethical committee. The samples were initially collected to determine reference intervals [25] which indicated activation of the CAS of COC, which therefore has been investigated more thoroughly. In Denmark blood donors are healthy volunteers, without any apparent illness, donating blood without payment. The donors filled out a questionnaire about their age, height, weight, and smoking status. The formulation of COC and other ongoing hormone treatment were registered. Seven women were treated with pure progestogen contraceptives and were therefore excluded. Of the remaining 62 women, 30 were treated with COC and 32 were not. There was not enough plasma for all the investigations from all women, and therefore, the number of participants for each investigation was, in some instances, lower, confer Table 2.

2.2 | Blood collection

Venous blood was collected from the antecubital vein using a 16-gauge needle into 8.2 mL, 3.2% (w/v) trisodium citrate Monovette tubes (Sarstedt). The first tube was discarded. To reduce contact activation, the plunger was pulled backward as the blood flowed into the Monovette tube, thus forming a minimal vacuum concurrently with the blood flow. Platelet-poor plasma was obtained by centrifugation twice at 2500 g for 15 minute at 20 °C within 2 hours after collection and stored at −80 °C until analysis.

2.3 | Analyses and reagents

We used ELISA's to determine concentrations ofFXII [26], PK [11], HK [27], and cHK [28]. Concentrations of C1-esterase inhibitor (C1-inh)

TABLE 1 Baseline characteristics.

Baseline characteristics	COC users	Nonusers
N	30	32
Age, y, median(range)	26.0 (19.0-35.0)	37.5 (19.0-55.0)
BMI, kg/m^2, median (range)	24.4 (18.9-33.2)	27.3 (20.4-39.9)
Active smoker	1 (3.3%)	2 (6.3%)
Ethinyloestradiol dose (20 μg/30 μg)	3/26[a]	-
COC generation (2nd/3rd/4th)	26/3/1	-
Other hormonal treatment	0 (0.0%)	13[b] (37.5%)

[a]No information for one (*n* = 1) of the subjects (can be either 20 or 30 μg)
[b]Hormonal intrauterine device (*n* = 12) and local intravaginal progestogel (*n* = 1).

were determined with commercial C1-inh antibodies (Siemens Healthcare Diagnostics Products GmbH) using the BN II analyzer (Siemens Healthcare Diagnostics Products GmbH).

The kallikrein generation assay used for the determination of endogenous kallikrein potential (EKP), peak kallikrein concentration, and time-to-peak, was performed according to Biltoft et al. [6] In short, the Calibrated Automated Thrombin generation method [29] was modified using a fluorogenic substrate for kallikrein (instead of for thrombin), and initiating the reaction by colloidal silica (instead of tissue factor), resulting in a quantification of the overall kallikrein generation capacity of CAS.

2.4 | Statistical analysis

Between group differences were tested by the Mann–Whitney U-test and the results are presented as median, and 25-, and 75-percentiles in the table and as box-whisker plots in the figure. All differences were also tested parametrically (Student's *t*-test), showing virtually the same results, but because not all the distributions, assessed by histograms and quantile-quantile plots, were Gaussian, the nonparametric results are shown.

Mean differences in the measured coagulation parameters in nonusers and COC users, adjusted for age and body mass index (BMI), were estimated using linear regression models. The models were checked by diagnostic plots of the residuals.

3 | RESULTS

3.1 | Baseline characteristics

The characteristics of the 2 groups of women are presented in Table 1. The COC users were younger and had a lower BMI than the nonusers.

3.2 | Biochemical measurements

The results of the measurements of CAS are shown in Table 2, and Figure 1 depicts the distributions. FXII was significantly increased, whereas C1inh, the main inhibitor of FXIIa and PKa, was significantly decreased in COC users. In contrast, PK and HK, were not significantly different between the groups. (Figure 1, Table 2)

The capacity of CAS was significantly increased in COC users demonstrated by an increased EKP and peak PKa concentration and a

TABLE 2 Results of the CAS measurements .

	Median (25p-75p)		
Contact activation parameter	+ COC (n = 30)	− COC (*n* = 32)	*P* value
Factor XII (mg/L)	38.4 (32.5-42.7) n = 28	28.9 (26.8-34.8) *n* = 27	<.0003
Cleaved high molecular weight Kininogen (μg/ml)	2.02 (1.67-2.55) n = 30	1.07 (0.90-1.28) *n* = 32	<.0001
C1-esterase inhibitor (g/L)	0.20 (0.18-0.22) n = 30	0.23 (0.21-0.24) *n* = 32	<.0003
Endogenous kallikrein potential (nmol/L × min)	1860 (1690-2185) n = 28	1500 (1306-1785) *n* = 27	<.004
Peak kallikrein concentration (nmol/L)	1433 (1236-1630) n = 28	987 (842-1216) *n* = 27	.0001
Time-to-peak (min)	0.8 (0.8-0.9) n = 28	1.2 (1.0-1.8) *n* = 27	<.0001
Prekallikrein (μg/ml)	19.1 (17.5-24.7) n = 28	20.4 (17.5-22.8) *n* = 27	.98
High molecular weight kininogen (%)	112.5 (100.0-124.3) n = 28	114.0 (95.5-130.5) *n* = 27	.62

CAS, contact system; COC, combined oral contraceptives users; +COC, COC users; -COC, non COC users

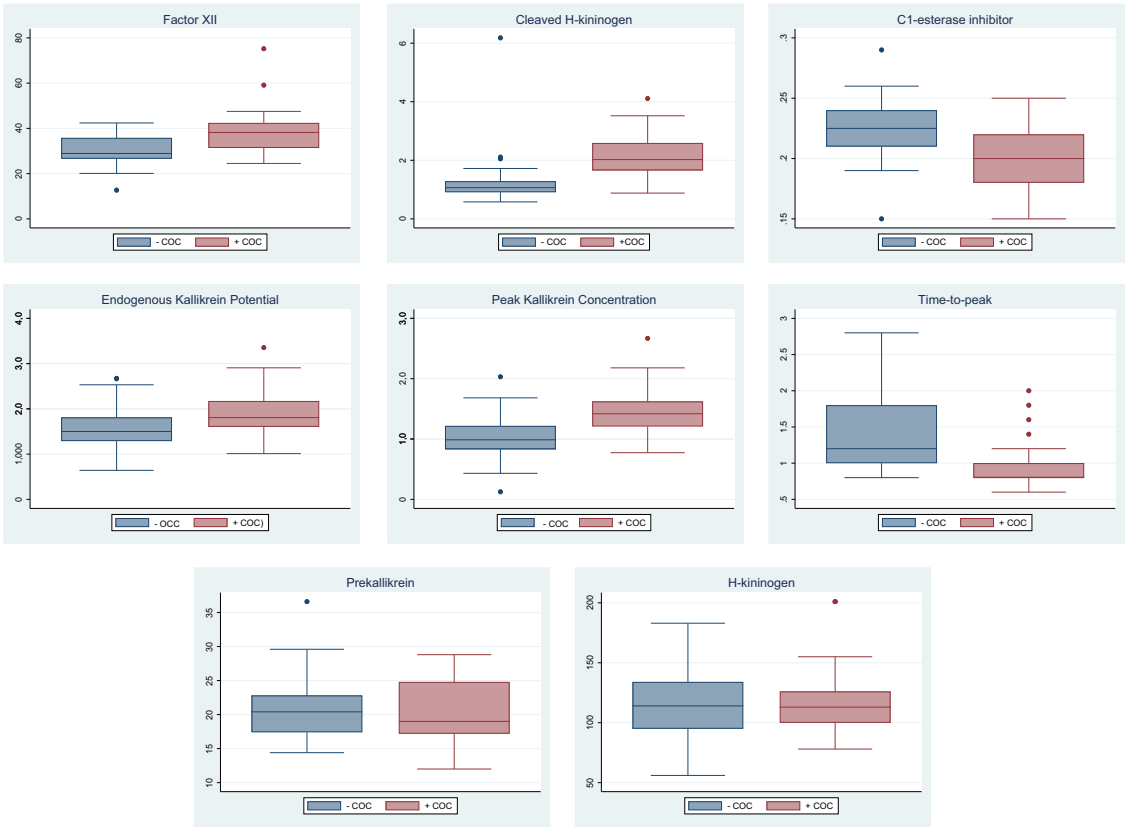

FIGURE 1 Summary of the results presented in box and whiskers plots showing median, 25th and 75th percentile, 5th and 95th percentiles, supplemented with outliers. COC, combined oral contraceptives; +COC, COC users; −COC, nonusers.

shorter time-to-peak. cHK, a marker of CAS activation, was also significantly increased in COC users.

The crude and adjusted estimated differences in the measured coagulation parameters are shown in Table 3. Apart from PK and HK, which did not show statistically significant differences between the groups, all estimated differences in the measured CAS parameters remained significant in COC users compared with nonusers after adjustment for age and BMI; neither age nor BMI significantly affected the results.

4 | DISCUSSION

Our study, based on measurements of various components of CAS in 62 women, where the sampling technique was performed with a minimal activation of CAS itself, indicates that several factors involved in CAS are affected during treatment with COC. The combined effect results in an increased CAS capacity in COC users, as demonstrated by increased EKP, and, furthermore, the increased plasma concentration of cHK indicates an increased activity of CAS *in vivo* in COC users. Of the proteins in the system, FXII was increased, and the inhibitor C1inh was decreased in COC users. All the differences were highly statistically significant ($P < .005$). PK and HK were not significantly different.

The effect of COC on CAS has only sparsely been investigated. A few older articles based on relatively small study populations described a significant elevation of FXII [30–35] in accordance with the present results. Recently, Stocco et al. [36] found only a nonsignificant increase in FXII levels in COC users compared with a control group. Inconsistent results are reported regarding PK and C1inh levels, showing elevations [30,32] or no significant change [31,33] of PK, and a decrease [31,34,35], as in the present study, or no change [30] of C1inh in COC users compared with controls. In accordance with our results Fossum et al. [32] and Wessler [34] also measured HK without any significant effect of COC. A recent study by Palarasah et al. [28], found an increased level of cHK in women receiving COC. To our knowledge, the present study is the first focusing on the dynamic interactions of the CAS proteins and the total capacity of CAS in women receiving COC, and the specimens were sampled in tubes with a low level of contact activation [25], and uniformly in the 2 groups. EKP has, to our knowledge, not been measured in COC users before.

The combination of the results is illustrated in Figure 2. Our results do not only show an increased potential of CAS, because the activator FXII is increased and C1inh is decreased, but also demonstrate that the capacity of CAS is increased. The increased level of cHK indicates higher CAS activity *in vivo* in COC users than controls. HK is a glycoprotein with cofactor functions for both the coagulation system leading to thrombin generation, as well as the kallikrein pathway. Conversion of HK to cHK induced by PKa, results in cleavage of domain 4 of HK liberating the vasoactive peptide

TABLE 3 Linear regression analysis with coagulation parameters as the dependent variable, estimated differences for COC users compared with nonusers.

Contact activation parameter	Estimate (crude)	95% CI	Estimate (adjusted for age)	95% CI	Estimate (adjusted for age and BMI)	95% CI
FXII	8.72	3.93-13.51	9.68	4.19-15.18	10.83	5.76-15.90
cHK	0.82	0.38-1.26	1.05	0.55-1.55	1.09	0.58-1.60
C1inh	−0.025	−0.037 to 0.012	−0.028	−0.042 to 0.013	−0.024	−0.038 to 0.001
EKP	370	114-626	383	89-679	425	136-713
Peak kallikrein concentration	458	244-672	422	177-668	459	–
Time-to-peak	−0.50	−0.76 to −0.24	−0.48	−0.78 to 0.19	−0.52	−0.81 to 0.23
Prekallikrein	−0.12	−2.72 to 2.48	−0.34	−3.33 to 2.66	−0.30	−3.35 to 2.75
HK	3.15	−12.60 to 18.91	6.23	−11.84 to 24.31	7.13	−11.21 to 25.46

CI, confidence interval; FXII, coagulation factor XII; cHK, cleaved high molecular weight kininogen; C1inh, C1-esterase inhibitor; EKP, endogenous kallikrein potential; Peak, peak kallikrein potential; PK, prekallikrein; HK, high molecular weight kininogen

bradykinin which is an inflammatory mediator [9,37–39], and therefore cHK is a marker for an activation of CAS.

The kallikrein generation assay [6] assesses the total capacity of CAS, measuring the amount of PKa being generated (EKP), as well as the speed of the reaction (time-to-peak) and the peak PKa concentration after activation of CAS. The kallikrein generation assay is sensitive to the plasma levels of FXII, PK, and C1-inh [6].

The study implies, therefore, that the changes in CAS in COC users may subsequently lead to a potentially higher coagulation activity and fibrin formation promoted by CAS. This may be a contributing factor to the increased coagulability in COC users.

The importance of the effect of CAS in normal hemostasis has been discussed during the last decades. FXII was previously considered to be without importance for coagulation *in vivo*, because hereditary FXII deficiency in humans is not associated with severe bleeding [2,40]. Pauer et al. [41] and Iwaki et al. [42], however, showed that FXII deficiency in mice was thrombo-protective but that the mice retained a normal hemostatic capacity, indicating that FXII could be involved in the pathologic thrombus formation process, without affecting the hemostatic capacity. In 2012 Renné et al. [43] described several contact activators mediating FXII activity, including platelet polyP (an inorganic polyphosphate released upon platelet activation [43–45]), misfolded protein aggregates, and mast cell heparin, which were thought to be of relevance in the development of thrombotic and inflammatory conditions. These findings indicate that CAS is of importance for the risk of thrombus formation, as also shown in eg, cancer and liver diseases [46–48].

In accordance with these findings, the idea that inhibition of CAS may be anticoagulant without increasing the risk of bleeding has emerged [38]. Especially inhibitors of FXIa have been developed as new thromboprophylactic drugs which have shown promising properties, and they are presently tested in clinical trials [49–53].

Thus, a higher activity of the CAS among COC users may be a contributing thrombotic change in addition to the well-known changes in the coagulation system.

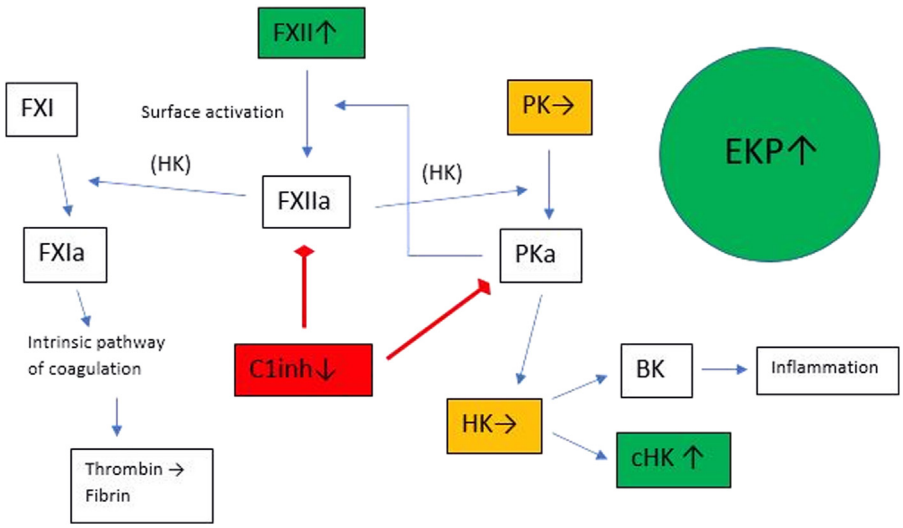

FIGURE 2 An overview of the impact of combined oral contraceptives (COC) on the contact system. Red arrows represent inhibition and blue arrows represent activation. Study results are represented in green boxes (increase), red boxes (decrease), and yellow boxes (no changes). FXI, coagulations factor XI; FXIa, activated coagulations factor XI; FXII, coagulation factor XII; FXIIa, activated coagulation factor XII; C1inh, C1-esterase inhibitor; PK, prekallikrein; PKa, kallikrein; HK, high molecular weight kininogen; cHK, cleaved high molecular weight kininogen; BK, bradykinin; EKP, endogenous kallikrein potential.

A limitation of this study is the relatively small number of participants within the 2 groups and the study was not primarily designed for this investigation. The fact that there are differences between the groups, considering age and BMI, could potentially affect the results. However, the results are statistically significant with clear differences between the groups, and adjustments for age and BMI, using multiple regression analyses did not affect the results significantly; thus, indicating a negligible effect.

The women in the COC-group use various COC formulations. More uniform results might have been obtained if identical ethinyloestradiol doses and COC generations were administered to all subjects. The time of administration of COC or day in the menstrual cycle was not registered, which is another limitation of the study; however, only relatively minor differences during the cycle of various other hemostatic components have been described. [54] Furthermore, the potential variation of these factors will mainly increase the variation within the groups, and consequently, it would be more difficult to demonstrate a difference.

Ethnicity was not registered, but in Denmark the vast majority of the population, and also the participants, are Caucasian; thus the results cannot necessarily be extrapolated to other ethnicities.

This study can be recognized as a kind of a pilot study indicating an effect of COC on CAS. A prospective study addressing the effect of COC on CAS in the same persons before and after the start of COC should be performed to confirm the results of our study.

5 | CONCLUSION

The findings of this study indicate that COC users have an increased capacity of CAS compared with nonusers. This capacity may be a procoagulant factor contributing to the increased thrombotic risk caused by COC.

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ETHICS STATEMENT

Ethics statement All participants provided informed consent. According to Danish legislation and agreement with the organization of blood donors, an investigation similar to this is allowed to be performed when the samples are anonymized without formal approval from an ethical committee.

AUTHOR CONTRIBUTIONS

S.R.K., J.B.G., and J.J.S. designed the study. J.J.S., J.B.G., and YP. constructed the assays and made the analyses. J.S., I.L.G., and S.R.K. interpreted data. J.S. and S.R.K. wrote the paper. All authors contributed to the critical revision of the manuscript and approved the final version to be published.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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