REVIEW

MECHANISMS IN ENDOCRINOLOGY

Endogenous sex steroids and cardio- and cerebro-vascular disease in the postmenopausal period

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Abstract

Objective: Cardio- and cerebro-vascular diseases are two leading causes of death and long-term disability in postmenopausal women. The acute fall of estrogen in menopause is associated with increased cardiovascular risk. The relative contribution of androgen to this risk is also being recognized. The use of more sensitive assays for estradiol measurement and the study of receptor and carrier protein gene polymorphisms have provided some new information on the clinical relevance of endogenous sex steroids. We provide an update on the role of endogenous sex steroids on cardio- and cerebro-vascular disease in the postmenopausal period.

Design and methods: We performed a PubMed search using the terms ‘endogenous estrogen’, ‘androgen’, ‘cardiovascular disease’, ‘cerebro-vascular disease’, ‘stroke’, ‘carotid artery disease’, and ‘subclinical atherosclerosis’.

Results: The majority of studies show a beneficial effect of endogenous estrogen on the vasculature; however, there are a few studies reporting the contrary. A significant body of literature has reported associations of endogenous estrogen and androgen with early markers of atherosclerosis and metabolic parameters. Data on the relevance of endogenous sex steroids in heart disease and stroke are inconclusive.

Conclusions: Most studies support a beneficial role of endogenous estrogens and, probably, an adverse effect of androgens in the vasculature in postmenopausal women. However, the described associations may not always be considered as causal. It is possible that circulating estrogen might represent a marker of general health status or alternatively reflect the sum of endogenous androgens aromatized in the periphery. Elucidating the role of sex steroids in cardio- and cerebro-vascular disease remains an interesting field of future research.

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Introduction

Coronary heart disease (CHD) and stroke represent two leading causes of death and long-term disability in Western societies. Premenopausal women enjoy an innate protection that is lost at the menopausal transition, when their cardio- and cerebro-vascular risk increases to reach that of the male population. This has long been considered to be due to the abrupt fall of endogenous estrogen production. However, studies from the Women’s Health Initiative (WHI) evaluating the role of hormone replacement therapy (HRT) could not provide consistent evidence of a beneficial effect of HRT on primary and secondary cardio- and cerebro-vascular protection (1, 2). The timing of HRT initiation has been hypothesized as a factor modulating estrogens’ actions on the vasculature. Given the increased life span, women are becoming a growing body of the population to spend a

significant period at an age associated with increased cardio- and cerebro-vascular risk.

Older studies focusing on the role of endogenous estrogens in the postmenopausal period did not show a significant association between circulating estrogen and cardiovascular disease (CVD) or CVD risk factors (3, 4, 5). After the publication of the WHI studies and the negative findings of HRT on cardio- and cerebro-vascular risk, the importance of endogenous sex hormones in cardio- and cerebro-vascular disease was rather neglected. Lately, the development of ultrasensitive assays has allowed the measurement of endogenous estradiol with appreciable precision even at the very low levels seen in the postmenopausal period. This advent of the laboratory techniques has renewed the interest to expand our current knowledge and investigate the relevance of endogenous sex hormones in cardio- and cerebro-vascular disease.

In this paper, we attempt to provide a succinct clinical update of the current literature regarding the role of endogenous estrogen and androgen in women in the peri- and postmenopausal period. We performed a search in the PubMed database since 1990 using the terms 'estrogen', 'endogenous estrogen', 'androgen', 'CVD', 'cerebro-vascular disease', 'subclinical atherosclerosis', 'carotid artery disease', and 'stroke'. We included studies published in English assessing women in the peri- and postmenopausal period. Evaluating the effect of exogenous estrogens was beyond the scope of this review and, therefore, evidence from HRT studies is not included.

Risk of cardio- and cerebro-vascular disease

Several studies have evaluated the relationship of endogenous sex steroids with CVD, whereas fewer data are available for cerebro-vascular disease. Women experiencing an acute stroke are older compared with men. Stroke severity and mortality rates are higher in elderly women compared with men, possibly due to the increased prevalence of cardioembolic stroke associated with more severe neurological damage and degree of handicap (6, 7). In the prospective population-based study of Barrett-Connor & Goodman-Gruen (3) on 651 postmenopausal women, endogenous estrogen levels were not associated with the risk of developing CVD. Cauley *et al.* (8) studied 87 postmenopausal women undergoing diagnostic coronary angiography and found no significant difference in estrone levels between women with and without coronary artery disease (CAD). Similar results were reported by the Swedish study of Nilsson *et al.* (9) who evaluated 195 women aged 71–80 years followed for 8 years; no difference in estradiol levels was found with respect to the development of CVD.

Concerning cerebro-vascular disease, endogenous estradiol was recently identified as an indicator of stroke risk, especially in women with central adiposity (10). In a smaller case–control study on women with peripheral arterial disease, Price *et al.* (4) found no significant difference in estrogen levels between patients and the age-matched control group.

In the study of Chen *et al.*, 99 women with CVD were matched 1:2 for age with a control group. Although in the initial model estradiol levels were associated with higher risk for CVD, this relationship did not remain significant after adjusting for hypertension, body mass index (BMI), and lipid concentration (11). Likewise, Rexrode *et al.* (12) found similar estradiol levels in non-HRT users with CVD and the age-matched controls.

Concerning endogenous androgens, Barrett-Connor & Goodman-Gruen (3) did not find a significant association with the risk of developing CVD and the same was shown in another study on peripheral arterial

disease (4). In the above-mentioned study of Rexrode *et al.*, CVD patients had higher free androgen index (FAI) compared with the control group. However, after adjusting for BMI, hypertension, and other CVD risk factors, this association lost its statistical significance (12). A positive association between endogenous androgen and CVD risk was reported in two recent studies. In the cross-sectional study of Patel *et al.* evaluating 344 women aged 65–98 years, total testosterone was associated with a threefold greater risk of CHD. In addition, a significant association was found between total and free testosterone and an adverse metabolic profile, i.e. insulin resistance and abdominal obesity (13). In accordance were the findings of Page-Wilson *et al.*, who studied 200 nondiabetic postmenopausal women, 98 of them with CVD and 102 serving as matched controls. The FAI was significantly higher in women with CVD compared with controls and was also associated with HbA1c in the multivariate analysis (14).

The polycystic ovary syndrome (PCOS) is the most frequent endocrinopathy associated with hyperandrogenemia and CVD risk factors in women of reproductive age (15). Markopoulos *et al.* (16) and Puurunen *et al.* (17) recently reported that increased androgen production and a proinflammatory profile persist in PCOS women in their menopausal transition. In a meta-analysis by de Groot *et al.* (18), PCOS women were recognized to harbor a twofold increased risk for arterial disease (CHD and stroke) compared with non-PCOS subjects, independent of their BMI. Furthermore, Shaw *et al.* studied 390 postmenopausal women followed for 5 years; 104 of them presented clinical features of PCOS, i.e. irregular menstrual cycles premenopausally and current biochemical hyperandrogenemia. It was shown that 79% of women with features of PCOS experienced a 5-year CVD event-free survival compared with 89% of non-PCOS women, and this finding was independent of hypertension and diabetes status, as well as other CVD risk factors (19). However, there are also studies reporting similar CVD morbidity and mortality rates in PCOS subjects and control populations (20).

In contrast to studies linking androgens to an adverse CVD profile, there are data supporting an inverse relationship of endogenous androgen with carotid artery disease. In the case–control study of Debing *et al.* in 56 patients undergoing carotid artery endarterectomy and 56 age-matched controls, it was found that total testosterone and $\Delta 4$ -androstenedione levels were lower in the patients' group compared with the controls. The inverse association of circulating androgens with carotid atherosclerosis remained significant even after controlling for classical CVD risk factors (21). These results were reproduced in the same year by Montalcini *et al.* (22). Furthermore, Golden *et al.* (23) also showed a negative association

of total testosterone levels with the risk for carotid atherosclerosis.

It appears that the association of circulating sex steroid levels with CVD in the postmenopausal period is complex, and it is difficult to draw definite conclusions or prove an etiological relationship. In their population-based study on septuagenarian women with CVD, Naessen *et al.* (24) suggested that the increased aromatase index (estradiol to testosterone ratio) in the study population might represent a physiological response counteracting the vascular alterations in the atherosclerotic process. Furthermore, in a prospective, 12-year follow-up study on 639 postmenopausal women, Laughlin *et al.* (25) showed a U-shaped association of bioavailable testosterone with the development of CHD, where women at both low and high levels of testosterone were at a significantly increased risk of CHD. It might be the case that endogenous androgens, at a certain range, have a beneficial action on the female cardiovascular system. In another study, Braunstein *et al.* found that androgen and estrogen levels correlated closely in women with suspected CAD, even after adjusting for BMI and insulin resistance. This highlights the need to take into consideration and control for the independent effect of each sex steroid hormone (26).

In summary, the majority of reports in the current literature – briefly presented in Table 1 – do not support a significant link between endogenous sex steroids and the risk of CVD. It is clear that the androgenicity characterizing PCOS women even in the postmenopausal period appears to be an independent CVD risk factor; however, this is a more complex disease. It is possible that variation of hormonal levels from a certain range, either up- or downwards, is related to the development of atherosclerotic changes.

Severity and mortality of cardio- and cerebro-vascular disease

There are only a few studies evaluating the role of endogenous sex steroids on the severity and mortality in CVD and even fewer in cerebro-vascular disease. One of the first reports comes from Phillips *et al.*, who studied 60 postmenopausal women undergoing coronary angiography. The authors found a significant positive association of free testosterone levels with the degree of CAD (27). Our group has recently shown a positive association of Δ 4-androstenedione with stroke severity on admission in a population of 302 acute stroke women (28). In this study, Δ 4-androstenedione was also an independent predictor of short-term mortality, even after adjusting for traditional risk factors for stroke.

Furthermore, we showed an independent positive association of circulating estradiol with stroke severity, short-term mortality, and handicap in the same stroke patients' cohort (29). Except for the circulating levels, sensitivity to sex hormone actions as indicated from receptor gene polymorphism studies may also have some importance. For example, we recently reported a significant relationship of the androgen receptor and the estrogen receptor α gene polymorphism with the severity of CAD in postmenopausal women undergoing coronary angiography (30, 31).

With respect to CVD mortality, evidence in the current literature is rather contradictory and inconclusive. The prospective studies of Barrett-Connor & Goodman-Gruen (3) and Nilsson *et al.* (9) did not show an association of endogenous estrogen and androgen levels with CVD mortality. In the recent large study of Sievers *et al.* (32) assessing 2914 women followed for 4.5 years, it was demonstrated that women with the

Table 1 Cumulative results of studies investigating associations of endogenous estrogen and androgen levels with cardio- and cerebro-vascular disease risk in postmenopausal women (reference in parenthesis).

Reference	Study type	Number of participants	Result
(3)	19-year FU, population-based	651	No association of E and A with CVD risk
(8)	Case-control	87	No association of E with CHD risk
(9)	8-year FU, population-based	195	No association of E with CVD risk
(10)	Prospective case-control	415	Positive association of E with increased stroke risk
(4)	Case-control	90	No association of E and A with peripheral arterial disease
(11)	Prospective case-control	297	No independent association of E with CVD risk
(12)	Case-control	400	No independent association of E and A with CVD risk
(13)	Cross-sectional	344	Positive association of total testosterone with threefold higher CHD risk
(14)	Case-control	200	Positive association of A with CVD
(21)	Case-control	112	Inverse association of A with carotid atherosclerosis
(22)	Cross-sectional	101	Inverse association of A with carotid atherosclerosis
(23)	Case-control	364	Inverse association of total testosterone with carotid atherosclerosis
(25)	12-year FU, population-based	639	U-shaped association of A with CHD risk

FU, follow-up; E, estrogen; A, androgen; CVD, cardiovascular disease; CHD, coronary heart disease.

lowest testosterone levels are at increased risk of all-cause mortality and CVD, an association that was independent of traditional risk factors. An interesting finding comes from the population-based, 9-year follow-up study of Maggio *et al.* (33) on 509 women older than 65 years. In this study, higher estradiol levels were an independent determinant of all-cause mortality after adjusting for age, insulin resistance, inflammatory factors, and comorbidities, in line with our recent findings (29). The authors support the hypothesis that circulating estrogen might be part of an adaptive process or represent a marker of general health and metabolic status (33). A synopsis of the current literature on the associations of sex steroids with the severity and mortality of cardio- and cerebro-vascular disease is presented in Table 2.

The bioavailability of circulating sex steroids is regulated by the sex hormone-binding globulin (SHBG) concentration. Several studies have evaluated possible associations between CVD and circulating SHBG levels (12, 14, 23, 27). The majority of them has shown an inverse relationship, although this was significantly attenuated following adjustment for BMI and other CVD risk factors. Currently, there is increased interest in clarifying whether SHBG has a direct, independent association with CVD, as it is also strongly associated with insulin resistance. Studies on *SHBG* gene polymorphisms have shown an association of the *SHBG* gene promoter polymorphism with the severity of CAD, as well as early markers of atherosclerosis in postmenopausal women (34, 35). However, discussing studies on the clinical relevance of *SHBG* in cardio- and cerebro-vascular disease is not part of this review and the reader is referred to recent reviews (36, 37).

One could conclude that endogenous sex steroids might serve as surrogate markers of the severity of underlying disease and as a useful adjunct to identify earlier subjects at increased risk of an adverse CVD outcome. The observed associations of sex steroid levels with disease severity and mortality do not necessarily suggest a causative relationship but may represent the effect of the disease on the metabolic status.

Early markers of atherosclerosis

The ultrasonographic evaluations of carotid intima-media thickness (IMT), carotid-femoral pulse wave velocity (PWV), and flow-mediated dilation of brachial artery (FMD) are technically easy, cheap, and most importantly noninvasive and radiation-free methods to estimate arterial atherosclerosis, arterial stiffness, and early endothelial changes in otherwise healthy individuals. In the last decades, these methods have been widely studied as markers of early atherosclerosis.

In the study of Creatsa *et al.* (38) on 120 healthy postmenopausal women, estradiol levels were not related to the aforementioned vascular parameters, whereas Ouyang *et al.* (39) did not find a significant correlation of estradiol levels with common carotid IMT or coronary calcification. Similarly, in a subgroup of the Multi-Ethnic Study of Atherosclerosis (MESA) assessing 881 postmenopausal women, abdominal aortic calcification quantified by computerized tomography was unrelated to estrogen concentrations (40). Recently, in a cross-sectional study on 312 postmenopausal women, a negative correlation of circulating estradiol with PWV was reported, but this association was attenuated in the multivariate model (41). Munir *et al.* (42) reported similar findings in a cross-sectional study on 126 perimenopausal women; estrogen levels were not independently associated with the presence of coronary and aortic atherosclerosis. On the other hand, there are a few studies showing a favorable role of endogenous sex steroids on the vasculature. In a small case-control study on 33 women with CHD and 17 controls, Li *et al.* (43) reported a positive correlation of estradiol with FMD, and also in the multivariate model. Wildman *et al.* (44) showed an inverse relationship of estradiol levels with common carotid artery adventitial layer diameter, even after adjusting for age and other CVD risk factors, whereas no association was found with the other sex steroids. In the study of Jeon *et al.* (45) on 436 postmenopausal women, higher estradiol levels were independently related to a lower probability of having coronary artery calcification.

Table 2 Summary of studies investigating associations of endogenous estrogen and androgen levels with the severity and mortality of cardio- and cerebro-vascular disease in postmenopausal women (reference in parenthesis).

Reference	Study type	Number of participants	Result
(28)	Cross-sectional	302	Positive association of A with stroke severity and adverse outcome
(27)	Cross-sectional	60	Positive association of free testosterone with the degree of CAD
(29)	Cross-sectional	302	Independent positive association of E with stroke severity, mortality, and handicap
(3)	19-year FU, population-based	651	No association of E and A with CVD mortality
(9)	8-year FU, population-based	195	No association of E with mortality
(32)	4.5-year FU, prospective cohort	2914	Negative association of testosterone with all-cause mortality and CVD event
(33)	9-year FU, population-based	509	Independent positive association of E with all-cause mortality

A, androgen; CAD, coronary artery disease; E, estrogen; FU, follow-up; CVD, cardiovascular disease.

Concerning the evidence of endogenous androgens, in the study of Creatsa *et al.* (38), FAI was positively associated with PWV independently of age, smoking, and BMI. These findings agree with those of the large cross-sectional MESA study, where testosterone was independently associated with common carotid IMT after correcting for age, BMI, and insulin resistance. In the same study, there was no significant relationship of endogenous androgens with coronary calcification (39). In a small study on 26 postmenopausal women, FAI was positively related to acetylcholine doses for maximal vasodilation, a surrogate marker of early endothelial dysfunction (46). Few data support an association of endogenous androgens with improved endothelial function. In the study of Montalcini *et al.* (47) on 60 postmenopausal women, testosterone was independently positively related to FMD in the multivariate regression model. Additionally, an inverse association, independent of CVD risk factors, was demonstrated between $\Delta 4$ -androstenedione and free testosterone levels and carotid IMT in a study on 101 women, 48 of which were postmenopausal (48). It should, however, be noted that there are several reports that do not show a significant relationship between endogenous androgens and subclinical atherosclerosis. The study of Calderon-Margalit *et al.* (49) did not find any link between testosterone and free testosterone and the presence of coronary artery calcification or carotid IMT. Similarly, in the MESA study, endogenous androgens were not related to the

presence of aortic calcification (40). Furthermore, in the study of Akishita *et al.* (50), there was no significant association of sex steroid levels with the brachial FMD. Similar findings were recently reported by Munir *et al.* (42) concerning the association of androgen levels with the presence of coronary and aortic atherosclerosis.

Current literature, in its majority, suggests a favorable role of endogenous estrogens and an adverse role of androgens on the vasculature. However, there is a substantial body of clinical studies showing no significant association of endogenous sex steroids with early atherosclerotic changes, implying that if a role of endogenous estrogen and androgen levels in the atherosclerotic process is finally confirmed, its magnitude is modest. A summary of these studies is presented in Table 3.

Metabolic parameters

Coagulation–inflammation

The menopausal transition is characterized by an increase in inflammatory factors and proatherogenic molecules. With regard to the hemostatic system, Sowers *et al.* (51) have studied 3302 women and reported that lower estradiol levels were associated with higher levels of plasminogen activator inhibitor-1 and tissue plasminogen activator, whereas there was no significant correlation with fibrinogen, factor VII-c, and high-sensitivity C-reactive protein (CRP). At the same

Table 3 Summary of studies evaluating the association of endogenous estrogen and androgen levels with early markers of atherosclerosis in postmenopausal women (reference in parenthesis).

Reference	Study type	Number of participants	Result
Estrogen			
(38)	Cross-sectional	120	No independent relationship of E with cIMT and PWV
(39)	Cross-sectional	1947	No association of E with cIMT and coronary calcification
(40)	Cross-sectional	881	No association of E with abdominal aortic calcification
(41)	Cross-sectional	312	No independent association of E with PWV
(42)	Cross-sectional	126	No association of E with the presence and extent of coronary and aortic atherosclerosis
(43)	Case–control	50	Positive relationship of E with FMD
(44)	Cross-sectional	483	No association of E with cIMT, but inverse relationship with common carotid adventitial layer diameter
(45)	Cross-sectional	436	Higher E levels linked to 75% lower risk of coronary artery calcification
(50)	Cross-sectional	115	No association of E with FMD
Androgen			
(38)	Cross-sectional	120	Positive association of FAI with cIMT and PWV
(39)	Cross-sectional	1947	Positive association of A with cIMT but not with coronary calcification
(40)	Cross-sectional	881	No association of A with abdominal aortic calcification
(42)	Cross-sectional	126	No independent association of A with the presence and extent of coronary and aortic atherosclerosis
(46)	Cross-sectional	26	Positive relation of FAI with endothelial dysfunction
(47)	Cross-sectional	101	Positive correlation of A with FMD
(48)	Cross-sectional	101	Inverse relation of A with cIMT
(49)	20-year follow-up cohort	1629	No association of A with cIMT and coronary artery calcification
(50)	Cross-sectional	115	No association of A with FMD

E, estrogen; cIMT, carotid intima-medial thickness; PWV, pulse wave velocity; FMD, flow-mediated dilatation; FAI, free androgen index; A, androgen.

time, Sutton-Tyrrell *et al.* (52) demonstrated an inverse correlation of estradiol levels with insulin resistance, hemostatic, and inflammatory markers in a population of 3297 pre- and peri-menopausal women. A significant independent positive association between estradiol and CRP concentrations was identified in the population study of Störk *et al.* (53). Folsom *et al.* (54) also reported a positive association of estrone levels with CRP concentration in a study on 57 postmenopausal women; however, controlling for obesity attenuated the findings.

With respect to the role of endogenous androgens, in the study of Maturana *et al.* (55) performed on 53 postmenopausal women, testosterone levels were related to CRP, BMI, as well as endothelin-1 levels, which support associations with a proatherogenic profile. In accordance were the findings of the study of Sutton-Tyrrell *et al.* (52). In addition, CRP concentration was positively associated with FAI and $\Delta 4$ -androstenedione levels in the studies of Störk *et al.* (53) and Folsom *et al.* (54) respectively. However, there are also clinical data showing the opposite; in the cross-sectional study of Joffe *et al.* (56), testosterone was negatively associated with CRP, even after adjusting for the classical CVD risk factors. In the study of Bell *et al.* including 234 postmenopausal women, testosterone levels were not an independent determinant of CRP and lipid concentrations (57).

It appears that the decrease in estrogen levels accompanying the menopausal transition and the androgenic milieu are linked to a proinflammatory profile promoting atherosclerosis. However, given the heterogeneity of studies and the inconsistency of the described associations, one cannot exclude the possibility that these could be biased by alterations in BMI, insulin sensitivity, and age-related comorbidities accompanying transition to menopause. Given the availability of accurate analytical methods, studies measuring specific inflammation markers, such as proinflammatory cytokines rather than the less specific CRP, and evaluating their relevance with sex hormones seem indicated.

Diabetes mellitus

There is considerable clinical evidence supporting a positive relationship of endogenous sex steroids with the risk of developing diabetes mellitus (DM). One of the first studies was Khaw & Barrett-Connor (58) who studied 848 nondiabetic postmenopausal women and found a positive correlation of free testosterone with fasting plasma glucose, independent of age, BMI, and smoking. Circulating estrogen and androgen, i.e. testosterone and $\Delta 4$ -androstenedione, were higher in diabetic postmenopausal women compared with the control group in the study of Phillips *et al.* (59), and this finding was later reproduced by Korytkowski *et al.* (60). In accordance, the odds of DM were significantly increased with higher testosterone levels in the study of Oh *et al.* (61),

including 233 community-dwelling postmenopausal women. Goodman-Gruen & Barrett-Connor (62) showed that postmenopausal women with impaired glucose tolerance or DM had significantly higher bioavailable testosterone and estradiol levels compared with the control group. Ding *et al.* performed a nested case-control study on women health professionals with 359 incident cases of DM, matched 1:1. Both estradiol and testosterone levels were significantly associated with the risk of developing DM, even after adjustment for other metabolic parameters (63), in accordance with the study of Phillips *et al.* (59). In the large cross-sectional study of Golden *et al.* (64), bioavailable testosterone and estradiol levels were positively associated with the presence of diabetes; however, the relationship of testosterone was attenuated in the multivariate analysis. In the similarly designed study of Kalyani *et al.*, women in the higher quartiles of bioavailable testosterone and estradiol were at an increased risk of DM development. After adjustment for BMI and insulin resistance, however, this relationship remained significant for estradiol but not for testosterone (65).

The follow-up of patients with PCOS provides insight on the long-term effect of androgenicity on glucose metabolism. The incidence of DM was significantly higher in PCOS subjects compared with the control group in a 14-year follow-up (66). Similarly, in a study on 149 PCOS women aged 15–25 years after ovarian wedge resection, an increased occurrence of DM was reported (67). In the study of Margolin *et al.* (68) on 104 postmenopausal women, the presence of PCOS was significantly related to DM. Furthermore, in the Dutch follow-up study on PCOS women, the prevalence of diabetes in middle-aged women was four times higher compared with the corresponding age group of the general population (69). These findings are in line with previous reports by Wild *et al.* (20) and Dahlgren *et al.* (70).

In the field of DM, it seems that available evidence consistently supports a positive relationship of endogenous estrogen and androgen levels with the risk of developing DM.

Lipid profile

With respect to the link between endogenous sex steroids and lipid profile, data appear inconclusive. In the early studies of Shelley *et al.* (5) and Berge *et al.* (71), estradiol and testosterone were not related to lipid concentration. On the contrary, Lambrinoudaki *et al.* (72) reported an association of higher free estradiol index with an unfavorable lipid profile in healthy postmenopausal women, and this was independent of age, BMI, and insulin resistance. These findings were in the same direction as those of the population-based study of Haffner *et al.* (73), where estrone levels were inversely related to the HDL/total cholesterol ratio.

Furthermore, Mudali *et al.* (74) reported similar results in a case-control study on 328 postmenopausal women. In the MESA study, Vaidya *et al.* (75) also found that higher estradiol levels were related to a more atherogenic lipoprotein particle profile. On the other hand, in the study of Shakir *et al.* (76), estradiol correlated inversely with total cholesterol concentration both in women with or without the metabolic syndrome. The authors suggested that the effect, if any, of endogenous sex steroids on CVD markers differs according to the underlying metabolic and cardiovascular status and this is also supported by Sowers *et al.* In their prospective study on 2863 participants, they showed a modest association of lipoprotein α with estradiol levels in CHD-free women; however, no association was found in women with self-reported CHD (77). Furthermore, Mesalić *et al.* (78) reported a favorable profile in postmenopausal women with higher estrogen levels, the latter correlating positively with HDL and negatively with VLDL and triglyceride concentrations.

Most studies support positive associations of endogenous androgens with a proatherogenic lipid profile (72, 73). In addition, Svendsen *et al.* (79) evaluated 121 overweight postmenopausal women and showed an independent negative association of testosterone with HDL concentration. Later, van Beek *et al.* (80) reported an independent positive association of $\Delta 4$ -androstenedione and testosterone with VLDL-triglyceride concentration in postmenopausal women. Free testosterone levels correlated inversely with adiponectin concentration in the study of Milewicz *et al.* (81), but no association was found with estradiol. Contrary to the previous findings, in their large cross-sectional study, Shakir *et al.* (76) found that testosterone levels were positively associated with HDL in women without metabolic syndrome.

It is not easy to draw a definite conclusion on the relationship of endogenous sex steroid levels with lipid profile. It is hypothesized that the variable associations reported depend on the underlying cardiometabolic status of the studied population.

Blood pressure

Even though arterial hypertension is a well-established CVD risk factor, there are a few studies with conflicting results evaluating the clinical relevance of endogenous sex steroid levels with blood pressure (BP). In the longitudinal study of Shelley *et al.* (5), estradiol was not significantly related to diastolic BP values. In agreement were the more recent studies of Szmilowicz *et al.* (82) and Masi *et al.* (83). Of note, in the study of Szmilowicz *et al.*, patients had discontinued antihypertensive therapy before hormonal evaluation. However, two studies, one including 253 postmenopausal women and the other with 20 postmenopausal diabetic women and 29 controls, reported a positive association of

testosterone levels with BP values (59, 73). In the previously mentioned study of Shakir *et al.*, the variable effect of sex steroids according to the presence or absence of metabolic syndrome was shown also for BP. In women with metabolic syndrome, $\Delta 4$ -androstenedione was positively associated with systolic BP, and estradiol negatively related to diastolic BP. In women without the syndrome, $\Delta 4$ -androstenedione levels again correlated positively with systolic BP, whereas estradiol now appeared to have a positive association with systolic BP (76). Additionally, in the study of Phillips *et al.* (84), free testosterone levels were an independent determinant of systolic BP values among postmenopausal women, whereas estradiol levels were similar between hypertensive and normotensive women.

Although available data on the role of endogenous sex steroids on BP are limited, the findings of the aforementioned studies suggest a positive link between endogenous androgen and BP. A limiting factor in the accuracy of the results of the previous studies is the use of antihypertensive medication at the time of sex hormone measurement in most, but not in all (82), studies. Larger, adequately powered and homogeneous studies would possibly aid to clarify the clinical relevance of estrogen and androgen levels in BP tuning.

Obesity-insulin resistance-metabolic syndrome

Insulin resistance and abdominal obesity are the hallmarks of metabolic syndrome tightly associated with CVD. In two recent population-based studies, testosterone was positively related to body fat mass. In the study of Janssen *et al.* (85) on 359 women, this association was independent of age and CVD risk factors, whereas in the work of Rariy *et al.*'s (86) model, adjustment for estradiol attenuated this association. Transition to menopause was accompanied by an increase in $\Delta 4$ -androstenedione levels and FAI in the study of Mesch *et al.* (87); FAI was also associated with a higher waist circumference measurement, insulin resistance, and an adverse lipid profile. This agrees with the previous work of Tufano *et al.* in 44 obese women, half of whom were in the postmenopausal period. FAI was positively related to waist circumference and was significantly higher in menopausal women compared with the premenopausal group (88). In an earlier case-control study of Hauner *et al.* (89) in women undergoing coronary angiography, testosterone was positively associated with the waist:hip ratio, an index of central adiposity, as well as with insulin levels. A positive association of hyperinsulinemia with total testosterone and FAI was also demonstrated in a study on 104 peri- and postmenopausal women, and this was independent of age, BMI, and time since menopause (90). In the work of Phillips *et al.* (91), it is suggested that free testosterone might be the underlying mediator

of both visceral adiposity and insulin resistance in the metabolic syndrome.

Some clinical data suggest a link between androgenicity and the presence of metabolic syndrome. In the study of Golden *et al.* (92) on 362 postmenopausal women, women in the highest FAI quartiles had fivefold greater odds of meeting the criteria of metabolic syndrome compared with those in the lowest quartiles. In the same line, Maggio *et al.* (93) showed that Italian women with metabolic syndrome had higher age-adjusted bioavailable testosterone levels in comparison with women without the syndrome. In the 9-year follow-up study with 949 participants investigating the course of menopausal transition, Janssen *et al.* (94) found that an increase in bioavailable testosterone increased the odds of developing metabolic syndrome. Similarly, in the study of Weinberg *et al.* (95), postmenopausal women in the highest FAI tertile had a significantly increased risk of the metabolic syndrome, which was observed even after adjustment for BMI and other CVD risk factors. Regarding endogenous estrogen, there are various reports showing a positive relationship with adiposity markers (96). This is not an unexpected finding, as a significant source of estradiol in postmenopausal women is that which is derived from the aromatization of testosterone in the adipose tissue. In the cross-sectional study of Lukanova *et al.* (97), estrogen and testosterone levels were increased with higher BMI in healthy postmenopausal women and this was recently confirmed in the study of Freeman *et al.* (98). In agreement, in the study of Olson *et al.* (99) performed on 207 postmenopausal women undergoing angiography for suspected ischemia, estradiol levels were significantly associated with waist circumference measurement and BMI in the regression analyses. However, in the study of Janssen *et al.* (94), estradiol levels did not appear to have a significant relationship with the risk of developing metabolic syndrome.

With respect to PCOS-associated hyperandrogenemia and its relevance with the metabolic syndrome, useful information is derived from the follow-up studies of PCOS subjects. In the study of Schmidt *et al.* (100), postmenopausal women with PCOS showed increased clustering of features of the metabolic syndrome, such as hypertension and hypertriglyceridemia, compared with the control population. Similar results were found in the studies of Margolin *et al.* (68) and Dahlgren *et al.* (70) reporting an increased prevalence of central obesity, diabetes, hypertension, and dyslipidemia in PCOS postmenopausal women. Furthermore, in the long-term follow-up study of Hudecova *et al.* (101), the prevalence of the metabolic syndrome was significantly higher in middle-aged women with a previous diagnosis of PCOS compared with control subjects, independently of the PCOS phenotype or the persistence of PCOS in the reevaluation.

According to the majority of current data, both estrogen and androgen appear to be positively

associated with abdominal obesity and the metabolic syndrome. The peripheral aromatization of androgens to estrogens in adipose tissue should be taken into consideration when interpreting the results of various clinical studies. Furthermore, one should note that the metabolic syndrome is the integral of various factors of inflammation, lipid metabolism, and coagulation, all in a complex interplay with sex hormones. Therefore, identifying the independent effect of each sex steroid on CVD and CVD risk factors can often be puzzling.

Conclusion

In this review, we tried to provide a literature update on the role of endogenous sex steroids on cardio- and cerebro-vascular disease. Clinical studies investigating associations between endogenous estrogen and androgen and vascular alterations in the peri- and postmenopausal period show rather inconsistent results. It is obvious that, although some relationship may be identified, in most studies, the design does not allow its characterization as being causal. Furthermore, the population size is heterogeneous in various studies and a direct comparison of their findings may not be appropriate. Another caveat when trying to draw conclusions is that the mean age of the studied women, which translates in a different time as menopause, varied among the reports that were reviewed. In addition, differences in race and ethnicity should be taken into account in the interpretation of the clinical findings. The majority of the previously mentioned studies concern white population. Of note, in the multi-ethnic MESA study, there was a significant difference in the circulating sex hormone levels between the different races; however, the described associations between sex hormones and markers of atherosclerosis were not modified by ethnic background (39).

It may well be that circulating sex steroid levels represent an index of the general health status. Metabolic features, like adiposity, dyslipidemia, and insulin resistance, often encountered in the postmenopausal age, are a common confounder. As adipose tissue is a significant source of estradiol in postmenopausal women, it is likely that associations with estradiol reflect an association with adrenal and ovarian androgens peripherally aromatized to estrogens.

Well-designed clinical studies and animal models will provide further insight as to whether and how endogenous sex steroids affect the cardio- and cerebro-vascular tissue or whether, alternatively, they represent a surrogate marker of the underlying disease. Clarifying the unique role of estrogen and androgen in cardio- and cerebro-vascular disease is an interesting subject of research. Future research will expand our knowledge, and transferring new information from bench to bedside may have significant implications in the clinical setting.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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