RMMG
Revista Médica de Minas Gerais

ORIGINAL ARTICLE

Effect of the use of female hormones on platelet volume indices: evidence from ELSA-Brasil

Efeito do uso de hormônios femininos sobre os índices de volume plaquetário: evidências do ELSA-Brasil

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ABSTRACT

Introduction: Platelet volume indices (PVI) are obtained using haematology analysers at a low cost. Studies have suggested that these indices might be good markers of platelet function in women using oral contraceptives (OC) or hormone therapy (HT). This study aims to investigate the association of PVI with both OC and HT use (current or past) in women. **Methods:** Cross-sectional study carried out with 609 women of childbearing age and 640 going through menopause from the baseline of ELSA-Brasil. We used analysis of variance and the Bonferroni post-test to compare the PVI means according to the use of OC and HT, considering $p<0.05$ as statistically significant. Results: The mean (SD) age of participants of childbearing age was 45.1 (5.0) years, and of those going through menopause 58.3 (6.5) years. No significant differences were observed in the mean values of PVI between non-users, ex-users, and current users of OC and HT. **Conclusion:** PVI were not associated with the current or past use of OC and HT in women over 35 years of age.

Keywords: Oral contraceptive; Hormone therapy; Medium platelet volume.

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RESUMO

Introdução: Os índices de volume plaquetário (IVP) são obtidos por meio de analisadores hematológicos de baixo custo. Estudos têm sugerido que esses índices podem ser bons marcadores da função plaquetária em mulheres em uso de contraceptivos orais (CO) ou terapia hormonal (TH). **Objetivo:** Este estudo tem como objetivo investigar a associação dos IVP com o uso de CO e TH (atual ou passado). **Métodos:** Estudo transversal realizado com 609 mulheres em idade fértil e 640 em menopausa da linha de base do ELSA-Brasil. Utilizou-se a análise de variância (ANOVA) e o pós-teste de Bonferroni para comparar as médias dos IVP segundo o uso de CO e TH, considerando $p<0,05$ como estatisticamente significativo. **Resultados:** A idade média (desvio padrão) das participantes em idade fértil foi de 45,1 (5,0) anos, e das que estão na menopausa, 58,3 (6,5) anos. Não foram observadas diferenças significativas nos valores médios dos IVP entre não usuárias, ex-usuárias e usuárias atuais de CO e TH. **Conclusão:** Os IVP não se mostraram associados ao uso atual ou passado de CO e TH em mulheres acima de 35 anos.

Palavras-chave: Contraceptivo oral; Terapia hormonal; Volume plaquetário médio.

Supporting sources:
FAPEMIG, CNPq e CAPES.

Clinical Trial Registration (if applicable):
Not applicable.

Conflict of Interests:
The authors declare that they have no conflicts of interest.

Received on: 13 Abril 2022
Approved on: 18 Março 2023
Publication Date: August 08, 2023.

DOI: 10.5935/2238-3182.2023e33109-en

Introduction

Thromboembolic disorders have serious complications in the short and long term and are among the main causes of morbidity and mortality worldwide[1].

Studies have shown that women who use oral contraceptives (OC) or hormone therapy (HT) are at higher risk of developing thromboembolic disorders than those who do not[2,3]. The use of OC has been reported to cause a state of hypercoagulability caused by increased hepatic production of coagulation factors (fibrinogen and factors 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)[3]. Similarly, HT seems to promote increased levels of factors VII and X and fibrinogen, decreased antithrombin levels, and resistance to APC[4]. However, studies have suggested that all these effects appear to be nullified with discontinuation of use, although these findings are still inconclusive[3,5].

Because platelets have pro-inflammatory and pro-thrombotic functions, they play an important role in the development of thromboembolic disorders. It is suggested that the increase in platelet volume indices (PVI), such as mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (P-LCR), could signal a state of greater platelet reactivity, which predisposes to hypercoagulability[6]. If this were indeed the case, PVI could assist in detecting the risk of possible cardiovascular complications caused by female hormones, which would support the search for evidence for their clinical use.

Thus, given the advantage of these indices being easily obtained using haematology analysers at low cost, this study aimed to investigate whether the (current or past) use of OC and HT is associated with PVI, regardless of confounding factors, in a sample of Brazilian women of childbearing age or in menopause.

Methods

We conducted this cross-sectional study using data from the baseline (2008-2010) of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a prospective multicentre cohort composed of 15,105 civil servants aged between 35 and 74 years from teaching and research institutions in six Brazilian capitals[7].

Of the 2,351 women participating in the ELSA-Brasil baseline from the states of Minas Gerais and Rio Grande do Sul (these centres were selected because they used the same PVI measurement methods), 1,149 were of childbearing age (those who reported menstruating at the time of the interview) and 1,202 were in menopause (those who reported no longer menstruating due to natural causes for at least 12 months at the time of the interview)[7].

Participants who did not provide information about PVI and the use of OC or HT (n=1,102) were excluded. Although participants using antithrombotic medications [n=46 (3.7%)] are usually excluded from studies that

address haemostasis-related parameters, analysis with and without these participants showed no difference in the results, and therefore, they remained in this study. Thus, the sample of the present study was composed of 609 women of childbearing age (48.8%) and 640 women going through menopause (51.2%) (Figure 1).

Participants who reported using OC or HT at the time of the interview were classified as current users of these medicines, those who used OC or HT in the past but did not use them at the time of the interview were classified as ex-users, and those participants who reported never having used OC or HT were classified as non-users.

PVI were measured using XE 2100 D haematology analysers (Sysmex, Kobe, Japan). The time between collection and the test procedure was up to 2 hours[8]. The quality of the results was validated by internal quality procedures and participation in the proficiency of programs of the Brazilian Society of Pathology and Laboratory Medicine and the College of American Pathologists. An equivalence test between the analysers previously used was carried out to guarantee the interchangeability of the results[9]. Intra- and inter-assay coefficients of variation were <1.6% and <2.2% for PVI, respectively.

The study covariables were age (continuous, in years), diabetes (defined by means of self-report of diabetes or treatment with insulin or hypoglycemic agent or by laboratory values of fasting glucose ≥126mg/dL, or glucose ≥200mg/dL after the tolerance test or glycated haemoglobin ≥6.5%), hypertension (systolic blood pressure level ≥140mmHg and/or diastolic blood pressure level ≥90mmHg and/or use of antihypertensive medication), obesity (participants who had a body mass index ≥30kg/m^2 were defined as obese), ratio between total cholesterol (continuous) and high-density lipoprotein cholesterol (HDL-c) (continuous) [total cholesterol/HDL-c], triglycerides (continuous), and use of lipid-lowering drugs (categorised as no or yes).

The normality of continuous variables was assessed using histogram analysis. Continuous variables that showed a symmetrical distribution were described using mean ± standard deviation. For comparing means between the categories of use of OC and HT, analysis of variance and the Bonferroni post-test were used. The variables with asymmetric distribution were described using the median and interquartile range (Q1-Q3). For the comparison between the categories of use of OC and HT, the Kruskal-Wallis test was used. The association between the use of OC and HT and PVI was assessed using linear regression. Crude and adjusted β coefficients and their respective 95% confidence intervals (CIs) were estimated. 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 menopausal women and with PVI. A p-value <0.05 was considered significant. All analyses were performed using the Stata software version 14.0 (Stata, College Station, TX, US).

ELSA-Brasil was approved by the Research Ethics Committees of the participating institutions and by the National Committee for Research Ethics (CONEP 976/2006) of the Ministry of Health. All study participants signed an Informed Consent Form.

Results

Of the 609 participants of childbearing age (48.8%), 140 (23.0%) never used OC, 418 (68.6%) used them in the past, and 51 (8.4%) were currently using them. Of the 640 menopausal participants (51.2%), 324 (50.6%) never used HT, 227 (35.5%) used it in the past, and 89 (13.9%) were currently using it. The characteristics of the study population with respect to age, health conditions, lipid profile, history of thrombotic events, and use of antiplatelet/anticoagulant agents are shown in Table 1. Compared to ex-users and non-users, women of childbearing age currently using OC were younger (p<0.001) and had a lower total cholesterol/HDL-c ratio (p<0.001) and higher serum triglyceride levels (p<0.05). On the other hand, when compared to ex-users and non-users, menopausal women using HT were younger (p<0.001) and had lower serum triglyceride levels (p<0.001) and a lower prevalence of hypertension (p<0.05), obesity (p<0.05), and use of lipid-lowering drugs (p<0.05).

There were no significant differences in the mean values of MPV, PDW, and P-LCR between current users, ex-users, and non-users of OC or HT (Figure 2).

Current use and past use of OC and HT were not associated with PVI, and adjustment for confounding factors did not change the results (Table 2).

Discussion

This cross-sectional study, conducted with a sample of Brazilian middle aged adult women of childbearing age and women going through menopause, did not support the hypothesis that the mean PVI would be higher among women currently or past using OC compared to non-users or among women using HT compared to non-users.

Larger platelets are metabolically and enzymatically more active than smaller ones, as they contain more alpha granules, produce more thromboxane A2, and have high adhesion glycoprotein expression[10,11]. For this reason, increased PVI could theoretically be alternative markers capable of assisting in the early detection of possible cardiovascular complications resulting from the use of OC or HT.

A study on cardiovascular risk factors suggested a compensatory production of larger and more active platelets, in view of greater activation[12]. OC and HT promote a state of hypercoagulability due to their effects to increase hepatic production of clotting factors, reduce plasma levels of natural anticoagulants, and develop resistance to APC[3]. However, it is unclear whether therapy with OC or HT could induce the production of more reactive platelets, that is, of larger size. It is possible that such therapies alone are not able to promote a continuous production of larger platelets, which could explain the lack of association between the current use of OC and HT and PVI, as shown in our study.

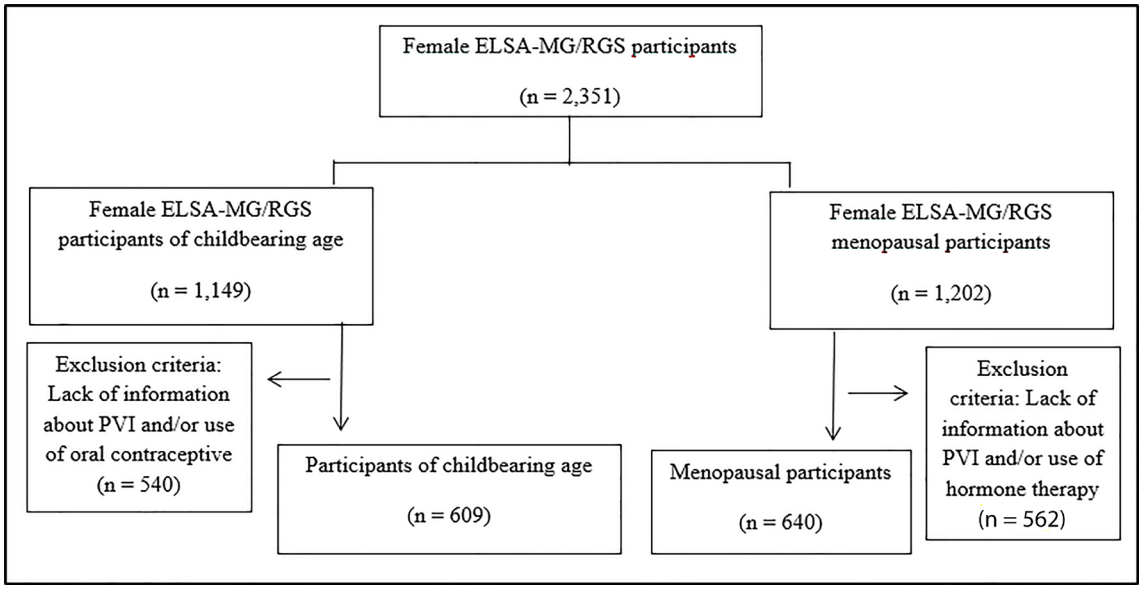

Figure 1. Scheme of the study population. HT, hormone therapy; PVI, platelet volume indices; OC, oral contraceptive; MG., Minas Gerais; RG, Rio Grande do Sul.

Although this is one of the first Brazilian studies to investigate the association between platelet markers and the use of OC and HT, researchers have already investigated this association in other countries. David et al.[13], already in the 1990s, in an intervention study in Belgium with 36 young, healthy women, analysed the effect of low-dose OC on platelets. Two identical groups were treated with Marvelon® (single-phase OC with ethinyl oestradiol and desogestrel) or Trigynon® (three-phase OC with ethinyl oestradiol and levonorgestrel) for a period of 6 months. There were no significant changes in the number of platelets or in the rate of platelet aggregation, which suggests the absence of reactive platelets due to the use of these drugs. An intervention study by Bulur et al. (2012)[14] investigated 95 women in Turkey and found no difference in platelet counts or MPV before and after 6 months of treatment with OC.

Regarding HT, Ranganath et al. (1996)[15], in an intervention study in England with 38 women with a mean age of 52.2 years, reported a significant increase in MPV after 6 weeks of treatment with HT [conjugated equine oestrogen (0.625mg/day) combined with L-norgestrel (75mg/day)]. In contrast, Teede et al. (2001)[16], in an intervention study in Australia with 32 women (median age of 50 years), did not observe a difference in MPV after six months of using HT (2 mg of oestradiol combined with 1 mg of norethisterone). Saraç et al. (2009)[17], also in an intervention study with 80 women in Turkey with a mean age of 52 years, found no difference from baseline in the platelet function of participants using HT after 6 months.

Although PVI indirectly estimate platelet function and are easily obtained at low cost using haematology analysers, studies investigating the association with the use of OC and HT are still scarce, especially observational, and contradictory[13-17]. Our study did not identify such an association. This lack of association suggests that PVI are not efficient in detecting reactive platelets resulting from the use of OC and HT, or even that this therapy is in fact not able to influence the production of platelets with greater volume, having a more significant effect on pro- and anticoagulant factors. However, a larger number of studies are needed to support these results.

It is also important to highlight that the ELSA-Brasil cohort includes women aged 35 years or older, i.e., at least 20 years of childbearing age are not covered by the study. The mean age of our participants (45.1 years) was considerably higher than other studies that evaluated the use of OC (<40 years). Thus, our OC prevalence is lower when compared to other studies[14], which may have limited our ability to find an association, if such a fact existed. The majority of the studies that found association between HT and PVI are intervention studies, which may explain the differences between our results and those.

Our results also demonstrated that the ex-users of OC and HT have similar PVI to non-users. In agreement with our results, Rosendaal et al. (2003)[5], in a literature review, and Grodstein et al. (1996)[18], in a cohort study, demonstrated that the risk of developing thromboembolic disorders resulting from the use of OC and HT, respectively, is cancelled after discontinuing use.

Our study has some limitations, such as the cross-sectional study design that does not allow inferring the temporality between exposure and outcome, i.e. even if the result were statistically significant, it would not be enough to infer that the use of female hormones induces the production of more reactive platelets. A considerable loss of the participants' PVI also occurred, which made it impossible to conduct stratified analyses regarding oestrogen concentration, type of progestogen, type of contraceptive (combined or not), dosage (continuous or with pause) and time of use of OC and HT, since these characteristics seem

Table 1. Characteristics of the study population according to the use of oral contraceptives or hormone therapy (n=1249): ELSA-Brasil (2008-2010).

Characteristics	Total (n=609)	Use of oral contraceptives			*p*-value	Total (n=640)	Use of hormone therapy			*p*-value
		Non-users (n=140)	Ex-users (n=418)	Current users (n=51)			Non-users (n=324)	Ex-usuárias (n=227)	Ex-users (n=89)	
Age (years)[a]	45.1 ± 5.0	45.6 ± 5.3	45.3 ± 4.8	42.3 ± 4.6	<0.001§¶	58.3 ± 6.5	56.5 ± 6.1	60.7 ± 6.2	58.6 ± 6.7	<0.001§¶
Health conditions[b]*										
Diabetes	49 (8.0%)	11 (22.4%)	34 (69.4%)	4 (8.2%)	0.993	112 (17.5%)	65 (58.0%)	3 8(33.9%)	9 (8.1%)	0.085
Arterial hypertension	127 (20.8%)	27 (21.3%)	90 (70.9%)	10 (7.8%)	0.830	247 (38.6%)	127 (51.4%)	101 (40.9%)	19 (7.7%)	0.001§¶
Obesity	103 (16.9%)	26 (25.2%)	69 (67.0%)	8 (7.8%)	0.600	133 (20.8%)	85 (63.9%)	39 (29.3%)	9 (6.8%)	0.005§¶
Lipid profile										
Total cholesterol/ HDL-c[a]	3.49 ± 0.9	3.68 ± 092	3.47 ± 0.83	3.14 ± 0.94	<0.001§¶	3.72 ± 1.0	3.78 ± 0.88	3.70 ± 0.95	3.55 ± 1.19	0.117
Triglycerides (mg/dL)[c]	90.0 (67-123)	93.0 (67-127)	85.0 (67-120)	107.0 (76-156)	0.0123§¶	105.5 (79.5-145)	115.0 (84.5-151.5)	105.0 (82-146)	82.0 (66-111)	<0,001§¶
Use of lipid-lowering drugs[b]					-					0.028§¶
No	585 (96.1%)	135 (23.1%)	399 (68.2%)	51(8.7%)		509 (79.5%)	271 (53.2%)	169 (33.2%)	69 (13.6%)	
Yes	2 4(3.9%)	5 (20.8%)	19 (79.2%)	0		131 (20.5%)	53 (40.5%)	58 (44.3%)	20 (15.3%)	

Legend: ELSA-Brasil = The Brazilian Longitudinal Study of Adult Health; HDL = High-density lipoprotein.
[a]ANOVA: values expressed as mean ± 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 ex-users of oral contraceptives.
¶Statistically significant difference between categories: current users and non-users of hormone therapy.
Source: Elaborated by authors.

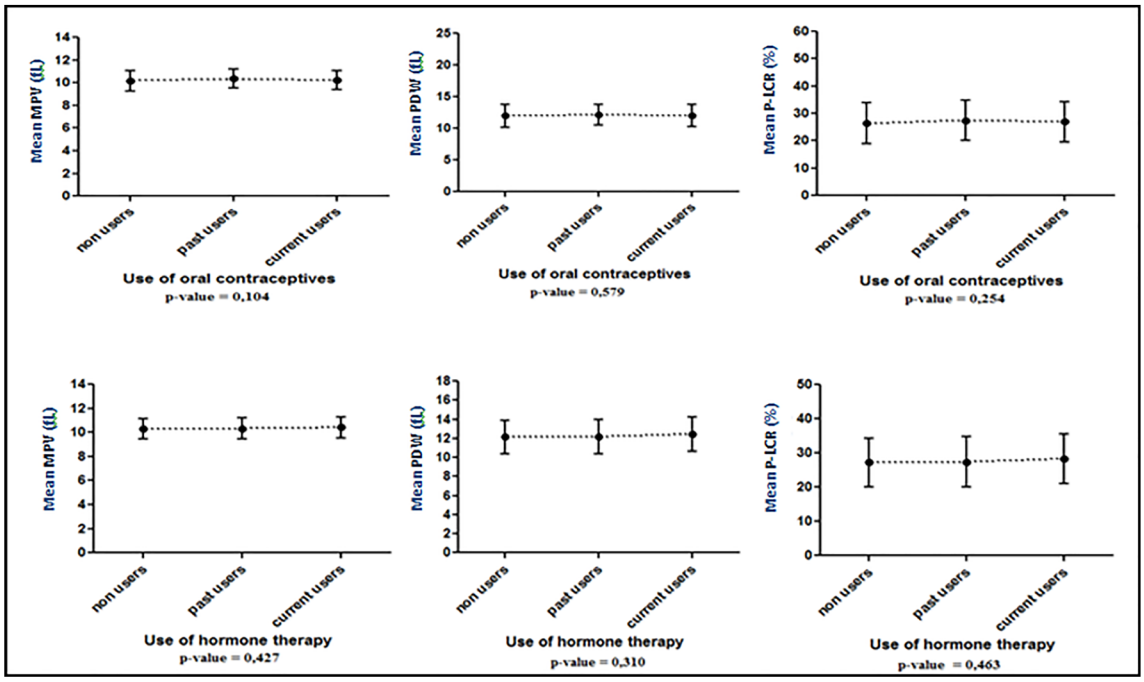

Figure 2. Distribution of the means of MPV, PDW, and P-LCR of participants of childbearing age (n = 609) and in menopause (n = 640) in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) from the states of Minas Gerais and Rio Grande do Sul, according to the categories of use of oral contraceptives and hormone therapy. For the analysis of variance, values were expressed as mean ± standard deviation.

to modify the association between OC and HT use and PVI. In addition, self-reported data, mainly regarding to past OC and HT use, may have been compromised due to possible difficulty in remembering past behaviours, such as the type and concentration of medicines used. Finally, the age of the participants in this study does not include the entire childbearing age group of the Brazilian population and contraceptive use. However, we do not believe that it could have compromised the results.

On the other hand, the present study also shows strengths, blood samples as well as laboratory tests were performed by trained and certified staff.

Conclusion

PVI, under the conditions of our study, were not associated with the use of OC and HT, suggesting that such parameters are not useful to evaluate circulating platelets that differ in size and haemostatic potential and that their use would not add to the assessment of the state of hypercoagulability caused by the use of female hormones in women over 35 years of age.

Acknowledgments

The authors thank FAPEMIG and CNPq/Brasil. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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 fellows 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.

Authors' Contribution

The manuscript was read and approved by all authors and the requirements for authorship, according to the ICMJE criteria, were met.

i. Thaís Resende Batista: work conception; data analysis and interpretation; article writing; final approval of the version to be published;

ii. Sandhi Maria Barreto: work conception; collection, analysis and interpretation of data; critical review of the article; final approval of the version to be published;

Table 2. Linear regression analyses of the relationship between the PVI (MPV, PDW, and P-LCR) and the use of oral contraceptives and hormone therapy in participants of childbearing age (n=609) and in menopause (n=640) of the ELSA-Brasil baseline (2008-2010), from the states of Minas Gerais and Rio Grande do Sul.

Variables	β coefficient (95%CI)														
	MPV					PDW					P-LCR				
	Model 0	Model 1	Model 2	Model 3	Model 4	Model 0	Model 1	Model 2	Model 3	Model 4	Model 0	Model 1	Model 2	Model 3	Model 4
Use of oral contraceptives															
Non-users	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ex-users	0.167 (0.003-0.331)*	0.166 (0.002-0.330)*	0.159 (-0.007-0.325)	-	-	0.164 (-0.165-0.493)	0.164 (-0.165-0.494)	0.150 (-0.182-0.481)	-	-	1.167 (-0.234-2.569)	1.161 (-0.242-2.564)	1.133(-0.285-2.552)	-	-
Current users	0.035 (-0.240-0.310)	0.020 (-0.284-0.299)	0.010 (-0.258-0.299)	-	-	0.031 (-0.520-0.582)	0.031 (-0.528-0.591)	0.035 (-0.528-0.591)	-	-	0.587 (-1.765-2.940)	0.509 (-1.876-2.895)	0.508(-1.968-2.552	-	-
Use of hormone therapy															
Non-users	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ex-users	0.037 (-0.110-0.184)	0.015 (-0.138-0.169)	-	0.010 (-0.143-0.1163)	0.016 (-0.139-0.171)	0.054 (-0.248-0.355)	0.008 (-0.307-0.324)	-	0.006 (-0.309-0.320)	0.004 (-0.314-0.323)	0.190 (-1.040-1.419)	0.043 (-1.244-1.333)	-	0.003 (-1.283-1.289)	0.016 (-0139-0.171)
Current users	0.134 (-0.069-0.338)	0.123 (-0.081-0.328)	-	0.119 (-0.085-0.323)	0.127 (-0.083-0.338)	0.324 (-0.092-0.742)	0.301 (-0.119-0.721)	-	0.298 (-0.120-0.718)	0.325 (-0.108-0.757)	1.074 (-0.626-2.773)	0.999 (-0.723-2.710)	-	0.961 (-0.749-2.671)	1.127 (-0.083-0.338)

Legend: CI = Confidence interval; MPV = Mean platelet volume; PDW = Platelet distribution width; P-LCR = Platelet large cell ratio; PVI = Platelet volume indices.
Model 0: univariate analysis. Model 1: model 0 adjusted for age. Model 2: model 1 adjusted for total cholesterol/high-density lipoprotein cholesterol and triglycerides. Model 3: model 2 adjusted for use of lipid-lowering drugs. Model 4: model 3 adjusted for triglycerides, diabetes, arterial hypertension, and obesity.
The adjusted for the hormone therapy analysis has stopped at model 2. *p<0.05.
Source: Elaborated by authors.

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iv. Antonio Luiz Pinho Ribeiro: work conception; collection, analysis and interpretation of data; critical review of the article; final approval of the version to be published;

v. Dora Chor: work conception; collection, analysis and interpretation of data; critical review of the article; final approval of the version to be published;

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References

1. Khan F, Tritschler T, Kahn SR, Rodger MA. Venous thromboembolism. Lancet. 2021 Jul;398(10294):64-77.
2. Høibraaten E, Abdelnoor M, Sandset PM. Hormone replacement therapy with oestradiol and risk of venous thromboembolism: a population-based case-control study. Thromb Haemost. 1999 Oct;82(4):1218-21.
3. Kluft C, Lansink M. Effect of oral contraceptives on haemostasis variables. Thromb Haemost. 1997 Jul;78(1):315-26.
4. Lowe G, Woodward M, Vessey M, Rumley A, Gough P, Daly E. Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45-64 years. Relationships to hormone replacement therapy. Thromb Haemost. 2000 Apr;83(4):530-5.
5. Rosendaal FR, Van Hylckama Vlieg A, Tanis BC, Helmerhorst FM. Estrogens, progestogens and thrombosis. J Thromb Haemost. 2003 Jul;1(7):1371-80.
6. Kaplan ZS, Jackson SP. The role of platelets in atherothrombosis. Hematol Am Soc Hematol Educ Program. 2011;2011:51-61.
7. Aquino EM, Barreto SM, Bensenor IM, Carvalho MS, Chor D, Duncan BB. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): objectives and design. Am J Epidemiol. 2012 Feb;175(4):315-24.
8. Maluf CB, Barreto SM, Vidigal PG. Standardization and reference intervals of platelet volume indices: insight from the Brazilian longitudinal study of adult health (ELSA-Brasil). Platelets. 2015;26(5):413-20.
9. Maluf CB, Silva IO, Vidigal PG. Understanding commutability: important quality requirement for clinical laboratories. J Bras Patol Med Lab. 2011;47(6):595-601.
10. Martin JF, Kristensen SD, Mathur A, Grove EL, Choudry FA. The causal role of megakaryocyte-platelet hyperactivity in acute coronary syndromes. Nat Rev Cardiol. 2012 Nov;9(11):658-70.
11. Vizioli L, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. Int J Clin Pract. 2009 Oct;63(10):1509-15.
12. Lotufo PA. Framingham score for cardiovascular diseases. Rev Med (São Paulo). 2008;87(4):232-7.
13. David JL, Gaspard UJ, Gillain D, Raskinet R, Lepot MR. Hemostasis profile in women taking low-dose oral contraceptives. Am J Obstet Gynecol. 1990 Jul;163(1 Pt 2):420-3.
14. Bulur S, Albayrak M, Bulur S, Keskin F, Köse SA, Aslantas Y, et al. Effect of combined oral contraceptive use on platelet volume in women at reproductive age. Clin Exp Obstet Gynecol. 2012;39(3):314-6.
15. Ranganath LR, Christofides J, Semple MJ. Increased mean platelet volume after oestrogen replacement therapy. Ann Clin Biochem. 1996 Nov;33(Pt 6):555-60.
16. Teede HJ, McGrath BP, Turner A, Majewski H. Effects of oral combined hormone replacement therapy on platelet aggregation in postmenopausal women. Clin Sci (Lond). 2001 Feb;100(2):207-13.
17. Saraç F, Saydam G, Sahin F, Oztekin K, Saygili F, Tüzün M, et al. Effects of hormone replacement therapy on insulin resistance and platelet function tests. Med Princ Pract. 2009;18(1):43-7.
18. Grodstein F, Stampfer MJ, Goldhaber SZ. Prospective study of exogenous hormones and risk of pulmonary embolism in women. Lancet. 1996 Oct;348(9033):983-7.

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