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electrocardiogram. e Body Mass Index (BMI) was calculated as weight/height (kg/m²). Furthermore, the women reported no signs of major symptomatology (clinical depression or any other mental disorder), and were also questioned about premenstrual symptoms.

Lipids and clotting factors measurement

Venous blood samples were drawn between 8-10 am, a er overnight fasting for at least 12 hours, three times at each cycle: at the follicular phase (FL), mid luteal phase (ML) and late luteal phase (LL), the latter corresponding to the premenstrual phase. e following lipidemic factors, haemostatic factors and clotting time tests were determined at each sample as follows.

Lipidemic factors

Total Cholesterol, LDL-Cholesterol, HDL-Cholesterol, Triglycerides, VLDL.

Factors of haemostasis

• Coagulant factor: Fibrinogen

• Fibrinolytic factor: Plasminogen

• Anticoagulant factors: Protein S, Protein C, ATIII

• Clotting time tests: APTT, PT.

Serum lipid concentrations were measured using biochemical analyzer ILAB 350 of company Instrumentation Laboratory. LDL cholesterol was calculated with the Friedewald equation [27].

Fibrinogen, plasminogen, ATIII, APTT, and PT were analyzed on a DADE-Behring analyzer (with reagents from the same company); protein S and protein C were measured using Eliza. Normal range of Protein S activity is 60-150% and that of Protein C activity is 65-140%.

Assessment of depression and hostility

Depressive symptomatology was assessed by the Zung Self-Rating Depression Scale, a 20-item self-report questionnaire, which is widely used as a screening tool encompassing a ective, psychological and somatic symptoms associated with depression [28]. e Questionnaire takes about 10 minutes to complete, and items are framed in terms of positive and negative statements. Each item is scored on a Likert scale ranging from 1 to 4. A total score is derived by summing the individual item scores and ranges from 25 to 80. e scores provide indicative ranges for depression severity that can be useful for clinical and research purposes.

Individuals also completed the Hostility and Direction of Hostility Questionnaire (HDHQ) [29]. is assessment instrument represents a measure of hostility and anger, and consists of ve subscales: (a) the urge to act out hostility (AH), (b) criticism of others (CO), (c) projected delusional or paranoid hostility (PH), (d) self-criticism (SC), and (e) delusional guilt (DG). e rst three subscales are summed to form an Extrapunitive score, and the other two are summed to yield an Intropunitive score. e direction of hostility was obtained from the following formula: [2x self-criticism+delusional guilt–(urge to act out hostility+criticism of others+projected delusional or paranoid hostility)]. Each subscale score is ranged from 0 to 11, so the total Hostility score ranges from 0 to 55 by summing the ve subscale scores. e women completed these questionnaires immediately before blood sampling at each phase of the cycle.

Statistical Analysis

Data are presented as mean ± SD. e relations between variables at each phase of the menstrual cycle were determined using the Pearson correlation coe cient (R). e Spearman rank correlation coe cient (RS) was also computed when normality assumption was not satis ed. Comparisons within cycle phases were performed by the one-way analysis of variance (ANOVA) for repeated measures followed by Bonferroni correction for multiple comparisons. When data were not normally distributed, the non-parametric Friedman test was used for comparisons within phases and the Wilcoxon Signed Ranks test for paired comparisons. All reported p-values were considered signi cant when less than 0.05.

Results

e clinical features of the women are shown in Table 1. Mean values ± SD of lipidemic and haemostatic factors, as well as of the psychometric scores evaluated in FL, ML, LL phases of the menstrual cycle are shown in Table 2. Tables 3-5 show the statistically signi cant correlations between lipids, haemostatic factors, hostility, and depressive symptoms respectively. ere were no statistically signi cant correlations between the tested lipids, the haemostatic factors and the Zung scores throughout the menstrual cycle phases.

Discussion

Haemostatic variables and lipids

A signi cant correlation between any haemostatic variable and lipids was detected at the LL phase only. Speci cally, HDL-C levels might be implicated in the reduction of the coagulation process while they are positively related to APTT. In addition, a positive association between brinogen and triglycerides, as well as between protein C and cholesterol/LDL was determined, while plasminogen, the only brinolysis-related parameter examined, was positively correlated with VLDL and triglycerides. Regarding the data so far, V. Giardiva et al. [3] focused on the relation between lipids and clotting factors at each phase of the menstrual cycle in 20 young women; in contrast to our results, no selective correlation was determined at speci c time points across the cycle, although the averaged concentrations of total cholesterol and brinogen were positively associated.

Among the studies concerning female subjects only, several detected an inverse association between HDL and brinogen in various sample groups, while a positive correlation was reported between brinogen and total cholesterol, LDL and triglycerides [1,2]. e dissimilar results, in comparison to our work, could be justi ed by the discrepancies with regard to the study samples and design. On the contrary, in ARIC study, authors reported a positive relation between proteins C with many elements of the lipid pro le, paralleling our results concerning the LL phase [30]. As mentioned before, our ndings concern the premenstrual phase only. ese data indicate that probably the speci c hormonal changes during this phase of the cycle might interfere in the connection between haemostasis and lipid pro le.

Clinical characteristics of subjects (n=59)	
Age	23.0 ± 2.8 (years)
Weight	60.1 ± 8.0 (kg)
Height	164.9 ± 6.2 (cm)
BMI	22.1 ± 2.7 (kg/m²)
Menstrual cycle duration (range)	27-33 (days)
Menstrual cycle duration	29 (days)

Table 1: Clinical characteristics of the individuals.

	Phase FL	PHASE ML	PHASE LL
Lipids			
Total cholesterol (mg/dL)	184.96 ± 30.94	183.90 ± 29.68	185.27 ± 24.78
Triglycerides (mg/dL)	65.52 ± 28.31	66.15 ± 19.28	61.47 ± 16.82
HDL-C (mg/dL)	54.66 ± 9.30	57.27 ± 9.49	60.00 ± 8.99
LDL-C (mg/dL)	117.40 ± 29.47	113.45 ± 29.60	113.08 ± 25.59
VLDL (mg/dL)	13.06 ± 5.62	13.22 ± 3.80	12.27 ± 3.37
Haemostatic factors			
PT (sec)	13.80 ± 1.29	13.88 ±1.72	13.92 ± 1.18
APTT (sec)	42.04 ± 4.91	44.81 ± 12.58	40.58 ± 5.93
Fibrinogen (mg/dL)	2.67 ± 0.76	3.12 ± 1.34	3.11 ± 1.39
AT III (mg/dL)	99.07 ± 13.97	100.91 ± 9.46	101.48 ± 10.11
PRC (mg/dL)	96.66 ± 17.95	95.07 ± 17.79	92.24 ± 18.05
PRS (mg/dL)	46.30 ± 14.88	53.60 ± 9.38	48.75 ± 12.01
Plasminogen (mg/dL)	91.78 ± 13.37	96.23 ± 14.06	91.48 ± 12.21
Depression (Zung self-rating scale scores)			
	47.69 ± 8.28	46.57 ± 8.96	46.16 ± 9.68
Hostility (Hostility and direction of hostility questionnaire scores)			
Urgue to act out Hostility (AH)	4.30 ± 1.90	4.06 ± 1.92	4.02 ± 2.14
Critisism of others (CO)	5.41 ± 2.06	5.48 ± 2.26	5.58 ± 2.70
Projected delusional or Paranoid Hostility (PH)	1.76 ± 1.37	1.93 ± 1.35	1.58 ± 1.42
Self Critisism (SC)	3.65 ± 1.96	3.57 ± 2.11	3.94 ± 2.19
Delusional Guilt (DG)	1.78 ± 1.27	1.71 ± 1.40	1.55 ± 1.22
Intropunitive Hostility	11.01 ± 5.74	10.53 ± 6.18	11.05 ± 5.98
Extrapunitive Hostility	11.33 ± 4.17	11.48 ± 4.34	11.16 ± 4.97
Direction of Hostility	-0.55 ± 5.25	-1.04 ± 5.26	0.28 ± 5.11
Total Hostility	16.93 ± 5.90	16.78 ± 6.57	16.69 ± 7.08

Notes:

Lipids and hostility

Several authors so far showed an association between low lipid levels or lipid lowering and hostility, however, this relation was not con rmed by other studies. In the present study, some elements of extrapunitive hostility (criticism of others and paranoid hostility) were negatively correlated to atherogenic elements such as LDL cholesterol and total cholesterol levels in the luteal phase only. No correlation was found between triglycerides, VLDL and HDL-C levels, with hostility, throughout the menstrual cycle.

Focusing on the data concerning female subjects, in a large cohort of Taiwanese women [8] as well as in healthy primiparous women [31], a negative correlation between total cholesterol and hostility was detected, while Rutledge et al. [11] showed a positive correlation between LDL cholesterol and 'anger out scores' in a sample group comprising middle aged women. However, a considerable number of reports do not support a consistent relation between these lipid elements and hostility [19,21]. e studies so far vary with regard to the sex and physical condition of the individuals, the examined lipidemic factors as well as the psychometric scales and the statistical methods the authors used. In contrast to these reports, the negative correlation between extroverted forms of hostility and atherogenic igriedents like LDL cholesterol and serum total cholesterol, observed in our study, shows selectivity for the luteal phase of the cycle.

Although various CHD risk behaviours have been determined in hostile persons [32], it has been proposed that hostility might directly

Psychometric Parameter	Lipidemic Factors	Cycle Phase			R[a] (p-value)	Rs (p- value)
		FL	ML	LL		
HDHQ CO	T Cholesterol			-		-0.35 (0.048)
HDHQ PH	T Cholesterol		-			-0.31 (0.044)
	LDL-C		-			-0.32 (0.041)

Notes: In ML phase, HDHQ PH was negatively correlated with total cholesterol (Rs=-0.31, p=0.045) as well as with LDL-C (Rs=-0.32, p=0.041). In LL phase, total

in uence lipid metabolism, probably through the modi cation of serotonergic neurotransmission. is was based on the observation of an association between measures of aggression and hostility, and changes in 5-HT function [6,33] and low lipid levels and alterations in 5-HT activity [6].

Haemostatic variables and hostility

Our ndings indicate that introverted aspects of hostility might decrease coagulation activity through an inverse e ect on brinogen levels in the FL phase. Moreover, the positive correlation between the PT test and some overt forms, as well as the total hostility scores support the hypothesis that hostility might weaken haemostasis in the particular cycle phase. In ML phase, it seems that hostility promotes a hypercoagulable state, while the clotting time tests were negatively correlated to some ingredients of hostility (i.e., delusional guilt as well as the Directionality of Hostility). Finally, in the premenstrual phase (LL), many elements of hostility were inversely correlated to the anticoagulant variables AT III, Pr C and Pr S. is might suggest a potential mechanism through which hostility enhance haemostasis in the speci c cycle phase.

In various reports concerning female groups, no statistically signi cant correlation between brinogen and hostility was detected, particularly when cardiovascular risk factors were taken into account [4,13,20]. Although these studies referred to healthy individuals, there were essential discrepancies in comparison to the present study, in the size and the age range of the sample, as well as the di erent statistical analysis the authors used. In addition, to the authors' knowledge, no investigation concerning the association of clotting time tests PT and APTT with hostility has been published so far.

As for the mechanism which underlies the link between hostility and haemostasis, it has been speculated that not only unhealthy lifestyle behaviours [32] but also neuroendocrine pathways are involved to this association [5]. Several authors investigate the involvement of hostile behaviour to the hyper activation of sympathetic nervous system [32,34] and the hypothalamic-pituitary-adrenocortical HPA axis [35] in order to provide insight into this relation. With respect to future research, these biological mechanisms could mediate the association between hostility and haemostatic alterations.

Lipids and depressive symptoms

In the present study, no statistically signi cant correlation was detected between the tested lipids and the Zung scores throughout the menstrual cycle phases, suggesting that the cycle e ect does not seem to in uence this relation.

e literature until now has revealed contradictory results, probably due to di erent study samples, and techniques employed by the authors. In agreement with our study, two reports concerning young and healthy individuals found no correlation between total Cholesterol and depression scores [17,19]. Similar results were shown in hospitalized depressed patients [36] as well as in postmenopausal women [8]. On the contrary, there is a paucity of studies which support a relation between low cholesterol levels and depression; in a study group comprised of healthy middle aged Swedish women, authors detected an inverse relation between plasma cholesterol and severity of depressive symptoms. A main discrepancy with our study was the use of a di erent depression measure scale, as well as the di erent age range, and ethnicity of the study sample [37]. An inverse association between depression and total cholesterol, as well as the ratio total cholesterol/ HDL- cholesterol, was also reported concerning healthy young adult women. Likewise, the author used a di erent depression scale which, in comparison to the Zung questionnaire, could be characterized as a trait, rather a state indicator of depression [12]. A similar negative correlation has been shown in other study samples, comprising healthy women in the initial puerperal period [38], as well as in the postpartum period [31], healthy and obese postmenopausal women [39] and subjects with major depression [9]. Regarding HDL-C, some studies have revealed an inverse association with depression, focusing on healthy young [19] and middle aged women [37] as well as on subjects diagnosed with major depression [9]. e di erent age range, the unlike psychometric assessment, as well as the di erent mental condition of the above sample groups, could justify the dissimilar results. In accordance with our work, many other studies, including various study groups, have not concluded to a consistent relation between HDL-C and depression, either as a personality trait, or as a psychological state at the given moment [12,17,36]. With respect to the association between LDL-C or triglycerides and depression, the majority of the studies are consistent with a lack of an association, paralleling our results [9,17,19,36,37]. e hypothesis of a direct biological link between low lipid levels and depression remains unclear; parameters like the age, the poor physical health and the preexisting medical conditions of the study group possibly in uence the relations between these factors [12,40]. Additionally, relevant psychological variables like anxiety and hostility should be taken into consideration, while it is well known that depression o en co-occurs with these statuses [12]. Serotonin alterations have been involved in the interrelation between depressive symptoms and lipid changes; it was reported that low lipid levels may lead to reduction of central serotonergic activity [6]. Additionally, low serotonin concentrations have been detected in depressed subjects [41].

behaviour interfere with haemostatic activity: across the cycle in a non-consistent manner hostility might promote haemostasis process in the L phase, while weaken it in the FL phase. It is obvious di cult to evaluate clinically the signi cance of our ndings but it seems that in order to determine the interaction between lipid pro le, haemostatic activity and depression in apparently healthy young women, the in uence of the menstrual cycle may not have to be taken into consideration.

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