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Endothelial biology in the post-menopausal obese woman

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Abstract

Women generally have a reduced risk of cardiovascular disease (CVD). However, this protection of gender diminishes rapidly after menopause and with advancing age, particularly in obese women. Alterations in vascular function are thought to a key early step in the development of atherosclerosis. In this review, we will describe the features of endothelial dysfunction in the post-menopausal obese female and discuss the interplay of aging, estrogen withdrawal, and obesity. The objectives include (1) a review of endothelial biology and endothelial dysfunction, and (2) a discussion how the endothelial function is altered in the context of aging, hormonal changes and insulin resistance. The clinical consequences of endothelial dysfunction and CVD will also be reviewed.

Keywords

Endothelial function; nitric oxide; vasodilation; menopause; gender; vascular function; cardiovascular disease; obesity; aging; metabolic syndrome

Introduction

Women have a lower risk of cardiovascular disease (CVD), as compared to their male peers. However, this protection is lost after menopause and with advancing age, particularly in obese women. Cardiovascular (CV) mortality accounts for 37% of deaths in women.[1] Perturbations in endothelial biology and function are thought to a key early step in the development of atherosclerosis. Advancing age and estrogen withdrawal mediate changes in endothelial function. Endothelial function may also be adversely affected by adiposity. The incidence of obesity is rising in older adults; obesity affects one-third of middle age (40–59 years) and older adults (>60 years) in the US.[2] Additionally, increase in adiposity is strongly linked to metabolic syndrome (MetS) and obstructive sleep apnea (OSA), both of which may contribute to endothelial dysfunction. While age, menopause, obesity, MetS, and OSA are associated with an increase incidence of traditional CV risk factors, there is evidence that

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these clinical states may independently modify the risk of endothelial dysfunction and CVD beyond the traditional risk factors.

In this review, we will address endothelial dysfunction in the post-menopausal obese female and discuss the interplay of aging, estrogen withdrawal, and obesity with its associated metabolic changes. The objectives include (1) review of endothelial biology and endothelial dysfunction, and (2) discuss how the endothelial function is altered with healthy aging, menopause, with obesity, MetS, and OSA. Correlations between endothelial dysfunction and CVD will be highlighted.

Endothelial biology: Definition and measures of endothelial function

Endothelial cells play an important role in vascular function. They function to regulate and modify vascular tone, permeability, inflammation, thrombosis versus fibrinolysis, platelet and leukocyte adhesion, angiogenesis, and atherosclerosis progression. One of the critical mediators of endothelial function is nitric oxide (NO), which is the primary endothelial-derived autocoid. NO promotes vasodilation, inhibition of leukocyte adhesion and platelet aggregation, and inhibition of smooth muscle proliferation.

Many methods have been developed to evaluate and quantify endothelial function.[3] The endothelial regulation of blood flow represents the most reliable approach to study NO availability. Endothelial-dependent vasodilation (EDV) is most commonly assessed by performing vascular reactivity studies in response to flow-mediated dilation (FMD) of the brachial artery or by pharmacologic stimulation with intra-arterial infusions of acetylcholine. Other vascular reactivity studies include venous occlusion plethysmography assessment of forearm microcirculation and videocapillaroscopy assessment of nail-fold microcirculation. Smooth muscle mediated vasodilation is independent of the endothelium and is studied by administration of NO donors, such as nitroprusside. Alternatively, endothelial activation can be characterized by an increase in endothelial-derived inflammatory markers and circulating adhesion molecules such as soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion moledule-1(sVACM-1), E-selective, and C-reactive protein (CRP).

The loss of normal endothelial function, termed endothelial dysfunction, is thought to be the early step in the development of atherosclerosis and occurs prior to any structural changes in the arterial wall. The primary mechanisms of endothelial dysfunction include decreased production and availability of NO, increase in the inactivation of NO by reactive oxygen species (ROS), and reduced availability of vasodilators (prostacyclin and endothelium-derived hyperpolarizing factor) and increase in vasoconstrictors (endothelin-1, angiotensin II).[3]

Endothelial damage can be assessed by measuring markers such as von Willebrand factor (vWF), tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), circulating mature endothelial cells, endothelial progenitor cells, and endothelial microparticles. Measures of endothelial dysfunction should be differentiated from measures of structural damage to the endothelium. Measures of structural changes in the vasculature include carotid intimal medial thickness (IMT) or pulse wave velocity (PWV), which reflects arterial stiffness,

Evidence of endothelial dysfunction is present many years before the onset of coronary disease, peripheral arterial disease, or cerebral vascular disease.[4] It is also a prominent feature of traditional CV risk factors (diabetes (T2DM), hypertension, dyslipidemia, tobacco use) and it is associated with poor prognosis in patients with known cardiovascular disease (CVD).

Apart from the vasodilatory response of the endothelium, our group and others have focused on arterial t-PA release as an important marker of endothelial function.[5] In the arterial vasculature, t-PA in stored in small, dense granules present in the endothelium, and is released rapidly in response to specific biochemical stimuli, including bradykinin and substance P. Much like vasodilator function, arterial t-PA release in impaired by common CV risk factors, including cigarette smoking, hypertension, obesity and T2DM.[6] Again, similar to vasodilator function, stimulated t-PA release predicts risk of future CV events in patients with coronary artery disease.[7] Bradykinin is felt to be a major regulator of endogenous t-PA release. Since angiotensin converting enzyme (ACE) inhibitors block the degradation of bradykinin, it is not surprising to note that the administration of ACE inhibitors augments bradykinin-induced t-PA release in humans. Interestingly, ACE inhibition appears to enhance basal t-PA release in women but not in men.[8]

Aging and endothelial biology

Despite the epidemiologic evidence of the strong association of advancing age with CVD and traditional CV risk factors, there is likely an independent relationship between age and progressive changes in the vasculature. There is an age-related deterioration in endothelial function that is distinct from atherosclerosis. This topic has been reviewed by Viridius et al in Maturitas April 2010.[9] In brief, advancing age is associated with a decline in endothelial function. There is a reduction in NO availability and an increase in the formation of ROS. This is coupled with a reduction in the regenerative capacity of endothelial cells and an increase of endothelial cell apoptosis.

In the absence of CVD, advancing age is associated with a decrease in acetylcholine induced EDV, but age is not associated with nitroprusside induced endothelial-independent vasodilation.[10] In healthy adults, FMD to reactive hyperemia progressively declines with age ($r=-0.34$, $p<0.0001$), with 10 year delay in women as compared to men.[11] FMD was stable in women until their early 50's but declined 0.49%/year thereafter. In men, FMD declined progressively starting at age 40 years with 0.21%/year decline. These findings have been confirmed in the Framingham Heart Study.[12]

The delayed onset and more rapid progression of endothelial dysfunction in women suggest a biologic effect of estrogen withdrawal and menopause. In a cohort study comparing subjects at age 35 and 55 years, there was no significant difference in FMD or NTG-induced vasodilation in men between the two age groups. In comparison, older women had a significantly reduced FMD as compared to younger women (2.6% (SD 2.3) vs. 5.7% (SD 3.5) $p<0.001$).[13] Even in premenopausal women, there is evidence that endothelial dysfunction declines with age. In an Australian study of adults age 18–89 years old of which 22% were women younger than 30 years, capillary refill time increased by 3.3% for each 10 years after the third decade.[14] Starting in the fourth decade, there is a progressive reduction in endothelial NO production.[15] On a structural level, advancing age is associated with remodeling of vascular wall. There is luminal enlargement, thickening of the intima and media, and an increase in vascular stiffness.

In healthy aging, there is evidence of a decline in endothelial function and a change in structural properties of the vascular wall which is seen in both men and women. In women, these age-related vascular changes are altered by menopause.

Menopause, estrogen withdrawal, and endothelial biology

It is well established that pre-menopausal women have a lower incidence of CV risk factors and CVD than their male peers.[16] As women age, changes in estrogen levels and activity are important mediators of CV risk.

Estrogen modifies endothelial function through two primary mechanisms: (1) modulation of NO activity and (2) through attenuation of vascular response to injury.[17] First, estrogen promotes vasodilation through stimulation of eNOS and a decrease in NO synthase activity.[18] In men, administration of an agent that reduces the conversion of testosterone to estrogen is associated with a reductionin FMD of the brachial artery.[19] In post-menopausal women, administration of exogenous estrogen provokes acute vasodilation, in a dose-dependent and endothelial-dependent fashion.[20] In the absence of T2DM and CVD, estrogen administration was associated with an increase in FMD of the brachial artery (8.0± 3.9% vs. 15.1± 4.0%, $p<0.05$).[21] Second, estrogen acts at the level of the mitochondria in the vascular endothelium to promote oxidative phosphorylation and reduce mitochondrial production of ROS.[18] Beyond the effect of ROS on vasodilation, estrogen's actions on the mitochondria may mediate age-related changes in the vascular endothelium. A decrease in ROS may reduce the accumulation of ROS-induced mitochondrial DNA mutations which are thought to be associated with age-related disease.[22] The effect of estrogen on mitochondria provides some hypothetical arguments for administration of HRT early after menopause.

Estrogen receptors mediate estrogen action in the peripheral circulation. ER-α is the major mediator of the atheroprotective effect of estradiol and it promotes re-endothelialization after arterial injury. Variability in estrogen receptors alters endothelial function and the risk of CVD. Polymorphisms of the estrogen receptor, ER-α, are associated with attenuation of FMD of the brachial artery, an increase in arterial stiffness in older women, and are associated with premature CAD.[18] Interestingly, polymorphisms of ER- α alter the relationship of HRT with CV risk factors and CVD. With advancing age and methylation of the estrogen receptor, there is a decrease in the activity level of the estrogen receptor.[23]

Endothelial response to estrogen is modified with advancing age. For instance, the acute response to estrogen on stimulating eNOS declines with age.[24] There is also evidence of marked endothelial dysfunction with a decrease in FMD, an increase in carotid IMT, and an increase in LDL cholesterol. Effects are seen within months of estrogen withdrawal.[18]

Similarly, the association of estrogen with CVD is modified by age. Endogenous estrogen is protective against CVD in premenopausal women, Age-associated risk of CVD in women lags 10 years behind that of men, which is similar to the relationship that is seen in endothelial dysfunction.[25] Following menopause, there has been demonstration of active progression of atherosclerotic lesions.[26]

It is difficult to quantify the independent contribution of age or time since menopause to the development of endothelial dysfunction. In a population of post-menopausal women (mean age 53.5±7.8 years, mean time since menopause or HRT 5.3±5.4 yrs) with median Framingham risk score 1%, videocapillaroscopy of red blood cell velocity (RBCV) demonstrated an age-dependent decrease in baseline RBCV ($r=-0.27$ $p=0.02$) and in RBCV response to reactive hyperemia ($r=-0.29$, $p=0.02$).(23) Notably, a similar relationship was seen for time since menopause (baseline $r=-0.32$, $p=0.01$, reactive $r=-0.26$, $p=0.03$).

There is some evidence that exogenous estrogens reduce menopause-associated changes in endothelial function.[26] Post-menopausal women on HRT have an increased FMD response to estrogen as compared to post-menopausal women who are not taking HRT.[27] In study of post-menopausal women without CV risk factors (mean age 58 years, range 53–65), women not using HRT had impaired microvascular reactivity, while postmenopausal women on HRT (mean age 53 years, range 43–58) had similar endothelium dependent and endothelium independent vasoreactivity to premenopausal women.[28] Interestingly, the admisntistration of 17b-estradiol increases basal but not bradykinin-stimulated release of t-PA in young post-menopausal women.[29]

In the post-menopausal woman with increasing risk of CVD, it is difficult to separate the effect of vascular aging from the effect of estrogen withdrawal since biologic age and time since menopause are intrinsically linked. Epidemiologic studies suggest that estrogen protect women against heart disease. The Framingham Heart Study and the Nurses Health Study provided strong observational evidence that menopause and lack of endogenous estrogens are associated with an increased CVD risk.[25] In observational studies, postmenopausal women who used HRT soon after menopause for 10 or more years showed a 40–50% reduction in CV mortality.[30]

This benefit was not replicated in large randomized controlled trials of HRT in older postmenopausal women. Instead HRT use was associated with an increase in CV events.[31] The Women's Health Initiative (WHI) was a double blind prospective multicenter study of more than 27,000 American post-menopausal women (mean age 63, 13 years of hormone deprivation after menopause) and demonstrated no CV benefit with HRT. The hazard ratio for CVD was 0.95 (95% CI 0.70–1.16) for conjugated equine estrogens and 1.24 (95% CI 1.00–1.54) for conjugated equine estrogens plus medroxyprogesterone.[32]

Notably, in a secondary analysis of the WHI, there was a higher CV risk in women who began HRT >20 years after menopause. Conversely, there was a CV risk reduction seen in women age 50–59 years who were <10 years from menopause.[33] This hypothesis-generating analysis suggests that CV risk may be associated with time since menopause and duration of time without estrogen. As a secondary endpoint in an osteoporosis trial of 1458 post-menopausal women age 55±6 years randomized to HRT for 2–3 years with almost a decade of follow-up, the HRT arm had 46% reduction in CV mortality ($p<0.0001$).[34]

It has been proposed that if estrogen is given to an already damaged endothelium, its benefits may be diminished and its prothrombotic effects may predominate, thus promoting CVD rather than preventing it.[35] The endothelium may be considered damaged in the setting of advancing age, prolonged time since menopause or estrogen exposure, or known CVD or endothelial dysfunction. This paradox may offer a therapeutic target. In post-menopausal women treated with HRT who are stratified by time since menopause, FMD increased after estrogen administration, but amount of vasodilation was dependent on years since onset of menopause.[18] Endothelial function, as representative of the earliest stage of vascular disease, may represent a therapeutic decision point. Given that (1) endothelial function declines with age and withdrawal from estrogen, (2) estrogen maintains endothelial function, and (3) temporary break from estrogen may have detrimental effect, there may be a role for evaluation of endothelial function at menopause to identify women who may derive a CV benefit from HRT. Theoretically, early administration of estrogen may prevent down-regulation of and desensitization of estrogen receptors.

Obesity and endothelial biology

Obesity may be another key contributor to endothelial dysfunction in the post-menopausal woman. Weight gain is accelerated at menopause. Decline in estrogen secretion is associated with an increase in abdominal fat, which is associated with an increase in CV risk.

The WHO estimates estimate 1 billion people worldwide are overweight (BMI>25 kg/m2), of which 300 million are obese (BMI >30 kg/m2).[36] Both conditions are associated with an increase in CVD.[37] It is unclear if this association with CVD is directly caused by adiposity alone or if it is mediated by CV risk factors, such as hypertension, insulin resistance, and T2DM, which are strongly associated with obesity.

Obesity had traditionally been viewed as a passive increase in adipose tissue and body weight. Emerging evidence has re-conceptualized adipose tissue as a metabolically active

with capacity to promote inflammation.[38] There is direct evidence that adiposity is associated with an increase in biomarkers of endothelial inflammation and vascular oxidative stress.[39] Adipocyte activity executed through a number of mediators including leptin, adiponectin, and other adipokines, chemokines and cytokines.[40]

There is a hypothetical direct link between adipose tissue and vascular disease. Adipocyte activity may help drive and perpetuate a chronic sub-inflammatory state in obese individuals and contribute to endothelial dysfunction and to the development of CVD. In healthy individuals, reduction in FMD and acetylcholine-induced vasodilation, both dependent on normally functioning endothelium, is associated with increasing BMI, visceral/abdominal obesity, and the obese state.[41] Studies have demonstrated that peri-vascular fat deposits may alter local cytokine production and alter blood flow regulation.[42] In addition, insulin resistance and increased insulin level may alter NO/EDV. In overweight individuals, a lower baseline tissue perfusion has been linked to higher vasomotor tone.[43]

Recent studies have documented that weight loss, either through bariatric surgery or diet and exercise, is associated with an improvement in endothelial dysfunction. Weight loss is associated with an increase in EDV and a fall in inflammatory markers.[4445] Sustained weight loss of a modest 5–10% of body weight is associated with marked improvements in CV risk.[46]

Metabolic syndrome and endothelial biology

The metabolic syndrome is defined by the presence of central obesity, impaired fasting glucose (IFG), dyslipidemia, and hypertension[47], all of which can impair endothelial function.[12414849] The prevalence of MetS in the United States is 34.6% and in Europe is 17.8–30.4%.[5051] Its prevalence increases in overweight and obese individuals. Importantly, MetS is associated with increased CVD morbidity and mortality.[52] The high prevalence of metabolic syndrome (MetS) and obstructive sleep apnea (OSA) in obese individuals makes it challenging to assess the relative contributions of adiposity, MetS, and OSA to endothelial dysfunction in obesity.

Several studies of persons with MetS have described impairment in EDV, as assessed by acetylcholine administration and FMD.[535455] In the Framingham Offspring Study and in the PIVUS Study of elderly individuals, an increasing number of components of MetS is associated with progressively lower vasodilator function and more severe impairment in endothelial dysfunction.[5356] Central adiposity may be considered a driver of endothelial dysfunction in this population. In the PIVUS Study of 1016 elderly subjects, waist circumference was independently and more strongly associated with endothelial function, then other components of Mets, including blood pressure and triglycerides. [56]

MetS is associated with alterations in circulating adipokine levels with lower adiponectin and higher leptin levels.[5557] Alterations in adipokine levels appear to contribute to endothelial dysfunction.[58] When measuring circulating markers of endothelial dysfunction, MetS is associated with elevated plasma levels of sICAM [59], t-PA antigen[60], and PAI-1 antigen and activity.[60] In the context of MetS as a pro-inflammatory state, the level of CRP elevation is associated with greater impairment of FMD.[60] On a structural level, MetS is associated with an increase in carotid IMT and an increase in arterial stiffness, as measured by PWV.[61]

MetS, as a syndrome, and its individual components are associated with increased risk of endothelial dysfunction and CVD. Of the components of the MetS, central adiposity appears to be a strong driver of risk.

Obstructive sleep apnea and endothelial biology

OSA is also strongly associated with increasing weight. OSA is thought to affect 50–70% of overweight and obese individuals.[62] OSA is characterized as repetitive episodes of hypoxia and reoxygenation. Both obesity and OSA are associated with endothelial dysfunction, which predisposes to atherosclerosis. Additionally, both obesity and OSA are CV risk factors. OSA is associated with hypertension[63], ischemic stroke[64], and myocardial infarction[65].

Some part of the pathophysiological link between OSA and CVD is likely mediated through changes at the level of the endothelium, such as changes in NO bioavailability, increases in inflammation and oxidative stress, elevation in plasma levels of adhesion molecules, and reduction in endothelial repairative capacity.[6667] These markers of endothelial dysfunction are evident in the absence of CVD. It has been proposed that the repetitive episodes of hypoxia/reoxygenation may reduce endothelial NO production and increase ROS.[68] Additionally, there may be an increase in endothelial stress, as measured by nitrotyrosine expression.

In a trial of 71 obese patients undergoing sleep studies, OSA was associated with attenuation of eNOS expression/activity, increase in NFκB, a marker of inflammation, and a reduction in FMD. [69] Treatment with continuous positive airway pressure (CPAP) was associated with an improvement in markers of endothelial function.[69] In another study of 32 OSA patients with BMI-matched controls without CVD or traditional CV risk factors, subjects with OSA had at 59% reduction of endothelial expression of eNOS and 94% reduction in phosphorylated eNOS. There was a 5-fold higher expression of nitrotyrosine and COX-2. Similarly, abnormalities in markers of endothelial function and inflammation were significantly improved with CPAP treatment. In addition, FMD and levels of endothelial progenitor cells were lower in OSA subjects and increased with CPAP treatment.[70] In the obese patient, treatment of OSA may promote improvement in endothelial dysfunction, independent of weight loss.

Conclusion: clinical implications and unresolved questions

There are a number of factors which increase the likelihood of endothelial dysfunction, and thus CVD, in the post-menopausal woman. Age-related vascular changes coupled with the effect of estrogen withdrawal predispose to changes in endothelial biology. In the obese female, adiposity adversely affects normal endothelial function. Further, obesity is strongly associated with traditional CV risk factors, in addition to MetS and OSA.

Recognition of endothelial dysfunction offers an early stage for assessment of CVD risk and, theoretically, an early step for therapeutic interventions to reduce this risk. From randomized controlled trials, we have evidence that anti-oxidants and HRT fail to confer CVD benefit in populations that may already have a perturbed endothelium. At present, assessment of endothelial function is primarily a research tool. Hypothetically, its clinical utility may lie in evaluation of the state of endothelium in the peri-menopausal woman. It is unclear if early administration of HRT to individuals with normal endothelial function will confer benefit to prevent endothelial dysfunction or CVD. Theoretically, a similar hypothesis may be offered about early administration of anti-oxidants prior to the onset of vascular aging.

In contrast to aging and estrogen withdrawal, obesity with MetS, and OSA represent metabolic derangements which contribute to endothelial dysfunction and increased CVD risk in the post-menopausal obese woman. All of these are also associated with traditional

CV risk factors. Endothelial dysfunction can be improved with therapeutic lifestyle changes of diet, exercise, weight loss, in addition to pharmacologic therapy and CPAP.

The interplay of aging, estrogen withdrawal, and obesity with its associated risks contributes to changes in endothelial biology. A better understanding of perturbations in endothelial function along with advances in ways to promote healthy endothelium may decrease the burden of CVD in the post-menopausal obese female.

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Abbreviations

BMI	body mass index
CAD	coronary artery disease
CPAP	continuous positive airway pressure
CRP	C-reactive protein
CV	cardiovascular
CVD	cardiovascular disease
EDV	endothelial-dependent vasodilation
eNOS	endothelial nitric oxide synthase
ER	estrogen receptor
FMD	flow mediated dilation
HDL	high density lipoprotein
HRT	hormone replacement therapy
IFG	impaired fasting glucose
LDL	low density lipoprotein
MetS	metabolic syndrome
MI	myocardial infarction
NHS	Nurse’s Health Study
NO	nitric oxide
OSA	obstructive sleep apnea
PAI-1	plasminogen activator inhibitor-1
PWV	pulse wave velocity
ROS	reactive oxygen species
sICAM-1	soluble intercellular adhesion molecule-1
SMC	smooth muscle cells
sVACM-1	soluble vascular cell adhesion moledule-1
tPA	tissue plasminogen activator
T2DM	type 2 diabetes mellitus

vWF von Willebrand factor

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