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Review

Coronary heart disease in postmenopausal women; the role of endogenous estrogens and their receptors

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ABSTRACT

Coronary heart disease is the main cause of death in women. Women during reproductive years are at lower risk for coronary heart disease than men but this difference tends to disappear after the menopause. In this article, we briefly review the clinical and experimental data which highlight the protective role of endogenous estrogens in the pathogenesis of coronary heart disease focusing on women after the menopause. Furthermore, recent data about the molecular and biochemical mechanisms of estrogen action on the vasculature are presented.

Key words: Atherosclerosis, Cardiovascular disease, Coronary, Estrogens, Estrogen receptor, Gene polymorphism, Menopause

INTRODUCTION

Coronary heart disease (CHD) is a multifactorial disease, its expression probably being influenced by the interaction of genetic and environmental risk factors.[1] Especially in women, CHD is a leading cause of death, in fact more frequent than breast cancer, at all ages.[2] Several epidemiological studies indicate a higher incidence of the disease in postmenopausal women when compared to women of reproductive age.[3-6] In addition, postmenopausal women with CHD have more advanced coronary artery stenosis compared to premenopausal women.[7]

On the other hand, at a younger age and independently of differences in lifestyle, women are at lower risk for CHD than men.[8] However this disparity tends to disappear after menopause.[9] On this evidence, the hypothesis as to a protective effect of estrogens against atherosclerosis has been based. In accordance with this hypothesis, estrogen deprivation may play an important role in the appearance of early CHD in women.[10,11] There is also evidence that higher levels of androgens during the reproductive years may contribute to a higher risk for CHD,[12] the exact reverse being observed in men.[13]

This brief review will discuss some of the data on the importance of endogenous estrogens and their receptors for the cardiovascular system in women mostly after menopause.

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EPIDEMIOLOGICAL STUDIES

In a recent review of studies which examined the influence of diverse reproductive parameters on the

risk for CHD in postmenopausal women, de Kleijn et al reported that the factors which correlated with higher risk for CHD were the irregularity of the menstrual cycles, the number of abortions and, most importantly, the age at menopause.[14] The importance of age at menopause, both natural and surgical, for the development of CHD has been thoroughly studied during the last few decades.[4-6,15-17] Most of these studies showed that women with earlier menopause have a higher risk for CHD independently of other risk factors such as blood pressure, dyslipidaemia, obesity and smoking, these risk factors themselves being associated with menopause.[3,18,19] In a recent study by Saltiki et al it was found[20] that age at menopause was significantly lower in women who had 2 myocardial infarctions (MI) compared to those with 0 or 1 MI.

Similarly, CHD mortality has been investigated in relation to age at menopause, but this association was not always significant.[5,6,21-23] Van der Schouw et al,[24] in a large prospective cohort study of 12,115 postmenopausal women, showed that each year's delay in natural menopause results in a 2% decrease in risk of death from CHD events, and similar results were shown in another very recent study.[25] Jacobsen et al reported that earlier menopause is related to an increase in total mortality, while when age at menopause is over 53 years, cardiovascular disease mortality decreases as much as 60%.[21] However, an extremely delayed menopause may be associated with an increased CHD risk.[21] Another large epidemiological study showed an increased risk for CHD in women with earlier menopause, especially in smokers.[26] In a study in which women with higher estrogen levels (either premenopausal or postmenopausal receiving HRT) were compared to women with lower estrogen levels (postmenopausal), it was found that estrogen status may constitute an independent prognostic factor of morbidity and mortality in women presenting for stress testing for suspected CHD.[27]

In the literature there are few studies which have examined the role of age at menarche and the calculated total lifetime exposure to endogenous estrogen as a predictor for the risk of CHD. Saltiki et al[20] showed that postmenopausal women with a shorter lifetime exposure to endogenous estrogens are more likely to present MI (Figure 1). These findings are similar to those reported by de Kleijn et al, namely an inverse correlation of cardiovascular mortality with the length of exposure to estrogens and a 20% decrease in mortality from CHD in postmenopausal women with longer exposure to endogenous estrogens.[23] Jansen et al also showed a decrease in mortality when lifetime exposure to endogenous estrogens was more than 40 years compared to less than 33 years.[22]

The time that has elapsed since menopause has been more rarely examined in relation to the various clinical manifestations of cardiovascular disease.[16,20] This parameter combines the influence of both the age at menopause and the current age and is an index of the length of estrogen deprivation. In the study of Saltiki et al,[20] significant associations between the time that elapsed since menopause and several manifestations of coronary artery disease such as history of angina and myocardial infarction were found (Figure 2). Specifically, the correlation with myocardial infarction was independent of current chronological age, which is by itself a strong predisposing factor.

Finally, current estrogen levels have been examined in relation to the presence and severity of CHD in postmenopausal women,[28-30] but no associations have been found in the majority of the studies. Only one longitudinal study showed that low levels of

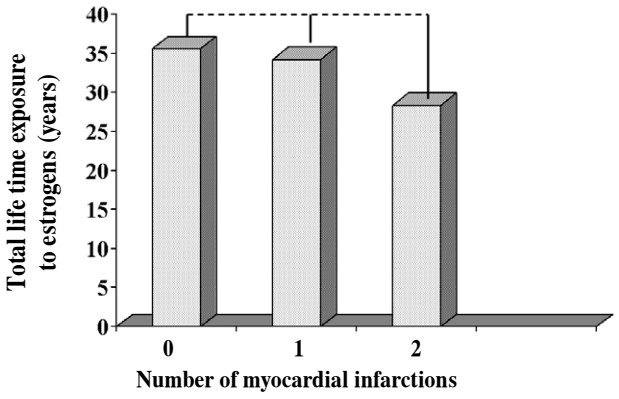

Figure 1. Total lifetime exposure to endogenous estrogens (age at menopause minus age at menarche) is inversely associated with myocardial infarctions in postmenopausal women undergoing coronary angiography (p=0.03, Kruskal Wallis test). Reprinted with permission from Maturitas.

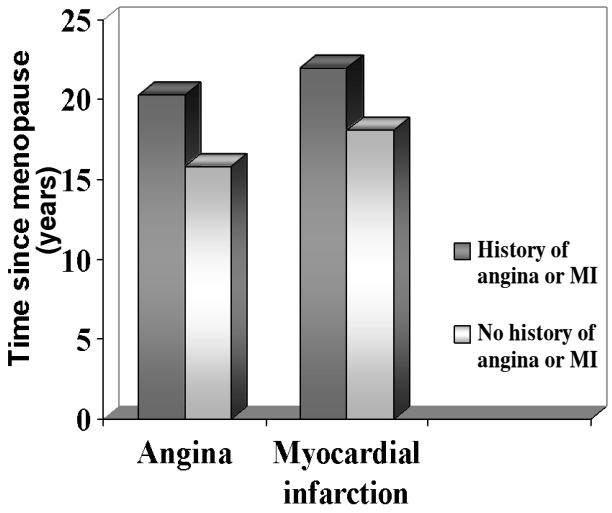

Figure 2. In 100 postmenopausal women undergoing coronary angiography, longer time interval since menopause is associated with history of angina or myocardial infarction (MI) ($p<0.03$ and $p<0.05$, respectively, t test). Reprinted with permission from Maturitas.

endogenous estrogens and relatively higher levels of androgens are associated with the risk for an acute myocardial infarction at menopause.[31] Current estrone levels have not been associated with a higher risk for CHD.[28,32] This is to be expected, as most postmenopausal women present with very low hormonal levels during menopause. It has been reported that premenopausal women with lower estrogen levels had more severe atherosclerosis in their vessels in the coronary angiography.[33] This provides some evidence of an increased risk for future coronary artery disease in women whose total exposure to estrogens has been lower during the reproductive years.

It appears that menopause by itself affects several classical predisposing factors for CHD[10,18,19,26,34-36] and is associated with an increase in triglyceride, total and low-density cholesterol (LDL) levels as well as with an increase in central fat deposition and insulin resistance.[18,36] The increase in central adiposity during menopausal transition is independent of the effect of total body adiposity and of age, as has been shown in several longitudinal and prospective studies.[18] In the study of Saltiki et al, positive associations of several predisposing factors such as hyperlipidaemia, diabetes mellitus, positive family history of CHD as well as insulin resistance (HOMA) with the severity of CHD in the angiography in postmenopausal women were found.[20] The associations of CHD severity with time since menopause remained significant when hyperlipidemia, measures of adiposity, insulin resistance and chronological age were taken into account.

In conclusion, it seems that shorter lifetime exposure to endogenous estrogens is an important risk factor for the presence and the severity of CHD, whereas endogenous estrogens appear to play a protective role for the cardiovascular system. Some of the implicated mechanisms will be analysed below.

ESTROGENS DURING MENOPAUSE

The main sources of estrogen in premenopausal women are the ovaries. During steroidogenesis, the theca cells produce androgens, which are aromatized to estrogens with the enzyme aromatase in the granulosa cells of the ovary. During menopause the main source of estrogen production is extragonadal. Estrogens are mainly produced by the adipose tissue which expresses the steroidogenic enzymes aromatase and 17βHSD. In postmenopausal women the predominant estrogen is estrone, which is 50-70% less active than 17β estradiol. Estriol is another circulating estrogen. The extragonadal production of estrogens is influenced by age and weight. Aromatase is also expressed in the endothelial cells and smooth-muscle cells of blood vessels. This fact suggests a paracrine or autocrine action of estrogens. The blood concentration of estrogens does not reflect the biologically active forms at the tissue level, as these are dependent on the local enzyme activity and the binding on the protein transporters such as sex hormone binding globulin (SHBG). The bioavailability and the functionality of estrogen receptors also play a major role in the tissue response to estrogens.[37]

MECHANISMS OF ESTROGEN ACTIONS

Estrogens act on cellular function through genomic and non-genomic mechanisms; the genomic effect is slower and is better characterized. The alteration of the expression of various genes resulting from estrogen action depends upon the activation of the two nuclear estrogen receptors ERα and ERβ which act as transcription factors.[38,39] The second

mechanism of estrogen action is non-genomic, as it is not dependent on changes in gene expression and occurs within minutes after estrogen binding with receptors situated on the cellular membrane. For example, the vasodilatation occurring rapidly after estrogen administration is attributed to a non-genomic effect. The effect of estrogens on the changes in the metabolic profile, on the immune process and on the response to vascular injury is dependent on genomic transcriptional activity[37,40] (Table 1).

Genomic actions and the role of estrogen receptors

Estrogen receptors (ER) are members of the superfamily of nuclear receptors; the androgen, progesterone and glucocorticoid receptors also belong to the same family. There are two different genes encoding ERs, the ERα and the ERβ genes located on different chromosomes. The classical ERα was cloned two decades ago. ERβ was cloned more recently. Functional estrogen receptors are present in the cardiovascular system. Various mRNA splice variants have been found in normal and atherosclerotic tissues, but the proteins which are encoded and their pathophysiological role are not well established.[41] They carry several functional domains characteristic for the receptors of this family. The two receptors have 53-96% homology in their different domains. These structural differences contribute to the variable affinity to various ligands and offer cellular specificity. Growth factors may also act as ligands to the ERs.[37,40,42]

In the absence of a ligand, the receptors are located within the cytoplasm, associated with cytoplasmic proteins which act as chaperones, like heat shock protein 90 (hsp 90). When free estrogen diffuses into the cell-target, these proteins dissociate from the receptor and several biochemical events, such as activation of ion channels and changes in the enzymatic activity, occur.[43] When the hsp dissociates from the receptor, the complex estrogen/estrogen receptor diffuses in the nucleus, where this complex is homodimerised or heterodimerised by either one ERα or one ERβ and then binds to the estrogen response element (ERE) of the DNA sequence, close to the responsive gene. The transcription process depends on the promoter of the gene and the various co-activators and co-repressors. Depending on the ligand, the complex acquires a special conformation which finally determines the binding of a certain co-activator to the promoter.[37,40,42] The complex interaction of co-regulators and homo- or hetero-dimers of ERs results in a highly specific response after transcriptional activation. An example of cardiovascular co-regulator specificity is the ste-

Table 1. Genomic influence of estrogens: estrogen-regulated genes which participate in the cardiovascular function.

Genes involved in vasodilation	**Genes related to lipid metabolism**
• Prostacyclin cyclooxygenase	• Lipoprotein lipase
• Prostacyclin synthase	• Apolipoproteins A, B, D, E, Lp(a)
• Endothelial NO synthase (eNOS)	• Leptin
Genes involved in vasoconstriction	**Genes related to the inflammatory process**
• Endothelin-1	• Vascular-cell adhesion molecule (VCAM-1)
• Angiotensin II receptor, type 1 (AT1)	• Cytokines (IL1, IL6, TNFα)
• Renin	• Cytokines receptors
• Angiotensinogen converting enzyme	**Genes related to coagulation and fibrinolysis system**
Genes involved in vascular remodeling and angiogenesis	• Fibrinogen
• Vascular endothelial growth factor (VEGF)	• Protein S
• Collagen	• Coagulation factors V, VII, IX, X
• Matrix metalloproteinase (MMP)	• Plasminogen-activator inhibitor 1 (PAI-1)
• E-Selectin	• Antithrombin III
• Growth Factors (TGFβ1, PDGF)	• Tissue plasminogen activator
	• Platelet-derived growth factor

roid receptor co-activator 3 (SRC3), which facilitates the estrogen-mediated vasoprotection from vascular injury.[44] The cellular environment and the nuclear receptor's and co-regulator's phosphorylations are also important for the specificity of the response of the target-tissue.[45] This phenomenon has been taken advantage of in clinical practice with the design of selective estrogen receptor modulators (SERMS), which act either as agonists or antagonists in various tissues depending on the co-activators which participate in the process.[46] Estrogens may regulate the transcription of genes lacking ERE by modulating the activity of other transcription factors such as activating protein 1 (AP1) and Nuclear factor kappa B (NF-κB).[47]

Estrogens act on the cardiovascular system[40,42,48] through their receptors ERα and ERβ, which are expressed in the endothelial cells[49] and on the smooth muscle cells of vessels.[50-52] ERα has been more thoroughly studied, most of the studies having shown the importance of this receptor for the atheroprotective effects of estrogens.[53] In premenopausal women with more severe atherosclerotic lesions in their vessels a lower expression of ERα has been found.[50] More recently there has been growing interest in the participation of ERβ in the physiology of the cardiovascular system.[54,55] It seems that ERβ is the receptor which is expressed to a larger degree on the endothelial cells and the smooth muscle cells of healthy and atherosclerotic vessels in both sexes.[52,54,55]

Apart from the level of the expression of the receptors, the functionality of these receptors has also been investigated at the tissue level. There are different isoforms of the estrogen receptors which are all expressed, but their significance is unknown.[56] Other studies have shown that each one of these receptors on its own has the ability to preserve the estrogen activity; in knockout mice for either the ERα or the ERβ gene, estrogen administration resulted in a decrease of the intima media layer thickness.[57,58] Finally, higher methylation of the ERα gene may play a role in atherosclerotic vessels.[59]

Genetic polymorphisms of the estrogen receptors may affect the tissue response, i.e. the tissue sensitivity to estrogen. In 1997, Sudhir et al reported an extreme example of dysfunction of the receptor in a young man who carried an inactivating mutation in the ERα gene causing severe estrogen resistance; this individual manifested premature atherosclerosis despite high circulating levels of estrogens.[60] Similarly, polymorphisms of the ERα and ERβ genes may affect the sensitivity of tissues to estrogens and could be related to a higher risk for CHD. This issue will be analyzed in the last section of this brief review.

Non-genomic actions

Apart from the genomic actions, estrogens also show rapid actions as has already been mentioned. In most of these actions the transcriptional machinery does not participate; in the non-genomic process, membrane receptors may be involved as well as cellular signaling pathways with the participation of ion channels (Ca and K), G proteins and G protein-coupled receptors (GPCRs), tyrosine kinases (PI3K) and MAP kinase cascades.[61]

These membrane receptors have not been well characterized, but there is evidence that they may be the same cytoplasmic receptors ERα and ERβ situated in caveolae on the cellular membrane.[62,63] Recently, it has been reported that an intracellular transmembrane G protein-coupled receptor acts as estrogen receptor probably mediating some of the rapidly occurring estrogen effects.[64] The non-genomic actions of estrogens are very critical for the cardiovascular system because they regulate the rapid vasodilation of coronary and other vessels. This vasodilation is achieved through the opening of Ca channels and activation of K channels, as well as through the secretion of vasoactive molecules like nitric oxide (NO) from the endothelium and the vascular smooth muscle cells.[61] Similarly, the rapid insulin secretion by the pancreatic β cells is possibly regulated by estrogen through a non-genomic action.[65] Recently, it has been reported that some non-genomic effects can be converted to genomic ones and to transcriptional activation with the mediation of cascades of cellular signaling pathways.[66]

DIRECT ACTIONS OF ESTROGENS ON THE VASCULATURE

Atherosclerosis is a chronic hyperplastic inflammation of the layers of the vessels and is influenced

by genetic, metabolic and hormonal factors.[67] During the early stage of the atherosclerotic process, the subepithelial trapping of oxidised LDL molecules that trigger the local reaction of inflammation play a major role. The result is the accumulation of macrophages, monocytes and T cells, the production of matrix and various enzymes such as metalloproteinases (MMPs) and the production of proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin 1 & 6 (IL-1 & 6), which mediate a Th1 response;[68] the final result is a rupture in the atherosclerotic plaque. Another important step is the calcification of the coronary vessels, which has a genetic element but is also influenced by hormonal factors.[54,69]

It seems that estrogens protect the vasculature at different levels. Through their direct actions, estrogens influence the evolution of the atherosclerotic lesions, whereas by their indirect actions they modulate various vasoactive, pro-inflammatory and metabolic factors as well as factors of the coagulation system[40,42,48,70] (Table 2).

There is evidence that the administration of estrogens is protective for the formation of the atherosclerotic plaque when administered to either healthy or hypercholesterolemic rats. Hodgin et al have shown that this protective action of estrogens is mediated by ERα in hypercholesterolemic transgenic Apo E knockout mice, whereas when these rats were double knockout for the Apo E and for the ERα gene, this protective effect was diminished.[71]

Estrogen deficiency also induces the calcification of atherosclerotic plaques. Postmenopausal women not receiving hormone replacement therapy (HRT) have more calcified atherosclerotic plaques in their coronaries than premenopausal or postmenopausal HRT users.[72] Aortic calcification increases when the time elapsed since menopause is longer.[73] Men have twice as many calcifications on their vessels as women until the age of 60, but after this age this gender difference attenuates, indicating the role that estrogen deficiency may play in vascular health.[74] It is interesting that the mechanism of vessel calcification is similar to that of bone formation. Paradoxically, the vessel calcification during menopause occurs inversely to the bone demineralization and postmenopausal osteoporosis.[75] It seems that in these two processes, various molecules such as metalloproteinases (MMPs) and osteoprotegerin may play an important role.[75] In their very recent study in autopsies of arteries of pre- and postmenopausal women, Christian et al[54] reported the critical role of ERβ in the atherosclerotic and calcification process; these receptors were found to a greater extent in atherosclerotic coronary vessels and were correlated with more severe lesions, independently of the chronological age of these women. One other point that should be mentioned is that estrogen receptor gene expression may be affected by DNA methylation, which occurs with aging thus contributing to the atherogenic process in the cardiovascular system.[59]

Estrogens also modulate the response to vessel injury with the mediation of both ERs.[42,53,57,58] Both ERs expression increases after vessel injury.[42,76] In animal models, ERβ appear to be important for the differences in the response to vascular ischemic injury between the two sexes.[77] Experiments in ERβ

Table 2. Mechanisms of estrogen action concerning the cardiovascular system.

Direct actions	**Genomic**
	• Vasodilation: increased NO production of endothelial cells and smooth muscle cells of vessels and myocardium
	• Protection from the formation and calcification of atherosclerotic plaques
	• Modulation of the response to vessel injury, antihyperplastic effect, re-endothelialization
	Non genomic
	• Rapid vasodilation through increased NO production and modulation of ion channels
	• Mitogen activity (MAP Kinases)
Indirect actions	
	• Lipid metabolism: Increase in HDL, triglycerides Decrease in LDL, oxidized LDL, LPα
	• Carbohydrate metabolism
	• Coagulation system
	• Proinflammatory factors and antioxidative effects

knockout mice show that acute myocardial infarction results in more severe cardiac dysfunction.[78]

Estrogens act positively on the endothelial cells. This angiogenic activity and re-endothelialization is mediated by ERα[53,79] and ERβ,[80] which increase the in vitro and in vivo local production of growth factors such as FGF, TGF and VEGF.[81] They also inhibit the apoptosis of endothelial cells in cell cultures.[82] On the other hand, they have antihyperplastic effects on vascular smooth muscle cells: they inhibit the proliferation of vascular muscle cells, thus affecting the thickness of the vessel wall.[40,42] The thickness of carotids intima media layer, an index of the risk for CHD, is increased in 45% of postmenopausal women while in only 16% of premenopausal women.[83]

Functional ERs are expressed in both animal and human cardiomyocytes and may play a role in gender differences concerning cardiac contractility, heart rate and myocardial hypertrophy[42] as well as in the myocardial protection of ischemic insults through inhibition of mitochondrial reactive oxygen species (ROS).[84]

Another very important action of estrogens is their vasodilatory action. This is mediated by the increase in the production of vasodilating molecules such as NO and prostacyclines, as well as by the decrease in vasoconstricting factors such as endothelin-1, renin, angiotensinogen converting enzyme and the down-regulation of the receptor of angiotensin AT1.[40,48] Transdermal estrogen administration in postmenopausal women with angina but normal vessels in the angiography increases vasodilation.[85] Female animals have a higher basic rate of NO production than males.[86] This increase in NO production is mediated by a rapid non-genomic as well as by a genomic mechanism through transcriptional activation of the NO synthase gene (eNOS) in the vessels.[87] The activation of eNOS is probably mediated by ERα, as in transgenic knockout mice for the ERα gene the NO levels are decreased.[88] In parallel, other signaling pathways and factors such as MAP kinases, PI3K/Akt and hsp90 participate in the activation of eNOS, showing how complicated this mechanism is.[87] The eNOS affects the function of other estrogen dependent tissues such as bone tissue and contributes to the prevention of bone loss.[89] NO attenuates the atherogenic process by decreasing the proliferation of vascular muscle cells. It also plays a central role in the inflammatory events, influencing the production of cytokines and decreasing the adhesion and accumulation of monocytes and platelets on the wall of the affected vessels.[87] Premature ovarian failure is associated with impaired endothelial function, which may contribute to the increased cardiovascular disease risk. Early initiation of hormone therapy may reverse this atherosclerotic process.[90,91]

What happens when atherosclerotic plaques already exist? In ovariectomized monkeys, estrogen administration decreases the formation of new plaques but has no beneficial effect on pre-existing ones.[92] Estrogen administration probably makes these plaques more destabilised as estrogens increase new vessel formation and the risk of haemorrhage. This may be one of the mechanisms through which HRT use is related to a higher risk of cardiovascular events during the first year of their use.[93,94]

INDIRECT SYSTEMIC ACTIONS OF ESTROGENS

Effects on metabolism of lipids and carbohydrates

During their reproductive years, women have lower levels of lipids and LDL than men while these levels increase after the menopause.[3,19,95] By contrast, the difference in HDL levels between men and postmenopausal women remains the same. After menopause, the increase in LDL, total cholesterol and Lp(a) levels is reversed with oral (p.o.) estrogen administration,[96] while triglycerides levels become worse.[96] The estrogens effects on lipid metabolism are mediated by ERα. It has been shown that polymorphisms of the ERα gene may influence the lipid response after HRT.[97,98]

Estrogens participate in both lipogenesis and lipolysis. At the transcriptional level they increase the hepatic expression of apoprotein genes and the LDL receptors and decrease the transcription of the lipoprotein lipase (LPL) gene through ERα. Thus, when estrogen levels decrease after the menopause, an increase of the LPL activity is observed and this probably contributes to the increase of free fatty acids (FFA) and the accumulation of abdominal fat.[99] There is also evidence that estrogen receptors

regulate the expression of other non sex steroid hormone nuclear receptors such as the peroxisome proliferator-activated receptor α (PPARα) and the liver X receptors (LXRs), which mediate various metabolic pathways relevant to cardiovascular disease.[42] By inhibiting lipogenesis, estrogens alter the expression of hormone-sensitive lipase.[100] On the other hand, through ERα and ERβ, estrogens are involved in the proliferation of adipocytes,[99] whereas their deprivation increases central obesity which is associated with a more atherogenic profile.[101] Plasminogen activator inhibitor-1 (PAI-1),[102] IL6 and CRP levels[70] often increase during menopause, while insulin resistance and several other components of the metabolic syndrome emerge;[18] all these factors may contribute to the increase in cardiovascular morbidity. Finally, levels of adiponectin, which is produced by adipocytes and whose role is protective for the metabolic syndrome, do not change during menopause.[103] Similarly, leptin levels do not correlate with menopausal status; there is, however, evidence that estrogens may act on the hypothalamus, influencing central sensitivity to leptin.[104,105]

Changes in proinflammatory factors and antioxidative effects

As has already been mentioned, atherosclerosis is a chronic inflammatory process. Evidence for such association is provided by several studies in animal models, reviewed by Hansson et al, in which a 10-fold higher lipoprotein accumulation was shown in the vessels of hypercholesterolemic animals compared to similar animals in which various genes important for the immune system had been knocked out.[67]

Some of the atheroprotective effects of estrogens are mediated through their interaction with the inflammatory process (Figure 3). Estrogens decrease the adhesion molecules, vascular cell and intracellular adhesion molecules (VCAM-1 and ICAM-1) and decrease the accumulation of leucocytes on the endothelium.[106] It has been found that monocytes, neutrophils and T and B lymphocytes express estrogen receptors, mostly ERα. In estrogen deficiency, these cells of the immune system are more active.[70] In vitro experiments have shown that estrogens affect the release of proinflammatory cytokines by these cells probably through the cross-talk of the ERs

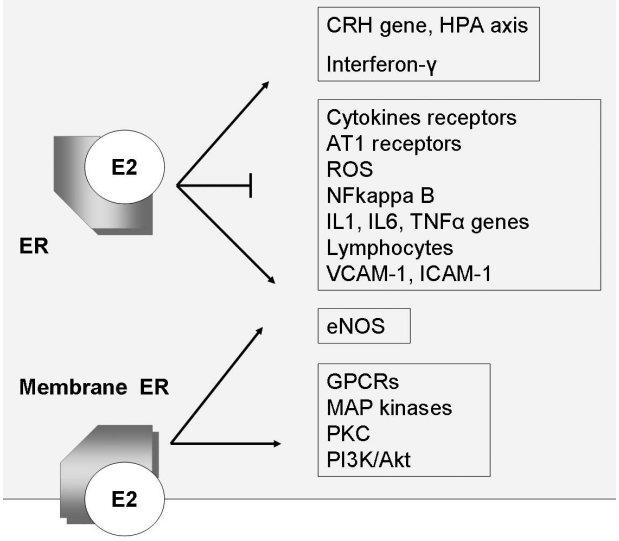

Figure 3. Effects of estradiol on the inflammatory process.

with NF-ϰB, thus resulting in inhibition of NF-ϰB activity.[70,107] It has been suggested that estrogen deficiency during the first years of menopause induces the production of various proinflammatory cytokines and the expression of their receptors such as IL6, IL1 and TNFα on the vessel wall.[70] In the model of ovariectomized Apo-E knockout mice, the expression of the IL6 gene and IL6 production are increased, while they decrease when estrogens are administered.[108] These alterations in cytokines affect the biosynthesis of several other molecules which are important for the pathogenesis of atherosclerosis, such as CRP (through IL6). CRP acts on the accumulation of monocytes in the atherosclerotic plaques[109] and is an index of inflammation and risk for new cardiovascular events.[110] Cytokines and CRP can stimulate NF-ϰB and angiotensin receptors AT1. As a result, oxidative stress is increased, smooth muscle cells proliferate and the production of metalloproteinases (MMPs) increases and participates in the destabilization and the rupture of atherosclerotic plaques.[110]

However, some of the data in the literature are conflicting. Several studies have shown that estrogens induce a decrease in anti-inflammatory cytokine levels such as IL4, IL10 and IL3 and also an increase of interferon-γ, causing a Th1 immune response which promotes atherosclerosis. It seems that estrogens affect the inflammatory response through many different pathways and modulate accordingly the

atherogenetic process.[111] On the other hand, clinical studies have shown an increase in CRP and IL-6 levels during p.o. HRT use, whereas transdermal administration does not influence these inflammatory factors. There are data in the literature which demonstrate that estrogens have atheroprotective effects when administered before the vascular damage occurs. It is thus possible that the adverse effects of oral estrogen on thrombosis and inflammation may predominate in the presence of pre-existing atherosclerotic lesions.[42,112,113]

Many in vitro and in vivo studies have shown antioxidant effects of estrogens. For example, estrogens inhibit the oxidation of LDL through both genomic and non-genomic mechanisms.[114] Inversely, estrogen deficiency induces the production of ROS, which participate in the oxidation of LDL molecules which then contribute to the formation of foam cells on the vessel wall, to the production of proinflammatory cytokines and to the increase of NO catabolism. Also during menopause, the expression of the angiotensin receptors is increased and this contributes further to ROS production.[70,110]

Finally, it has been speculated that the interaction of the hypothalamo-pituitary-gonadal axis with, for instance, the hypothalamic pituitary adrenal (HPA) axis might be involved in the atherosclerotic process: estrogens increase the expression of the corticotrophin releasing hormone (CRH) gene which affects the immune response.[115]

Estrogen effects on the coagulation system

Estrogens alter the transcription of genes coding for several proteins participating in the coagulation system. They affect fibrinogen and factors V, VII, IX X and TFPI[116] and they decrease the levels of antithrombin III, protein S and PAI-1.[116,117] Furthermore, estrogen receptors are expressed on platelets: they influence the migration, the adhesion and the aggregation of these cells and thus increase the thrombotic risk.[116] These effects may offer an explanation for the increased thrombosis events related to the p.o. use of HRT, which were reported in studies such as HERS[93,118] and WHI.[94,119] Transdermal administration carries a reduced risk.[120-122] In women taking HRT, estrogen dosage, medical history about the inherited hypercoagulable states frequently caused by factor V (Leiden) and prothrombin gene mutations, as well as a history of smoking, are well recognized factors influencing the thrombotic risk associated with HRT.[122,123]

POLYMORPHISMS IN THE ESTROGEN RECEPTOR GENES, ERα AND ERβ

ERα polymorphisms

Recently, there have been many studies performed in the general population about the effect of genetic variants of ERs, which may influence the tissue sensitivity to estrogens. The case of a young man who had an inactivating mutation in the ERa gene causing resistance to estrogen and also had premature atherosclerosis has already been mentioned.[60] Since this report, several studies have investigated possible associations between ERα single nucleotide polymorphisms and a variety of clinical and biochemical parameters predisposing to heart

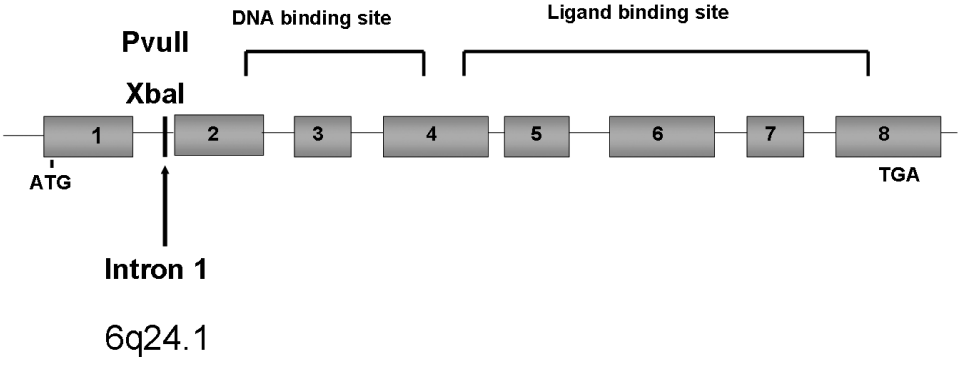

Figure 4. ERα gene and the two common polymorphic sites in intron 1: PvuII c.454-397 T>C and XbαI c.454-351 A>G.

disease, especially in men.[124-133] Most studies concern the PvuII c.454-397 T>C and XbαI c.454-351 A>G polymorphic sites in intron 1 of the ERα gene (Figure 4) which may be of functional importance (see below). Shearman et al in a subpopulation of the Framingham study showed that men with the PvuII C variant had a 3-fold increased risk for cardiovascular disease[125] and stroke.[134] In a different study, men carriers of the PvuII variant had more generalized atherosclerotic lesions and extensive calcification of the atherosclerotic plaques at autopsies,[135] while apparently healthy men with the polymorphism present premature coronary artery dysfunction.[133]

So far as women are concerned, we have recently found[136] similar associations to those shown in men, namely a positive correlation of two ERα variants of PvuII T>C and XbαI A>G with the severity of coronary artery disease, while the Rotterdam study showed conflicting results.[129] Finally, there are several studies which found no significant correlations between these ERα polymorphisms and the presence[132,137] or severity[138] of cardiovascular disease in either gender.

In the literature there are several studies which suggest that these polymorphisms of ERs may modify the sensitivity of various tissues to estrogens[38] and affect the clinical phenotype in other diseases such as breast cancer,[139] endometrial cancer,[140] endometriosis[141] and osteoporosis.[142] It is also possible that these variants may influence clinical parameters such as age of menarche and menopause,[143,144] blood pressure[145] and lipid levels[146] as well as their response to the HRT[98] or their association to tobacco use.[147]

There are several studies in the literature supporting the functional importance of these ERα polymorphisms on tissue sensitivity to estrogens.[98,148] It has been speculated that this intronic site of the variants, which is situated at a distance between 397 and 351 nucleotides from exon 2, might result in alternative splicing, thus modifying the gene's function, as has been reported for other genes.[149,150] It is also possible that this site might be the locus of attachment of a transcription factor, B-myb, which is nullified when nucleotide T is present, thus affecting the speed of transcription of the receptor gene.[98,148] Finally, it is possible that this polymorphic site is linked to some other locus, which could have a role in cardiovascular disease. It has further been reported that the PvuII intronic polymorphism is linked to the polymorphic TATA repeat site in the ER α promoter region.[151]

ERβ polymorphisms

Recently, a growing number of studies discuss the clinical significance of ERβ polymorphisms. Especially as regards the cardiovascular system, these variants appear to be associated with earlier presence of atherosclerosis,[137] as well as with lower LDL levels in women taking HRT[152] and higher HDL levels in women taking isoflavones.[153] They have also been associated with left ventricular hypertrophy in women[154] and with a history of arterial hypertension.[145,155] Pertinent experiments in animals showed analogous results; knockout mice for the ERβ gene develop hypertension, arterial dysfunction and chronic heart failure.[156,157] Also, polymorphisms of the androgen receptor (AR) and ERβ may influence circulating androgen levels in women[158] and thus affect indirectly the cardiovascular system. Finally, as is the case with ERα, ERβ may also affect clinical parameters such as age at menarche[159] or estrogen related diseases such as breast cancer[160] and osteoporosis.[161]

CONCLUSIONS

The in vivo and in vitro data that were presented in this short review show that estrogen is of particular importance for vascular health. Data from experimental studies or observations from the natural lack of endogenous estrogen occurring during menopause show that estrogen deprivation in women is associated with adverse effects on the cardiovascular system and with acceleration of atherosclerosis. On the other hand, HRT use was associated with a reduction of CHD risk by as much as 40-50%[162-165] in large epidemiological observational studies. This protective effect on the cardiovascular system was attributed mostly to the favorable effect on the lipid profile. Despite all these favorable effects, and despite the results of the large observational studies, the recent randomized prospective studies did not prove that exogenous estrogen has the expected protective effect. In HERS,[93] where HRT was administered to women with pre-existing coronary artery disease, an increased incidence of fatal cardiovascular events

and an increase of thromboembolic incidents were observed.[118] In the WHI,[94,119,166] the administration of a combination of conjugated estrogen with a progestogen was associated with an increased incidence of cardiovascular events as well as with an increase in the incidence of breast cancer; the administration of estrogens only in women with hysterectomy was also associated with an increase in thromboembolic incidents. It has been reported that the design of this study had intrinsic problems associated with the population group, their age, the distance since menopause, the presence of further risk factors for CHD as well as the presence of already established CHD.

How is it then possible to use the information from the experimental and epidemiological data showing a protective effect of estrogen in clinical practice? There are several questions that have to be answered. The first one is whether younger women might benefit from the use of HRT immediately after menopause. The second question concerns the compound, the dosage, the duration and the ideal and safest route of administration. Clearly, the careful design of further studies which will try to answer these questions is warranted, so that the apparent cardioprotective effects of endogenous estrogen may find some safe application in clinical practice for the benefit of women's health.

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