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## Subjects and Methods

Fifty-nine young students (mean age 23.0 ± 2.8 years) were participated, after exclusion from the analysis of women with missed data, (n=10). The women received written information on the purpose and procedures of the study and gave informed consent. All of them were interviewed and examined by an internist. All women were physically healthy, free of clinical evidence of cardiovascular disease and were not using medication (i.e., birth control pills or Selective Serotonin Reuptake Inhibitors); they reported regular menses ranging between 27 and 33 days and had no signs of acnea or hirsutism. Clinical examinations included weight, height, blood pressure and electrocardiogram. The Body Mass Index (BMI) was calculated as weight/height² (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. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

## Sex Hormones, Lipids, Clotting Factors Measurements

Venous blood samples were drawn between 8-10 am, after 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. The following steroid hormones, lipidemic and haemostatic factors and clotting time tests were determined at each sample as follows.

### Steroid hormones

Estradiol ($E_2$), Testosterone (T), Free Testosterone (FT), $\Delta_{4}$-Androstenedione ($\Delta_4$ A) and the ratios $T/E_2$, $FT/E_2$ and $\Delta_4 A/E_2$.

### Lipidemic factors

Total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triglycerides.

### Factors of haemostasis

Coagulant factor: fibrinogen; fibrinolytic factor: plasminogen; anticoagulant factors: protein S, protein C, ATIII; Clotting time tests: APTT, and PT.

## Measurement of Lipidemic and Coagulation Factors

Serum lipid concentrations were measured using biochemical analyzer ILAB 350 of company Instrumentation Laboratory. LDL cholesterol was calculated with the Friedewald equation [38].

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 Elisa. Normal range of Protein S activity is 60-150% and that of Protein C activity is 65-140%.

### Hormonal Assay

Serum samples were analyzed to determine the concentration of estradiol, total testosterone, free testosterone and Δ4-Androstenedione using a commercial radioimmunoassay kit purchased from DIACEL

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|---|---|---|---|
| | | | |
| E2-Estradiol | 23.97 ± 19.09 | 100.39 ± 68.23 | 57.83 ± 49.66 |
| Testosterone | 0.63 ± 0.15 | 0.76 ± 0.26 | 0.67 ± 0.17 |
| F- Testosterone | 2.27 ± 0.87 | 2.79 ± 1.17 | 2.26 ± 0.71 |
| Δ4 Androstenedione (ng/mL) | 1.95 ± 0.83 | 2.29 ± 0.78 | 2.07 ± 0.71 |
| T/E2 (× 10³) | 0.06 ± 0.07 | 0.02 ± 0.02 | 0.02 ± 0.02 |
| FT/E2 (× 10³) | 0.15 ± 0.13 | 0.06 ± 0.09 | 0.06 ± 0.05 |
| Δ4A/E2 (× 10³) | 0.13 ± 0.17 | 0.04 ± 0.05 | 0.05 ± 0.04 |
| | | | |
| Total cholesterol | 184.96 ± 30.94 | 183.90 ± 29.68 | 185.27 ± 24.78 |
| Triglycerides | 65.52 ± 28.31 | 66.15 ± 19.28 | 61.47 ± 16.82 |
| HDL-C | 54.66 ± 9.30 | 57.27 ± 9.49 | 60.00 ± 8.99 |
| LDL-C | 117.40 ± 29.47 | 113.45 ± 29.60 | 113.08 ± 25.59 |
| VLDL | 13.06 ± 5.62 | 13.22 ± 3.80 | 12.27 ± 3.37 |
| | | | |
| PT | 13.80 ± 1.29 | 13.88 ± 1.72 | 13.92 ± 1.18 |
| APTT | 42.04 ± 4.91 | 44.81 ± 12.58 | 40.58 ± 5.93 |
| Fibrinogen | 2.67 ± 0.76 | 3.12 ± 1.34 | 3.11 ± 1.39 |
| AT III | 99.07 ± 13.97 | 100.91 ± 9.46 | 101.48 ± 10.11 |
| PRC | 96.66 ± 17.95 | 95.07 ± 17.79 | 92.24 ± 18.05 |
| PRS | 46.30 ± 14.88 | 53.60 ± 9.38 | 48.75 ± 12.01 |
| Plasminogen | 91.78 ± 13.37 | 96.23 ± 14.06 | 91.48 ± 12.21 |
| | | | |
| | 47.69 ± 8.28 | 46.57 ± 8.96 | 46.16 ± 9.68 |
| | | | |
| 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 |

Mean values ± SD of the biological and psychometric parameters measured. E2: pg/mL, T:pg/dL, FT:pg/ml, Δ4-A:ng/ml, Total Chol, Trigl, HDL-C, LDL-C,VLDL: mg/dl, PT, APTT: sec, Fibrinogen, PrC, PrS, Plasminogen: mg/d.

Lipidemic factors: For the variables Triglycerides, Total Cholesterol, VLDL, HDL-C and LDL-C, a statistically significant difference was found only for HDL-C between phases FL and LL (p=0.009, Bonferroni correction). Hemostatic factors: For the variables PT, APTT, Fibrinogen, ATIII, Pr C, Pr S and plasminogen, a statistically significant difference was found only for Pr C between phases FL and LL (p=0.043 by the non-parametric Wilcoxon Signed Ranks test).

## Discussion

### Steroid hormones and lipids

Previous studies revealed that exogenously administered estrogens decrease the total cholesterol and LDL-C levels, increase the production ratio and the metabolic clearance rate of VLDL and increase the HDL-C levels [20-23]. Regarding the effect of exogenous androgens, the most settled and well documented findings until now seem to be the decrease in HDL-C and lipoprotein (a) levels in addition to a less well characterized decline of triglycerides and LDL-C [41,42]. In the present study, E2 does not seem to essentially modulate the lipid levels across the menstrual cycle with an exception for LDL-C, which is inversely correlated to E2 premenstrually. HDL-C seems to be negatively influenced by the ratio FT/E2 and FT, but during the ML period only.

With respect to the effect of endogenous sex hormones on blood lipids and lipoproteins throughout the menstrual cycle, the data so far are inconsistent; some investigators describe that the changes in sex hormones during the cycle induce cyclic modifications in lipid levels [33,34], while others do not confirm this relation [24,43,44]. The evaluation of the relevant studies has to be done with caution since in most studies sample sizes were small, while the age ranges as well as the variables tested (i.e., peak hormone concentrations versus mean value) were different compared to the present study. In the biocycle study [28] in which 259 premenopausal subjects participated, the authors in an effort to evaluate the association between E2 and lipids on the same day, used acute effects models and revealed an inverse association between the hormone and LDL paralleling our results in the premenstrual phase. In contrast to the bio-cycle study, we did not find any correlation between E2 and TC, HDL and TC/HDL ratios in any phase of the cycle. Two studies involving young and healthy premenstrual women failed to detect a significant correlation between these variables in any of the three menstrual cycle phases [44,45] while Mattson et al. [46], evaluating this relation in four time points across the cycle, determined various significant albeit different correlations in comparison to our study i.e., E2 levels were related to various lipid elements during the follicular, as well as the mid cycle phase, while no correlation was detected during the premenstrual phase [47-49].

Furthermore, Wall et al. [33] described a positive correlation between E2 levels and HDL-C by testing the correlation between peak E2 levels in the ovulatory phase, and lipids across the whole cycle. Finally, in a study concerning the follicular phase only, described significant correlations between E2 and various lipidemic factors (LDL-C, HDL-C, VLDL) [47].

Furthermore, according to our data, increased FT, and not the total concentration of T, appears to be significantly associated with increased total plasma triglycerides and VLDL at each phase of the menstrual cycle. The literature so far regarding this kind of association is limited and it encompasses studies with dissimilar sample groups. In a study, free Androgen Index, i.e., a marker of FT, was positively correlated with VLDL and LDL particles in a sample of 120 mid-life women [48]. Additionally, in a case control study involving 156 postmenopausal women with carotid atherosclerosis, FAI was also positively correlated to LDL-C as well as to triglyceride levels [26]. Other studies, however, involving postmenopausal women [27], healthy middle aged men [49,50] or men with CHD [51] and diabetes 2 [52,53] were not supportive of the above relations.

The positive interrelation of FT with serum triglycerides and VLDL along the menstrual cycle might support the hypothesis of a potentially atherogenic profile of the free fraction of this hormone in premenopausal women. The positive interrelation of FT with serum triglycerides and VLDL along the menstrual cycle may support the hypothesis of a potentially atherogenic profile of the free fraction of this hormone in premenopausal women. However, based on data so far, the effect of exogenous androgens on lipidemic factors may be different from endogenous ones.

In reference of estrogens, despite the discrepancy of some studies findings compared to ours, as well as the fact that, with the exception of HDL-C, we detected no significant difference for the rest of lipidemic factors levels throughout the cycle, there is still a need to define the phases of the cycle in which blood is collected when assessing the lipid profile in normal premenstrual women [46,54,55]. Additionally, more studies are needed in order to further clarify this issue.

the other hand, the sample characteristics in various studies appear to influence significantly this association.

### Sex hormones and hostility

Hostility is a multifaceted psychological construct comprising primarily cognitive and affective dimensions; it is thought to be a potentially 'toxic' characteristic of the type A personality, and has been related to premature mortality as well as to increased incidence of CHD [64,65]. Although hostility is improved by exogenous E2, as shown in several studies of postmenopausal women with racial differences [66,67] and chronically stressed older women-caregivers [68], according to our data, endogenous E2 does not seem to interfere with the expression of hostile behaviour across the menstrual cycle.

Regarding the exogenously administered androgens and hostility, a positive correlation between them has been reported [3,7,8,69]. In the present study total testosterone as, well as its free fraction, were positively correlated with the extroverted forms of hostility, particularly during the ML phase, while a positive association between the T/E2 ratio and the Direction of Hostility in the premenstrual phase was found. The existing studies are scant and contradictory: in some of them, focusing on small samples of healthy men [11,70], on criminals [10] and on female patients diagnosed with anorexia nervosa [9], a positive correlation between endogenous testosterone and hostility is shown, while in others no relation was found [17,71-76]. A possible explanation for the above discrepancies is the use of different questionnaires for the hostility assessment, as well as the different sample population characteristics.

The clinical significance of the present findings is difficult to estimate, but we can speculate that a) testosterone levels might interfere in a more complicated manner with the balance between physiological responding and emotional coping during stressful events than the hostility profile of the individual [75] and b) the ratio T/E2 might be a more essential parameter in the expression of hostile behaviour in the premenstrual phase of menstrual cycle than T or E2 alone. Dougherty et al. [77] investigated the relation between plasma testosterone levels and aggression, a behavioural ingredient of hostility, emphasizing the need to take into consideration the menstrual cycle phases when testing this interrelation. In the present study the findings are in accordance with those of Dougherty since the correlations between androgens and hostility differed markedly across the cycle.

Therefore, the positive correlation between androgens and hostility found in our study, depends either on the aspect of the hostility examined or on the phase of the cycle, showing a selectivity for the FL or the ML phases, since only one correlation was observed in LL phase.

In addition, we could speculate that exogenous androgens affect hostile behaviour in a similar way compared to endogenous androgens, while this is not the case with endogenous estrogens.

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