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ORIGINAL ARTICLE

Vascular Changes in Healthy Youngsters with and without Influence of Oral Contraception

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ABSTRACT

INTRODUCTION: Cardiovascular diseases are one of the leading causes of mortality worldwide. It is known to have a reduced incidence in premenopausal women compared to the opposite sex. This appears to be related to the female hormones and their fluctuation throughout the menstrual cycle.

Objectives: To evaluate and correlate behavior patterns of cerebral dynamics and arterial reactivity throughout the menstrual cycle and to identify possible differences caused by the use of oral contraceptives (OC).

METHODS: The sample consisted of 21 healthy, eumenorrheic and non-smoking young womens, belonging to the 18-21 age group. There were two distinct groups: 11 in the group without oral contraceptive (SCO) and 10 in the oral contraceptive group (CCO). Both groups performed an evaluation in the menstrual phase (MP), from the 1st to the 4th day, and in the ovulatory phase (OP), from the 12th to the 16th day. The third evaluation was performed in the late luteal phase (LLP) from the 26th to the 30th day, in the SCO group and in the OC pause, from the 21st to the 28th day, in the CCO group. Four noninvasive procedures were performed: blood pressure measurement, axillary temperature, carotid and right brachial artery sonography.

RESULTS: There were no statistically significant differences in carotid parameters, however, there were variations in the velocity of the systolic peak (VPS) of the common carotid artery (CCA) and internal carotid artery (ICA) and the ICA resistance index (IR), throughout the cycle. The SCO group showed a significant increase in FMD from MF to OF (p = 0.023), decreasing in LL (p = 0.012). The CCO group demonstrated a relative stabilization of FMD values. In OP, there were statistically significant differences between the two groups in relation to the FMD value (p = 0.040).

CONCLUSIONS: Cyclic fluctuations of estrogen appear to influence cerebral vascular impedance and arterial reactivity but in different proportions. In conclusion, this study supports the idea of standardizing the timing of vascular testing in a premenopausal woman.

Key words: Eumenorrheic; Oral contraceptives; Carotid Arteries; Flow-mediated dilatation

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INTRODUCTION

Estrogen and progesterone (PG) are the main hormones involved in the development, maintenance and control of women's reproductive function. It produces several types of estrogen, being estradiol (17β-estradiol or E2) the most important in terms of regulation of the menstrual cycle, produced mainly by the ovaries[1].

Cardiovascular diseases (CVDs) are responsible for more than 40% of mortality in developed countries[2]. Premenopausal women benefit from a lower incidence of CVDs when compared with men of the same age; proportionality reached after menopause or even at higher risk[3,4]. This premise fits the role of estrogen, also present in men but at lower levels. The "cardioprotective" effect of E2 and

its role in CVDs is associated with the effect on vasculature, lipid metabolism and blood pressure (BP)[1,2,3,4].

Endocrine physiology of women

The popular term "period" refers to menstruation that all healthy women experience monthly. In scientific terms it corresponds to a physiological and cyclic desquamation of the woman's uterine wall in response to a set of hormonal and nervous interactions[5]. The term eumenorreic is used to define a normal and regular menstrual cycle. This means a menstrual blood flow of approximately 30 ml, not to exceed 80 ml, and whose duration varies from 4 to 6 days[5]. It is generally accepted that a normal duration of a menstrual cycle corresponds to the period between the first day of menstruation in a given cycle to the beginning of the next menstrual cycle, being approximately 28 days (acceptable variation of 25-30 days) [5]. Although there are different divisions of the menstrual cycle, the most general and accepted one is the separation into two main phases: follicular/preovulatory/proliferative phase and luteal/post-ovulatory/secretory phase[2,5]. Endocrine control during these phases is supported by positive and negative feedback mechanisms based complex hypothalamic, pituitary, ovarian (hypothalamic-pituitary-ovarian axis) interactions and uterine interactions[2,3].

In the absence of pregnancy E2 and PG levels are minimal, resulting in desquamation of the endometrium resulting in menstruation. Initially, an increase in follicle-stimulating hormone (FSH) is observed, a consequence of a pulsatile secretion of gonadotrophin releasing hormone (GnRH) by the hypothalamus. The increase in FSH will result in a recruitment of primordial follicles in which only one will follow for ovulation - dominant follicle. Around the 14th day of the cycle, the pre-ovulatory follicle produces an abrupt E2 that leads to an outbreak of FSH and luteinizing hormone (LH). These hormonal peaks cause a follicle maturation and consequent ovulation, starting a luteal phase with a duration of approximately 14 days. The follicle that released the ovule happens to be called corpus luteum/yellow body, responsible for the secretion of PG. If the ovule is not fertilized, the corpus luteum degenerates and decreases concentrations of E2 and PG, starting a new cycle[2,5].

Relationship between E2 e nitric oxide (NO)

The E2 presents several types of specific receptors (estrogen receptors, ER), distributed in both sexes by the various systems, with gene expression being different according to their location in the target cells. Two classical subtypes of receptors are known: ERα, more abundant, and ERβ[4,6]. In recent years a growing interest in this subject has led to a better understanding of the interaction between E2 and their receptors, their locations and other possible ligands. The modulatory action of E2 is made on the basis of genomic mechanisms (slow) and non-genomic mechanisms (fast)[7]. Through studies in animal and human models, McNeil et al[8] 1999, Stironeet et al[9] 2003 and Russel et al[10] 2000 tried to demonstrate the physiological functioning of these mechanisms. The hormone-receptor complex acts as a transcription cofactor in the genomic mechanism, by binding to specific regions of the DNA, occurring the regulation of gene expression, being a slow process[6,11]. On the other hand, the non-genomic mechanism is based on the interaction of E2 with the receptor bound to the plasma membrane of the target cell that rapidly activate intracellular signaling through second messengers[6,7].

In the vascular system, the main endogenous source of NO is a nitric oxide synthaseable endothelial enzyme (eNOS or NOS3) [12]. When activated, it stimulates the biosynthesis of NO in the endothelial cells, having as substrate the amino acid L-arginine necessitating the presence of two cofactors, oxygen and phosphate dinucleotide adenine nicotinamide (NADPH)[13]. This mechanism is correctly functioning in the arteries with a normal endothelium, being abolished in cases of endothelial dysfunction.

The NO is known to be a potent vasodilator, inhibitor of platelet aggregation and adhesion and proliferation of smooth muscle cells[4,6,12]. In fact, in response to an increase in E2 concentration throughout the menstrual cycle, an increase in the bioavailability of NO will occur and due to its vasodilatory functions a direct action on the arterial wall physiology is predicted, mirror with hormonal fluctuation, which may play a role in the pathophysiology of cardiovascular risk in women[14].

As in the peripheral circulation, E2 receptors are located in the cerebral vasculature, regulating cerebral vascular tone through the modulation of cellular functions. Also here, E2 facilitates the production of vasoactive substances derived from the endothelium, such as NO and others. E2 is classically known for its neuroactive properties[15]. The vasodilatory effect of estrogen in cerebral vessels has been confirmed in women with hormone replacement therapy (HRT), however, their influence on cerebral blood flow in humans is still uncertain[16,17]. As the concentration of E2 varies throughout the menstrual cycle, it may be expected that the flow conditions within the brain will be modified accordingly[18].

Hormonal contraceptives (HC)

There are several existing HC products that base their action on women's endocrine physiology. Oral contraceptive pill (OCP) is the most used method in the USA[19] not only for pregnancy prevention but also for therapeutic purposes. Its formula is based on the use of two synthetic hormones that mimic estrogen and progesterone endogenous, namely the ethinylestradiol (EE) and progestagens[19,20]. The OCP can be constituted by progestogen alone or the combination of estrogen and progesterone synthetics (combined OCP). These last are the most used in adolescence, based on the daily intake of 21 pills with single-phase, biphasic or three-phase formulations, followed by a pause of 7 days or intake of 7 inert pills[20]. The dosage of EE varies can be of high dose (≥ 50 mcg) or low dose (< 50 mcg)[19]. Its action is based on the inhibition of pulsatile secretion of GnRH, which consequently suppresses FSH and LH levels, avoiding follicular development and ovulation[21,22], leading to low and stable levels of E2.

Following the increasing use of these methods, the side effects began to emerge. Knowing that the E2 influences the cardiovascular system, a change in their concentrations by HC will have an influence on this system. Women who use HC have a higher risk for cardiovascular system disorders such as acute myocardial infarction, stroke, venous thromboembolism, pulmonary embolism and hypertension[14,20]. Although these side effects were reduced with the low dose OCP insertion, they were not eliminated.

Objective

This research has as objective to evaluate the behavior of carotid arteries and endothelial function throughout the menstrual cycle in healthy young female users and non-users of oral contraceptive (OC) and, thus, to identify different patterns of behavior.

METHODS

Population

The sample consisted of 21 young, clinically healthy, nulliparous and of reproductive-age, belonging to the 18-21 age group, all of them students from the Clinical Physiology Degree at the Coimbra

Health School. Participants were recruited through the disclosure of the study by the researchers themselves. The selection criteria were defined based on previously analyzed articles[18,24] and verified based on a questionnaire addressed to the participants, which excluded all those with antecedents that could influence the results. So, only nulliparous, considered healthy girls, with regular menstrual cycles in the last 6 months and menarche for at least 4 years were included. To avoid HC variability, only the young people whose contraceptive was the oral contraceptive pill were included. Exclusion criteria were alcohol abuse, smoking, diabetes, head trauma, cardiovascular and gynecological problems, current or previous pregnancy, irregular menstrual cycles, use of medication during the sample collection and in the last 3 months. As a fundamental requirement, young women with oral contraceptives (OC) did not interrupt their intake during the evaluation period.

Study Design

They were divided into two groups: a group of 10 young people using oral contraceptive (CCO) and a group of 11 young people who did not use a hormonal contraceptive (SCO). As they all had regular menstrual cycles, the length of the menstrual cycle was standardized for a period of 28 days, starting on the first day of menstruation. The stipulation and marking of the evaluations was based on the menstrual calendar and accordingly to the hourly availability of the participants. Thus, before the start of the collection, with the help of the researchers, the young women made a forecast of the evaluation phases. These were subject to 3 moments of evaluation corresponding to 3 phases of the menstrual cycle that differentiated between the 2 groups. Both groups performed an evaluation in the menstrual phase (MP), from the 1st to the 4th day, and in the ovulatory phase (OP), from the 12th to the 16th day. The third evaluation was performed in the late luteal phase (LLP), from the 26th to the 30th day, in the SCO group and from the 21st to the 28th day, in the CCO group, in the pause of the contraceptive pill.

Each participant was informed of all the essential information about the study and guaranteed the confidentiality of the data obtained in order to sign free and informed consent to enable their participation in the study.

Procedure

Each young woman was asked not to perform vigorous exercise and not drink alcohol and caffeine at least 12 hours before each test. All the young women rested for a period of 5-10 minutes in dorsal decubitus position. The laboratory was low lit, silent and temperature controlled (22º / 23ºC). In order to avoid inter-observer errors all exams were performed by the same investigator who was not aware of the phase of the menstrual cycle.

We started the collection with the measurement of the BP by a certified measuring device, Riester ® model Ri-Champion N. In the first evaluation the BP was measured in both arms and if there were no significant differences, the remaining evaluations were always performed on the left arm and the mean value of three measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) was recorded. At the same time, the axillary temperature was measured in all evaluations using a digital thermometer, Ri-gital ®.

After ECG monitoring, we started the carotid evaluation using a GE ® Vivid T8 / Vivid T8 Pro ultrasound. A linear L6-12 probe was used at a frequency of 6-13 MHz. In the first evaluation, it was performed bilaterally and if no significant differences were verified, the left side was used in the remaining evaluations. The measurement sites in the arteries were standardized and fixed in order to avoid further variations, according to the articles found[18,23]: starting at the common carotid artery (CCA), the sample volume was placed 1-2 cm below the bifurcation, followed by the carotid artery (ECA) and internal carotid artery (ICA), with reference to 1-1.5 cm and 1.5-2 cm distal to the bifurcation, respectively. For analysis, information on the intima-media thickness (IMT) of CCA, systolic and diastolic diameter, systolic peak velocity (VPS) and endodiastolic velocity (VED) of CCA, ICA and ECA were collected. The mean velocity was then calculated according to the equation (VPS + 2*VED) /3 for each of the arteries. The resistance index (RI) and the pulsatility index (PI) were calculated based on the currently accepted formulas[24,25].

Finally, the ultrasound study of the upper limb was followed, where all evaluations were performed on the right arm. With a slight abduction of the arm, a mark 3-5 cm above the antecubital fossa was made, in order to guarantee the fixation of the evaluation site. To facilitate the location of the artery, color flow mapping and basal diastolic diameter of the brachial artery (DBAB) were used, with reference to the R wave peak of the electrocardiogram. The distance between the proximal and distal limits of the intima was measured. Once the basal diameter was obtained, the armband previously placed distally at the measurement site was inflated for 5 minutes at suprasystolic pressure. After 60 seconds of rapid deflation of the armband, the post hyperaemic diastolic diameter of the brachial artery (DPHAB) was measured - Figure 1. All measurements were taken 3 times at the same reference point and the mean was recorded as the final value. After that, flow-mediated dilation (FMD) was calculated based on the formula (DPHAB-DBAB)/DBAB*100[26].

Statistical Analysis

The data obtained was transferred and grouped in a database built in the program Excel 2013 (Microsoft Office, Redmond, WA) and then imported and statistically analyzed in the program SPSS Statistics version 25 for Windows (IBM, Armonk, NY).

The Shapiro-Wilks test was used to confirm the distribution of continuous variables. These were checked for variance homogeneity using the Levene test. Simple descriptive statistics were used for the general characterization of the population and the distribution of the variables. Continuous variables were presented as mean ± standard deviation (SD) and as absolute frequency (percentage) for qualitative variables. The ANOVA test was used for repeated samples to evaluate the behavior of the continuous variables in the different phases of the menstrual cycle, both in the total sample and in the groups constituted for the analysis. The Greenhouse-Geisser correction was applied whenever sphericity was violated, and the Bonferroni adjustment for degrees of freedom was used in multiple comparisons to locate the significant effects of a factor. The Student's t-test for independent samples was used for the comparisons of continuous variables between groups, during the 3 evaluation phases. Qualitative variables were compared using the chi-square test (χ^2) and Fisher's exact test, as appropriate.

The statistical significance (p) criterion used was a value of $p \leq 0.05$ for a confidence interval of 95%.

RESULTS

Descriptive Analysis

Twenty-two young women were recruited with an average age of 19.81 ± 0.87. From this initial sample, a young woman belonging to the CCO group was excluded because she presented hypercholesterolemia from the age of 6 years and unknown mild

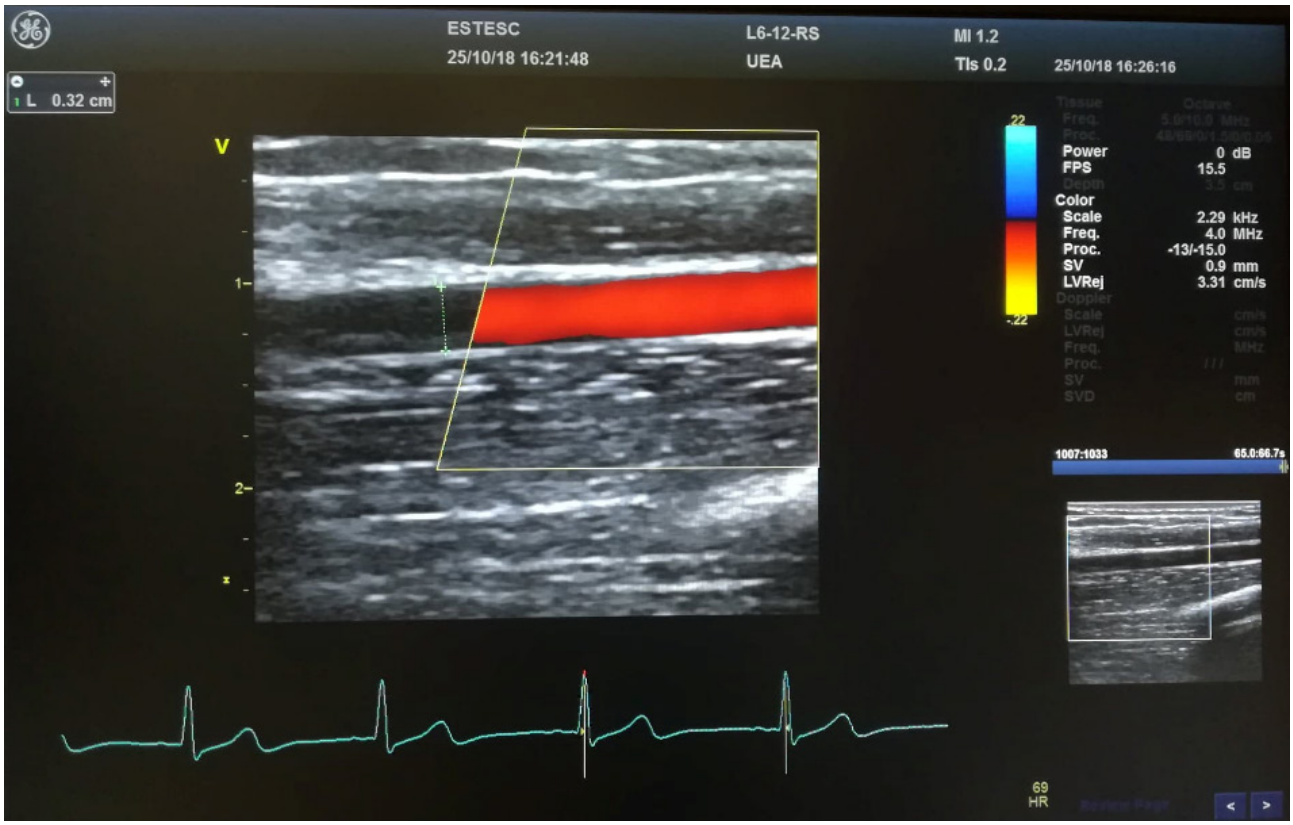

Figure 1 Two-dimensional image of the right brachial artery.

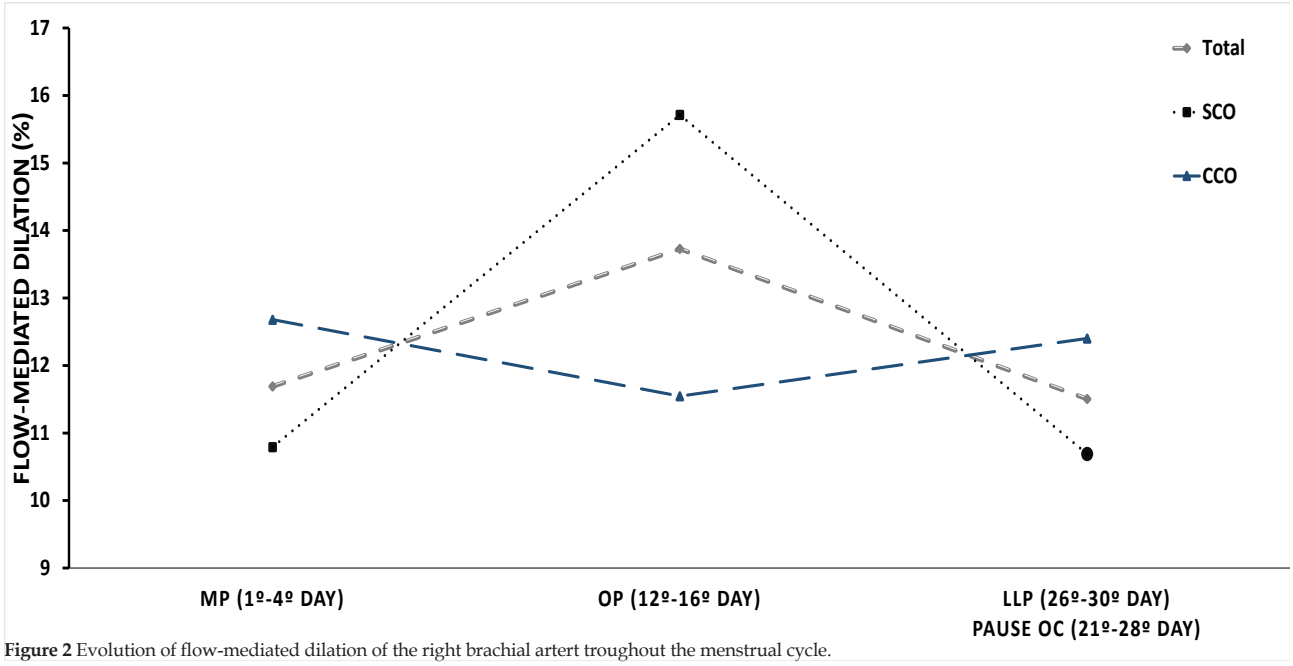

Figure 2 Evolution of flow-mediated dilation of the right brachial artert troughout the menstrual cycle.

arterial hypertension. So, 21 healthy young women with regular menstrual cycles of 28 days were included, considering the possible variation up to 2 days. Table 1 shows the mean values ± SD of the anthropometric and hemodynamic data that characterize the sample.

The total sample is characterized by a body mass index (BMI) of 21.33 ± 1.87 kg /m² in which only one young woman belonging to the CCO group was overweight. All participants had a menarche age for at least 4 years. None of the participants were on prolonged consumption of relevant medication. All the young members in the contraceptive group took combined monophasic OCs for at least 1 year. None of the participants were smokers or had any relevant disease. In terms of cardiovascular antecedents (CVAs) of direct parents: 12 answered affirmatively (7 SCO and 5 CCO) and 42% reported hypertension, 33% AMI, 8% diabetes, 42% stroke, 8% hypercholesterolemia. Most were not regular users of soft drinks (90.5%) and more than half do not attend any type of sport regularly (66.7%), standing out a former federate in non-practicing swimming since the age of 14. About 81% of participants say they consume alcohol but on an infrequent scale. In relation to caffeine consumption, 38.1% reported consuming 2 cups of coffee a day. The

hemodynamic data of the total sample are within normal values and there were no significant differences between the two groups under study.

Evolutionary analysis of the total sample

No statistically significant differences were found in any of the parameters of the total sample over the 3 evaluation phases in the same participant. The respective mean values ± SD of the variables evaluated are presented in Table 2. The SBP and DBP were lower in LLP. The VPS value of the CCA has the lowest value in the OP, reaching the maximum value in the LLP. Similar to CCA behavior, VPS value of the ICA reached its lowest value in OP, increasing to its maximum value in LLP. Although not statistically significant, the FMD (p = 0.186) increases from MP to OP, decreasing in LLP to values close to baseline.

Intra-group evolutionary analysis

Table 3 shows the results on the SBP, DBP, HR, temperature, carotid parameters and FMD of the SCO group, during the 3 assessment phases.

No statistically significant differences were observed in SBP, DBP, HR, axillary temperature and carotid parameters. However, axillary temperature showed a tendency to increase throughout the evaluations, reaching its highest value in LLP. Also, although not statistically significant, we verified the lowest VPS value of the CCA in the OP increasing in the LLP, similar behavior to the VPS of the ICA and ECA. The RI value of the ICA decreases from MF to OP, reaching the highest value in the LLP.

We observed statistically significant differences in the value of FMD throughout the menstrual cycle, in percentage and absolute

Table 1 Anthropometric and hemodynamic characterization of the sample.

Parameters	Total (n = 21)	Group SCO (n = 11)	Group CCO (n = 10)	p (c/OC vs. s/OC)
Age, years	19.81 ± 0,87	19.91 ± 0.94	19.70 ± 0.82	0.597
Weight, kg	58.71 ± 6.06	58.36 ± 4.55	59.10 ± 7.64	0.789
Height, m	1.66 ± 0.06	1.66 ± 0.05	1.66 ± 0.07	0.889
BMI, kg/m²	21.33 ± 1.87	21.17 ± 1.54	21.51 ± 2.24	0.691
Menarche, anos	12.29 ± 1.23	12.09 ± 0.83	12.50 ± 1.58	0.461
CVs antecendent				0.670
Yes, % (n)	42.9% (9)	64.6% (7)	50.0% (5)	
No, % (n)	57.1% (12)	36.4% (4)	50.0% (5)	
SBP, mmHg	115.10 ± 8.33	115.68 ± 7.95	114.47 ± 9.12	0.748
DBP, mmHg	68.492 ± 5.35	69.21 ± 4.84	67.70 ± 6.02	0.531
HR, bpm	72.05 ± 10.30	71.36 ± 9.62	72.80 ± 11.48	0.758
Temperature, C°	36.11 ± 0.61	36.07 ± 0.72	36.16 ± 0.48	0.751
IMT, mm	0.429 ± 0.046	0.436 ± 0.051	0.420 ± 0.042	0.433

Table 2 Global evolution of the parameters of the total sample, along the menstrual cycle.

Parameters	MP (1°-4° dia)	OP (12°-16° dia)	LLP (26°-30° dia)	p
SBP, mmHg	115.103 ± 8.330	113.762 ± 8.726	113.016 ± 8.529	0.384
DBP, mmHg	68.492 ± 5.348	67.794 ± 6,218	66.937 ± 6.358	0.344
HR, bpm	72.05 ± 10.298	70.857 ± 7.786	75.857 ± 9.329	0.097
Temperature, C°	36.114 ± 0.606	36.286 ± 0.408	36.362 ± 0.591	0.094
IMT, mm	0.429 ± 0.046	0.429 ± 0.046	0.448 ± 0.068	0.353
CCA VPS, cm/s	103.494 ± 19.613	101.754 ±15.713	108.300 ± 15.883	0.220
CCA VED, cm/s	22.773 ± 5.455	20.136 ± 5.040	21.687 ± 4.373	0.111
CCA PI	1,623 ± 0.242	1.729 ± 0.243	1.709 ± 0.233	0.107
CCA RI	0.775 ± 0.058	0.799 ± 0.053	0.796 ± 0.047	0.080
ICA VPS, cm/s	90.693 ± 19.143	88.336 ± 17.466	96.849 ± 19.703	0.073
ICA VED, cm/s	29.178 ± 7.761	26.247 ± 5.409	28.054 ± 4.986	0.223
ICA PI	1.247 ± 0.256	1.308 ± 0.334	1.342 ± 0.178	0.390
ICA RI	0.674 ± 0.074	0.686 ± 0.121	0.705 ± 0.051	0.417
ECA VPS, cm/s	70.764 ± 13.074	67.339 ± 15.034	68.294 ± 15.003	0.599
ECA VED, cm/s	7.649 ± 7.695	6.219 ± 3.313	6.664 ± 4.119	0.612
ECA PI	2.270 ± 0.419	2.290 ± 0.388	2.275 ± 0.344	0.972
ECA RI	0.894 ± 0.090	0.900 ± 0.067	0.899 ± 0.055	0.937
FMD, %	11.691 ± 5.135	13.728 ± 4.734	11.504 ± 2.882	0.186
FMD absolute, mm	0.321 ± 0.127	0.381 ± 0.125	0.324 ± 0.080	0.152

Table 3 Evolution of variables along the menstrual cycle, in the group without oral contraception.

Parameters	MP (1°-4° day)	OP (12°-16° day)	Pause OC (21°-28° day)	p
SBP, mmHg	114.467 ± 9.118	113.433 ± 9.402	110.00 ± 7.362	0.209
DBP, mmHg	67.700 ± 6.019	67.733 ± 6.629	65.400 ± 5.887	0.288
HR, bpm	72.80 ± 11.478	68.00 ± 5.716	74.100 ± 8.504	0.264
Temperature, C°	36.160 ± 0.479	36.400 ± 0.362	36.520 ± 0.270	0.078
CCA VPS, cm/s	102.910 ± 20.037	102.534 ± 18.702	105.618 ± 16.965	0.818
CCA VED, cm/s	23.822 ± 5.315	20.288 ± 4.936	21.980 ± 3.242	0.079
CCA PI	1.576 ± 0.203	1.724 ± 0.238	1.672 ± 0.136	0.065
CCA RI	0.765 ± 0.053	0.799 ± 0.052	0.790 ± 0.030	0.070
ICA VPS, cm/s	90.199 ± 20.373	89.930 ± 16.041	96.196 ± 15.210	0.442
ICA VED, cm/s	28.304 ± 7.073	24.531 ± 4.514	27.484 ± 4.693	0.389
ICA PI	1.268 ± 0.331	1.403 ± 0.278	1.362 ± 0.190	0.415
ICA RI	0.677 ± 0.96	0.719 ± 0.074	0.711 ± 0.054	0.293
ECA VPS, cm/s	74.197 ± 9.321	66.518 ± 12.467	63.076 ± 17.065	0.110
ECA VED, cm/s	9.374 ± 10.994	5.032 ± 3.237	5.553 ± 3.060	0.229
ECA PI	2.236 ± 0.582	2.414 ± 0.361	2.296 ± 0.371	0.463
ECA RI	0.880 ± 0.128	0.921 ± 0.051	0.902 ± 0.055	0.437
FMD, %	12.679 ± 7.067	11.546 ± 4.176	12.401 ± 3.199	0.863
FMD absolute, mm	0.340 ± 0.171	0.320 ± 0.114	0.340 ± 0.084	0.914

Table 4 Evolution of the parameters of the group with oral contraception, along the menstrual cycle.

Parameters	MP (1°-4° day)	OP (12°-16° day)	LLP (26°-30° day)	p
SBP, mmHg	115.682 ± 7.950	114.061 ± 8.515	115.758 ± 8.908	0.521
DBP, mmHg	69.212 ± 4.836	67.848 ± 6.145	68.333 ± 6.720	0.519
HR, bpm	71.36 ± 9.615	72.545 ± 9.234	77.455 ± 10.153	0.207
Temperature, C°	36.073 ± 0.724	36.182 ± 0.436	36.218 ± 0.765	0.680
CCA VPS, cm/s	104.025 ± 20.184	101.045 ± 13.336	110.737 ± 15.224	0.238
CCA VED, cm/s	21,820 ± 5.655	19.997 ± 5.370	21.420 ± 5.349	0.634
CCA PI	1.667 ± 0.276	1.733 ± 0.259	1.742 ± 0.298	0.625
CCA RI	0.785 ±0.062	0.800 ± 0.057	0.801 ± 0.059	0.581
ICA VPS, cm/s	91.142 ± 18.944	86.886 ± 19.331	97.443 ± 23.819	0.160
ICA VED, cm/s	29.972 ± 8.601	27.806 ± 5.878	28.572 ± 5.410	0.565
ICA PI	1.228 ± 0.176	1.223 ± 0.370	1.323 ± 0.173	0.507
ICA RI	0.672 ± 0.053	0.656 ± 0.1495	0.700 ± 0.050	0.485
ECA VPS, cm/s	67.643 ± 15.530	68.085 ± 17.631	73.038 ± 11.666	0.392
ECA VED, cm/s	6.080 ± 1.994	7.297 ± 3.137	7.675 ± 4.812	0.419
ECA PI	2.302 ± 0.208	2.178 ± 0.393	2.256 ± 0.334	0.484
ECA RI	0.907 ± 0.032	0.881 ± 0.076	0.896 ± 0.058	0.406
FMD, %	10.792 ± 2.433	15.712 ± 4.476	10.689 ± 2.422	0.001 *
FMD absolute, mm	0.305 ± 0.072	0.436 ± 0.112	0.309 ± 0.077	0.002 *

risky to evolve into a possible relationship. Krejza et al[18] (2001) affirmed that when exposed to high concentrations of E2 leads to variations in the flow velocity of the carotid arteries.

FMD is a noninvasive technique that provides information on endothelial function, a marker of vascular health[26]. As already

Table 5 Comparation of pressures, heart rate and temperature between groups.

Parameters		MP (1°-4° day)	p (SCO vs. CCO on MP)	OP (12°-14° day)	p (SCO vs. CCO on OP)	LLP (26°-30° day) Pause OC(21°-28° day)	p (SCO vs. CCO on LLP/Pause OC)
SBP, mmHg	SCO	115.68 ± 7.95	0.748	114.061 ± 8.515	0.874	115.758 ± 8.908	0.125
	CCO	114.47 ± 9.12		113.433 ± 9.402		110.00 ± 7.362	
DBP, mmHg	SCO	69.21 ± 4.84	0.531	67.848 ± 6.145	0.967	68.333 ± 6.720	0.303
	CCO	67.70 ± 6.02		67.733 ± 6.629		65.400 ± 5.887	
HR, bpm	SCO	72.05 ± 10.30	0.758	72.545 ± 9.234	0.309	77.455 ± 10.153	0.425
	CCO	72.80 ± 11.48		68.00 ± 5.716		74.100 ± 8.504	
Temperature, °C	SCO	36.11 ± 0.61	0.751	36.182 ± 0.436	0.230	36.218 ± 0.765	0.253
	CCO	36.16 ± 0.48		36.400 ± 0.362		36.520 ± 0.270	

Table 6 Comparation of systolic and diastolic diameters of carotid arteries, between groups.

Parameters		MP (1°-4° day)	p (SCO vs. CCO on MP)	OP (12°-14° day)	p (SCO vs. CCO on OP)	LLP (26°-30° day) Pause OC(21°-28° day)	p (SCO vs. CCO on LLP/Pause OC)
ACC VPS, cm/s	SCO	104.025 ± 20.184	0.900	101.045 ± 13.336	0.835	110.737 ± 15.224	0.475
	CCO	102.910 ± 20.037		102.534 ± 18.702		105.618 ± 16.965	
ACC VED, cm/s	SCO	21.820 ± 5.655	0.415	19.997 ± 5.370	0.899	21.420 ± 5.349	0.778
	CCO	23.822 v 5.315		20.288 ± 4.936		21.980 ± 3.242	
ACC PI	SCO	1.667 ± 0.276	0.405	1.733 ± 0.259	0.931	1.742 ± 0.298	0.506
	CCO	1.576 ± 0.203		1.724 ± 0.238		1.672 ± 0.136	
ACC RI	SCO	0.785 ± 0.062	0.460	0.800 ± 0.057	0.954	0.801 ± 0.059	0.586
	CCO	0.765 ± 0.053		0.799 ± 0.052		0.790 ± 0.030	
ACI VPS, cm/s	SCO	91.142 ± 18.944	0.914	86.886 ± 19.331	0.701	97.443 ± 23.819	0.889
	CCO	90.199 ± 20.373		89.930 ± 16.041		96.196 ± 15.210	
ACI VED, cm/s	SCO	29.972 ± 8.601	0.635	27.806 ± 5.878	0.172	28.572 ± 5.410	0.630
	CCO	28.304 ± 7.07		24.531 ± 4.514		27.484 ± 4.693	
ACI PI	SCO	1.228 ± 0.176	0.734	1.223 ± 0.370	0.226	1.323 ± 0.173	0.631
	CCO	1.268 ± 0.331		1.403 ± 0.278		1.362 ± 0.190	
ACI RI	SCO	0.672 ± 0.053	0.891	0.656 ± 0.1495	0.243	0.700 ± 0.050	0.654
	CCO	0.677 ± 0.096		0.719 ± 0.074		0.711 ± 0.054	
ACE VPS, cm/s	SCO	67.643 ± 15.530	0.262	68.085 ± 17.631	0.818	73.038 ± 11.666	0.132
	CCO	74.197 ± 9.321		66.518 ± 12.467		63.076 ± 17.065	
ACE VED, cm/s	SCO	6.080 ± 1.994	0.340	7.297 ± 3.137	0.120	7.675 ± 4.812	0.248
	CCO	9.374 ± 10.994		5.032 ± 3.237		5.553 ± 3.060	
ACE PI	SCO	2.302 ± 0.208	0.728	2.178 ± 0.393	0.169	2.256 ± 0.334	0.798
	CCO	2.236 ± 0.582		2.414 ± 0.361		2.296 ± 0.371	
ACE RI	SCO	0.907 ± 0.032	0.515	0.881 ± 0.076	0.176	0.896 ± 0.058	0.804
	CCO	0.880 ± 0.128		0.921 ± 0.051		0.902 ± 0.055	

Table 7 Comparation of carotid parameters between groups.

Parameters		MP (1°-4° day)	p (SCO vs. CCO on MP)	OP (12°-14° day)	p (SCO vs. CCO on OP)	LLP (26°-30° day) Pause OC(21°-28° day)	p (SCO vs. CCO on LLP/Pause OC)
ACC DD, mm	SCO	5.264 ± 0.330	0.639	5.127 ± 0.427	0.880	5.218 ± 0.343	0.908
	CCO	5.264 ± 0.469		5.100 ± 0.389		5.200 ± 0.371	
ACC SD, mm	SCO	6.082 ± 0.334	0.398	5.909 ± 0.558	0.729	6.045 ± 0.356	0.350
	CCO	5.920 ± 0.514		5.830 ± 0.464		5.880 ± 0.434	
ACI DD, mm	SCO	4.427 ± 0.249	0.618	4.482 ± 0.417	0.840	4.391 ± 0.324	0.639
	CCO	4.560 ± 0.829		4.530 ± 0.648		4.300 ± 0.535	
ACI SD, mm	SCO	4.727 ± 0.329	0.633	4.800 ± 0.471	0.776	4.709 ± 0.404	0.900
	CCO	4.870 ± 0.914		4.880 ± 0.776		4.740 ± 0.687	
ACE DD, mm	SCO	3.618 ± 0.555	0.724	3.509 ± 0.547	0.233	3.636 ± 0.639	0.079
	CCO	3.530 ± 0.574		3.240 ± 0.443		3.170 ± 0.492	
ACE SD, mm	SCO	3.900 ± 0.598	0.854	3.827 ± 0.604	0.155	3.864 ± 0.679	0.149
	CCO	3.850 ± 0.633		3.470 ± 0.490		3.470 ± 0.495	

value (p = 0.001 and p = 0.002, respectively). There was an increase in their value from MP to OP (p = 0.023), decreasing later to near baseline in LLP (p = 0.012).

In the CCO group, the mean values ± SD along the menstrual cycle of the parameters also analyzed in the SCO group and already mentioned above are shown in Table 4 (Apendix). No statistically significant differences were observed in any of the variables.

Comparative analysis between the SCO group and the CCO group

Table 5 shows the average values ± SD of SBP, DBP, HR and axillary temperature for both groups, as well as the significance value of the difference between them at each moment. There were no relevant variations in these parameters throughout the evaluations.

Table 6 shows the systolic and diastolic diameters of the carotid arteries for both groups and the significance value of the difference between them at each moment. There were no significant changes in the diameters during the 3 evaluations.

DISCUSSION AND CONCLUSION

No studies were found in the literature that simultaneously evaluated the carotid arteries and FMD. However, many articles that study the two strands individually were found. In the present study, the right brachial artery was used in order to evaluate the vasodilatation after a period of hyperemia of the distal limb - FMD. When using carotid ultrasound, we tried to evaluate the carotid flow and, indirectly, the cerebral flow. The standardization of the various moments of evaluation was based on specific phases of the endocrine / hormonal behavior of the woman throughout her monthly cycle. Thus, in the SCO group, the first evaluation should be performed on the days when the hormone levels are lower, corresponding to the MP from the 1st to the 4th day, the second sample should be on the days when the levels of E2 are the highest, corresponding to OP from day 12 to day 16 and the third assessment on days when E2 levels were low again, on the LLP from the 26th to the 30th day. For the CCO group, the first sample was collected at the time of the start of contraception from day 1 to day 4 in order to evaluate its initial effect, the second evaluation on the days when ovulation was supposed to occur, from the 12th to the 16th day and the last collection on the days of the OC pause, from the 21st to the 28th day.

All evaluations started with the measurement of BP and axillary temperature. An influence of E2 on BP is reported in women who do not use OC. Adkison et al[14] (2010) demonstrated that SBP and DBP decreased approximately 4 mmHg in LLP for 10 to 14 days in eumenorrheic women who did not use OC. Although we may consider this reduction in BP to be substantial, it has been estimated that a reduction of SBP by only 3 mmHg can lead to a reduction in cardiac mortality by 5-9% and stroke by 8-14%[27]. In our study, no significant variations were found in relation to BP in the SCO group, possibly due to the young age of the participants and to the fact that none of the participants included had high BP values. This may also justify the fact that the CCO group does not present significant changes in BP, although its elevation is described in young women using OC because of the presence of EE that exacerbates hepatic angiotensinogen production and, based on the renin system -angiotensin-aldosterone, causes an increase of BP[19]. The fact that young women are still relatively recent users of OC may be another argument in favor, so it is foreseeable that the increase in years of contraception will make their effects more evident. Regarding axillary temperature, it shows, though not significantly, a tendency to increase throughout the evaluations, reaching its highest value in LLP, possibly running as an argument in favor of the occurrence of ovulation.

When we analyzed the variables obtained by the carotid ultrasound study, we did not find statistically significant changes in each group individually and in the comparison between groups. This behavior may be justified by the young age group and the reduced moments of evaluation. Possibly with a more advanced age section and with more collections throughout the cycle, statistically significant values could be reached. However, we found some trends that, although not statistically significant, could alert to possible fluctuations in agreement with the hormonal changes. In the SCO group, the ICA RI value decreases from MP to OP, increasing later to values above baseline. Krejza et al[23] (2003) when studying a group of healthy and non-users of OC during twelve days of the menstrual cycle, found a statistically significant decrease in the late follicular phase when compared to the basal values, raising the hypothesis of the RI variation of ICA due to E2 fluctuations. As RI is a measure of resistance of the distal vascular area to the insonation site, an increase in peripheral resistance will lead to a decrease in VED and an increase in VPS, according to mechanisms of reflection of pressure waves produced by distal arterioles and by cardiac compensating mechanisms, in order to compensate for the increase in resistance. It is known that there are numerous components that influence and change the impedance of a stable system and that can change VPS and VED and consequently RI, specifically cardiac output[23]. In our study, since there were no significant changes in HR and BP, we believe that there was no relevant change. Vascular compliance may also influence vascular impedance[23]. With the measurement of the systolic and diastolic diameters of the carotid arteries, in the present study, we verified that there were no significant variations throughout the menstrual cycle and, so it is possible to think that the cerebrovascular compliance remains without considerable modifications. Thus, we can think that the increase of the E2 concentration with the late follicular phase and OP causes a decrease in the cerebral vascular impedance and in this way leads to a reduction of the RI. It is thought that the ER are present in endothelial cells and muscle cells of arteries and cerebral arterioles but not in the arteries carótidas[18,23]. The stimulation of these receptors by E2 will increase the secretion of vasoactive substances such as NO, prostacyclin and prostaglandin, leading to a relaxation of the vascular musculature. Several investigators[18,23] also affirm the possibility of a distinct behavior when exogenous estrogen is present either in the form of OCP or in HRT. In our study, we evaluated young women using combined monophasic OCP, with a relative stabilization of RI throughout the menstrual cycle which in relation to the one presented previously, meets the expectations, since the contraceptive pill will keep circulating levels of E2 stable.

Consciously, a “blind” comparison between carotid artery flow velocities and hormonal variations can be considered risky due to a set of factors related to both the lack of rigorous methodology and the lack of knowledge of the exact momentum of the menstrual cycle. This may explain why many researchers do not report differences in ICA flow velocity between the follicular and luteal phases of the cycle. However, several studies[28,29] have already demonstrated a variation of cerebral blood flow throughout the menstrual cycle but few are those that have studied in the carotid study and cerebral hemodynamics reason why its pathophysiological mechanisms are still not known. In fact, in our study, no statistically significant variations were found of VPS of CCA and ICA throughout the menstrual cycle, with only fluctuations occurring, admitting it is still

mentioned, vascular endothelial and smooth muscle cells have ER that upon their binding, increase the bioavailability of NO. Studies[23,28] demonstrated an increase in NO concentrations from the early follicular phase to the late follicular phase, returning to the basal levels in the luteal phase. In view of this, it is hypothesized that the increase of E2 production during the late / ovulatory follicular phase results in a greater opportunity for its binding to its receptors and thus increase the bioavailability of NO, a potent vasodilator and modulator of vascular reactivity[14]. Several investigators[14,30,31] when studying groups of healthy premenopausal young people with regular menstrual cycles and non-users of OC considered that an increase in vascular reactivity during the late/ovulatory follicular phase was a result of an increase in the bioavailability of NO secondary to a higher concentration of E2. Also in our study we obtained concordant results. In the SCO group, the FMD value increased significantly from MP to OP and later returned to basal values. In the CCO group, considerable stability was expected in the value of FMD, since OC prevents the occurrence of ovulation and the hormonal peak of E2. In fact, in our study we observed a relative stabilization of the FMD values and there wasn't increase in vascular reactivity in OP, unlike the SCO group.

As already mentioned, women at menopause achieve a CVDs risk similar to or greater than men. Although age, per se, has harmful effects on the vasculature, the reduction of E2 with menopause may add an aggravating factor of CVDs in postmenopausal women, making it difficult to distinguish the different contributions[32]. About 70% of the beneficial effects of E2 focus on vasodilation, decreased homocysteine levels, decreased levels of fibrinogen and lipoprotein A and their positive effects on the development of atherosclerosis[33]. So, together with all the mechanisms triggered by the physiological aging of cells, there is a further development of cardiovascular disease.

Based on these visible effects of E2, HRT seems to be a plausible solution for the reduction of vascular dysfunction potentiated by menopause. Although for many decades this therapy remained famous among women and the clinical community, it was largely challenged as of 2002, as a result of the publication of a study by the Women's Health Initiative Research Group (WHI)[34], indicating that the long-term risks of HRT outweigh the benefits and that this risk varies as HRT is based on the use of estrogen alone or in combined use of it with progesterone. These risks include increased incidence of breast cancer, endometrial cancer, early onset coronary disease, pulmonary embolism and stroke. In the absence of a clear consensus, other studies have argued that there is no clear evidence of an increase in these risks trying to rest clinicians and patients regarding the use of this therapy and that it should always be considered or adapted to the clinical profile of the woman[35].

In conclusion, our results are in agreement with the available studies that demonstrate the possible influence of E2 on arterial response and cerebral hemodynamics. It is likely that fluctuations of this hormone in the cerebral blood flow maybe change and/or serve as a pathophysiological or therapeutic basis for events such as stroke and some forms of epilepsy and migraine triggered by menstruation. These linking mechanisms are still not properly established, since the research in this area is scarce. Similarly, the improvement of arterial function in OP seems to be partly due to an increase in NO secondary to an increase in E2 concentration. These data highlight the importance of standardizing the timing of vascular testing in premenopausal women because the results may vary depending on the stage of the cycle it is in. Following this line of reflection, the phase of the "correct" menstrual cycle to assess cardiovascular risk in a premenopausal woman without hormonal interposition would be from the 1st to the 7th day of the menstrual cycle, already described in some guidelines[26]. Also in BP, the phase of the menstrual cycle should be taken into account in its measurement and interpretation in clinical context.

Limitations of the study

Using the calendar method and standardizing the menstrual cycle as 28 days, according to the literature, the respective phases of the cycle were estimated which vary from woman to woman. However, accurate knowledge of the duration of each cycle, of each specific phase and of the exact time of ovulation is difficult without the use of specific control methods that were not possible to achieve. In addition, a daily assessment of the participant would be necessary to evaluate its evolution, which was impossible both due to lack of resources and schedule incompatibility. With this awareness and through a detailed selection of the young people and with the greatest possible rigor, the three assessment phases were marked. With the appropriate resources and a multi-center contribution, it would be interesting to evaluate this issue on a wider scale taking into account several physiological and non-physiological variables, accompanying women's menstrual years.

REFERENCES

1. Pedrosa D, Rezende L, Silva I, Rangel L, Gonçalves W, Graceli J. Efeitos benéficos do estrogénio no sistema cardiovascular. Prespectivas. 2009; **3(12)**: p190-196.
2. Messinis IE, Messini CI, Dafopoulos K. Novel aspects of the endocrinology of the menstrual cycle. *Reprod Biomed Online.* 2014 Jun; **28(6)**: 714-22. [DOI: 10.1016/j.rbmo.2014.02.003]; [PMID: 24745832].
3. Franz WB 3rd. Basic review: endocrinology of the normal menstrual cycle. *Prim Care.* 1988 Sep; **15(3)**: 607-16. Review. [PMID: 3054966].
4. dos Santos RL, da Silva FB, Ribeiro RF Jr, Stefanon I. Sex hormones in the cardiovascular system. *Horm Mol Biol Clin Investig.* 2014 May; **18(2)**: 89-103. [DOI: 10.1515/hmbci-2013-0048]; [PMID: 25390005.
5. Reed BG, Carr BR. The Normal Menstrual Cycle and the Control of Ovulation. 2018 Aug 5. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, Hershman JM, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE,New M, Perreault L, Purnell J, Rebar R, Singer F, Trence DL, Vinik A, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. [PMID: 25905282].
6. White RE. Estrogen and vascular function. *Vascul Pharmacol.* 2002 Feb; **38(2)**: 73-80. Review.
7. Usselman CW, Stachenfeld NS, Bender JR. The molecular actions of oestrogen in the regulation of vascular health. *Exp Physiol.* 2016 Mar; **101(3)**: 356-61. [DOI: 10.1113/EP085148]; PMID: 26778523].
8. McNeill AM, Kim N, Duckles SP, Krause DN, Kontos HA. Chronic estrogen treatment increases levels of endothelial nitric oxide synthase protein in rat cerebral microvessels. *Stroke.* 1999 Oct; **30(10)**: 2186-90. [PMID: 10512927].
9. Stirone C, Chu Y, Sunday L, Duckles SP, Krause DN. 17 Beta-estradiol increases endothelial nitric oxide synthase mRNA copy number in cerebral blood vessels: quantification by real-time polymerase chain reaction. *Eur J Pharmacol.* 2003 Sep 30; **478(1)**: 35-8. [PMID: 14555182].
10. Russell KS, Haynes MP, Sinha D, Clerisme E, Bender JR. Human vascular endothelial cells contain membrane binding sites for estradiol, which mediate rapid intracellular signaling. *Proc Natl Acad Sci U S A.* 2000 May 23; **97(11)**: 5930-5. [PMID: 10823945].

11. Kypreos KE, Zafirovic S, Petropoulou PI, Bjelogrlic P, Resanovic I, Traish A, Isenovic ER. Regulation of endothelial nitric oxide synthase and high-density lipoprotein quality by estradiol in cardiovascular pathology. *J Cardiovasc Pharmacol Ther.* 2014 May; **19(3)**: 256-68. [DOI: 10.1177/1074248413513499]. Epub 2014 Jan 12. Review. [PMID: 24414281].
12. Stuehr DJ. Enzymes of the L-arginine to nitric oxide pathway. *J Nutr.* 2004 Oct; **134(10 Suppl)**: 2748S-2751S; discussion 2765S-2767S. [DOI: 10.1093/jn/134.10.2748S]. Review. [PMID: 15465779].
13. Van Cauwenberge JR. [Hormonal contraception]. *Rev Med Liege.* 1992 May; **47(5)**: 221-36. Review. French.
14. Adkisson EJ, Casey DP, Beck D, Gurovich AN, Martin JS, Braith RW. Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. *Exp Biol Med (Maywood)*. 2010 Jan; **235(1)**: 111-8. [DOI: 10.1258/ebm.2009.009186]; [PMID: 20404025].
15. Raz L. Estrogen and cerebrovascular regulation in menopause. *Mol Cell Endocrinol.* 2014 May 25; **389(1-2)**: 22-30. [DOI: 10.1016/j.mce.2014.01.015]; [PMID: 24472522].
16. Gangar KF, Vyas S, Whitehead M, Crook D, Meire H, Campbell S. Pulsatility index in internal carotid artery in relation to transdermal oestradiol and time since menopause. *Lancet*. 1991 Oct 5; **338(8771)**: 839-42. [DOI: 10.1016/0140-6736(91)91500-t]; [PMID: 1681213].
17. Penotti M, Nencioni T, Gabrielli L, Farina M, Castiglioni E, Polvani F. Blood flow variations in internal carotid and middle cerebral arteries induced by postmenopausal hormone replacement therapy. *Am J Obstet Gynecol.* 1993 Nov; **169(5)**: 1226-32. [DOI: 10.1016/0002-9378(93)90287-s]; [PMID: 8238189].
18. Krejza J, Mariak Z, Huba M, Wolczynski S, Lewko J. Effect of endogenous estrogen on blood flow through carotid arteries. *Stroke*. 2001 Jan; **32(1)**: 306. [PMID: 15981152].
19. Brito MB, Nobre F, Vieira CS. Hormonal contraception and cardiovascular system. *Arq Bras Cardiol.* 2011 Apr; 96(4): e81-9. [DOI: 10.1590/S0066-782X2011005000022]; [PMID: 21359483].
20. Colquitt CW, Martin TS. Contraceptive Methods. *J Pharm Pract.* 2017 Feb; **30(1)**: 130-135. [DOI: 10.1177/0897190015585751]. Epub 2016 Jul 8. [PMID: 26033795].
21. Sondheimer SJ. Oral contraceptives: mechanism of action, dosing, safety, andcefficacy. *Cutis*. 2008 Jan; **81(1 Suppl)**: 19-22. Review. [PMID: 18338654].
22. Rivera R, Yacobson I, Grimes D. The mechanism of action of hormonalcontraceptives and intrauterine contraceptive devices. *Am J Obstet Gynecol.* 1999 Nov; **181(5 Pt 1)**: 1263-9. [DOI: 10.1016/s0002-9378(99)70120-1]; [PMID: 10561657].
23. Krejza J, Siemkowicz J, Sawicka M, Szylak A, Kochanowicz J, Mariak Z, Lewko J, Spektor V, Babikian V, Bert R. Oscillations of cerebrovascular resistance throughout the menstrual cycle in healthy women. *Ultrasound Obstet Gynecol.* 2003 Dec; **22(6)**: 627-32. [DOI: 10.1002/uog.907]; [PMID: 14689537].
24. Pourcelot L. Applications cliniques de l'examen Doppler transcutane. *Coloques de l'Inst Natl Sante Rech Med* 1974; **12**: 1376-1380. DNL: 76112087.
25. Gosling RG, King DH. Arterial assessment by Doppler-shift ultrasound. *Proc R Soc Med* 1974; **67**: 447-9. [PMID: 4850636].
26. Thijssen DH, Black MA, Pyke KE, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol.* 2011; **300(1)**: H2-H12. [DOI: 10.1152/ajpheart.00471.2010]; [PMID: 20952670].
27. Rosendorff C, Black HR, Cannon CP, Gersh BJ, Gore J, Izzo JL Jr, Kaplan NM,O'Connor CM, O'Gara PT, Oparil S; American Heart Association Council for High Blood Pressure Research; American Heart Association Council on Clinical Cardiology; American Heart Association Council on Epidemiology and Prevention. Treatment of hypertension in the prevention and management of ischemic heart disease: a scientific statement from the American Heart Association Council for High Blood Pressure Research and the Councils on Clinical Cardiology and Epidemiology and Prevention. Circulation. 2007 May 29; **115(21)**: 2761-88. Epub 2007 May 14. Erratum in: Circulation. 2007 Jul 31; 116(5): e121. [DOI: 10.1161/CIRCULATIONAHA.107.183885]; [PMID: 17502569].
28. Brackley KJ, Ramsay MM, Broughton Pipkin F, Rubin PC. The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound. *Ultrasound Obstet Gynecol*. 1999 Jul; **14(1)**: 52-7. [DOI: 10.1046/j.1469-0705.1999.14010052.x]; [PMID: 10461339].
29. Matteis M, Troisi E, Monaldo BC, Caltagirone C, Silvestrini M. Age and sex differences in cerebral hemodynamics: a transcranial Doppler study. *Stroke*. 1998 May; **29(5)**: 963-7. [DOI: 10.1161/01.str.29.5.963]; [PMID: 9596243].
30. Williams MR, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K,Komesaroff PA. Variations in endothelial function and arterial compliance during the menstrual cycle. *J Clin Endocrinol Metab*. 2001 Nov; **86(11)**: 5389-95. [DOI: 10.1210/jcem.86.11.8013]; [PMID: 11701712].
31. Hashimoto M, Akishita M, Eto M, Ishikawa M, Kozaki K, Toba K, Sagara Y, Taketani Y, Orimo H, Ouchi Y. Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. *Circulation*. 1995 Dec 15; **92(12)**: 3431-5. [DOI: 10.1161/01.cir.92.12.3431]; [PMID: 8521564].
32. Novella S, Dantas AP, Segarra G, Medina P, Hermenegildo C. Vascular Aging in Women: is Estrogen the Fountain of Youth? *Front Physiol.* 2012 Jun **6(3)**: 165. eCollection 2012. [DOI: 10.3389/fphys.2012.00165]; [PMID: 22685434].
33. Antunes S, Marcelino O, Aguiar T. Fisiopatologia da Menopausa. *Rev Port Clin Geral* 2003; **19**: 353-7. [DOI: 10.32385/rpmgf.v19i4.9957]
34. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*. 2002 Jul 17; **288(3)**: 321-33. [DOI: 10.1001/jama.288.3.321]; [PMID: 12117397].
35. McNeil M. Menopausal Hormone Therapy: Understanding Long-term Risks and Benefits. *JAMA*. 2017 Sep 12; **318(10)**: 911-913. [DOI: 10.1001/jama.2017.11462. [DOI: 10.1001/jama.2017.11462]; [PMID: 28898364].