Cardiometabolic Risk Factors and Endogenous Sex Hormones in Postmenopausal Women: A Cross-Sectional Study

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Abstract

Context: It is uncertain which cardiovascular risk factors are associated with sex hormone levels in postmenopausal women.

Objective: This work aimed to investigate the association between cardiometabolic risk factors and sex hormones in a cross-sectional, observational population study.

Methods: In this Swedish population study, participants were physically examined from 2002 to 2004, and endogenous sex hormones were analyzed by liquid chromatography–tandem mass spectrometry. Women aged 55 years or older with estradiol levels below 20 pg/mL and not using any hormonal therapy were eligible for inclusion in the study (N = 146). Variable selection and bootstrap stability analyses were performed and linear regression models presented, with each of the 8 hormones as outcome variables.

Results: Body mass index (BMI) was positively associated with estradiol ($\beta = 0.054$, $P < .001$), but negatively associated with 17-α-hydroxyprogesterone ($\beta = -0.023$, $P = .028$). Waist-to-hip ratio (WHR) was negatively associated with dihydrotestosterone ($\beta = -2.195$, $P = .002$) and testosterone ($\beta = -1.541$, $P = .004$). The homeostatic model assessment of insulin resistance was positively associated with androstenedione ($\beta = 0.071$, $P = .032$), estradiol ($\beta = 0.091$, $P = .009$), estrone ($\beta = 0.075$, $P = 0.009$), and 17-α-hydroxyprogesterone ($\beta = 0.157$, $P = .001$). Age was positively associated with testosterone ($\beta = 0.017$, $P = .042$). C-reactive protein showed an inverse association with progesterone ($\beta = -0.028$, $P = .037$). Lower low-density lipoprotein cholesterol was associated with higher estradiol levels ($\beta = -0.093$, $P = .049$), whereas lower triglycerides were associated with higher concentrations of dihydrotestosterone ($\beta = -0.208$, $P = .016$).

Conclusion: In postmenopausal women, WHR was strongly inversely associated with androgens, while BMI was positively associated with estrogens.

Key Words: sex steroid hormones, postmenopausal period, menopause, chromatography mass spectrometry gas liquid, body composition

Abbreviations: BMI, body mass index; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; RIA, radioimmunoassay; TGs, triglycerides; WHR, waist-to-hip ratio.

In addition to their fundamental role in reproduction physiology, sex hormones may be important for cardiovascular health [1]. Premenopausal women are less vulnerable to cardiovascular diseases than men, whereas the incidence of cardiovascular diseases in women increases markedly after the menopausal transition [1, 2]. This may be a consequence of estrogen deficiency, leading to an increase in cardiovascular risk factors such as an adverse lipid profile [3], weight gain and hypertension [4], and endothelial dysfunction [5, 6]. Nevertheless, higher endogenous estrogen levels in postmenopausal women are known risk indicators for developing insulin resistance [7], breast cancer [8], endometrial cancer [9], and stroke [10]. On the other hand, higher estradiol levels in postmenopausal women have been associated with less adverse vascular remodeling both in the MSHeart Study [11] and in the Study of Women's Health Across the Nation Study (SWAN) [12]. There is also evidence that different patterns of estrogen decline during the menopausal

transition are associated with different levels of markers of atherosclerosis, such as the presence of carotid plaque [13]. Studies based on radioimmunoassay (RIA) measurements of sex hormone levels have shown a positive association between body mass index (BMI) and estrogen levels [14-17], whereas there are contradictory findings regarding androgens. One observational study showed that waist circumference was positively associated with levels of androgens [18], whereas other studies have found no association between waist-to-hip ratio (WHR) and testosterone [19]. Yet another study including both premenopausal and postmenopausal women (mean age, 47.5 years) found a negative association between visceral fat accumulation and dihydrotestosterone (DHT) [20]. A review investigating 13 prospective studies found that all sex hormone levels were higher in obese postmenopausal women compared to lean postmenopausal women [17].

RIA-based measurements are not suitable for the measurement of hormones in the lower range, that is, in postmenopausal women, children, or hypogonadal men, because of low precision and sensitivity [21]. Few population-based studies have used state-of-the art methods for sex steroid measurements in women, and there is a need for studies investigating how different variables are associated with endogenous sex hormones measured with more precise methods such as mass spectrometry [21, 22]. Thus, the aim of this study was to investigate the association between risk factors for cardiovascular disease and 8 different sex hormones—17-α-hydroxyprogesterone, estrone, DHT, estradiol, dehydroepiandrosterone (DHEA), androstenedione, testosterone, and progesterone—measured with mass spectrometry in postmenopausal women.

Materials and Methods

Study Population
To investigate the development of hypertension and type 2 diabetes in a longitudinal design, a cohort study was conducted using the Vara-Skövde cohort, a sex-balanced, random sample of 2816 individuals living in southwestern Sweden during 2002 to 2005. The study design has been described in more detail elsewhere [23]. Using the study population, we analyzed 8 different sex hormones (17-α-hydroxyprogesterone, estrone, DHT, estradiol, DHEA, androstenedione, testosterone, and progesterone) with a validated high-sensitivity liquid chromatography–tandem mass spectrometry assay [24] in 179 women aged 55 years and older at the first study visit and who also participated in the second visit. Eligible for the present cross-sectional study were women who did not use systemic hormone replacement therapy. Because no self-reported information regarding menopausal status was available, we selected participants that were aged 55 years or older. After excluding women with estradiol concentrations greater than or equal to 20 pg/mL [25], 146 women were included in the present study (Fig. 1). The Regional Ethical Review Board in Gothenburg, Sweden, approved the study (registration Nos. 161-92 and 036-12), and all participants gave their written consent to participate.

Physical Examination
Specially trained research nurses assessed study participants, measuring waist circumference and blood pressure in the supine and standing positions. Body weight was measured with participants in light clothing without shoes, and waist and hip
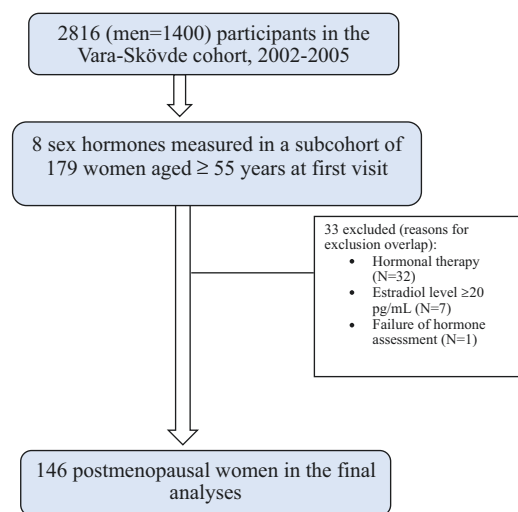
Figure 1. Study flowchart showing the study design and inclusion process.

circumferences were measured. BMI and WHR were calculated. Validated questionnaires were used to obtain information on leisure time physical activity [26, 27]. Furthermore, information about smoking habits, alcohol intake [28], medical history, and medications was obtained from the participants. Diabetes mellitus and hypertension were defined based on World Health Organization [29] and Joint National Committee [30] recommendations.

Laboratory Analyses
Fasting venous blood samples were drawn in the morning and 2 hours after a 75-g oral glucose load. All blood samples were immediately frozen at −82 °C. Serum concentrations of estradiol, estrone, DHEA, DHT, androstenedione, progesterone, testosterone, and 17-α-hydroxyprogesterone were assessed with a validated high-sensitivity liquid chromatography–tandem mass spectrometry assay in 2018 [24] in the described subgroup of 179 participants. Blood lipids, creatinine, high-sensitivity C-reactive protein (hsCRP), and concentrations of insulin and glucose (at fasting) were measured. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the equation (fasting insulin × fasting glucose)/22.5 in participants without insulin therapy [31].

Statistical Analyses
Descriptive statistics were used to describe the study population. Distribution of levels of sex hormones was analyzed separately for each hormone. Normality tests were performed with the Shapiro-Wilk method [32].

Levels of sex hormones were used as outcomes in linear regression models after being log-transformed to improve the model fit. The improvements were particularly notable for testosterone, DHT, progesterone, estradiol, and 17-α-hydroxyprogesterone. Backward variable selection with Akaike information criterion was used to choose the set of cardiometabolic variables that could best explain the hormone levels. The cardiometabolic variables included BMI, smoking, WHR, diabetes, hypertension, alcohol consumption, leisure time physical activity, age, low-density lipoprotein (LDL), hsCRP, HOMA-IR, creatinine, triglycerides

(TGs), and homeostatic model assessment of β-cell function. Among the methods of variable selections and criteria, the use of backward variable selection with Akaike information criterion has been recommended [33]. Variable selection generally introduces additional uncertainty in terms of the selected set of variables, the coefficient of the selected variables, and the SE of the selected variables. This means that the variable selection results may differ because of small perturbations in samples. To reduce this uncertainty, bootstrap was used to perform stability investigations. The basic idea of bootstrap stability investigation is to draw N resamples from the original data set and to repeat variable selection in each of the resamples. In this study, we drew 1000 sets of resamples. Bootstrap inclusion frequency quantifies how likely an independent variable is selected. Thus, 50% inclusion means that in the variable selection results in the 1000 sets of resamples, the variable has been selected 500 times. Variables that had greater than 50% inclusion in the bootstrap procedure and showed a significant association with the outcome were selected in the final multiple regression model. All statistical analyses were conducted using R version 4.0.2.

Results

Characteristics of the Study Population

Of the 2816 participants in the Vara-Skövde cohort, 146 postmenopausal women were included in this prospective cohort study (see Fig. 1). The descriptive characteristics of the study population are shown in Table 1. The mean age was 64.3 years (SD 5.2); 12% had type 2 diabetes, and about 31% had hypertension. The mean BMI was 28 (SD 5.1), and about 70% of the study population reported a low level of daily physical activity. The Shapiro-Wilk normality tests showed a P value of less than .001 for all hormones (Table 2), meaning that all hormones had higher mean values than median values. All hormones were thus positively skewed, which is illustrated in Fig. 2.

Sex Hormones and Cardiovascular Risk Factors

Multivariable linear regression analyses were then performed to investigate the association between the concentration of sex hormones in postmenopausal women and risk factors for cardiovascular disease (Table 3). Two participants were excluded from all the regression analyses because of extreme hsCRP values. For the analysis of 17-α-hydroxyprogesterone, 3 participants were excluded, and for progesterone, 1 participant was excluded because of extreme values in the measurement. Further details on the bootstrap stability analysis are available in the supplementary material [34].

Association Between Sex Hormones and Body Composition Variables/Glycemic Traits

After the variable selection analyses, BMI showed a significantly positive association with estradiol (β = 0.054, P < .001) and estrone (β = 0.015, P = .057), whereas BMI was negatively associated with androstenedione (β = –0.016, P = .075) and 17-α-hydroxyprogesterone (β = –0.023, P = .028). WHR showed a negative association with DHT (β = –2.195, P = .002) and testosterone (β = –1.541, P = .004). High HOMA-IR was associated with high levels of androstenedione (β = 0.071, P = .032), estradiol (β = 0.091, P = .009), estrone (β = 0.075, P = .009), and 17-α-hydroxyprogesterone (β = 0.157, P = .001). Type 2 diabetes was found to have a negative association with 17-α-hydroxyprogesterone, although not statistically significant in the regression analysis (β = –0.321, P = .099).

Association Between Sex Hormones and Age, High-sensitivity C-reactive Protein, and Blood Lipids

Age was significantly positively associated with testosterone (β = 0.017, P = .042), whereas an inverse nonsignificant relationship was seen with DHEA (β = –0.020, P = .059). hsCRP was significantly negatively associated with progesterone (β = –0.028, P = .037). Lower LDL was associated with higher levels of estradiol (β = –0.093, P = .049), whereas lower TGs showed significant association with higher concentrations of DHT (β = –0.208, P = .016).

Discussion

In this observational study, we found a strong association between risk factors for cardiovascular disease and sex hormones in postmenopausal women measured with mass spectrometry. More specifically, body composition variables (ie, BMI and WHR) were shown to best explain the variance in the levels of specific sex hormones. Here, we found a strong, significantly positive association between BMI and 17-α-hydroxyprogesterone and between BMI and estradiol, whereas WHR was significantly negatively associated with DHT and testosterone.

Body Composition and Sex Hormones

In the present study, testosterone as well as DHT were negatively associated with WHR. Although similar results have been reported both in premenopausal and postmenopausal

Table 1. Characteristics of the study population

	Mean (SD)
Age, y	64.3 (5.17)
BMI	28.1 (5.09)
WHR	0.85 (0.08)
LDL, mmol/L	3.72 (0.93)
Triglycerides, mmol/L	1.42 (0.64)
hsCRP, mg/L	3.54 (5.77)
HOMA-IR	1.81 (1.41)
HOMA-BC	61.2 (29.7)
Alcohol consumption, g/wk, n = 138	25.4 (42.3)

	N (%)
Physical activity n = 137	
Sedentary	8 (5.5)
Low	104 (71.2)
Moderate	25 (17.1)
Intense	0 (0)
Type 2 diabetes	18 (12.3)
Hypertension	45 (30.8)
Daily smoking	16 (11)

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-BC, homeostatic model assessment of β-cell function; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; WHR, waist-to-hip ratio.

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Table 2. Concentrations of sex hormones in postmenopausal women in the Vara-Skövde cohort (N = 146)

Sex hormone	Mean	SD	Median	Test of normality Shapiro-Wilk	
				Statistic	P
17-α-Hydroxyprogesterone, pg/mL	380.35	383.85	304.84	0.487	< .001
Androstenedione, pg/mL	619.58	284.19	554.59	0.909	< .001
DHEA, pg/mL	2843.18	1739.60	2406.19	0.890	< .001
DHT, pg/mL	47.47	32.12	39.19	0.854	< .001
Estradiol, pg/mL	5.04	3.07	4.25	0.875	< .001
Estrone, pg/mL	24.15	9.94	21.72	0.932	< .001
Progesterone, pg/mL	49.50	32.21	40.89	0.743	< .001
Testosterone, pg/mL	257.81	150.14	220.76	0.761	< .001

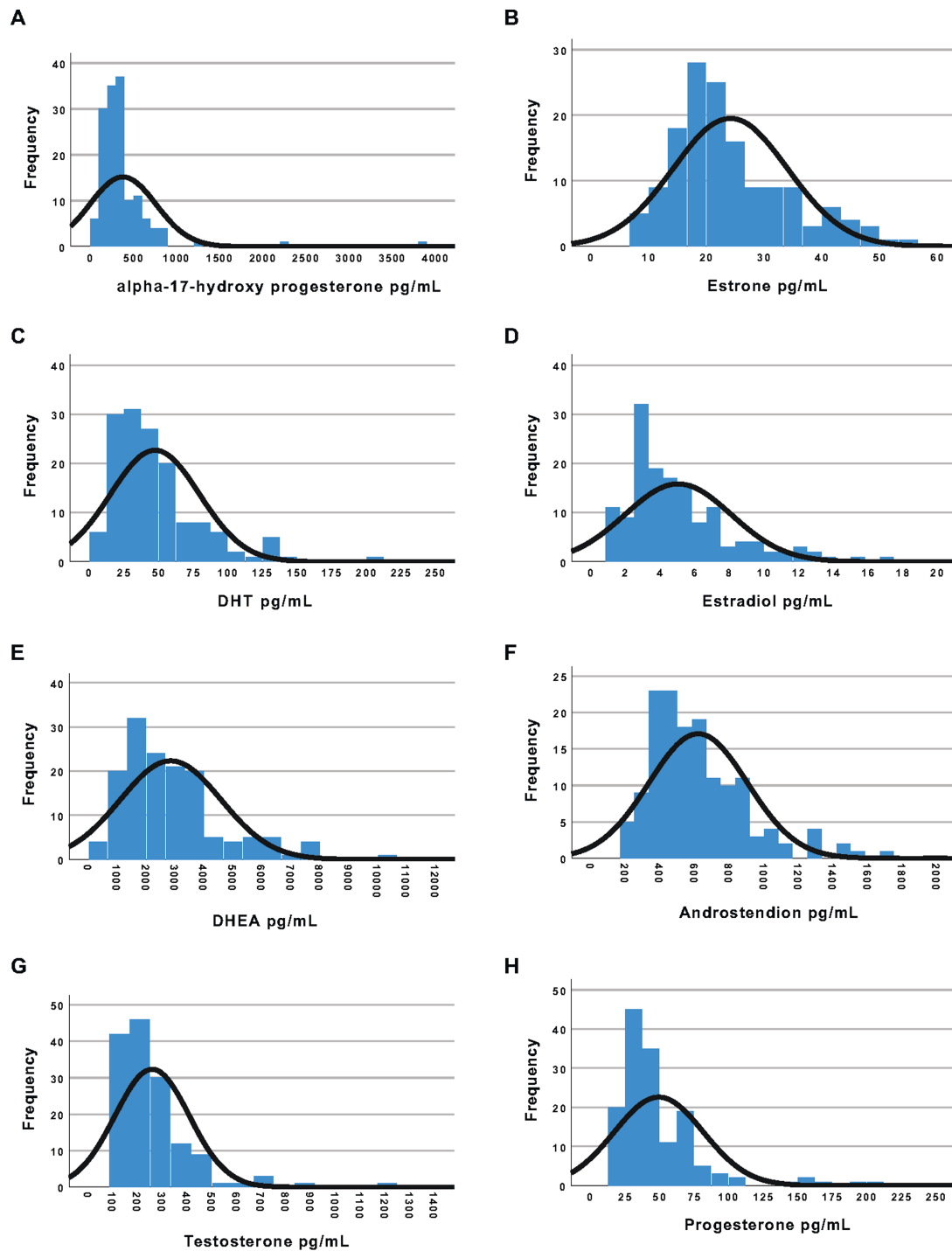Abbreviations: DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

women [35, 36], another study demonstrated contradictory findings concerning this association [20]. As most of these studies used RIAs, which are less precise when measuring sex hormones, their findings might be partially explained by type 1 errors. Two studies using mass spectrometry [19, 20] found no significant association between testosterone and WHR. However, the low number of participants included in these studies combined with the fact that not all participants in the studies were postmenopausal can suggest type 2 error in these studies. Similarly, Côté et al [20] showed a negative association between BMI and DHT, which is a potent androgen derived from testosterone, and variables of visceral adiposity measured with computed tomography, which is in line with our findings. Although the evidence is not entirely consistent, and there is uncertainty in the direction of causality, there are studies indicating that androgens can stimulate lipolysis in adipose tissues both in men and women [37, 38], a mechanism that may explain the negative association seen in this study. Still, the conversion from androgens to estrogens by aromatization in the fat tissue might also contribute to the low levels of androgens observed in individuals with high WHR.

This study used BMI and WHR to describe body composition, both of which were strongly associated with 6 of the 8t studied hormones. These findings are in agreement with a recent study conducted by Marchand et al [19] containing approximately 100 postmenopausal women in which estradiol and estrone showed a positive association with BMI, waist circumference, total body fat mass, and body fat percentage, but not with WHR. Data from the Multi-Ethnic Study of Atherosclerosis (MESA) show similar results, with a positive association between estradiol (by RIA) and visceral fat in postmenopausal women [39]; however, in the Diabetes Prevention Program visceral fat loss was not associated with change in estradiol [40]. The activity of enzymes of aromatization, converting androgens to estrogens in the peripheral fat tissue, is proportional to body fat mass, and thus higher levels of body fat mass may result in higher estradiol and estrone levels, explaining the strong association found in this study and also in previous studies [19, 41]. Furthermore, 17-α-hydroxyprogesterone showed a significantly negative association with BMI in our study, which to our knowledge has not been previously described elsewhere and might need further investigation to be confirmed.

Inflammation

We observed a significant inverse association between hsCRP and progesterone levels. Previous observational studies have come to different results regarding this association. Crandall et al [42] investigated 623 postmenopausal women with regard to sex hormones (measured with RIA technique) and cardiovascular risk factors, but found no significant associations between CRP and progesterone when adjustments were made for confounding factors. An observational study in premenopausal women found a positive association between CRP and progesterone levels [43]; however, owing to the several metabolic changes that occur during the menopausal transition, this may not be the case in postmenopausal women. In a randomized controlled trial, 133 early postmenopausal women were treated either with synthetic progesterone or placebo. No significant change in hsCRP between the groups was observed [44]. Yet another recent cross-sectional study observed a positive association between progesterone and hsCRP in postmenopausal women living in China, and stated further that hsCRP mediates the association between progesterone and obesity [45].

hsCRP has been used as a proxy for inflammation in many studies [46], of which the Women's Health Study was among the first, revealing a strong positive association between hsCRP levels and risk of death from myocardial infarction, stroke, or coronary heart disease in more than 28 000 postmenopausal healthy women [47]. Even so, hsCRP is not specific to inflammatory states only, and other inflammatory markers (eg, interleukin-6) might have shown different results in our study. There is evidence that progesterone may have an anti-inflammatory effect on different cells of the immune system, such as an inhibitory effect on natural killer cells and T cells [48, 49], and that synthetic progesterone inhibits the activation of inflammatory pathways in mucosal sites [50], but the research is not entirely clear on this point [51]. However, the fact that the progesterone receptor is found in several tissues, including many cells of the immune system, suggests that progesterone may have a role in the immune response, as described in a recent review by Azeez et al [49]. Even though progesterone may have anti-inflammatory properties, the direction of causality may also be the opposite, given that inflammatory states, which can also be part of a systemic disease, have inhibitory effects on the hypothalamic-pituitary-adrenal axis, resulting in a downregulation of androgen production

Figure 2. The distribution of endogenous sex hormones in 146 postmenopausal women. DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.

[52]. This may also be the case for progesterone deriving from the adrenal cortex. Estrogen, however, has been positively associated with hsCRP in postmenopausal women in previous studies [19, 53] when using both RIA and mass-spectrometry assays. However, studies using the RIA method should be interpreted with caution, since analyses of estrogen and perhaps also other sex hormones with RIA methods seem to be possibly interfered with by CRP [54]. This may result in a type 1 error in associations between sex hormones measured with RIA and CRP that are not seen when using mass-spectrometry assays.

Glycemic Traits and Blood Lipids

Here, we showed a strongly significant association between high levels of HOMA-IR and 17-α-hydroxyprogesterone, which is in agreement with a recent study in which women with high levels of 17-α-hydroxyprogesterone at baseline had an increased risk of glycemic deterioration after approximately 6 years of follow-up [55]. Similarly, high levels of 17-α-hydroxyprogesterone have been shown to predict type 2 diabetes in mouse models and humans [56]. Furthermore, high levels of HOMA-IR were associated with high levels of

Table 3. Multivariable linear regression analyses investigating the association between risk factors for cardiovascular disease and concentration of sex hormones in postmenopausal women

	β	95% CI	*P*	Standardized estimate
17-α-Hydroxyprogesterone				
HOMA-IR	0.157W	0.062 to 0.252	.001	0.224
Type 2 diabetes	−0.321	−0.704 to 0.062	.099	−0.108
BMI	−0.023	−0.044 to −0.002	.028	−0.120
Androstenedione				
HOMA-IR	0.071	0.006 to 0.136	.032	0.101
BMI	−0.016	−0.034 to 0.002	.075	−0.084
DHEA				
Age, y	−0.020	−0.040 to 0.001	.059	−0.101
DHT				
WHR	−2.195	−3.586 to −0.804	.002	−0.175
Triglycerides	−0.208	−0.377 to −0.040	.016	−0.137
Estradiol				
BMI	0.054	0.035 to 0.072	< .001	0.279
HOMA-IR	0.091	0.023 to 0.160	.009	0.130
LDL	−0.093	−0.185 to 0.000	.049	−0.083
Estrone				
BMI	0.015	0.000 to 0.030	.057	0.077
HOMA-IR	0.075	0.019 to 0.131	.009	0.107
Progesterone				
hsCRP	−0.028	−0.055 to −0.002	.037	−0.093
Testosterone				
Age, y	0.017	0.001 to 0.033	.042	0.086
WHR	−1.541	−2.579 to −0.504	.004	−0.123

The table presents the explanatory variables selected in the variable selection and showing greater than 50% inclusion in the bootstrap stability investigation. Dependent variable: sex hormone.
Abbreviations: BMI, body mass index; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; WHR, waist-to-hip ratio.

androstenedione. Few studies have investigated this association using RIA methods to assess the concentration of sex hormones, which could have led to a type 2 error [57].

Some independent associations between lipids and sex hormones were also found. In particular, high levels of TGs were significantly associated with low levels of DHT. To our knowledge, this is a novel finding that has not previously been described. However, a negative association between DHT and LDL has been previously shown [19]. These findings might both be related to the strong association between fat mass and DHT, but further studies are needed.

Strengths and Limitations

The strength of this study is its design, with a high participation rate around 70% and thorough physical examinations performed by trained nurses during the study visit. By examining a number of biometric variables with validated methods, we were able to perform variable selection to identify variables that best explained the variation in hormonal levels. Another strength is the use of mass-spectrometry technique, which is the gold standard especially when measuring steroid hormones in the lower range, as in postmenopausal women. According to Mayo Clinic Laboratories [25], the sex hormone concentrations shown in the present study were within the reference values for postmenopausal women,

showing high external validity of the method used in this study. Furthermore, we included bootstrap validation to perform stability investigations, which adds further strength to the methodology. A formal power analysis was not conducted; however, the ratio between the sample size (n = 146) and the number of independent variables (ie, n = 14) in each regression analysis was above 10, which is above the commonly used rule of thumb described in the literature, that is ie, the ratio should at least be greater than 10 to achieve an acceptable power [33, 58].

Owing to the lack of information about menopausal status, a limitation of this study was the use of age 55 years and older as a proxy for menopause. Although this could lead to a misclassification of menopause, we excluded participants with estradiol values that were not compatible with postmenopausal concentrations. Another limitation is that the cohort consisted mainly of women of Swedish origin and may not be fully generalizable to all postmenopausal women. However, the results of our study are very much in line with previous research including participants of different geographic or ethnic origins and could therefore be seen as representative of a large portion of postmenopausal women. The observational design of this study did not permit the investigation of the direction of the associations found, and due to the cross-sectional nature of the study, it is not possible to draw conclusions about causality.

It is obvious that the menopausal transition is a vulnerable period in women's lives with regard to the increase in cardiometabolic risk factors. This study about endogenous sex hormones and their associations with certain risk factors adds knowledge to previous results from studies using the RIA method. This knowledge may become important for cardiovascular risk stratification of women.

Conclusion

Various cardiometabolic factors, in particular body composition variables, were found to be associated with sex hormone levels in postmenopausal women. Our results suggest that traditionally male hormones such as testosterone and DHT decrease with increasing WHR, while estradiol and estrone increase with increasing BMI. Notably, we also found a negative association between progesterone and hsCRP. Further investigations with longitudinal observations might shed light on the importance of hormonal levels for female cardiovascular health.

Disclosures

The authors have nothing to disclose.

Data Availability

Data can be obtained on request, and requests should be directed to the corresponding author. Owing to privacy regulations, all data may not be available for public distribution.

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