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Review Article

The effects of estrogen and hormone replacement therapy on platelet activity: a review

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Abstract: Many studies have shown that an increase in cardiovascular disease in women is related to hormonal changes occurring particularly after menopause with increasing age. While the results of large clinical trials reporting no benefit of hormone replacement therapy (HRT) in cardiovascular disease have been known for some time, there is an increasing body of knowledge regarding the various mechanisms by which estrogen modulates platelet function that could in part explain the higher cardiovascular risk occurring in postmenopausal women and potential benefits of HRT on cardiovascular health. Our review summarizes our current knowledge regarding the effect of endogenous and exogenous estrogen on platelet activity, which can help researchers design future studies. We collected information from 21 peer-reviewed articles published from 1993 to 2021. Studies have indicated that postmenopausal women have higher platelet activity than premenopausal women, which can increase the risk of thrombo-embolic events and cardiovascular disease. Although some studies have reported pro-thrombotic effects of estrogen replacement therapy such as increased platelet activation and adhesion, other studies demonstrated decreased platelet aggregation by inhibiting GP IIb/IIIa receptor expression. This is mediated by estrogen receptors on the platelet membrane in a non-genomic manner and suggests an opportunity for the usage of estrogen replacement therapy with subtle changes in the formulation and route, particularly if started early after menopause. The effect of estrogen on platelet activity is promising as an important factor in reducing the risk of cardiovascular events, warranting further investigation.

Keywords: Cardiovascular disease, coronary artery disease, atherosclerosis, menopause, estrogen, hormone replacement therapy, estradiol, estrone, estriol, E2, HDL, osteoporosis, platelet aggregation, GP IIb/IIIa, antiplatelet therapy, estrogen receptor

Introduction

Atherosclerosis can be caused by numerous factors such as age, risk factors, lifestyle, and diet. Both males and females share most of the classic risk factors for the development of cardiovascular disease (CVD). According to numerous studies, the onset of CVD in women lags roughly 10 years behind that of men [1]. These studies have also indicated that the pathophysiology of CVD in women by way of atherosclerosis is correlated with age and decreasing levels of the sex hormone estrogen seen in menopausal women [2]. Cardiovascular disease in women has strongly been associated with the loss of endogenous estradiol [1]. Due to the large number of women affected by CVD each year, increasing research has begun to deduce a possible mechanism by which estrogen might convey possible cardiovascular protection for women.

However, hormone replacement therapy in large, randomized trials has failed to show any cardiovascular benefit unless it is started early [3, 4]. Hormone replacement therapy (HRT) in the form of conjugated equine estrogens (CEE) or synthetic conjugated estrogens have been around for numerous years to help alleviate the symptoms of menopause such as hot flashes, sleep disturbances, and mood lability. Research has also indicated a possible secondary benefit of HRT, namely the suppression of CVD in postmenopausal women. Basic science and

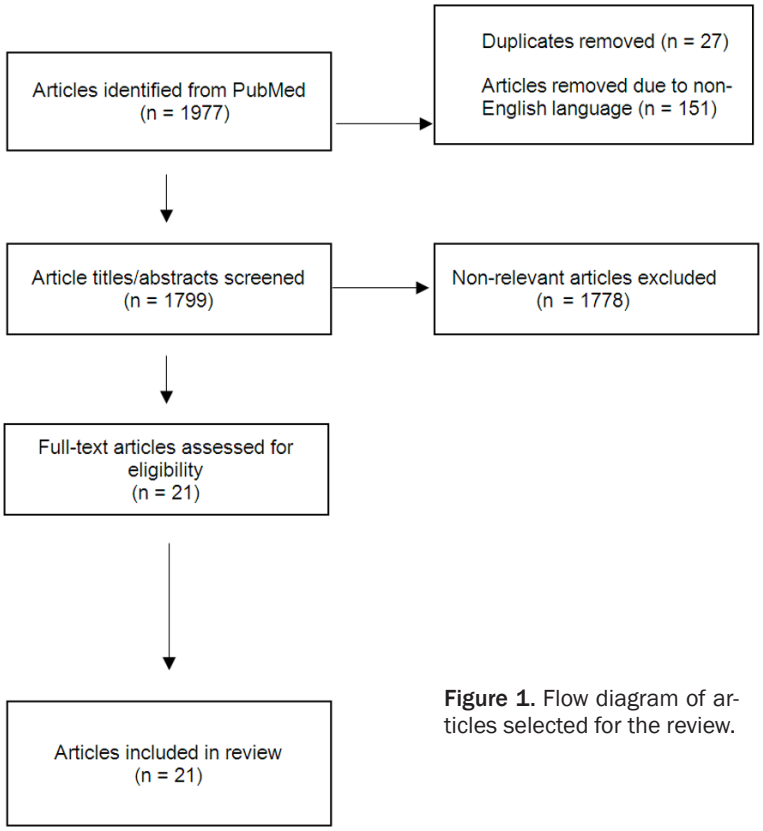

Figure 1. Flow diagram of articles selected for the review.

animal studies have suggested that estrogen therapy could be beneficial. The female cynomolgus monkey has many reproductive characteristics similar to female humans such as a 28-day menstrual cycle with cyclic changes in plasma concentrations of estradiol, follicle-stimulating hormone, luteinizing hormone, and progesterone and the occurrence of menopause. Compared to their male counterparts, premenopausal cynomolgus monkeys present significantly higher plasma concentrations of HDL cholesterol, much like premenopausal women. In addition, the likelihood of coronary artery atherosclerosis in male versus female monkeys is analogous to that of humans. When premenopausal female monkeys had oral contraceptives added to their experimental diet based on their body weight and caloric requirements, like humans, these monkeys had less atherosclerosis and a lower incidence of thrombosis. Postmenopausal monkeys receiving HRT did not have an increased incidence of arterial thrombosis compared to untreated controls. However, the effect of HRT in humans has been negative in protecting from cardiovascular events as mentioned earlier. The cause of this discrepancy is not known. In fact, HRT reduces risk factors partially due to its beneficial effects on lipoproteins [5].

The purpose of this review is to summarize the available literature regarding the effects of naturally occurring endogenous estrogen and the addition of exogenous estrogen on platelet activity in postmenopausal women which may explain the favorable cardiovascular effects seen with early estrogen replacement in post hoc analyses of large clinical trials. This paper will discuss in detail the mechanism of estrogen's interaction with platelets and platelet receptors such as glycoprotein IIb/IIIa (GP IIb/IIIa) that can lead to decreased platelet activation and aggregation based on findings from basic science and clinical studies.

Methods

A review of the literature was performed by searching the PubMed database using the following keywords alone or in combination: "estrogen", "hormone replacement therapy", "platelets", "cardiovascular disease", "thromboembolism", and "postmenopausal". Inclusion criteria for this review consisted of peer-reviewed articles published from January 1, 1993 to September 30, 2021 (**Figure 1**). Articles that were not in English and/or not relevant to the effects of HRT on platelet activity and cardiovascular health of postmenopausal women were excluded. Information was synthesized from 21 peer-reviewed articles, including previous literature reviews as well as experimental and observational studies, and key findings were extracted by the researchers (**Tables 1** and **2**).

The role of platelet binding in cardiovascular disease

Cardiovascular disease begins as atherosclerosis. Atherosclerosis is a process of accumula-

Table 1. Summary of original study characteristics

Study	Design	N	HRT	Outcome measures	Follow-up (years)
Aldrighi et al. [13]	Cross-sectional	37	--	P-selectin and GP IIb/IIIa expression, plasma [TXB2]	--
Bar et al. [16]	Quasi-experimental	51	oral CEE + MPA	Platelet aggregation, ATP release	0.25
Gu et al. [28]	Quasi-experimental	30	--	Platelet surface P-selectin and αIIbβ3 integrin expression	0.5
Hodis et al. [3]	RCT	643	oral 17β-estradiol + micronized progesterone vaginal gel	CIMT progression, CT measures of coronary artery calcium, plaque, and stenosis	7.5
Jayachandran et al. [19]	Cross-sectional	146	--	Platelet count, P-selectin and GP IIb/IIIa expression, microparticles from platelets, granulocytes, monocytes, and endothelium	--
Khoudary et al. [35]	RCT	474	oral CEE or transdermal 17β-estradiol	Coronary artery calcification progression, CT-based paracardial and epicardial adipose tissue	4
Kim et al. [32]	In vitro experiment	--	17β-estradiol	vWF and IL-8 release, platelet adhesion	--
Miller et al. [31]					
Nakano et al. [14]	Quasi-experimental	18	oral CEE	Platelet aggregation, staining for GP IIb/IIIa, intraplatelet Ca^{2+}, cAMP, cGMP, nitrite/nitrate	0.08
Roshan et al. [27]	Cross-sectional	49	--	Platelet surface P-selectin expression and PAC-1 levels	--
Rossouw et al. [34]	RCT	27,347	oral CEE + MPA	HR for CHD and stroke	6.8
Smith et al. [36]	Case-control	384	oral CEE or estradiol	Incident VT, MI, or ischemic stroke	--
Stachowiak et al. [18]	Quasi-experimental	92	transdermal 17β-estradiol + NETA	Platelet count, GP IIb/IIIa expression	0.25
Thijs et al. [30]	RCT	60	oral E2 + trimegestrone or dydrogesterone	P-selectin and GP 53 levels	0.25
Toh et al. [4]	RCT	16,608	oral CEE + MPA	Adherence-adjusted HR, CHD-free survival	5.6
Valéra et al. [17]	Mouse model	--	implanted estradiol pellet	Bleeding time, platelet aggregation	--

ATP, adenosine triphosphate; CEE, conjugated equine estrogens; CHD, coronary heart disease; CIMT, carotid artery intima-media thickness; CT, computed tomography; GP, glycoprotein; HR, hazard ratio; HRT, hormone replacement therapy; MI, myocardial infarction; MPA, medroxyprogesterone acetate; NETA, norethisterone acetate; PAC-1, pro-caspase activating compound; RCT, randomized controlled trial; TXB2, thromboxane B2; VT, venous thrombosis.

Table 2. Summary of review articles included

Primary author	Articles retrieved	Number of articles included	Endpoint	Conclusions
Del Principe et al. [29]	1986-2015	104	Effect of estrogen on megakaryocyte and platelet functions	- Estrogen promotes megakaryocytopoeisis and can modify platelet function via ERβ
Hashemzadeh et al. [2]	1986-2020	39	Effects of HRT on cardiovascular system	- Estrogen increases coronary blood flow and myocardial ischemia while decreasing vascular resistance - Greater platelet activity seen in postmenopausal vs. premenopausal women
Leng and Bray [15]	1974-2005	46	Effect of sex hormones on platelet activity	- Platelet activity can be enhanced or inhibited by estrogen and progesterone in vivo - Inconsistent results based on varying routes, formulations, and assays
Miller et al. [8]	1979-2006	94	Effect of estrogen on platelet activity and thrombotic risk with aging	- Thrombotic cardiovascular events increase in postmenopausal women - Estrogen affects platelet function in a genomic and non-genomic manner
Samsioe [33]	1973-2002	22	Primary and secondary prevention of CVD by HRT	- HRT should not be given solely for CVD prevention - Limited evidence for exclusion of women with increased CVD risk or withdrawal of HRT in case of a cardiovascular event

CVD, cardiovascular disease; GP, glycoprotein; GPCR, G-protein coupled receptor; HRT, hormone replacement therapy.

tion of white blood cells within the arterial wall, combined with cholesterol accumulation and inflammation leading to plaque formation. Over time, the growing plaque within the arterial wall and lumen can cause narrowing and plaque rupture, leading to ischemia or myocardial infarction by platelet aggregation [7].

Platelets are responsible for hemostasis and coagulation. The life span of a platelet is about 10 to 12 days on average. The phenotype of the platelets in circulation alters as hormone levels change over the life span, largely because they reflect genomic influences during development from megakaryocytes in the bone marrow. In a study of women with a family history significant for coronary artery disease (CAD), younger women had less fibrinogen binding to platelets compared to women who were older than 48 years of age [8]. This suggests a possible linkage between hormonal status and platelet activity since the average age of menopause is about 53 years. Additionally, there is a lower incidence of CVD among premenopausal women compared to their male counterparts of similar age. However, the incidence of thrombosis increases exponentially at menopause. It is implied that the sex steroid hormone estrogen is deeply related to the functions of platelets [8].

There are a large variety of transmembrane receptors located on the surface of platelets. These receptors include glycoprotein toll-like receptors, integrins ($\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_{IIb}\beta_3$, and $\alpha_V\beta_3$), leucine-rich repeated receptors, seven-membered G protein-coupled receptors, immunoglobulin superfamily proteins (GP VI, FcγRIIA), tyrosine kinase receptors, C-selectin receptors, and various other types (**Figure 2**) [7].

Glycoprotein IIb/IIIa (GP IIb/IIIa) is one of the many integrin receptors located on the surface of platelets. It is also one of the most abundant receptors on platelets with roughly 80,000 receptors per platelet, composing about 15% of total surface protein. In terms of structure, GP IIb/IIIa is a transmembrane heterodimer composed of α_2 and β_3 subunits. This receptor is responsible for platelet aggregation and plays a crucial role in hemostasis. Therefore, GP IIb/IIIa is a major target for numerous pharmaceutical companies in the development of antiplatelet medications [9]. Inactive platelets have GP IIb/IIIa receptors that have decreased affinity for their ligands, including fibrinogen, von Willebrand factor (vWF), and prothrombin. Upon platelet activation, GP IIb/IIIa can bind vWF, fibrinogen, and prothrombin [7].

For platelet aggregation to occur, platelet adhesion to the vessel wall is necessary. GP IIb/IIIa binds irreversibly to the immobilized fibrinogen, allowing time for platelets to stabilize and adhere to the vessel wall. Once bound to the vessel wall, platelets recruit and bind to other platelets, forming a hemostatic plug [5]. Platelet aggregation is affected by shearing forces that result from the high velocity of blood flow in coronary vessels. At shear rates of 1,000 to 10,000 s^{-1}, platelets are capable of binding to one another via vWF receptors. At shear rates greater than 10,000 s^{-1}, platelet aggregation may occur without activation if soluble vWF is present. However, without platelet activation via the GP IIb/IIIa receptor, platelets will be unstable [5].

The activation of GP IIb/IIIa is tightly regulated through outside-in and inside-out signaling to prevent uncontrolled platelet aggregation and unwanted thrombus formation [10]. Platelet activation is caused by various factors such as thromboxane A_2, ADP, subendothelial collagen, and thrombin [9]. All of these triggering agonists result in an increased intracellular calcium concentration ($[Ca^{2+}]_i$) via Ca^{2+} release from intracellular stores and an influx of Ca^{2+} from the extracellular environment through channels in the plasma membrane. Increased $[Ca^{2+}]_i$ initiates and organizes the steps of cellular activation of platelets such as shape change and inside-out activation of the GP IIb/IIIa receptor [11].

During inside-out signaling, adhesion receptors and GP IIb/IIIa receptor agonists such as ADP and thrombin activate cytoplasmic proteins that interact with the cytoplasmic tails of GP IIb/IIIa. The cytoplasmic tails (containing α_2 and β_3 subunits) are connected by electrostatic and hydrophobic bonds, which must be completely disrupted for the full activation of the receptor. The displacement of these bonds is facilitated by talin-H, which then displaces the bonds holding the α_2 and β_3 subunits together [5]. Displacement of the α_2 and β_3 subunits results in exposure of binding sites that are further involved in platelet activation. The clustering of integrins via various ligands and activation-

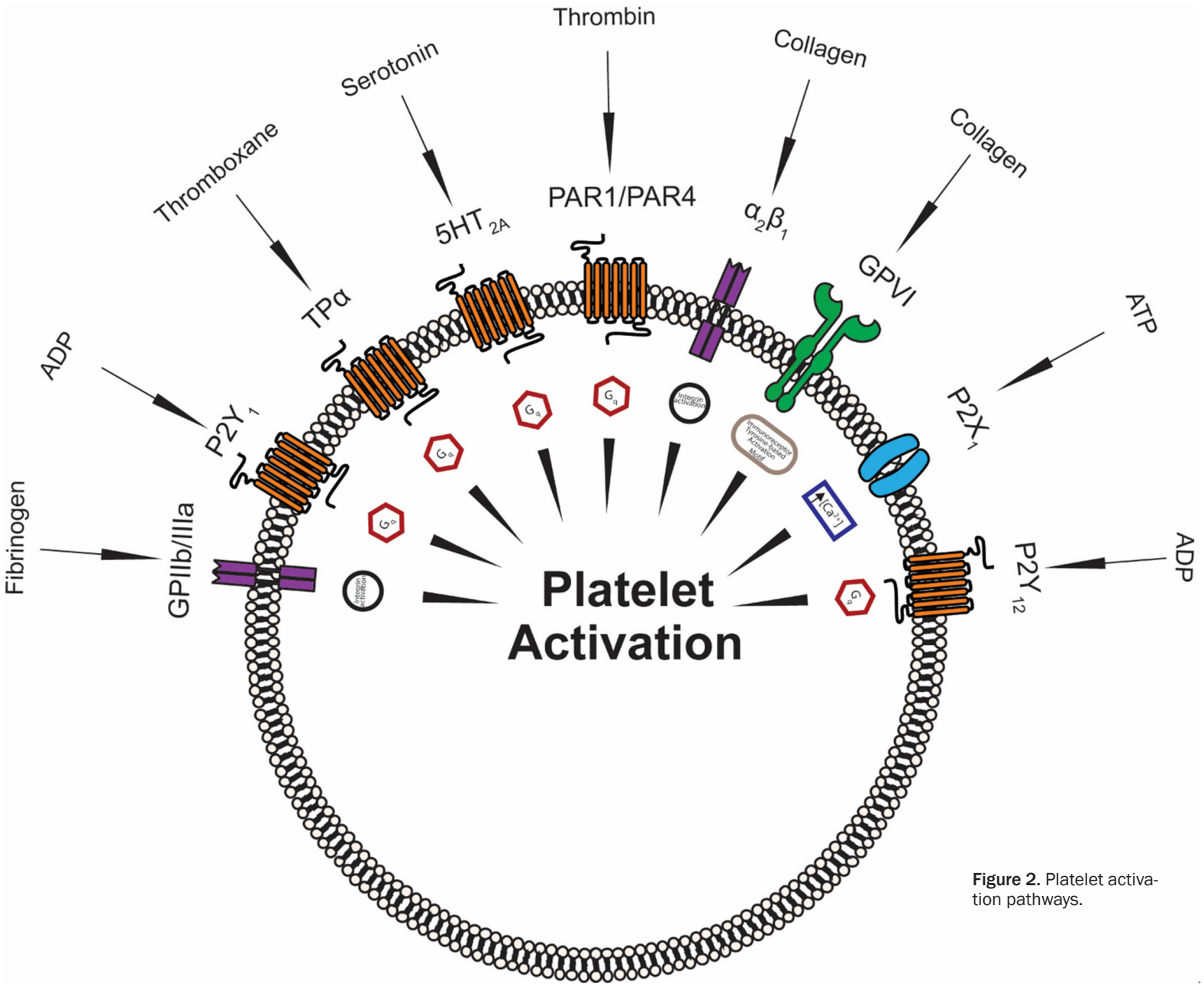

Figure 2. Platelet activation pathways.

dependent mechanisms is the start of outside-in signaling. The density of integrins increases near a soluble ligand at the platelet's surface and leads to the clustering and activation of tyrosine kinases, thereby allowing for platelet adhesion via phosphorylation of tyrosine kinase. The successful completion of inside-out and outside-in signaling enables platelets to form cross-linkages between themselves and surrounding platelets via fibrinogen, allowing for long chains of platelets to form [5, 12].

The effect of estrogen and estrogen receptors on platelets

The inhibition of the platelet receptor GP IIb/IIIa located on the surface of platelets has been an important target for pharmaceutical entities attempting to combat CVD [9]. As adhesion receptors and soluble G protein-coupled receptor agonists promote the activation of platelets, prostacyclin (PGI_2) and nitric oxide (NO) promote the inactive state via cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) production, respectively [5]. Estrogen has been shown to have an anti-atherosclerotic effect by modulating platelet activity through the release of vasodilatory substances such as NO [13]. This may in part explain the higher rates of adverse cardiovascular events seen with estrogen depletion in postmenopausal women.

Numerous studies have indicated that the sex hormone estrogen and hormone replacement therapies might also inhibit activation of the GP IIb/IIIa receptor, thereby reducing platelet aggregation. In a study carried out by Nakano, it was determined that HRT in the form of CEE did reduce the activity of platelets stimulated by thrombin by inhibiting Ca^{2+} influx and increasing cAMP [14]. Flow cytometry studies showed no significant change in GP IIb/IIIa expression in women who were treated with CEE. After treatment with CEE, there was a noticeable reduction in thrombin-induced Ca^{2+} influx, whereas the baseline $[Ca^{2+}]_i$ and thrombin-induced release of calcium from platelets' internal stores remained unchanged. This result is consistent with numerous other studies indicating that 17-β-estradiol can inhibit Ca^{2+} entry from the extracellular space. After four weeks of treatment in this HRT study, cAMP levels increased dramatically, but the cGMP levels increased minimally. These results suggest that the suppressed function of platelets and cardiovascular benefit of HRT is conveyed predominantly by cAMP [10].

Many studies have implied that the beneficial effects of HRT may be dependent on the type of hormones used. A 2005 study analyzed the direct effects of HRT utilizing washed platelets to analyze the intrinsic effects of the therapy on platelets. The results showed that HRT (in the form of CEE) in ovariectomized mice increased platelet sensitivity to collagen peptides [15]. At least 10 different estrogens (17-β-estradiol, estrone, 17-α-estradiol, equilin, etc.) have been identified in CEE formulations, with the three most common being estrone, equilin, and 17-α-dihydroequilin. All CEE are biologically active even in the unconjugated form and are capable of binding to recombinant human estrogen receptors alpha (ERα) and beta (ERβ). The affinity for estrogen to interact with the ER is variable depending on the form of estrogen. E2 along with 17-β-dihydroequilin binds strongest to these receptors [9]. It has been postulated that the CEE formulations used have a profound effect on any potential benefits of HRT.

Another study on platelet activity in postmenopausal women receiving estrogen therapy has hinted at some possible beneficial effects of the treatment. This study included the observation of platelet aggregation and release of adenosine triphosphate (ATP) before and after treatment with CEE and progestogen in 51 postmenopausal women. The results were then compared with untreated patients. The outcome of the study was a significant reduction in adrenaline-induced platelet aggregation after the initiation of estrogen therapy in the estrogen-progestogen group [16]. These results suggest that estrogen therapy may inhibit the process of atherosclerosis through the suppression of platelet function [11].

Estrogen has been shown to act in a non-genomic manner using a multitude of ERs. In-vitro studies have indicated that E2, although at much higher levels than seen physiologically, inhibited platelet aggregation [15]. Despite this in-vitro effect, E2 at physiologic concentrations has been shown to increase intracellular Ca^{2+} and lower the threshold for GP IIb/IIIa activation via ERs. This effect seems to be lost after prolonged exposure to physiologic concentra-

tions of estrogen [15]. According to a study conducted using animal models including rabbits and pigs, platelet activity was shown to decrease after exposure to exogenous E2 [8]. In addition, another study on mice compared the effect of endogenous and exogenous estrogens on platelet activity [2]. Prolonged E2 therapy was shown to inhibit platelet aggregation and decrease the risk of thromboembolism, while naturally occurring estrogens did not interfere with the function of platelets [2]. Furthermore, it has been shown that chronic hormone therapy with E2 significantly decreases platelet aggregation and increases bleeding time. Platelets from hormone-treated mice also failed to aggregate when exposed to thrombin [17]. Stachowiak et al. compared two groups of postmenopausal women, one of which received estradiol and progesterone, while the other received estradiol, progesterone, and low-dose aspirin [18]. Their study has demonstrated that after three months, the applied hormonal therapy resulted in a decrease in platelet count ($P = 0.004$) as well as a decrease in the expression of GP IIb/IIIa ($P = 0.022$. There were no statistically significant changes in the studied hemostatic parameters in the second group of postmenopausal women with the addition of low-dose aspirin [18].

However, other studies have reported prothrombotic effects of endogenous and exogenous estrogen on platelet activity. In a study by Jayachandran et al. which grouped postmenopausal women by high (>40 pg/mL) or low (<20 pg/mL) endogenous estrogen levels, the high-estrogen group demonstrated greater GP IIb/IIIa expression [19]. No significant difference was found in platelet counts or P-selectin expression on platelet membranes, an important mediator of platelet rolling and adhesion to endothelial cells in sites of inflammation or injury [20]. However, the low-estrogen group had higher levels of circulating microparticles from platelets, granulocytes, monocytes, and endothelial cells [19]. Platelet-derived microparticles (PMPs) in particular, released from activated platelets, can promote inflammation and thrombosis by inducing the release of inflammatory mediators such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) from monocytes and by expressing tissue factors which activate Factor VII and subsequently the extrinsic coagulation pathway, among other mechanisms [21, 22]. PMPs have been found in higher levels in patients with atherosclerosis [23-26]. This may in part explain the increased risk of CVD and adverse cardiovascular events seen in postmenopausal women. Other underlying causes that have been suggested by studies include higher levels of P-selectin expression on the platelet surface as a marker of increased platelet activation in postmenopausal women compared to premenopausal women [27, 28]. This may increase the risk of atherosclerosis and thrombogenesis after menopause. Platelet activity seems to be sensitive to hormonal changes, which may in part be explained by the presence of ERs on platelet membranes [29].

Conversely, one study reported lower levels of P-selectin as well as GP IIb/IIIa expression in postmenopausal women at baseline compared to premenopausal women, even after adjusting for potential confounding variables in cardiac risk [13]. Gu et al. reported a significant decrease in platelet P-selectin expression after six months of HRT [28], while another study demonstrated increased levels of P-selectin after three months of HRT [30]. A more recent study by Miller et al. showed no change in platelet P-selectin levels after two years of HRT [31]. The conflicting results regarding the effects of estrogen therapy on platelet activation may be due to variation in the route and formulation of HRT used as well as the timing of P-selectin measurement.

A 2021 study by Kim et al. revealed another mechanism by which estrogen therapy may affect platelet activity. Human umbilical vein endothelial cells treated with 17β-estradiol demonstrated increased release of vWF and interleukin-8 (IL-8) from Weibel-Palade bodies mediated by the nuclear ERα [32]. Additionally, platelet adhesion to the endothelial cells was increased after E2 treatment [32]. These are key steps in promoting vascular inflammation and thrombus formation.

Positive clinical effect of early estrogen replacement

The reason for the lack of clinical benefit of HRT in large clinical trials has remained elusive despite many basic science and clinical studies demonstrating estrogen-related antiplatelet activity. However, newer studies have elucidat-

ed other mechanisms by which estrogen may affect platelet activity with some conflicting results. It is suspected that the timing and type of estrogen replacement therapy may play a role. The Heart and Estrogen/progestin Replacement Study (HERS) (n = 2,763) showed that postmenopausal women who received CEE plus medroxyprogesterone acetate (MPA) had no significant difference in coronary heart disease (CHD) risk compared to the placebo group. Post hoc analyses revealed a higher incidence of CHD events compared to the placebo during the first year of hormone therapy and a lower incidence in years three to five [33].

In the Women's Health Initiative (WHI), another randomized controlled trial (n = 16,608), postmenopausal women who received CEE with MPA had an increased risk of CHD than those who received a placebo [33]. Secondary analysis by Rossouw et al. showed that women who started on the therapy ≥10 years after menopause had a higher risk of coronary heart disease than the placebo group, while the <10 years postmenopausal subgroup had a lower risk [34]. Similarly, analysis of WHI data by Toh showed that CEE plus MPA increases the risk of CAD except in women who started on the therapy closer to menopause and continued using it for greater than six years [4]. Furthermore, in the Early versus Late Intervention Trial with Estradiol (ELITE), there was a lower mean increase in carotid artery intima-media thickness (CIMT) per year in women who initiated oral 17β-estradiol therapy within six years of menopause compared to women who had menopause more than 10 years prior [3].

In regards to the type of estrogen replacement therapy, analysis of the multi-center Kronos Early Estrogen Prevention Study (KEEPS) suggested that oral CEE in recently postmenopausal women may slow the accumulation of epicardial adipose tissue while transdermal 17β-estradiol may increase the progression of coronary artery calcification [35]. However, a 2014 population-based case-control study of postmenopausal women reported that oral CEE use was associated with an increased risk of venous thromboembolism and possibly myocardial infarction compared to oral estradiol use, albeit less than the control group [36]. Overall, these studies suggest the beneficial effect of hormone therapy if started early. This positive effect of estrogen and estrogen replacement therapy on cardiovascular disease could in part be explained by the estrogen-mediated decrease in GP IIb/IIIa expression and platelet activity as discussed above.

Based on clinical experience, postmenopausal patients who are not taking estrogen are at higher risk for myocardial infarction and other adverse cardiac events. This effect could in part be related to the effect of estrogen on platelets in addition to the positive effect of estrogen on the lipid profile. The importance of starting estrogen early after menopause to have the most positive effect on cardiovascular disease is an important point that needs further investigation.

Conclusion

Estrogen has been shown to reduce platelet activity. Studies have suggested that hormone replacement therapies may inhibit activation of the GP IIb/IIIa receptor, thereby reducing platelet aggregation, explaining in part the beneficial effect of estrogen replacement therapy on cardiovascular disease if started early as found in many clinical trials. Although some studies have reported pro-thrombotic effects of estrogen therapy such as increased platelet activation and adhesion, there is an opportunity for usage of estrogen therapy in the early postmenopausal period with subtle changes in the formulation and route. The effect of estrogen on platelet activity as one of the important factors in reducing cardiovascular events is promising, warranting further investigation.

Disclosure of conflict of interest

None.

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