Research, Society and Development, v. 10, n. 9, e12910917888, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i9.17888

Association between the use of female hormones and the thrombin generation: Cross-sectional analysis of the Longitudinal Study on Adult Health (ELSA-Brasil)

Associação entre o uso de contraceptivos orais e terapia hormonal e a geração de trombina: Análise transversal do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil)

Asociación entre uso de anticonceptivos orales y terapia hormonal y generación de trombina: Análisis transversal del Estudio Longitudinal de Salud del Adulto (ELSA-Brasil)

Received: 07/05/2021 | Reviewed: 07/11/2021 | Accept: 07/12/2021 | Published: 08/03/2021

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Abstract
Introduction: The thrombin generation assay (TGA) assesses the risk of developing thrombotic events. The aim of this study was to investigate the association between the use of oral contraceptives (OCs) and hormone therapy (HT) with the TGA and resistance to activated protein C (APC). Methods: Cross-sectional study with women from the Longitudinal Study of Adult Health (ELSA-Brasil). TGA was performed by the CAT method. Results: There was an association between all parameters of the TGA and the use of OCs and an association of lag time and peak time with the use of HT. Conclusion: The TGA allows us to assess the hypercoagulability status and may be useful in the management of female hormones users.
Keywords: Contraceptives, oral; Hormone replacement therapy; Blood coagulation tests.

Resumo
Introdução: O teste de geração de trombina (TGT) avalia o risco de desenvolvimento de eventos trombóticos. O objetivo deste estudo foi investigar a associação entre o uso de contraceptivos orais (COs) e a terapia hormonal (TH)

com a TGT e a resistência à proteína C ativada (PCa). Métodos: Estudo transversal com mulheres do Estudo Longitudinal de Saúde do Adulto (ELSA-Brasil). A TGT foi realizada pelo método CAT. Resultados: Houve associação entre todos os parâmetros do TGT e o uso de COs e associação do tempo de latência e pico com o uso de TH. Conclusão: A TGT permite avaliar o estado de hipercoagulabilidade e pode ser útil no manejo de usuárias de hormônios femininos.
Palavras-chave: Anticoncepcionais orais; Terapia de reposição hormonal; Testes de coagulação sanguínea.

Resumen
Introducción: La prueba de generación de trombina (TGT) evalúa el riesgo de desarrollar eventos trombóticos. El objetivo de este estudio fue investigar la asociación entre el uso de anticonceptivos orales (AO) y la terapia hormonal (TH) con TGT y la resistencia a la proteína C activada (CaP). Métodos: Estudio transversal con mujeres del Estudio Longitudinal de Salud del Adulto (ELSA-Brasil). La TGT se realizó mediante el método CAT. Resultados: Hubo asociación entre todos los parámetros TGT y el uso de CO y asociación de latencia y tiempo pico con el uso de TH. Conclusión: TGT permite la evaluación del estado de hipercoagulabilidad y puede ser útil en el manejo de mujeres usuarias de hormonas.
Palabras clave: Anticonceptivos orales; Terapia de reemplazo de hormonas; Pruebas de coagulación sanguínea.

1. Introduction

The use of hormones by women is a historically known event, be it for family planning purposes or for the relief of vasomotor symptoms of menopause and prevention of osteoporosis. Approximately 100 million women worldwide use oral contraceptives (OCs) (EPIUN, 2011) and it is estimated that in the early 21st century, approximately 15 million American women were using hormone therapy (HT) (Rozenberg, 2013). OCs and HT have a desired action on the reproductive organs, vasomotor symptoms, and osteoporosis, however they can also have negative effects on lipid and carbohydrate metabolism, in addition to hemostatic changes (OMS, 2003; Vigo, 2011).

Studies have shown that women who use OCs or HT are at a higher risk of developing thromboembolic diseases (TED) when compared to those who do not use (Grodstein, 1996; Hoibraaten, 1999; Lidegaard, 2011; Vlieg, 2009). The use of OCs has been reported to cause a state of hypercoagulability caused by increased hepatic production of coagulation factors (fibrinogen, VII, VIII, IX, X, XII and XIII), reduction in natural anticoagulants (protein S and antithrombin) and development of acquired resistance to activated protein C (APC) (Olivieri, 1995; Rosing, 1997; Vieira, 2007). Similarly, HT seems to promote increased levels of factors VII, IX, X and fibrinogen, decreased antithrombin, and development of resistance to APC (Lowe, 2000; Notelovitz, 1975). However, studies have suggested that all of these effects appear to be nullified with discontinuation of use, although these findings are still inconclusive (Grodstein, 1996; Rosendaal, 2003).

The development of TED in OC and HT users is a rare event, but one that presents a serious and potentially fatal clinical outcome (Heit, 2005; Heit, 2016). For this reason, screening for women who have hereditary or acquired thrombophilia before the indication for the use of OCs and HT is essential. However, the blood coagulation tests available at the clinic (Prothrombin Time, Activated Partial Thromboplastin Time and D-dimer) only evaluate coagulation superficially and are not able to identify states of hypercoagulability (Lecut, 2015). In this sense, the thrombin generation assay (TGA) has emerged as a possibility of screening and identifying thrombophilia in women candidates for the use of OCs and HT.

The TGA has been developed and improved for decades, and aims to assess the coagulation process more widely (Macfarlane, 1953; Pitney, 1953). It is a technique capable of assessing hemostasis globally, covering the stages of initiation, amplification and propagation of coagulation. Thus, it is able to reflect the risk of developing hemorrhagic and thrombotic events by assessing all components and/or conditions of the hemostatic process, including coagulation factors, natural anticoagulants, fibrinolysis and resistance to APC (Pitney, 2014).

Therefore, the present study aims to investigate the association between the use (current and past) of OCs and HT and TGA, as well as resistance to APC in women of childbearing age and menopause in the Longitudinal Study of Adult Health (ELSA-Brasil), from the state of Minas Gerais.

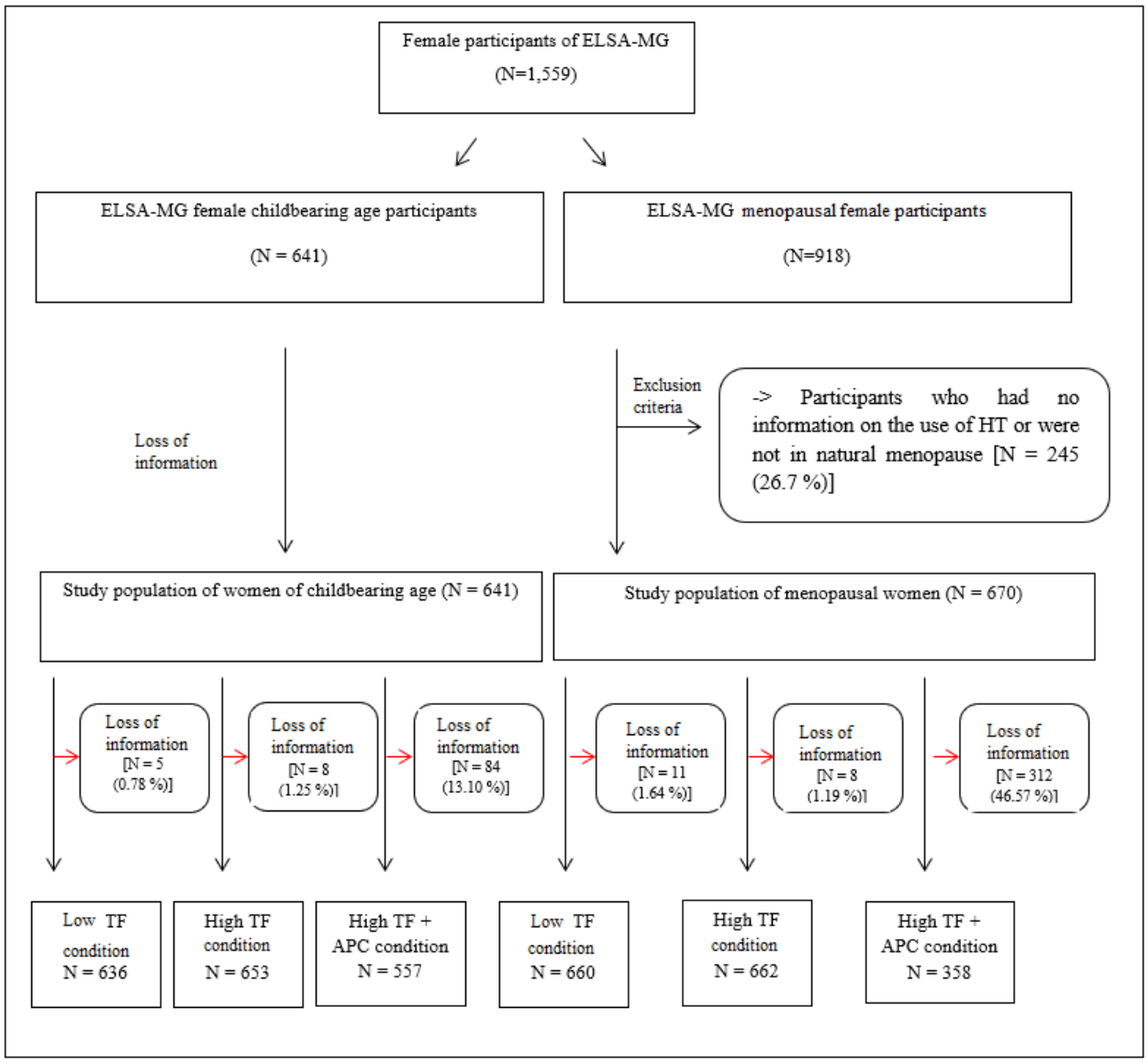
2. Methodology

Study population

This is a cross-sectional study that used data from the baseline (2008-2010) of the Longitudinal Study of Adult Health (ELSA-Brasil), a prospective multicenter cohort composed of 15,105 public servants aged between 35 and 74 years from teaching and research institutions in six Brazilian state capitals (Aquino, 2012; DCTMS, 2009).

Of the total of 1,559 women participants of the baseline ELSA-Brasil from Minas Gerais, 641 were of childbearing age (those who at the time of the interviews reported that they still menstruate) and 918 were menopausal (those who declared they did not menstruate due to natural causes) for at least twelve months at the time of the interview) (Figure 1) (Aquino, 2012). Participants who reported using OCs or HT at the time of the interview were classified as current users of these medications. Those who used OCs or HT in the past but at the time of the interview no longer used them, were classified as ex-users, while those participants who reported never having used them, were classified as non-users of these medications.

Figure 1: Flowchart of the study population. *Due to issues inherent to TGT, there was a loss of information in the three conditions and the number of participants analyzed in each condition differed.



Source: Authors.

Participants with a past history of thrombosis or embolism [women of childbearing age: N = 14 (2.2%); menopausal women: N = 25 (3.7%)], with medical self-report of cirrhosis or hepatitis [women of childbearing age: N = 46 (7.2%); menopausal women: N = 35 (5,7%)], or use of antiplatelet drugs and/or anticoagulants [women of childbearing age: N = 5 (0.8%); menopausal women: N = 42 (6.3%)], are generally excluded from studies investigating the association between the use of OCs and HT and hemostatic markers. However, after analysis with and without these participants, we did not see any difference in the results, and so we chose to keep them in this study.

ELSA-Brasil was approved by the Research Ethics Committees of the participating educational and research institutions and also by the National Research Ethics Committee (CONEP 976/2006) of the Ministry of Health. All participants signed the Free and Informed Consent Form before conducting interviews, exams, and measurement.

Plasma samples

The collection of venous blood samples was performed in the morning, after 12-14 h of fasting, following the procedures of the Clinical & Laboratory Standards Institute (CLSI) (CLSI, 2007). Venipuncture was performed in vacuum tubes containing 1 volume trisodium citrate 0.105M to 9 volumes blood (BD Vacutainer System/Greiner tubes) identified with bar codes. The samples were centrifuged at 2,500g for 15 minutes. Subsequently, the plasma supernatant corresponding to the platelet-poor plasma (PPP) was separated and stored in an ultra-freezer at -80 ºC until thrombin generation measurements were performed.

Thrombin generation assay

For realization of the TGA, a control plasma pool was prepared to normalize the endogenous thrombin potential (ETP) of the participants' samples, to calculate the normalized APC sensitivity ratio (nAPCsr), as well as to use as internal quality control and for the determination of inter-assay variability. PPP was obtained by mixing 170 samples from female participants of ELSA-Brasil from the state of Minas Gerais who met the following criteria: C-reactive protein ≤ 3mg/dL (to exclude acute diseases), without the use of female hormones and antithrombotic drugs potentially interfering with the hemostatic mechanism. Control plasma pool was added in duplicate to all plates.

The TGA was performed using 96-well plates, where 80 µL of PPP from the sample to be tested or from the control sample pool, and 5 µL of the HNBSA buffer were added to each well in which sensitivity to APC was not tested. In parallel for each sample, a well was added for the calibrator, consisting of 80 µL of PPP, 5 µL of HNBSA, and 20 µL of the thrombin calibrator reagent (STAGO®). Once the plate was filled with the plasma samples to be tested and their respective calibrators, the plate was placed on the fluorimeter (Fluoroskan Ascent, Thermo Laboratories®) for incubation at 37ºC for 10 minutes. After incubation, 20 µL of the TF in high and low concentrations were added only to the wells with the test samples of PPP and the control sample pool: PPP Reagent High (STAGO®) or PPP Reagent Low (STAGO) ®, respectively. In the wells in which a high concentration of TF was added to assess sensitivity to APC, 5 µL of APC were also added. Subsequently, 20 µL of the fluorescent substrate (Fluca-Kit - STAGO®) was dispensed in all wells by the fluorimeter dispenser, making a total of 125 µL in each well. The plate reading began immediately and continued for 60 minutes. Thrombinoscope® software was used to construct the time curve (min) versus thrombin concentration (nM) and to calculate the TGA parameters. The formation of this curve can be observed kinetically on the computer screen.

The four parameters evaluated by the TGA were the lag time (which corresponds to the time to start the formation of thrombin); the peak of thrombin generation (which corresponds to the maximum concentration of thrombin formed); the time to reach the peak of thrombin generation (time to peak); and the ETP (which corresponds to the area on the TGA curve, i.e., all thrombin formed during the 60 minutes of evaluation).

It is important to note that the TGA was performed under three conditions: in the Low TF condition (in which the low TF concentration was used as the trigger for the reaction); in the High TF condition (in which the high TF concentration was used as the trigger for reaction) and in the High TF + APC condition (in which the high TF concentration was used as the trigger for the reaction and was added to the APC).

For the determination of the nAPCsr, the control plasma pool was titrated with concentrations of 10 nM, 15 nM, and 20 nM APC to determine the concentration that inhibited approximately 90 % of TG. After performing the titration, the concentration of 15 nM APC was used in the TGA to determine the ETP with and without APC from the tested sample and from the control sample pool.

The nAPCsr was subsequently calculated using the following formula:

$$nAPCsr = \frac{(ETP + APC / ETP - APC)\ sample}{(ETP + APC / ETP - APC)\ control\ pool}$$

Where ETP + APC corresponds to the ETP value with the addition of APC, and ETP - APC, the ETP value without the addition of APC.

Covariables

Age was assessed on a continuous scale and schooling was categorized into (≤ complete elementary school, complete high school, and complete higher education).

Diabetes was defined by self-reported medical diagnosis of diabetes or use of medication to treat diabetes, or fasting glucose ≥ 126 mg/dL, or glucose tolerance test ≥ 200 mg/dL, or glycated hemoglobin ≥ 6.5%. Arterial hypertension was defined by self-reported medical diagnosis of arterial hypertension, or use of medication to treat hypertension, or pressure levels ≥ 140/90 mmHg. The lipid profile was defined through the levels of LDL and HDL cholesterol and triglycerides; the use of lipid-lowering drugs refers to those used in the two weeks prior to the interview. The prevalent cardiovascular disease was built from the self-report of the medical diagnosis of the following comorbidities: acute myocardial infarction, angina, congestive heart failure, cerebrovascular accident, and myocardial revascularization. Self-reported medical diagnoses of thromboembolic events and liver disease were also evaluated. Obesity was assessed using anthropometric measurements of weight and height, and participants who had a body mass index (BMI) ≥ 30 kg/m2 were defined as “obese”.

Health-related behaviors were determined by participants’ self-reporting. Leisure-time physical activity was obtained through the International Physical Activity Questionnaire (IPAQ) and defined as light, moderate, and strenuous. Participants who performed physical activity for three days or more, for 20 minutes per day with vigorous intensity; or performed physical activity for three days or more, for 30 minutes with moderate intensity and/or walking; or performed physical activity for more than five days, achieving a minimum of at least 600 metabolic equivalent of the task (MET) minutes per week with moderate or vigorous walking intensity; or performed physical activity for more than three days, achieving a minimum of at least 1500 MET minutes per week and vigorous walking intensity, were included in the moderate physical activity group. The strenuous physical activity group included participants who performed physical activity seven days a week, equivalent to ≥ 3000 MET with moderate or vigorous walking intensity. All individuals who did not meet the criteria for moderate or strenuous activity were classified in the light physical activity group.

Excessive consumption of alcoholic beverages was assessed and defined by the type of drink usually consumed, frequency of consumption, and consumption patterns. The information obtained in the questionnaire was summarized and

defined in grams of alcohol consumed per week. Excessive consumption of alcoholic beverages was defined as ≥140 g per week. Participants who declared they had smoked at least 100 cigarettes in their lifetime were considered smokers. The category "currently smokes" was composed of participants who still smoked at the time of the interview. Those who reported having smoked throughout their lives but who did not smoke at the time of the interview were classified as ex-smokers.

Statistical analysis

The normality of continuous variables was assessed using histogram analysis. Continuous variables that showed a symmetrical distribution were described using mean ± standard deviation. For the comparison of means between the categories of use of OCs and HT, the ANOVA test and the Bonferroni post-test were used. Variables with asymmetric distribution were described using the median and interquartile range (Q1-Q3). For the comparison between the categories of use of OCs and HT, the Kruskal-Wallis test was used.

Categorical variables were described using proportions. To compare proportions between the different groups of use of OC and HT, the Chi-square test was used. The independent association between the use of OC and HT and TG was assessed using linear regression. The gross and adjusted β and their respective 95 % confidence intervals (95 % CI) were estimated. For the two study populations (women of childbearing age and menopausal women), the gross β (model 0) was initially estimated. Among women of childbearing age, age was entered in model 0 (model 1), and levels of HDL-c cholesterol and LDL-c cholesterol (model 2), triglyceride levels (model 3), triglyceride and LDL-c cholesterol (model 4), triglyceride levels, and HDL-c cholesterol (model 5) were inserted in model 1. Among menopausal women, after adjusting for age (model 1), the model was adjusted by BMI and triglycerides (model 2). The variables used as adjustment were those associated with both the use of OC, among women of childbearing age, and the use of HT, among women in menopause, such as TG. The value of $p < 0.05$ was considered significant. All analyses were performed using Stata software version 14.0.

3. Results

Of the 641 participants of childbearing age, 151 (23.5 %) never used OCs, 437 (68.2 %) used in the past and 53 (8.3 %) currently use them. Regarding the 670 menopausal participants, 339 (50.6 %) never used HT, 242 (36.1 %) used in the past and 89 (13.3 %) currently use. The characteristics of the study population with regard to age, education, health conditions, lipid profile, health-related lifestyle habits, past history of thrombotic events and liver disease, and use of antiplatelet/anticoagulant agents are shown in Table 1.

Table 1: Characteristics of the study population according to the use of oral contraceptives or hormonal therapy (N = 1311). ELSA-Brasil (2008-2010).

		Use of OCs					Use of HT			
Characteristics	**Total (N=641)**	**Non-user (N=151)**	**Ex-user (N=437)**	**Current user (N=53)**	**p-value**	**Total (N=670)**	**Non-user (N=339)**	**Ex-user (N=242)**	**Current user (N=89)**	**p-value**
Age (years)[a]	45.1±5.0	45.8±5.3	45.1±4.8	42.2±4.6	<0.001[§¶]	58.2±6.6	56.4±6.2	60.8±6.2	58.4±6.6	<0.001[§¶ǂ]
Schooling[b]					-					-
Complete Higher Education	401 (62.6%)	94 (62.3%)	271 (62.0%)	36 (67.9%)		392 (58.6%)	177 (52.2%)	146 (60.3%)	69 (77.5%)	
Complete High School	231 (36.0%)	55 (36.4%)	159 (36.4%)	17 (32.1%)		218 (32.5%)	124 (36.6%)	74 (30.6%)	20 (22.5%)	
Complete Elementary School	9 (1.4%)	2 (1.6%)	7 (1.6%)	0		60 (8.9%)	38 (11.2%)	22 (9.1%)	0	
Health conditions[b]*										
Diabetes	49 (7.6%)	13 (8.6%)	32 (7.3%)	4 (7.5%)	0.876	122 (18.2%)	67 (19.8%)	45 (18.6%)	10 (11.2%)	0.175
Arterial Hypertension	136 (21.2%)	28 (18.5%)	97 (22.2%)	11 (20.7%)	0.636	260 (38.8%)	133 (39.2%)	109 (45.0%)	18 (20.2%)	<0.001
Cardiovascular Disease	5 (0.8%)	0	5 (1.1%)	0	-	35 (5.3%)	22 (6.5%)	13 (5.4%)	0	-
Obesity	108 (16.8%)	27 (17.9%)	73 (16.7%)	8 (15.1%)	0.550	138 (20.6%)	89 (26.2%)	40 (16.5%)	9 (10.1%)	0.003

Lipid Profile [a]										
HDL Cholesterol (mg/dL)	59.5±13.6	57.2±13.5	59.5±13.0	65.9±16.4	<0.001[§¶]	61.7±14.3	61.0±13.6	62.6±14.5	61.5±16.4	0.455
LDL Cholesterol (mg/dL)	119.9±30.8	123.7±30.1	120.3±31.0	105.8±27.9	0.001[§¶]	134.8±35.3	135.6±30.5	136.7±38.0	127.0±43.2	0.076
Triglycerides (mg/dL)	104.7±57.5	103.7±55.9	102.5±56.9	125.9±63.4	0.019[§¶]	120.9±64.9	127.4±68.9	120.5±63.0	97.3±46.5	<0.001[§¶]
Use of hypolipidemics[b]					-					0.019
No	612 (95.5%)	143 (94.7%)	416 (95.1%)	53 (100.0%)		531 (79.3%)	283 (83.5%)	179 (74.0%)	69 (77.5%)	
Yes	29 (4.5%)	8 (5.3%)	21 (4.9%)	0		139 (20.7%)	56 (16.5%)	63 (26.0%)	20 (22.5%)	
Smoker[b]					0.858					0.109
No	592 (92.4%)	141 (93.4%)	402 (92.0%)	49 (92.4%)		581 (86.7%)	285 (84.1%)	215 (88.8%)	81(91.0%)	
Yes	49 (7.6%)	10 (6.6%)	35 (8.0%)	4 (7.6%)		89 (13.3%)	54 (15.9%)	27 (11.2%)	8 (9.0%)	
Leisure time physical activity[b]					0.779					0.103
Physically inactive	480 (75.3%)	115 (76.7%)	327 (75.4%)	38 (71.7%)		482 (72.0%)	260 (76.7%)	161 (66.5%)	61 (69.3%)	
Moderate physical activity	112 (17.6%)	26 (17.3%)	77 (17.7%)	9 (17.0%)		151 (22.6%)	63 (18.6%)	66 (27.3%)	22 (25.0%)	
Strenuous physical activity	45 (7.1%)	9 (6.0%)	30 (6.9%)	6 (11.3%)		36 (5.4%)	16 (4.7%)	15 (6.2%)	5 (5.7%)	
					-					-

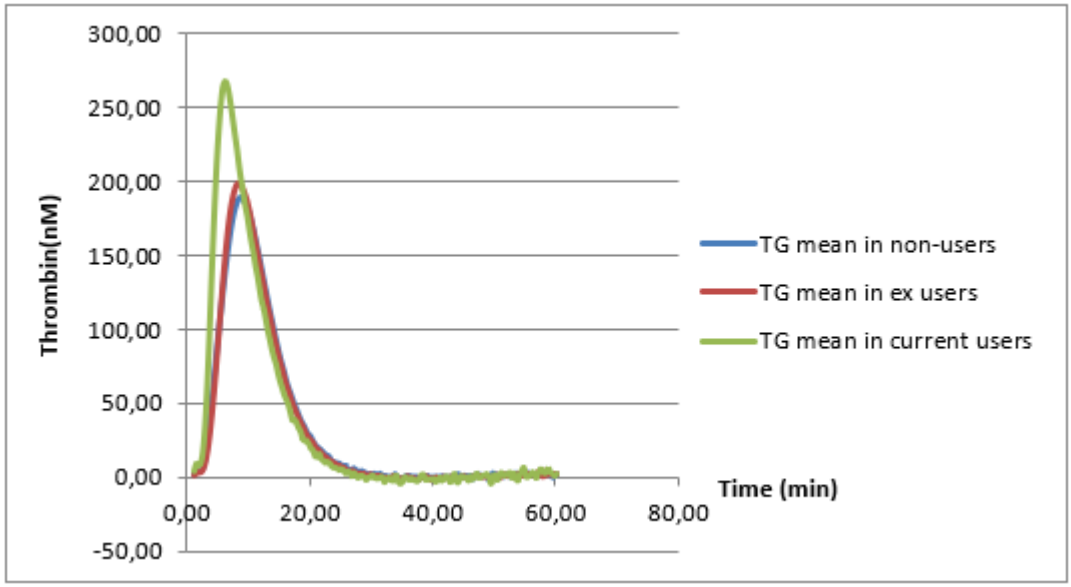
Previous history of thrombotic event[b]										
No	627 (97.8%)	146 (96.7%)	428 (97.9%)	53 (100.0%)		644 (96.3%)	323 (95.6%)	232 (95.9%)	89 (100.0%)	
Yes	14 (2.2%)	5 (3.3%)	9 (2.1%)	0		25 (3.7%)	15 (4.4%)	10 (4.1%)	0	
Previous history of liver disease[b]					0.291					0.397
No	595 (92.8%)	140 (92.7%)	403 (92.2%)	52 (98.1%)		635 (94.3%)	480 (71.6%)	55 (8.2%)	100 (14.9%)	
Yes	46 (7.2%)	11 (7.3%)	34 (7.8%)	1 (1.9%)		35 (5.7%)	17 (2.5%)	4 (0.6%)	14 (2.1%)	
Use of antiplatelet and/or anticoagulant[b]					0.311					0.395
No	633 (99.2%)	149 (100.0%)	431 (98.8%)	53 (100.0%)		628 (93.7%)	322 (94.9%)	224 (92.6%)	82 (92.1%)	
Yes	5 (0.8%)	0	5 (1.2%)	0		42 (6.3%)	17 (5.1%)	18 (7.4%)	7 (7.9%)	

[a]ANOVA: values expressed as mean and standard deviation. [b]Chi-square: values expressed in proportions. [c]Kruskal-Wallis: values expressed as median and interquartile range (Q1-Q3). *Reference category is the absence of disease. [§]Statistically significant difference between categories: current users and former users of OCs or HT. [¶]Statistically significant difference between categories: current users and non-users of OCs or HT. [ǂ]Statistically significant difference between categories: former users and non-users of HT or OCs. OCs: Oral contraceptives. HT: hormone therapy.

Compared to ex-users and non-users, women of childbearing age who are OC users were younger (p <0.001), had higher levels of HDL cholesterol (mg/dL) (p <0.001) and triglycerides (mg/dL) (p = 0.019), and lower levels of LDL cholesterol (mg/dL) (p = 0.001). In contrast, for menopausal women compared to former users, those currently using HT were younger (p <0.001), had a lower prevalence of hypertension (p <0.001) and obesity (p = 0.003), and lower levels of triglycerides (p <0.001).

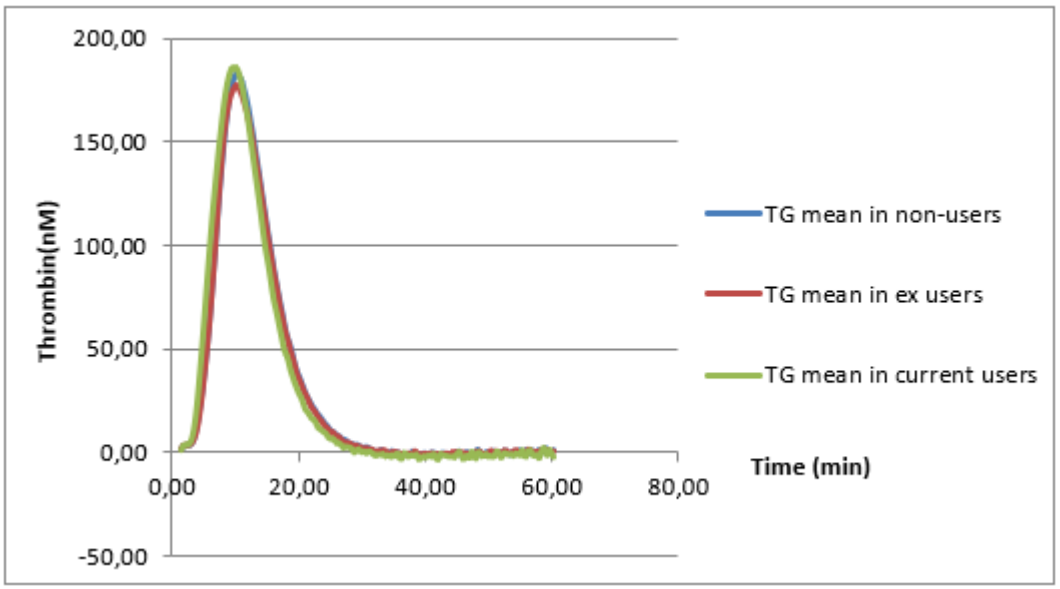
The parameters evaluated in the Low TF condition can be seen in Table 2. The current users of OCs presented lower lag time and time-to-peak averages (4.02 min ± 1.63 and 7.11 min ± 2. 38) than former users of OCs (5.10 min ± 1.68 and 8.95 min ± 2.55; p <0.001) and non-users (5.23 min ± 1.77 and 9.08 min ± 2.62 ; p <0.001). In relation to the average values of peak and ETP, we observed higher values in the current users of OCs (356.61 nM ± 83.35 and 2298.56 nM.min ± 418.28) in relation to the former users (274.94 nM ± 81.49 and 1946.28 nM.min ± 369.58; p <0.0001) and non-users of OCs (274.17 nM ± 87.29 and 1939.77 nM.min ± 357.36; p <0.0001) (Figure 2). The current users of HT had a lower lag time average (5.84 min ± 1.96) than the former HT users (6.80 min ± 2.40, p = 0.001) and non-users (6.79 min ± 2.39, p=0.001) and a lower mean peak (9.54 nM ± 2.73) than former users (10.58 nM ± 3.19, p = 0.014) and non-users of HT (10.60 nM ± 3.25, p = 0.014). There was no significant difference in the parameters evaluated between ex-users and non-users of OCs and HT (figure 3).

Figure 2: Thrombin generation (TG) as a function of time in the Low TF condition in women of childbearing age (N = 641).



Source: Authors.

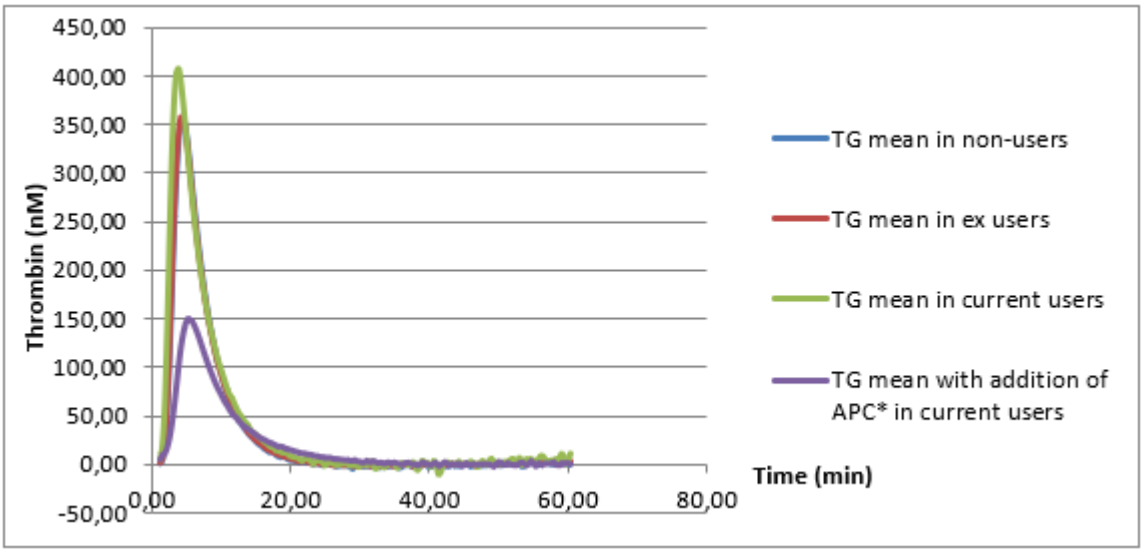
Figure 3: Thrombin generation (TG) as a function of time in the Low TF condition in menopausal women (N = 670).



Source: Authors.

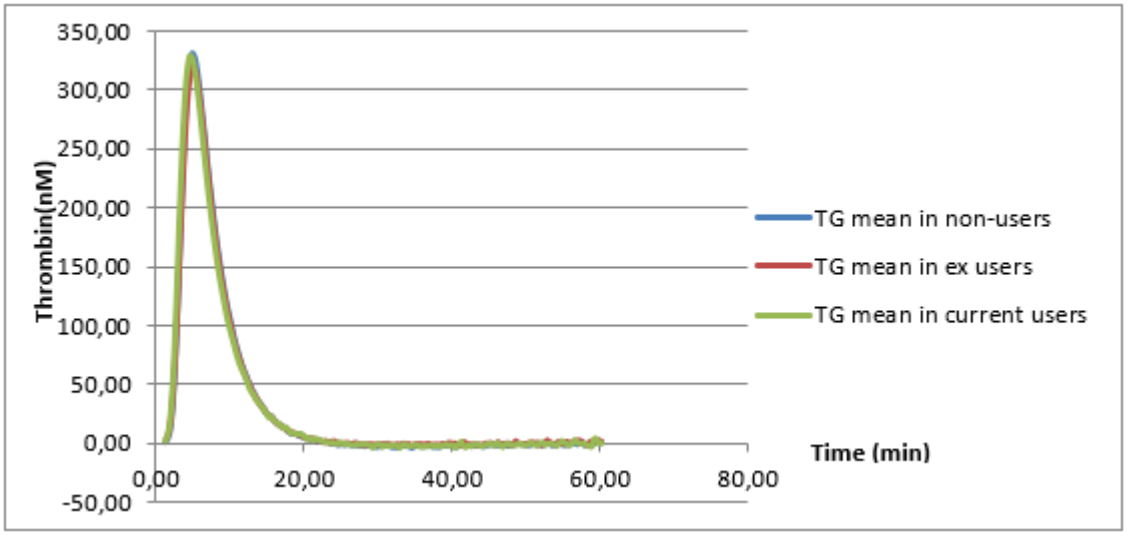
The parameters evaluated in the High TF condition are shown in Table 3. The current users of OCs presented lower mean lag time and time-to-peak values [1.85 min (1.36-2.00) and 3.88 min ± 0.63] than former users [2.13 min (1.67-2.33) and 4.36 min ± 0.82; p = 0.001] and non-users [2.26 min (2.00-2.33) and 4.37 min ± 0.66; p <0.001]. In relation to the average peak and ETP values, we observed higher values in the current users of OCs (434.39 nM ± 50.14 and 2335.73 nM.min ± 351.54) in relation to the former users (387.68 nM ± 53.22 and 2073 , 59 nM.min ± 349.25, p <0.001) and non-users of OCs (390.82 nM ± 56.59 and 2072.38 nM.min ± 350.01, p <0.001) (figure 4). The current users of HT had a lower mean time-to-peak value (4.65 min ± 0.88) than the former users (4.99 min ± 0.99, p = 0.015) and non-users of HT (4.97 min ± 0.99, p = 0.015). There was no significant difference in the parameters evaluated between ex-users and non-users of OCs and HT (Figure 5).

Figure 4: Thrombin generation (TG) as a function of time in the High TF condition in women of childbearing age (N = 641). *APC: activated protein C.



Source: Authors.

Figure 5: Thrombin generation (TG) as a function of time in the High TF condition in menopausal women (N = 670).



Source: Authors.

To assess the development of acquired resistance to APC, we analyzed nAPCsr. We observed that it was higher in current users of OCs (2.54 ± 1.27) compared to former users (1.40 ± 0.83, p = 0.001) and non-users (1.37 ± 0.91, p = 0.0001). There was no significant difference in values between former users and non-users of OCs (Figure 4). Regarding women in menopause, it was not possible to assess nAPC.

Table 2: Comparison of the parameters of thrombin generation assay in the Low TF condition, according to the categories of use of oral contraceptives (N = 636) and hormonal therapy (N = 660).

	Use of OC				Use of HT			
Parameters	**Non-users (n=151)**	**Ex-users (n=433)**	**Current users (n=52)**	**p-value**	**Non-users (n=335)**	**Ex-users (n=236)**	**Current Users (n=89)**	**p-value**
Lagtime (min)	5.23±1.77	5.10±1.68	4.02±1.63	<0.001§¶	6.79±2.39	6.80±2.40	5.84±1.96	0.001§¶
Time to peak (min)	9.08±2.62	8.95±2.55	7.11±2.38	<0.001§¶	10.60±3.25	10.58±3.19	9.54±2.73	0.014§¶
Peak (nM)	274.17±87.29	274.94±81.49	356.61±83.35	<0.001§¶	280.73±93.74	276.64±89.63	281.46±88.72	0.848
ETP (nM.min)	1939.77±357.36	1946.28±369.58	2298.56±418.28	<0.001§¶	1899.21±384.60	1864.81±369.48	1891.39±387.45	0.560

ANOVA: values expressed as mean and standard deviation. §Statistically significant difference between categories: current users and ex-users. ¶Statistically significant difference between categories: current users and non-users. OC: oral contraceptive; HT: hormone therapy. Source: Authors.

Table 3: Comparison of the parameters of thrombin generation assay in the High TF condition, according to the categories of use of oral contraceptives (N = 633) and hormonal therapy (N = 662).

	Use of OC				Use of HT			
Parameters	**Non-users (n=150)**	**Ex-users (n=431)**	**Current Users (n=52)**	**p-valor**	**Non-users (n=337)**	**Ex-users (n=236)**	**Current Users (n=89)**	**p-value**
Lagtime (min)	2.26 (2.00-2.33)	2.13 (1.67-2.33)	1.85 (1.36-2.00)	<0.001§¶	2.33 (2.00-2.67)	2.33 (2.00-2.70)	2.33 (1.96-2.67)	0.062
Time to peak (min)	4.37±0.66	4.36±0.82	3.88±0.63	<0.001§¶	4.97±0.99	4.99±0.99	4.65±0.88	0.015§¶
Peak (nM)	390.82±56.59	387.68±53.22	434.39±50.14	<0.001§¶	373.55±62.33	362.96±63.13	365.88±59.19	0.126
ETP (nM.min)	2072.38±350.01	2073.59±349.25	2335.73±351.54	<0.001§¶	2033.78±362.48	1983.08±343.92	1987.72±382.29	0.208
nAPCsr*	1.37±0.91	1.40±0.83	2.54±1.27	<0.001§¶	-	-	-	-

*nAPCsr: Normalized activated protein C sensitivity ratio (ETP with APC / ETP without APC of the participant / ETP with APC / ETP without APC of the control pool). Kruskal-Wallis: values expressed as median and interquartile range (Q1-Q3). ANOVA: values expressed as mean and standard deviation. §Statistically significant difference between categories: current users and ex-users. ¶Statistically significant difference between categories: current users and non-users. OC: oral contraceptive; HT: hormone therapy. Source: Authors

Even after adjustment for confounding factors (age, LDL-c cholesterol, HDL-c cholesterol and triglycerides), the current use of OCs remained associated with TGA parameters and past use remained unassociated, as can be seen in Table 4.

Regarding the current use of HT, after adjustment for confounding factors (age, BMI, and triglycerides), an association was observed between the current use of HT with the parameters lag time and the time to peak under Low and High conditions. Past use remained unassociated (Table 5).

Table 4: Linear regression analysis of the relationship between thrombin generation assay parameters (lag time, ETP, peak, and time to peak) in Low, High, and High + APC conditions and the categories of use of OCs in participants of childbearing age (N = 641) from the ELSA-Brasil (2008-2010) baseline, from Minas Gerais

TGA Conditions	Parameters		Models	Childbearing aged women		
				Non-users	Ex-users	Current Users
LOW	Lag time	β Coefficient (IC 95%)	Model 0	-	-1.346 (-0.450 - 0.180)	-1.212 (-1.749 - -0.677) §
			Model 1	-	-0.125 (-0.440 - 0.191)	-1.161 (-1.705 - -0.617) §
			Model 2	-	-0.089 (-0.403 - 0.225)	-1.001 (-1.552 - -0.451) §
	Time to Peak		Model 0	-	-0.126 (-0.601 - 0.348)	-1.966 (-2.773 - -1.158) §
			Model 1	-	-0.115 (-0.590 - 0.361)	-1.907 (-2.727 - -1.087) §
			Model 2	-	-0.068 (-0.542 - 0.405)	-1.694 (-2.525 - -0.864) §
	Peak		Model 0	-	0.772 (-14.641 - 16.186)	82.439 (56.215 - 108.663) §
			Model 1	-	1.000 (-14.449 - 16.451)	83.604 (56.954 - 110.253) §
			Model 3	-	1.064 (-14.080 - 16.208)	74.278 (47.933 - 100.623) §
	ETP		Model 0	-	6.514 (-62.623 - 75.352)	352.789 (235.674 - 469.904) §
			Model 1	-	7.231 (-61.775 - 76.237)	356.447 (237.419 - 475. 476) §
			Model 4	-	10.634 (-55.892 77.160)	326.582 (209.742 - 443.421) §
HIGH	Lag time	β Coefficient (IC 95%)	Model 0		-0.129 (-0.280 - 0.026)	-0.409 (-0.668 - -0.149) §
			Model 1	-	-0.119 (-0.272 - 0.034)	-0.366 (-0.629 - -0.103) §
	Time to Peak		Model 0		-0.015 (-0.159 - 0.129)	-0.491 (-0.736 - -0.245) §
			Model 1	-	-0.013 (-0.158 - 0.131)	-0.481 (-0.730 - -0.231) §
			Model 2	-	0.004 (-1.391 - 0.148)	-0.402 (-0.653 - -0.150) §
	Peak		Model 0	-	-3.137 (-13.152 - 6.877)	43.563 (26.565 - 60.566) §
			Model 1	-	-3.104 (-13.143 - 6.935)	43.738 (26.462 - 61.014) §
			Model 4	-	-2.510 (-12.140 - 7.119)	40.384 (23.511 - 57.256) §
	ETP		Model 0		1.165 (-63.920 - 66.249)	263. 350 (152.865 - 373.835) §
			Model 1	-	1.689 (-63.551 - 66.929)	266.081 (153.812 - 378.350) §
			Model 4	-	5.407 (-57.645 - 68.459)	250.691 (140.214 - 361.168) §
HIGH + APC	nAPCsr	β Coefficient (IC 95%)	Model 0		0.032 (-0.146 - 0.212)	1.175 (0.874 - 1.475) §
			Model 1	-	0.035 (-0.145 - 0.215)	1.188 (0.883 - 1.492) §
			Model 5	-	0.022 (-0.158 - 0.201)	1.081 (0.776 - 1.396) §

Model 0: univariate analysis between lag time, ETP, peak, and time to peak in Low, High, and High + APC conditions and OC use categories. Model 1: model 0 adjusted for age. Model 2: model 1 adjusted for LDL-c cholesterol and HDL-c cholesterol. Model 3: model 1 adjusted for triglycerides. Model 4: model 1 adjusted for LDL-c cholesterol and triglycerides. Model 5: model 1 adjusted for HDL-c cholesterol and triglycerides. §p-value <0.05. OCs: oral contraceptives, TGA: thrombin generation.assay Low condition: condition in which the generation of thrombin was evaluated with a low concentration of tissue factor. High condition: condition in which the generation of thrombin was evaluated with a high concentration of tissue factor. Condition High + APC: condition in which the generation of thrombin was evaluated with a high concentration of tissue factor in the presence of activated protein C.
Source: Authors.

Table 5: Linear regression analysis of the relationship between thrombin generation assay parameters (lag time, ETP, peak, and time to peak) in Low and High conditions and the categories of use of HT in menopausal participants (N = 670) of ELSA-Brasil baseline (2008-2010), from Minas Gerais.

				Menopause women		
TGA Conditions	**Paramters**		**Models**	**Non-users**	**Ex-users**	**Current users**
LOW	***Lag time***	**Coeficiente β (IC 95%)**	Model 0	-	0.008 (-0.383 - 0.399)	-0.948 (-1.496 - -0.399)
			Model 1	-	0.032 (-0.376 - 0.442)	-0.936 (-1.488 - -0.385)
			Model 2	-	0.024 (-0.389 - 0.437)	-0.949 (-1.513 - -0.385)
	Time to Peak		Model 0	-	-0.025 (-0.552 - 0.502)	-1.066 (-1.807 - 0.326)
			Model 1	-	0.101 (-0.450 - 0.652)	-1.010 (-1.753 - -0.267)
			Model 2	-	0.556 (-0.500 - 0.612)	-1.103 (-1.862 - -0.343)
	Peak		Model 0	-	-4.093 (-19.383 - 11.197)	0.726 (-20.729 - 22.181)
			Model 1	-	-8.043 (-24.015 - 7.928)	-1.058 (-22.588 - 20.473)
			Model 2	-	-2.867 (-18.667 - 12.943)	10.655 (-10.932 - 32.243)
	ETP		Model 0	-	-34.396 (-97.750 - 28.957)	-7.820 (-96.720 - 81.078)
			Model 1	-	-20.307 (-86.521 - 45.906)	-1.460 (-90.721 - 87.801)
			Model 2	-	8.991 (-55.424 - 73.406)	64.029 (23.926 - 151.985)
HIGH	***Lag time***	**Coeficiente β (IC 95%)**	Model 0	-	-0.038 (-0.145 - 0.069)	-0.194 (-0.344 - -0.445)
			Model 1	-	-0.029 (-0.141 - 0.083)	-0.190 (-0.341 - -0.040)
			Model 2	-	-0.018 (-0.131 - 0.095)	-0.163 (-0.317 - -0.009)
	Time to peak		Model 0	-	0.014 (-0.149 - 0.178)	-0.318 (-0.548 - -0.088)
			Model 1	-	0.048 (-0.124 - 0.219)	-0.302 (-0.533 - -0.071)
			Model 2	-	0.061 (-0.111 - 0.234)	-0.258 (-0.493 - -0.022)
	Peak		Model 0	-	-10.588 (-21.075 - -0.102)	-7.665 (-22.378 - 7.038)
			Model 1	-	-8.197 (-19.173 - 2.779)	-6.603 (-21.371 - 8.165)
			Model 2	-	-5.043 (-15.963 - 5.876)	0.655 (-14.233 - 15.544)
	ETP		Model 0	-	-50.698 (-110.487 - 9.090)	-46.056 (-130.004 - 37.892)
			Model 1	-	-29.532 (-91.912 - 32.847)	-36.427 (-120.532 - 47.678)
			Model 2	-	-3.914 (-64.818 - 56.989)	22.297 (-60.873 - 105.468)

Model 0: univariate analysis between lag time, ETP, peak, and time to peak in Low and High conditions and HT use categories. Model 1: model 0 adjusted for age. Model 2: model 1 adjusted for body mass index and triglycerides. §p-value <0.05. HT: hormone therapy. TGA: thrombin generation assay. Low condition: condition in which the generation of thrombin was evaluated with a low concentration of tissue factor. High condition: condition in which the generation of thrombin was evaluated with a high concentration of tissue factor.
Source: Authors.

4. Discussion

The present study aimed to investigate the association between the use (current and past) of OCs and HT and the generation of thrombin, as well as resistance to APC in women of childbearing age and menopause, from ELSA-Brasil, of the

state of Minas Gerais.

With regard to women of childbearing age, we observed that both TGA and acquired resistance to APC were higher among current users of OCs compared to former users and non-users, even after adjustment for confounding factors. Among non-users and ex-users of OCs, there was no statistically significant difference in any of the evaluated parameters. With regard to menopausal women, we observed that after adjustment for confounding factors, the parameters of TGA, lag time, and time to peak were associated with the current use of HT. However, among non-users and ex-users of HT, there was no statistically significant difference in any of the parameters evaluated even after adjustment for confounding factors.

The state of hypercoagulability of current users of OCs was characterized by a shortening of lag time and time-to-peak, and an increase in peak and ETP. The shortening of lag time and time-to-peak in OC users compared to ex-users and non-users reflects the accelerated generation of thrombin, while the increase in peak and ETP expresses the greater amount of thrombin generated during the process of coagulation. Although ETP is the parameter that most correlates with the patient's clinical condition as it is a parameter capable of reflecting the entire blood coagulation process (initiation, amplification, propagation, and action of natural anticoagulants), in our study the other parameters were also shown to be associated with the use of OCs and consequently with the state of hypercoagulability. Zia et al. (2015) and Glintborg et al. (2015) in intervention studies, also demonstrated the association between the state of hypercoagulability and the parameters lag time, ETP, and peak; however, they did not evaluate the time-to-peak.

It is important to note that the highest TG in participants using OCs was observed both in the Low TF condition and in the High TF condition. Therefore, our findings suggest that the effect of OCs on blood coagulation occurs jointly over both intrinsic and extrinsic pathways and/or over the common pathway. This finding corroborates the results of other studies that have already demonstrated that the use of OCs is associated with an increase in coagulation factors both in the intrinsic pathway, FXII, FIX, and FVIII, as well as in the extrinsic pathway, FVII, and the common pathway, FX (Olivieri, 1995; Rosing, 1997; Vieira, 2007).

A case-control study carried out in the United Kingdom (Vlieg, 2009) and a cohort carried out in Denmark (Lidegaard, 2011) demonstrated that OC users are at higher risk of developing TED than non-users. Our results corroborate these findings, since we found a higher TG in current users compared to ex-users and non-users of OCs. With the increase in the amount of thrombin generated, a key molecule in the formation of thrombi, our results may suggest a possible mechanism for the development of TED in OC users.

In contrast, a proposed mechanism to explain the increase in TG in current users of OCs, refers to the development of acquired resistance to APC, which can be evaluated by nAPCsr. In our study, nAPCsr was higher among current users compared to former users and non-users of OCs. This finding is able to explain, at least in part, the higher TG in current users of OCs, because the action of OCs on TG can occur due to its effect on the decrease of levels of protein S, a non-enzymatic cofactor of APC, leading to an inefficient inhibition of factors Va and VIIIa (Vliet, 2008). OCs can also lead to a decrease in the levels of the tissue factor pathway inhibitor (TFPI), increasing the plasma procoagulant potential and making it difficult to inhibit the generation of thrombin via the action of APC. In addition, the inadequate inhibition of FXa by decreased levels of TFPI can generate an increase in the levels of this factor, which is also responsible for protecting FVa from inactivation by APC (Tchaikovski, 2010).

In agreement with our results, two other cross-sectional studies, by Rosing et al. (1997) (N = 154) and Alhenc-Gelas et al. (2004)] (N = 174) found that OC users have significantly higher nAPCsr than non-users. In addition, they also observed that the nAPCsr of users of OCs with third generation progestogens was significantly higher than that of users of OCs with second generation progestogens, as well as those who used OCs containing cyproterone acetate, having a higher nAPCsr than

those that use OCs with second or third generation progestogens. Additionally, Ruhl et al. (2014) in a prospective longitudinal study, found an increase in nAPCsr compared to baseline, after one, two, and three months of using OCs.

Our results also showed that ex-users of OCs have TG and nAPCsr similar to non-users. This finding suggests that the increase in TG and the development of acquired resistance to APC caused by the use of OCs remains only during the period of use, i.e., it is reversible. This result agrees with previous studies that investigated the past use of OCs in the development of thromboembolic events, even if these are still scarce (after a wide review of the literature we found only four, two are review articles), and their results are not conclusive. Vessey et al. (1986) in a cohort study, reported that there was no difference in the incidence of deep vein thrombosis (DVT) and pulmonary thromboembolism between groups of former users and non-users of OCs. Subsequently in literature reviews, Hannaford (2000) and Rosendaal et al. (2003) concluded that the risk of TED is quickly eliminated after stopping the use of OCs. Additionally, Stampfer et al. (1990) in a cohort study, demonstrated in multivariate analysis that ex-users of OCs have a relative risk of 0.80 (CI: 0.64 to 0.99) for coronary disease and 0.85 (CI: 0.72 to 1.00) for fatal cardiovascular disease compared to non-users. In addition, it has been demonstrated in a meta-analysis with thirteen studies that past use of OCs has little or no impact on the risk of cardiovascular disease.

The shortening of the lag time in the Low condition and the time-to-peak in the High condition in current HT users seem to reflect an accelerated generation of thrombin, which suggests a possible state of hypercoagulability, although we have not observed an association of ETP and peak (parameters that best correlate with the clinic) with the current use of HT. Scarabin et al. (2015) in a cross-sectional study carried out in France, evaluated the parameters lag time, ETP, and peak. They observed that current users of oral HT had lower lag time and higher ETP and peak compared to non-users. In contrast, current users of transdermal HT did not show a statistically significant difference in these parameters compared to non-users. Similarly, Canonico et al. (2010) in another cross-sectional study also carried out in France, found no statistical difference in the parameters lag time, ETP, and peak between current users and non-users of transdermal HT. The time-to-peak parameter was not evaluated by the studies.

Previous studies have already shown that oral HT activates the coagulation cascade, while transdermal HT has little or no effect on hemostasis (Canonico, 2010; Gutthann, 1997). In addition, randomized trials showed substantial resistance to APC among postmenopausal women who used oral HT, but not in users of transdermal HT (Grodstein, 1996; Oger, 2003).

Given that in our study it was not possible to separate users of oral HT from users of non-oral HT, it is possible that the hemostatic changes caused by the use of oral HT were diluted by the use of transdermal HT, which may justify, at least in part, the absence of association between the ETP and peak parameters with TGA.

From these results it is also possible to suggest that the hemostatic changes caused by the use of HT are less pronounced than those resulting from the use of OCs. Roach et al. (2011) carried out a case-control study in the Netherlands and observed that users of HT have a lower risk of developing TED than OC users. According to their results, current OC users have a 7.5 times greater risk of developing DVT and 5.3 times greater risk of pulmonary embolism (PE) when compared to non-users. In contrast, users of oral HT have a 1.8 times greater risk of developing DVT and 1.5 times greater risk of PE. And users of non-oral HT have a 1.6 times higher risk of DVT and 0.8 times of PE.

Our results also showed that ex-users of HT have similar TG to non-users. This finding suggests that the increase in TG caused by the use of HT remains only during the period of use, i.e., it is reversible. After extensive research in the literature, we found no studies that investigated the effect of past use of HT in menopausal women on hemostatic markers or development of thromboembolic diseases, therefore our study appears to be the first or one of the first to investigate such an association.

Our study has some limitations. First, it is important to note that unlike a randomized clinical trial, participants in

observational studies using OCs and HT (current or past) may have higher generation of thrombin and nAPCsr for reasons unrelated to the use of hormones. However, even after adjustment for potential confounding factors, the association between TG and the use of OCs and HT remained. In this sense, we do not believe that the differences found in relation to age and lipid profile for women of childbearing age, and in relation to age, health conditions, and lipid profile for menopausal women, may compromise the results found. It is important to emphasize that we expected TG to be associated with these classic cardiovascular risk factors (diabetes, hypertension, cardiovascular disease (CVD), obesity, and smoking), however we believe that due to the characteristics of our population (healthier and younger), we were unable to identify them.

Continuing on the study design, the transversal character does not allow making any causal inference, since this design does not guarantee temporality. Second, because the average age of our population is higher than that of other studies that investigated exposure to the use of OCs and less than that of other studies that investigated the use of HT, the category of current users of OCs and HT presented a reduced number of participants. In addition, in the course of the study possible losses occurred, which made it impossible for stratified analyses of estrogen concentration, progestogen generation, and time of use of OCs to be carried out. Additionally, in the interviews performed by ELSA-Brasil, the menopausal participants were not asked about the type of HT (oral or transdermal) and for this reason this type of analysis could not be performed either.

Third, we have not investigated hereditary resistance to APC, caused by the mutation of coagulation factor V (Factor V Leiden). Thus, it is not possible to state that the resistance to APC found is only acquired through the use of OCs. However, in Brazil, this mutation is present in only 2% of the population, and for this reason, we do not believe that this has been a problem (1995). Fourth, due to possible losses that occurred during the study, we were unable to assess resistance to APC in menopausal women, considering that we observed different losses (analyses not shown) of the TGA parameter, nAPCsr, in relation to age, education, health conditions, lipid profile, and health-related lifestyle habits.

Fifth, it is important to highlight that Dargaud et al. (2007), in a study of standardization and normalization of TGA by the CAT® method (automated calibrated method of thrombogram®), recommends the preparation of the sample through double centrifugation for 15 minutes to 2,500 g to obtain platelet-poor plasma. However, in large epidemiological studies such as ELSA-Brasil, part of the samples are stored for the performance of new procedures in supplementary studies, such as the TGA presented in the present study. Thus, a standard centrifugation was performed that followed the study's quality assurance and control criteria, which was through a single centrifugation for 15 minutes at 2,500g. For this reason we cannot guarantee that the plasma used in our experiments was in fact low in platelets. However, Tripodi (2020) comments in its article that many samples for general use are centrifuged only once and can be used later for TGA. Thus, he suggests that the plasma samples that were centrifuged for 15 minutes at 3,000g are acceptable. Based on that, as our samples were centrifuged at 2,500xg for 15 minutes, it is likely that they do not contain many residual platelet fragments. The increase in residual platelet fragments contributes to greater potential to generate thrombin. However, this increase is not different between the comparison groups (users and non-users of female hormones). Thus, we do not believe that our results have been impacted. Longitudinal analysis from the ELSA-Brasil study can contribute to evaluate whether TGA values are associated with clinical conditions in the Brazilian female population.

In contrast, the present study also presents the benefit of being one of the first studies to evaluate the TGA using the CAT® method in users of OCs and HT in a sample of Brazilian adult women. Since thrombin is a protein that plays a key role in the hemostatic process, as it performs pro-coagulant, anticoagulant, and anti-fibrinolytic functions, the TGA makes it possible to evaluate the process of initiation, amplification and propagation of coagulation, being able to reflect the risk of development of hemorrhagic and thrombotic events by evaluating all the components and/or conditions of the hemostatic process (Lecut, 2015). In this sense, the TGA presents potential as a possible marker of hypercoagulable state, which will

allow its use in early screening for the risk of developing CVD and hereditary or acquired thrombophilia in women who wish to use OCs and HT.

5. Conclusion

An association between the current use of OCs, TG and resistance to APC was observed in this work. An association was also observed between the current use of HT and TG. However, former users of OCs and HT had TG and resistance to APC similar to non-users. These findings suggest that the parameters of the TGA are useful for assessing hemostatic parameters, and therefore, can assist in decision making regarding the use and monitoring of OCs and HT.

More studies should be carried out in order to assess the efficiency of TGA in determining hemostatic changes in OCs and HT users, which may serve as an indication of its efficiency in monitoring CVD.

Acknowledgments

The authors thank FAPEMIG and CNPq/Brasil.

Funding

The authors thank the staff and participants of the ELSA study for their important contributions. We thank the Brazilian agencies FAPEMIG (grants nº APQ-03578-13 and APQ-00211-14) and CNPq/Brazil (grant nº 457624/2014-0) for funding this study. The ELSA-Brasil baseline study was supported by the Brazilian Ministry of Health (DECIT – Science and Technology Department) and the Brazilian Ministry of Science and Technology (FINEP, Financiadora de Estudos e Projetos and CNPq, National Research Council), grants 01 06 0010.00 RS, 01 06 0212.00 BA, 01 06 0300.00 ES, 01 06 0278.00 MG, 01 06 0115.00SP, 01 06 0071.00 RJ. S. M. B. and M.G.C. are research fellow of the National Research Council (CNPq, grant nº 300159/99-4). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

Alhenc-gelas, M., et al. (2004). Impact of progestagens on activated protein C (APC) resistance among users of oral contraceptives. *Journal of Thrombosis and Haemostasis*, 2: 1594–1600.

Aquino, E. M., et al (2021). Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): objectives and design. *Am J Epidemiol*, 4, 315-24.

Arruda, V. R., et al. (1995). Factor V Leiden (FVQ 506) is common in a Brazilian population. *American Journal of Hematology*, 49:242-243.

Brummel-Ziedins, K. E., & Wolberg, A. S. (2014). Global assays of hemostasis. *Curr Opin Hematol*, 21(5):395–403.

Canonico, M., et al. (2010). Activated protein C resistance among postmenopausal women using transdermal estrogens: importance of progestogen. Menopause: *The Journal of The North American Menopause Society*. 17, 1122/1127.

CLSI, 2007. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; approved standard-sixth edition. CLSI Document H3-A6, Wayne (PA): CLSI.

Compiled by Earth Policy Institute from U.N. Population Division. World Contraceptive Use 2011, wall chart (2011) .www.google.fr/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CCMQFjAA&url=http%3A%2F%2Fwww.earthpolicy.org%2Fdatacenter%2Fxls%2Fhighlights26_all.xls&ei=FZ0WVKPMOdTvaKXzgqgP&usg=AFQjCNF10wNQtG2aoPUgkK9YRymHRxb5cw&sig2=ZpqOHEY2CTVVkIA 9WUrS4Q&bvm=bv.75097201,d.d2s.

Dargaud, Y., et al. T (2007). Effect of standardization and normalization on imprecision of calibrated automated thrombography: an international multicentre study. *Journal of Haematology*, 139, 303–309.

Departamento de Ciência e Tecnologia do Ministério da Saúde (2009). ELSA Brasil: maior estudo epidemiológico da América Latina. *Revista de Saúde Pública*, v. 43, p. 1.

Glintborg, D., et al. M (2015). Increased thrombin generation in women with polycystic ovary syndrome: A pilot study on the effect of metformin and oral contraceptives. *Metabolism Clinica and Experimenta*, l6 4:1272–1278.

Grodstein, F., et al (1996). Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet*, 348, 983-987.

Grodstein, F., et al (1996). Prospective study of exogenous hormones and risk of pulmonary embolism in women. *Lancet*, 348:983-7.

Grodstein, F., et al (1996). Posmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N Engl. J. Med*, 335, 453-461.

Gutthann, S. P., et al. (1997). Hormone replacement therapy and risk of venous thromboembolism: population based case-control study. *BMJ*, 314:796-800.

Hannaford, P. (2000). Cardiovascular events associated with different combined oral contraceptives: a review of current data. *Drug Saf.* 22(5):361-71.

Heit, J. A., et al. (2016). The epidemiology of venous thromboembolisam. *J Thromb Thrombolysis*, 41(1):3–14.

Heit, J. A. (2005). Venous thromboembolism: disease burden, outcomes and risk factors. *J Thromb Haemost*, 3, 1611–17.

Hoibraaten, E., et al. (1999). Hormone replacement therapy with estradiol and risk of venous thromboembolism: a population- based case-control study. *Thromb Haemost*, 82, 1218-21.

Lecut, C., et al. (2015). Is there a place for thrombin generation assay in routine clinical laboratory? *Ann Biol Clin (Paris)*, 73(2):137–49.

Lidegaard, O., et al. (2011). Risk of venous thromboembolism from use of oral contraceptives containing different progestogens and oestrogen doses: Danish cohort study, 2001-9. *BMJ*, 343: d6423.

Lowe, G., et al. (2000). Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45–64 years. Relationships to hormone replacement therapy. *Thromb Haemost*, 83, 530–5.

Macfarlane, R. G., & Biggs, R. (1953). A thrombin generation test; the application in haemophilia and thrombocytopenia. *J Clin Pathol*, 6(1):3–8.

Notelovitz, M., & Greig, H. B. W. (1975). The effect of natural oestrogen on coagulation. *S Afr Med J*, 49, 101.

Oger, E., et al (2003). Differential effects of oral and transdermal estrogen/progesterone regimens on sensitivity to activated protein

C among postmenopausal women: a randomized trial. *Arterioscler Thromb Vasc Biol*, 23:1671-1676.

Olivieri, O., et al (1995). Resistance to activated protein C in healthy women taking oral contraceptives. *Brit J Haematol*, 91: 465-70.

Organização Mundial da Saúde (2003). *Anticoncepcionais Orais: o que há de novo.*

Pitney, W. R., & Dacie, J. V. (1953). A simple method of studying the generation of thrombin in recalcified plasma; application in the investigation of haemophilia. *J Clin Pathol*, 6(1):9–14.

Roach, R. E. J., et al. (2011). The risk of venous thrombosis in women over 50 years old using oral contraception or postmenopausal hormone therapy. *Journal of Thrombosis and Haemostasis*, 11: 124–131.

Rosendaal, F. R., et al. (2003). Estrogens, progestogens and thrombosis. *Journal of Thrombosis and Haemostasis*, 1, 1371–1380.

Rosing, J., et al (1997). Oral contraceptives and venous thrombosis: different sensitivities to activated protein C in women using second- and third-generation oral contraceptives. *Br J Haematol*, 97: 233-8.

Rozenberg, S., et al. (2013). Postmenopausal hormone therapy: risks and benefits. *Nat Rev Endocrinol*, 9,216-27.

Ruhl H, Schroder L, Muller J, Sukhitashvili S, Welz J, Kuhn WC, Oldenburg J, Rudlowski C, Potzsch B (2014). Impact of Hormone-Associated Resistance to Activated Protein C on the Thrombotic Potential of Oral Contraceptives: A Prospective Observational Study. *Plos One*, 9.

Scarabin, P. Y., et al. (2015). Increased thrombin generation among postmenopausal women using hormone therapy: importance of the route of estrogen administration and progestogens. *Menopause: The Journal of The North American Menopause Society.* 18, 873/879.

Stampfer, M. J., et al. (1990). Past use of oral contraceptives and cardiovascular disease: a meta-analysis in the context of the Nurses' Health Study. *Am J Obstet Gynecol.* Jul;163(1 Pt 2):285-91.

Tchaikovski, S. N., & Rosing, J. (2010). Mechanisms of Estrogen-Induced Venous Thromboembolism. Thrombosis Research 126, 5–11.

Tripodi A (2020). Usefulness of thrombin generation. *Hämostaseologie*, 40: 509-514.

van Vliet, H. A., et al (2008). Different effects of oral contraceptives containing different progestogens on protein S and tissue factor pathway inhibitor. *J Thromb Haemost*, 6: 346–51.

Vessey, M., et al. (1986). Oral contraceptives and venous thromboembolism: findings in a large prospective study. *BMJ.*

Vieira, C. S., et al. (2007). Female hormones and hemostasis. *Rev Bras Ginecol Obstet.* 29, 538-47.

Vigo, F., et al. (2011). Progestógenos: farmacologia e uso clínico. *Femina*, 39

Vlieg, van H., et al. (2009). The venous thrombotic risk of oral contraceptives, effects of oestrogen dose and progestogen type: results of the MEGA case-control study. *BMJ*, 339:b2921.

Zia, A., et al. (2015). Hypercoagulability in adolescent girls on oral contraceptivesglobal coagulation profile and estrogen receptor polymorphisms. *Am. J. Hematol*. 90:725–731.