Changes in the Profile of Lipoprotein Subfractions Associated with Hormone Replacement Therapy

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Objective – *To report the effects of 2 regimens of hormone replacement therapy during the postmenopausal period on the profile of the major lipoprotein subfractions (HDL, LDL, and VLDL).*

Methods – *We carried out a cohort study in 38 postmenopausal patients who were starting their hormone replacement therapy due to gynecological indications, for a period of 12 weeks. Analysis of lipoprotein subclasses was performed through nuclear magnetic resonance spectroscopy.*

Results – *Hormone replacement therapy cause an increase in the proportion of larger subfractions of VLDL and HDL (p=0.008 and 0.03, respectively) and in the proportion of larger particles of VLDL due to a 36% increase in the levels of larger particles (p=0.004), concomitantly with a 15% reduction in the levels of smaller particles (p=0.04). In regard to HDL, the increase occurred only a 17% increase in the levels of larger particles (p=0.002). No significant change occurred in the distribution pattern of LDL subfractions.*

Conclusion – *The proportion of larger subfractions of VLDL and HDL increases after hormone replacement therapy. The increase in the proportion of larger particles of VLDL occurs due to an increase in the levels of the larger subclasses concomitantly with a reduction in the smaller particles. However, an increase in the proportion of larger particles of HDL occurs only due to an increase in the levels of the larger subfractions.*

Key words: lipoproteins, hormone replacement therapy, coronary heart disease

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Despite the evidence, at the present time challenged by the results of the first 2 randomized clinical trials of secondary prevention in ischemic heart disease [1,2], that estrogen may protect against coronary heart disease, the method by which this protective effect can be mediated has never been properly explained. Among the proposals, the estrogen impact on the lipid profile has always been highlighted, with a reduction in LDL-cholesterol and an increase in HDL-cholesterol. However, some studies have shown that only 25% to 50% of risk reduction could be attributed to this change [3-5]. In the last decade, addition of progestin has been routinely recommended in postmenopausal hormone replacement to reduce the risk of endometrial carcinoma induced by estrogen [6]. In observational studies [7-9], this practice seems not to have attenuated the cardioprotective effect of postmenopausal estrogen therapy.

In recent years, has gained relevance that each group of lipoproteins – high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) – is formed by very heterogeneous and different particles in their association with coronary heart disease [10-12]. Based on this, increasing importance has been attributed to the influence of the distribution pattern of subfractions of each of these major lipoproteins in coronary risk. Therefore, individuals with similar serum levels of LDL-cholesterol and HDL-cholesterol may also have very different levels of risk for coronary heart disease because of differences in the distribution of subclasses. Currently, evidence exists that for a certain level of LDL-cholesterol, patients with a profile of lipoprotein subclasses in which small and dense LDLs (pattern B) predominate have a substantially higher risk of developing coronary heart disease and acute myocardial infarction than those patients in whom large and floating LDLs (pattern A) predominate [10-14]. Likewise, subclasses of HDL measured by electrophoresis show a striking difference in their associations with coronary heart disease, as follows: the 3 largest subclasses (HDL_{2b}, HDL_{2a}, and HDL_{3a}) show the expected inverse correlation with progression and seve-

rity of the disease, but the 2 smallest subclasses (HDL_{3b} and HDL_{3c}) show a positive correlation [15,16].

Only some studies considered the possibility that the cardioprotective mechanism of postmenopausal hormone replacement therapy could also be mediated by changes imposed on the profile of these subfractions [17-19].

We report a cohort study aiming to assess the effects of an estrogen-progestin replacement regimen and another of estrogen replacement, classically used in the period following menopause, on the distribution of the subfractions of the major lipoproteins – HDL, LDL, and VLDL. These subfractions were determined by nuclear magnetic resonance to test the hypothesis that hormone replacement therapy may lead to a less atherogenic distribution profile of the lipoprotein subfractions.

Methods

We carried out a cohort study on postmenopausal patients from the Outpatient Care Clinics of Climacterium of the Gynecological Service of the Irmandade da Santa Casa de Misericórdia de Porto Alegre, who had gynecological indication for initiating hormone replacement therapy. Postmenopausal patients of any age referred to the ambulatory clinics due to symptoms of climacterium or for treatment or prevention of osteoporosis were accrued. After complete gynecological assessment, including mammography and endometrial evaluation, these patients were referred for hormone replacement therapy during the period from July '97 to December '97. Two regimens of hormone replacement were used out of those usually employed in the outpatient clinics. The regimens comprised equine conjugated estrogens at the dosage of 0.625mg associated with 2.5mg of medroxyprogesterone (Premelle®), administered daily in a continuous way for those patients with an intact uterus, and equine conjugated estrogens at the dosage of 0.625mg daily (Premarin 0.625®) used isolated for hysterectomized patients. Patients on isolated or combined estrogen therapy who effectively used the medication for more than 75% of the days were enrolled in the same group (group of hormone replacement therapy) because, after the statistical analysis, no difference was found between the 2 forms of therapy in regard to repercussions on the profile of distribution of the subclasses of lipoproteins. The patients who did not use the medication for at least 75% of the days were excluded from the treatment group. Those patients with less than 50% adherence or who did not use the medication for at least 4 weeks when returning at the 12th week became part of the control group (group C). The patients with an intermediary level of adherence to treatment were considered as lost and excluded from the study. Of the 44 postmenopausal patients who were effectively enrolled, 7 were included in group C because of the already cited reasons. Two patients did not return and 4 used the medication during 50% to 75% of the days; therefore, they were considered as lost. The group of hormone replacement therapy comprised 31 patients, 26 with combined estrogen-progestin therapy, and 5 with isolated estrogen therapy. Group C comprised 7 patients.

Right after arriving at the laboratory, the frasks with withdrawn blood were centrifuged for 15 minutes at 1,600 G for plasma separation. The plasma was then frozen in a special freezer at the temperature of -75°C. Measurements of the subfractions of lipoproteins were performed at Wake Forest University School of Medicine, Winston-Salem, NC, USA, in a Brucker WM-250 spectrometer. The basis of the analysis through nuclear magnetic resonance is that, in plasma, each particle of lipoprotein, within a diameter band, irradiates a distinct signal of nuclear magnetic resonance, which is proportional to the total concentration of the lipid mass. Quantification is obtained through a 3-step process comprising measurement of the plasma lipid spectrum through nuclear magnetic resonance, followed by splitting, and computerized calculation using special software. The entire process lasts approximately 1 minute, and an excellent degree of accuracy and precision was demonstrated in validation tests. A strict concordance exists between distribution of the LDL and HDL subclasses determined by electrophoresis and nuclear magnetic resonance [20-23]. In addition to chylomicrons, this method quantifies 15 different subclasses of lipoproteins as follows: 6 subclasses of VLDL, 1 of IDL, 3 of LDL, and 5 of HDL, exploring natural differences of proton nuclear magnetic resonance spectra exhibited by lipoprotein particles of different sizes, without requiring reagents, and using minimum manipulation.

The significance level was ≤ 0.05 in the 2-tailed test. Analysis of efficacy was performed for the patients who effectively used the medication. The basal differences between the groups, in regard to demographic and biochemical variables, were analyzed with the Student # test for independent samples when continuous variables were considered, and with the chi-square test for categorical variables. For each major lipoprotein (VLDL, LDL, and HDL), the subfractions were grouped into large and small for analysis of their distribution. In regard to VLDLs, the V_6, V_5, and V_4 subfractions (corresponding to the largest VLDLs) constituted the large VLDL subgroup, and V_3, V_2, and V_1 (corresponding to the smallest VLDLs) constituted the small VLDL subgroup. In regard to LDLs, the L_3 and L_2 subfractions (corresponding to large, floating, and intermediate LDLs) constituted the large LDL group, and L_1 (corresponding to small and dense LDLs) constituted the small LDL group. Of the 5 HDL subfractions, H_5 and H_4 (corresponding to HDL_{2b} and HDL_{2a}) constituted the large HDLs, and H_3, H_2, and H_1 (corresponding to HDL_{3a}, HDL_{3b}, and HDL_{3c}) constituted the small HDL group. Values within each group of subfractions were transformed in percentage of the total sum of lipoprotein subfractions for analysis of their distribution. For analyzing the results of the variations of the profile of subfractions from the week 0 to week 12, we used the Student *t* test for samples paired for each group.

Results

Basal data related to age, years after menopause, race,

weight, body mass index, blood pressure, reason for searching the outpatient care clinic of climacterium, alcohol ingestion, coronary risk factors, and use of medication are shown in table I and do not differ in the 2 groups.

During the 12-week follow-up, no significant change occurred in the mean weight of the patients in both groups. Two patients in the group using hormone replacement therapy and 1 patient in the control group started regular physical exercise practice (p=0.46).

Basal and final distribution profiles of lipoprotein subfractions in the different groups studied, according to grouping for analysis, are shown in table II. The corresponding variation of this distribution is depicted in figure 1.

A significant increase in the proportion of the larger VLDL subfractions (large VLDLs comprising V_6, V_5, and V_4) occurred in regard to the smaller subfractions (small VLDLs comprising V_3, V_2, and V_1) in the hormone replacement therapy group (p<0.008). In this group, the proportion of larger particles of this lipoprotein increased from 47% to 57% in the 12 weeks of treatment, and the proportion of smaller particles decreased correspondingly from 53% to 43%. Group C showed no variation in the proportion of particles.

In regard to LDL subfractions, no significant variation in the proportion of large (corresponding to L_3 and L_2) and small (corresponding to L_1) LDLs occurred in both groups during the 12 weeks of treatment.

Table I – Basal characteristics of patients in the different groups

Basal characteristics	Group C (n=7)	Group HRT (n=31)	p
Age in years, mean ± SD	54±4.1	53±6.2	0.44
Years after menopause, mean ± SD	4.2±1.6	4.3±3.7	0.97
Race, number (%)			1.00
White	7 (100)	30 (97)	
Black	0	1 (3)	
Weight in kg, mean ± SD	69.3 ±11.7	65.6±10.8	0.43
Body mass index in g/m², mean ± SD	26.9±4.7	26.2±4.4	0.73
Blood pressure in mm Hg, mean ± SD			
Systolic	122±9.5	121±14.8	0.74
Diastolic	77±7.5	81±10.3	0.40
Glucose in mg/dL, mean ± SD	87±11	90±18	0.71
Reason for outpatient care clinic referral, number (%)			1.00
Climacteric syndrome	6 (86)	28 (90)	
Preventive treatment for osteoporosis	1 (14)	3 (10)	
Coronary risk factors, number (%)			
Hypertension	4 (57)	11 (35)	0.40
Diabetes	0	1 (3)	1.00
Current smoker	1 (14)	5 (16)	1.00
Obesity - BMI >30kg/m² *	2 (29)	9 (29)	1.00
Sedentary lifestyle	4 (57)	20 (65)	1.00
Family history of ischemic heart disease	1 (14)	4 (13)	1.00
Total cholesterol >240mg/dL	1 (14)	7 (23)	1.00
LDL-cholesterol >160mg/dL	1 (14)	5 (16)	1.00
HDL-cholesterol <35mg/dL	2 (28)	3 (10)	0.22
Triglycerides >200mg/dL	1 (14)	4 (13)	1.00
Alcohol consumption, number (%)			
Occasional or frequent	0	0	
Medication use, number (%)			
Diuretics	3 (43)	4 (13)	0.09
Beta-blockers	0	3 (10)	1.00
Corticosteroids	1 (14)	0	0.18

SD- standard deviation; * - criteria for obesity according to Wood et al [37].

In regard to HDLs, a significant increase in the proportion of large HDLs (corresponding to H_5 and H_4, or HDL_{2b} and HDL_{2a}) occurred as compared with small HDLs (corresponding to H_3, H_2, and H_1, or HDL_{3a}, HDL_{3b}, and HDL_{3c}) in the group of hormone replacement therapy (p<0.025). This resulted from an increase in the larger subfractions from 46% to 50% and correspondingly reduction in the smaller subfractions from 54% to 50%. Group C showed no variation in the proportion of particles.

Analyzing the changes occurring in plasma levels of VLDL subclasses to verify how alterations in proportion occurred after hormone replacement therapy, we observed a 36% increase in the larger VLDL subfractions (p<0.004) concomitantly with a 15% reduction in the smaller VLDL subfractions (p<0.036). In regard to HDL, a 17% increase in the larger subfractions (p<0.002) occurred with no change in the smaller subfractions (Table III and fig. 2).

Discussion

Even though the clinical relevance of the heterogeneity of the lipoprotein subfractions has been difficult to assess because of the extremely laborious nature of the methods for determining the subfractions of lipoproteins, differences in association with coronary heart disease suggest that data on lipoprotein subfractions may improve the evaluation of coronary risk. This situation is similar to

Table II – Distribution of lipoprotein subfractions (% of total ± standard deviation) in the two groups

Groups of subfractions	Group C (n=7)			Group HRT (n=31)		
	Week 0	Week 12	p	Week 0	Week 12	p
Large VLDLs	51±15	50±6	0.83	47±18	57±15	0.008*
Small VLDLs	49±15	50±6	0.83	53±18	43±15	0.008*
Large LDLs	67±21	65±22	0.69	80±17	79±17	0.814*
Small LDLs	33±21	35±22	0.69	20±17	21±17	0.814*
Large HDLs	37±23	37±22	0.75	46±19	50±31	0.03*5
Small HDLs	63±23	63±22	0.75	54±22	50±21	0.03*5

*p≤0.05; Week 0 - basal measurement; Week 12 - measurement after 12 weeks ; p- Student *t* test for paired variables.

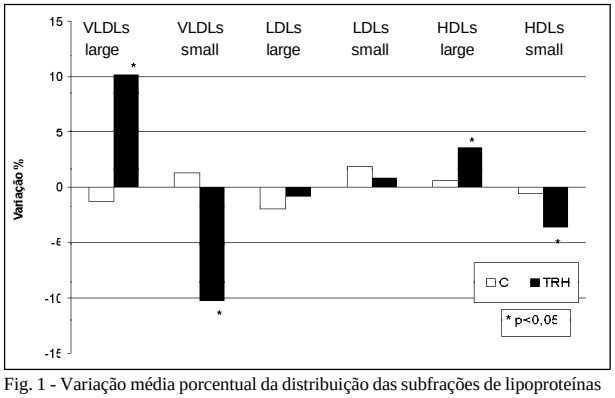

Fig. 1 - Variação média porcentual da distribuição das subfrações de lipoproteínas entre a 12ª semana e a basal nos dois grupos.

that observed in the '50s and '60s, when data on cholesterol levels of lipoproteins could better predict the coronary risk than the total cholesterol serum levels isolated.

Most methods usually used for separating and quantifying subfractions of lipoproteins include techniques such as ultracentrifugation, electrophoresis, or chromatography, which are very laborious and slow and, therefore, become unfeasible for clinical research on a large scale or for assessment of coronary risk in the general population. A recently validated method that employs nuclear magnetic resonance spectroscopy is a more precise, rapid, and less expensive alternative as compared with the already existing methods for assessing and handling coronary risk based on measurements of subfractions [21]. This technique explores the natural spectral difference that exists between the subfractions of each lipoprotein, depending only on the size of the particles. The technique avoids the steps of physical separation, the use of reagents, and employs a totally automated process, thus providing a high rate of efficiency and precision in the evaluation. This allows the simultaneous calculation of the concentrations of each of the 16 lipoprotein subclasses, 1 being chylomicron, 6 of VLDL, 1 of IDL, 3 of LDL, and 5 of HDL [24].

Despite the great methodological difference between nuclear magnetic resonance and the other methods used to quantify lipoproteins, some studies have shown a proximity between the sizes of LDL and HDL particles measured by nuclear magnetic resonance and by electrophoresis with gel gradient [20,25]. As no appropriate and high-resolution technique was available for measuring VLDL subfractions, only a few studies could separate VLDL subfractions that effectively represented distinct subclasses [21,26,27]. Therefore, comparison of nuclear magnetic resonance with other methods is impaired in this regard.

In our study, we observed an increase in the proportion of larger VLDLs as compared with that of smaller VLDLs due to an increase in the level of larger particles and a concomitant reduction in smaller particles. Another study [28] using the same method has already shown a reduction in the smaller VLDL subfractions after hormone replacement therapy. In the literature, only a few studies report the effect of hormone replacement therapy on the profile of VLDL subfractions, because of the difficulties in separating these subfractions by the conventional techniques used prior to nuclear magnetic resonance. Estrogens seem to increase hepatic synthesis of VLDL-triglycerides forming particularly large VLDLs, rich in triglycerides, acting directly upon the hepatocyte [29]. Most VLDLs are withdrawn from circulation directly by the liver without being metabolized to small VLDL or LDL. In this way, the elevation in triglycerides induced by estrogens is supposed to be less harmful than that present in other clinical situations [30]. The increase in the proportion of larger VLDL subfractions was similar in patients using isolated or combined estrogen therapy, which could be expected because progestins do not block the metabolic effects of estrogen in the hepatocyte. Whether large VLDLs actually produce small and dense LDLs [22] in case of no inhibition of the hepatic lipase by estrogen, with the increase in VLDLs caused by hormone replacement therapy, a greater amount of small and dense, more atherogenic, LDLs could be produced, which in reality does not occur [30].

No change was observed in the distribution of LDL subfractions in the present study. Even though an induction to a predominance of larger and less atherogenic (pattern A) particles could be expected because of the supposed cardioprotective effect, a few studies have found an opposite trend [17,31]. This action of reducing the larger and less dense LDLs in detriment to the smaller and denser particles could be due to the fact that the former are more easily captured by hepatic receptors of LDL, whose action is stimulated by estrogens [32].

The increase in the proportion of larger subfractions of HDL found agrees with data in the literature [28,33]. An increase in the proportion of larger particles (H_5 and H_4, corresponding to HDL_{2b} and HDL_{2a}) as compared with that of smaller particles (H_3, H_2, and H_1, corresponding to HDL_{3a}, HDL_{3b}, and HDL_{3c}) occurred due to an increase in the larger subfractions with no changes in the levels of the smaller fractions, which is in accordance with data in the literature [29,30,34,35]. The catabolism of HDL_2 is mediated by hepatic lipase, a lipolytic enzyme present in the endothelial cells lining the hepatic sinusoids and highly specific for HDL_{2b}, the larger subfraction of HDL [36]. The effects of sexual steroids on the levels of HDL, particularly HDL_2, are believed to be mediated by changes in the activity of hepatic lipase [33], because the increase in HDL_2 with the use of estrogen is associated with a

Table III – Plasma levels of the groups of VLDL and HDL ubfractions (mg/dL ± standard deviation) and their variation (%) in the group HRT

Groups de subfractions	Correspondence	Plasma levels			Variation (%)
		Week 0	Week 12	p	
Large VLDLs	V6, V5 e V4	49±34	67±41	0.004*	36
Small VLDLs	V3, V2 e V1	51±23	43±17	0.04*0	-15
Large HDLs	H5 and H4 (HDL_{2b} and HDL_{2a})	28±18	33±19	0.002*	17
Small HDLs	H3, H2 and H1 (HDL_{3a}, HDL_{3b} and HDL_{3c})	28±7	28±99	0.744*	0

*p≤0.05; Week 0 – basal measurement; Week 12 – measurement after 12 weeks; p – Student *t* test for paired variables.

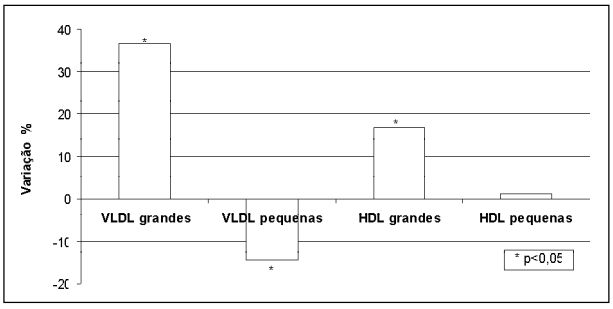

Fig. 2 - Variação média porcentual dos níveis médios das subfrações de lipoproteínas entre a 12ª semana e a basal no grupo TRH.

reduction in the activity of this enzyme. On the other hand, addition of a progestin has an inverse effect, which is more marked as the androgenic effect increases (the effect of medroxyprogesterone is low), and it may even reverse the increase in HDL_2 and the reduction in the activity of the hepatic lipase [34].

Clinical significance of the changes imposed by hormone replacement therapy in the profile of lipoprotein subfractions should be better evaluated in further larger clinical trials.

In the present study, in addition to the possibility of potential contamination of the cohort model by confounding factors, the control group was composed of patients not adhering to the treatment. As all patients starting hormone replacement therapy in the outpatient care clinics had precise medical indications for it (climacteric symptoms or osteoporosis), from the ethical point of view they were not amenable to being directly allocated to the control group. Even though all basal variables observed were not significantly different in both groups, a tendency to a higher prevalence of hypertension occurred in the control group, as did HDL below 35mg/dL and the use of diuretics. This and the known fact that the adherence factor in many studies may be associated with healthier habits may have interfered with the final result of the study.

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