Menopause is defined by the World Health Organization as the permanent cessation of menses as a result of the loss of ovarian follicular function or of surgical removal of ovaries. The mean age for occurrence of natural menopause is around 50 years.

The hormonal changes that accompany menopause, particularly the decreased levels of estrogen hormones, have a great physiological impact. Estrogen deficiency has been associated with vasomotor symptoms, urogenital atrophy, and cognitive impairment, as well as increased risk of chronic degenerative diseases such as osteoporosis and Alzheimer’s disease.

MENOPAUSE AND CARDIOVASCULAR DISEASE

Cardiovascular disease remains the leading cause of death in the 21st century. Despite the advances in this area, it is still the main cause of death among women in developed countries. In the United States, over half a million women die of cardiovascular disease every year, exceeding the number of deaths among men and the total of the subsequent leading causes of death among women. Coronary arterial disease is responsible for most of the deaths by cardiovascular disease among women. Moreover, women have a less favorable prognosis than men with respect to a coronary event: 40% of the total coronary events in women are deadly, and 67% of sud-
den deaths occur in women without a history of coronary disease.\(^5\)

The prevalence of cardiovascular disease in premenopausal women is smaller than in postmenopausal women, when there is an exponential increase, causing the risk for women to equal that for men by the age of 70 years. This lag concerning the age period at which the frequency of cardiovascular events increases among women as compared to men has been ascribed to the actions of endogenous estrogen on the cardiovascular system, through mechanisms as yet not completely clarified.

The beneficial influence of hormone therapy (HT) during menopause on the cardiovascular comorbidities is not well-established. Observational studies have found a reduction of up to 50% in the risk of cardiovascular disease in postmenopausal women using HT.\(^7-9\) However, the results of 2 large randomized prospective studies refute the claims of cardioprotective effects of hormone therapy based on observational studies. The Heart and Estrogen/Progestin Replacement Study (HERS) was a randomized clinical trial designed to test the effectiveness of HT in the secondary prevention of coronary heart disease. The results indicate an increase in the coronary events within the first year of follow-up after acute myocardial infarction among patients treated with a combination of conjugated estrogens/menadione progesterone acetate at fixed doses and mean age of 66.7 years.\(^10\) Recently, the Women’s Health Initiative (WHI), a prospective study of primary prevention of cardiovascular disease, was interrupted early because a higher incidence of cardiovascular events was demonstrated among the women randomized for use of HT than in the group receiving placebo. In this study, although HT was associated with reduced risk of colorectal cancer and bone fracture, it was also associated with increased risk of breast cancer.\(^11\) Finally, in 2004 the results of the therapeutic arm of the WHI evaluating the isolated use of conjugated estrogens versus placebo among 10,739 hysterectomized postmenopausal women were published. There was an increased risk of cerebral vascular accident (HR = 1.39 CI: 1.10-1.77), but a decreased risk of hip fracture and null effect on the incidence of cardiovascular disease, as well as potential reduction in the incidence of breast cancer. Overall, the risk-benefit index was neutral.\(^12\)

The well-known risks for cardiovascular disease, such as systemic hypertension, smoking, obesity, sedentary lifestyle, dyslipidemia, stress, family history of cardiovascular disease, diabetes mellitus, and insulin resistance, are numerous.\(^5\) More recently, endothelial vascular dysfunction has become suspected as being associated with cardiovascular disease, as detailed below.

**THE ENDOTHELIUM**

The endothelium is a layer of cells lining all the vessels of the organism and placing itself between the blood and the vascular smooth muscle layer. The endothelial cells form the more exposed surface of the inner vascular layer, presenting selective permeability, having a nonthrombogenic character, exhibiting exuberant metabolic activity, and having the ability to produce several vasoactive substances.\(^13\)

The main actions exerted by the endothelium can be described as the following:

- **Selective permeability:** The endothelium forms a highly selectively permeable barrier, regulating the flow of nutrient substances, many biologically active molecules, and blood cells.\(^14\)
- **Maintenance of a balance between thrombosis and fibrinolysis:** The endothelium normally provides a nonthrombogenic surface due to its ability to form prostaglandin derivatives, especially prostacyclin, which is a potent vasodilator and an effective inhibitor of platelet aggregation.\(^15\) The endothelial cells also secrete agents (including plasminogen) that are effective in the lysis of coagula, but they also synthesize procoagulant agents such as Von Willebrand factor (vWF). Additionally, they produce thrombogenic substances such as coagulation factors, adhesion molecules, plasminogen activator inhibitor-1 (PAI-1), and tromboxan A\(_2\).\(^16\)
- **Inhibition of cell proliferation of vascular smooth muscles:** Preventing the migration of smooth muscle cells through direct mechanisms, such as synthesis of nitric oxide, which inhibits cell proliferation, and/or indirect ones (platelet action).\(^17,18\)
- **Active participation in immune response through the release of factors leading to active contraction, control of plasma extravasation, as well as increase in the expression of integrins, molecules of adhesion and secretion of cytokines, allowing the migration of monocytes, lymphocytes, and neutrophils and greater local phagocytic activity.**
- **Modulation of vascular tonus through the production of numerous vasoactive substances (Table 1).** The regulation of the vasomotor tonus is determined by a balance

<table>
<thead>
<tr>
<th>Table 1 - Endothelial-derived vasoactive factors(^21)</th>
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<tbody>
<tr>
<td><strong>Vasodilator Factors</strong></td>
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<tr>
<td>• Nitric oxide (NO)</td>
</tr>
<tr>
<td>• Endothelium-derived hyperpolarizing factor (EDHF)</td>
</tr>
<tr>
<td>• Prostacyclin (PGI(_2))</td>
</tr>
<tr>
<td>• Acetylcholine</td>
</tr>
<tr>
<td>• Bradykinin</td>
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</table>
of dilation and constriction. Nitric oxide (NO) is the main mediator of vasomotor tonus in physiological situations. Some stimuli, such as the dragging force produced by the pulsatile blood flow, the pressure of blood against the vascular wall, and the shear stress, contribute to the basal generation of NO.19–21

ENDOTHELIAL DYSFUNCTION

The term endothelial dysfunction is more frequently used to mean reduction in endothelium-dependent vasodilatation, associated with diminished bioactivity of local vasodilating factors, especially nitric oxide, but it probably includes other normal functions of the endothelium such as interaction with leukocytes, platelets, and regulatory substances. Currently, it is a consensus that endothelial dysfunction is the initial event in development of atherosclerosis.22 Several conditions such as aging, menopause, dyslipidemia, high blood pressure, and diabetes mellitus are associated with endothelial dysfunction.23–26

TECHNIQUES FOR EVALUATION OF ENDOTHELIAL FUNCTION

There are many techniques for investigating the endothelium, from those that focus on cellular and molecular aspects, through methods involving tissue culture and molecular biology tools, to clinical trials applied to human beings, using invasive and noninvasive procedures to evaluate endothelium-dependent vasodilatation, or the determination of plasmatic substances that indicate endothelial activation and damage (Tables 2 and 3).

Table 2 - Markers of endothelial function24,91

<table>
<thead>
<tr>
<th>Endothelial Function</th>
<th>Marker</th>
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<tr>
<td>Coagulation</td>
<td>• Fibrinogen</td>
</tr>
<tr>
<td></td>
<td>• vWF</td>
</tr>
<tr>
<td></td>
<td>• TXA₂</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>• t-PA</td>
</tr>
<tr>
<td></td>
<td>• PAI-1</td>
</tr>
<tr>
<td>Inflammation</td>
<td>• CRP</td>
</tr>
<tr>
<td></td>
<td>• E-selectin</td>
</tr>
<tr>
<td></td>
<td>• fibrinogen</td>
</tr>
<tr>
<td></td>
<td>• ICAMs and VICAMs</td>
</tr>
<tr>
<td></td>
<td>• IL-6</td>
</tr>
<tr>
<td>Vascular Tonus</td>
<td>• Plasma Markers</td>
</tr>
<tr>
<td></td>
<td>• ET-1</td>
</tr>
<tr>
<td></td>
<td>• NO</td>
</tr>
</tbody>
</table>

vWF: von Willebrand factor; TXA₂: tromboxan; A₂: tPA: tissue plasminogen activator; PAI-1: plasminogen activation inhibitor-1; CRP: C-reactive protein; ICAMs: intercellular adhesion molecules; VICAMs: vascular cell adhesion molecules; IL-6: interleukin 6; ET-1: endothelin-1; NO: nitric oxide

Intracoronary studies evaluating endothelium-dependent vasodilatation after infusion of acetylcholine are considered gold standard techniques in assessing endothelial function, but their invasive character precludes their large-scale use.22 Thus, taking into account that endothelial dysfunction is present at early stages of atherosclerosis and that it involves several arteries, noninvasive tests in the peripheral circulation have been increasingly used.23 Three techniques are particularly useful for estimating the endothelial dysfunction in peripheral circulation: ultrasonography of brachial artery, impedance pletismography, and dorsal hand vein compliance. Each of these techniques evaluates the endothelial function indirectly by measuring changes to the size of brachial artery, forearm veins, and back of the hand, respectively, after physical (active hyperemia) or pharmacological stimulation (bradykinin or acetylcholine).

ULTRASONOGRAPHY OF BRACHIAL ARTERY

Flow-mediated dilatation (FMD) can be measured through high resolution ultrasonography. This technique contrasts the changes in the diameter of the brachial artery in response to the increased flow induced by reactive hyperemia and sublingual nitrate. Vasodilating responses are expressed as a percentage of change to the size of the vessel (basal and following stimulation). In 1994 Celermajer et al28 studied more than 500 healthy individuals using FMD and demonstrated negative association between FMD and a variety of cardiovascular risk factors, such as smoking, hypertension, age, and hypercholesterolemia. Other studies have demonstrated a positive correlation between FMD and central hemodynamic parameters as well as the extent and prognosis of the coronary disease.29,30

Although FMD has been used in several studies, it has limitations related to environmental and individual factors, such as prandial state and arterial size.31 Also, problems related to reproducibility and intraobserver variation are mat-
ters of debate in the literature. Rossi et al. demonstrated variation coefficients of 3.3% and 12.4%, respectively, for basal size and after stimulation of the brachial artery.

There is as yet no consensus in literature about the parameters of normality for FMD. Ryliskyte et al. analyzed 115 individuals with low cardiovascular risk and found that the only independent predictors of FMD were age and vessel size. Therefore, these authors suggest that in analyzing results, normal ranges according to age and the caliber of the tested vessel must be taken into account.

**IMPELLATION PLETHYSMOGRAPHY AND DORSAL HAND VEIN COMPLIANCE**

Compliance and vein occlusion plethysmography uses a sensor of stretching to quantify variations in volume in the forearm or lower limb, taking into account the fact that variations in volume in these parts are dependent on the local blood flow variation. Forearm blood flow (FBF) measurements can be done following ischemic (reactive hyperemia) or pharmacologic stimulation, the latter requiring catheterization and drug infusion into brachial artery, which is an invasive procedure. Flow values can be calculated by manual or semi-manual analysis using specific software.

Forearm blood flow has been validated in several studies as a tool for evaluating the endothelial function. Diminished FBF is associated with increased thickness of the tunica media of the artery as well as presence of CAD and the risk factors associated with it. Forearm blood flow can also be used to monitor the changes to the endothelial function after interventions for cardiovascular risk factors.

Environmental and physiological variables, such as room temperature, age, race, hormonal state, phase of menstrual cycle, anxiety, and prandial state, can influence the magnitude of FBF responses. Variation in FBF values in a single individual can be about 12.9%, reflecting the influence of these variables. To minimize variability, the tests must be done in a silent room at a stable temperature, and the patient must be fasting.

The dorsal hand vein compliance technique uses measurements of venous diameter variations obtained through a linear transducer. Venodilation curves are obtained after infusion of acetylcholine or bradykinin (endothelium-dependent vasodilatation) and nitrate (endothelium-independent vasodilatation) as compared to basal curves. Regarding safety, risks, and accuracy, the dorsal hand vein compliance technique is comparable to impedance plethysmography, although it has the advantage of using vasoactive substances at lower doses, thus avoiding potential systemic confounders. Again, the results are influenced by environmental and physiological factors. Greater reproducibility and smaller intrapatient variability of the test are related to preconstriction of the tested vessel, ideally about 80% of the vein constriction in the basal state (ED<sub>80</sub>).

**ESTROGEN, MENOPAUSE, AND ENDOTHELIAL FUNCTION**

There is evidence of an association between endothelial dysfunction and reduced endogenous production of estrogens after natural or surgical menopause or premature ovarian failure (POF) in women with or without coronary arterial disease.

The actions of endogenous estrogens on the cardiovascular system can be mediated directly on the vessels or indirectly through the modulation of cardiovascular risk factors, as well as on the lipid profile (reduction of total cholesterol and LDL, increase in HDL), as already described more than 20 years ago. More recently, studies have also demonstrated an antioxidant effect by estrogen, reducing LDL oxidation in vivo and in vitro.

The direct effects of estrogen on the vascular system and which modulate the vascular tonus comprise 1) acute vasodilatation, increasing the synthesis and bioactivity of nitric oxide; 2) long-term modulation of vascular tonus, regulating the production of prostaglandins and expression of eNOS and the endothelin gene; 3) inhibition of endothelin-induced vasoconstriction; and 4) inhibition of sympathetic activity.

In addition to these actions on the vascular tonus, estrogen exerts an antiproliferative action on the vascular smooth layer. Also, it appears to have a major role in vascular remodeling, inhibiting the proliferation of the inner layer after injury and increasing the expression of contractile proteins in the myocardium.

In other tissues, such as the liver, estrogen can mediate both beneficial (expression of genes of apoproteins that improve the lipid profile) and adverse effects (increase in the expression of procoagulant factors and decrease of fibrinolytic factors).

From the clinical point of view, our group has recently reported, in postmenopausal women who were not on hormone therapy, a positive association between nitric oxide and the free estradiol level, confirming the influence of this steroid on the endothelial function.

On the other hand, androgen and sex hormone-binding globulin (SHBG) levels have been associated with risk of cardiovascular disease in pre- and postmenopausal women. An increase in circulating androgens appears to be associated with insulin resistance and a predictor of
diabetes mellitus. In a previous study, we demonstrated a positive association, independent of obesity, between testosterone levels and hyperinsulinemia in postmenopausal women with no clinical evidence of cardiovascular disease.

HORMONE THERAPY AND ENDOTHELIAL FUNCTION

Disturbances in endothelial function have an important role in the physiopathology of atherosclerosis, and several lines of evidence suggest that interventions in endothelial function could modify the progress rates of atherosclerotic disease and the risk of cardiovascular events. A number of studies have tested the impact of the use of HT on endothelial function using inflammatory and fibrinolytic/thrombogenic markers as well as functional methods.

Results from the main randomized studies, HERS 2 and WHI, indicate increased risk of venous thromboembolism with the use of HT. It is important to note that these studies have been criticized in at least 2 respects: patient selection, which included women who were on average 10 years older than the age at which HT is usually recommended (WHI), and the use in both studies of a regimen of HT with fixed drugs, oral administration route and doses.

Blood pressure control was achieved by administration of amlodipine at individually adjusted doses. Hormone therapy was introduced in a cyclic regimen (21 of 28 days) with percutaneous estradiol (1.5 mg/day) and vaginal micronized progesterone (100 mg/day).

Table 4 - Echocardiographic variables in 20 hypertensive postmenopausal women

<table>
<thead>
<tr>
<th></th>
<th>Before HT</th>
<th>6-month</th>
<th>12-month</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle posterior wall (mm)</td>
<td>9.1 ±0.4</td>
<td>8.3 ± 0.3</td>
<td>8.0 ± 0.2</td>
<td>0.042</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>71.3 ± 1.3</td>
<td>67.9 ± 1.2</td>
<td>69.4 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Interventricular septum (mm)</td>
<td>9.2 ± 0.4</td>
<td>8.9 ± 0.4</td>
<td>8.2 ± 0.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Blood pressure control was achieved by administration of amlodipine at individually adjusted doses. Hormone therapy was introduced in a cyclic regimen (21 of 28 days) with percutaneous estradiol (1.5 mg/day) and vaginal micronized progesterone (100 mg/day).
change in left ventricle mass, but a significant reduction in the thickness of the posterior wall of the left ventricle was observed71 (Table 4).

A number of studies have highlighted the effect of HT on endothelial inflammatory markers, such as decreased expression of adhesion molecules like ICAM-1, VICAM-1, and E-selectin.72-74

Studies using the oral route have shown an increase in the circulating levels of C-reactive protein (CRP), a well-known inflammatory marker.73-75 However, the clinical significance of these results has not been totally clarified. Other trials with non-oral routes reported, on the contrary, stability in CRP levels during HT.73,76

Acute non-oral use of estradiol may increase endothelium-dependent vasodilatation.77,78 Saitta et al79 compared the effects of using 17-β estradiol plus norethisterone, raloxifene, or placebo for 6 months in postmenopausal patients. Treated women in both groups showed a significant increase in endothelium-dependent vasodilation measured by ultrasonography of brachial artery and in the plasmatic nitrite/nitrate levels, which was not observed in placebo users. A significant increase in the NO metabolites levels has also been shown by Balci et al80 with the use of transdermal 17β estradiol, 100 mg/week for 3 months.

Although there is some evidence suggesting a favorable action of HT on vasomotor endothelial function, the long-term benefits on the natural history of atherosclerotic disease are as yet unknown. Ceballos et al81 showed a significant increase in endothelium-dependent vasodilation in menopausal patients treated with a combination of transdermal 17β estradiol and micronized progesterone, but this benefit was lost after 6 months of drug discontinuation.

Few studies in the literature have addressed the question of the effect of progestogens on cardiovascular risk, and despite the well-recognized benefits of estrogens, controversy surrounds the risks and negative aspects of combined estrogen and progestogen use in HT.

The vascular actions of progestins and progesterone are mediated by progestosterone receptors, expressed in endothelial cells and the vascular smooth muscle as well as through down-regulation of the estradiol receptor.82 Concerning progesterone, evidence suggests that the natural molecule facilitates the inhibitory effects of estrogen on vascular smooth muscle proliferation83 and may induce endothelium-dependent vascular relaxation.84,77 In addition, natural progesterone used in HT appears to preserve the beneficial actions of estrogen.8,70,71

Progestins present different pharmacological profiles according to their molecular structure, dosage, and to the presence of comorbidities. As with estrogens, the various progestins used in HT may differ significantly as to how closely they mimic their natural counterparts. For instance, progestin molecules with androgenic properties may antagonize estrogen-dependent beneficial effects on lipids,83 and a new molecule with antimineralocorticoid activity may reduce blood pressure in postmenopausal women with hypertension.84

Progestins added to estrogen therapy seem to increase inflammatory markers.72 In addition, medroxyprogesterone acetate associated with conjugated equine estrogens produces no effect85 or inhibits endothelium-dependent vasodilation stimulated by estrogens.86 These observations have been taken to explain, at least in part, the adverse results observed in the large prospective, randomized, placebo-controlled trials of combined HT—the WHI and HERS studies. However, the extent to which findings of these studies of medroxyprogesterone acetate and conjugated equine estrogens apply to other HT formulations is unclear at present.

ENDOTHELIAL DYSFUNCTION AND CARDIOVASCULAR DISEASE PREDICTORS IN MENOPAUSE

Data from prospective trials have been confirming the hypothesis that endothelial dysfunction precedes the emergence of chronic disorders. The MONICA/KORA study (Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Research in the Region of Augsburg), which involved more than 2000 patients, found an association between increased levels of E-selectin and I-CAM and increased risk of diabetes mellitus type 2 (DM-2).87 In a follow-up study of 840 postmenopausal women, Rossi et al82 showed an adjusted relative risk for DM-2 of 5.87 (95% confidence interval, 4.34-8.10) in patients in the lowest tertile of FMD (≤ 4.3).

In addition to effects on endothelial markers, changes in other factors of cardiovascular risk have been associated with the menopausal transition, such as the lipid profile,88 weight, and body fat distribution.89,90 The association between prevalence of cardiovascular risk during perimenopause and postmenopause and tunica media thickness (TMT) of the carotid was monitored in 314 women by Matthews et al88 In premenopausal patients, arterial and pulse pressure, LDL, HDL, triglycerides, and BMI values were predictors of TMT and the presence of atherosclerotic plaque after 5 years of menses cessation. In postmenopausal patients, only the increase in pulse pressure was a predictor of carotid intimal-medial thickness.91 These findings support the notion that women at higher risk of cardiovascular disease can be identified during premenopause, and it is in this period that strategies must be implemented to prevent the development of atherosclerosis in the postmenopausal years.
Endothelial dysfunction can be curtailed by nonpharmacological measures, such as physical activity\(^2\) and weight loss,\(^9\) or pharmacological ones, such as statins and angiotensin converting enzyme (ACE) inhibitor.\(^9\) The indication for HT use must be individualized, taking into account the presence of climacteric symptoms and their impact on inflammatory and coagulation markers, since long-term benefits regarding atherosclerotic vascular disease have not yet been determined.

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Menopause, estrogens, and endothelial dysfunction

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