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Original Research

Association of hyperandrogenaemia with hypertension and cardiovascular events in pre-menopausal women: a prospective population-based cohort study

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Abstract

Objective: The present study aimed to clarify the conflicting association of premenopausal hyperandrogenaemia (HA) with the development of hypertension and cardiovascular disease (CVDs) in women.

Design: A population-based cohort study including 5889 women.

Methods: The association of serum testosterone (T), sex hormone-binding globulin (SHBG), and free androgen index (FAI) at age 31 with blood pressure (BP) and hypertension (BP ≥ 140/90 mmHg and/or use of antihypertensive medication) at ages 31 and 46 and with CVDs (angina pectoris [AP] and/or acute myocardial infarction [AMI] $n = 74$, transitory cerebral ischaemia and/or stroke $n = 150$) and combined CVD events (AP, AMI, stroke, heart failure, or CVD mortality $n = 160$) by age 53 was investigated.

Results: T and FAI were positively associated with systolic and diastolic BP at ages 31 and 46 in the multivariable model. Compared to their lowest quartile, the highest quartiles of T and FAI were positively associated with hypertension at age 31 in the multivariable model. During the 22-year follow-up, FAI was positively associated with increased risk of AP/AMI (hazard ratio [HR]: 2.02, 95% CI: 1.06-3.85) and overall CVD events or mortality (HR: 1.54, 95% CI: 1.02-2.33) in the unadjusted models. However, the significance disappeared after adjusting for body mass index (BMI).

Conclusions: Women with HA at premenopausal age had an elevated risk of hypertension, and together with BMI, increased risk of CVD events and CVD mortality during the 22-year follow-up. However, because of several study limitations regarding ethnicity and BMI characteristics, a longer follow-up of this cohort and future studies in ethnically diverse populations are needed to verify the results.

Keywords: testosterone, blood pressure, hypertension, cardiovascular disease, hyperandrogenism, hyperandrogenaemia

Significance

The present cohort study provides novel information about the association of hyperandrogenaemia (measured by liquid chromatography-tandem mass spectrometry) with risk of elevated blood pressure, hypertension, and development of cardiovascular disease among premenopausal women. Our study shows that hyperandrogenaemia is independently, positively associated with elevated blood pressure and hypertension. During a 22-year follow-up, hyperandrogenaemia was also, together with body mass index, positively associated with an increased risk for future cardiovascular disease. These results indicate that women with hyperandrogenaemia should have blood pressure measured early and regularly and recommended for early treatment of elevated blood pressure. A longer-term follow-up of this cohort with postmenopausal data is needed to clarify long-term cardiovascular risks related to hyperandrogenaemia.

Introduction

Hypertension and cardiovascular diseases (CVDs) represent the leading cause of death worldwide.[1,2] Moreover, it is well known that the CVD risk profile exhibits sex-specific characteristics,[3,4] implying the involvement of sex hormones in CVD pathophysiology. Androgens are known to largely[5]—but also differentially—affect the female vascular system compared to that of men.[6] However, it remains unclear to what extent hyperandrogenaemia (HA), a common endocrine condition among women at fertile age with an estimated

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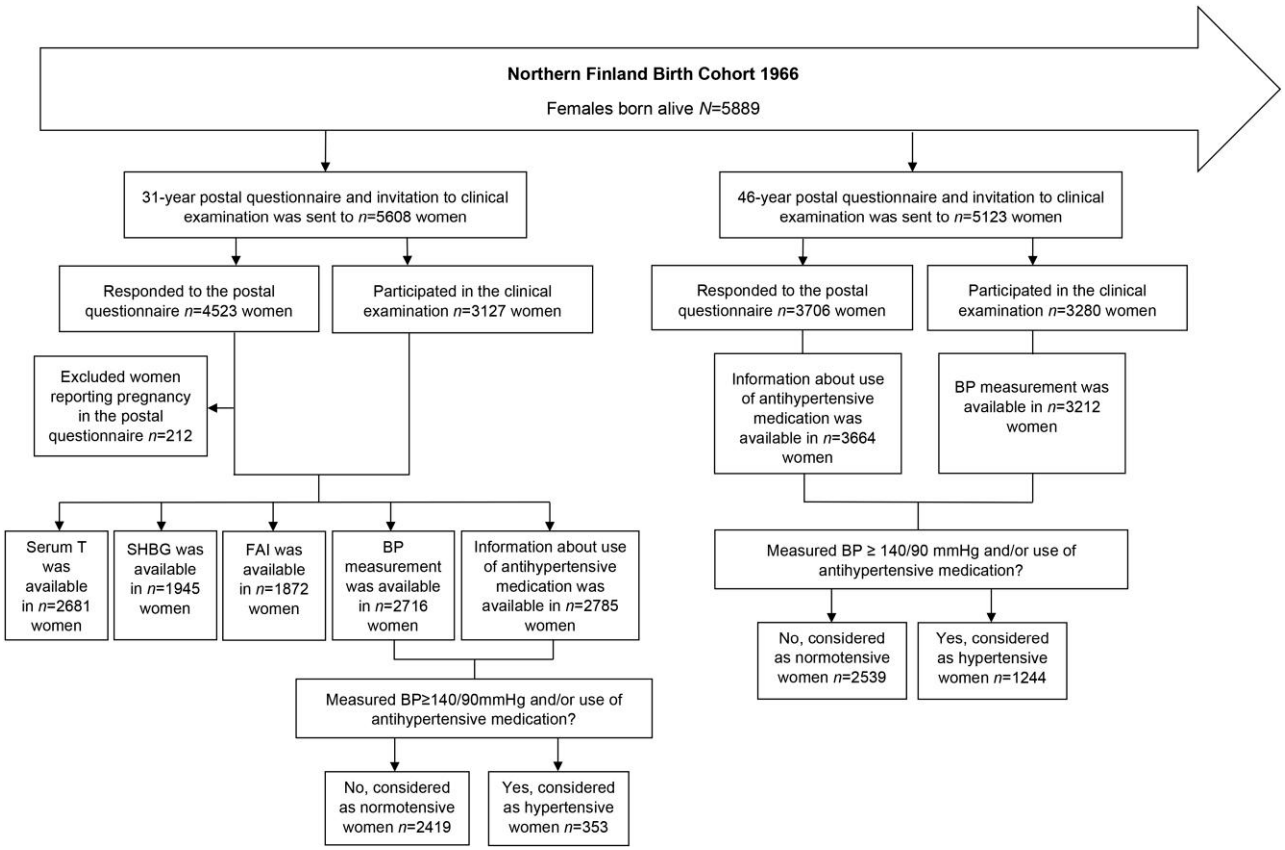


Figure 1. Flowchart of the study.

prevalence of 4%-10%,[7-9] contributes to the risk of developing hypertension and CVDs later in life.

To date, clinical studies of HA-related risk of elevated blood pressure (BP), hypertension,[10-16] and CVDs[17-26] in women have yielded contradictory results, as some studies have observed an increased risk of hypertension and CVDs in hyperandrogenic women,[10,13,14,18] whereas others have not.[11,12,16,26] These discrepancies may be partly explained by the different methods used to measure testosterone (T). The immunoassays employed in most studies lack the sensitivity and specificity of the more accurate liquid chromatography-tandem mass spectrometry (LC-MS/MS), considered to be the gold standard.[27] Other limitations of previous studies included cross-sectional design[11-14,16,24] or being targeted specifically in women with polycystic ovary syndrome (PCOS). Some studies have been confined to perimenopausal[14,17,25] or postmenopausal[12,15,18,22,24,26] women, or were performed in populations of women who varied significantly in terms of age, from early adulthood to late postmenopause.[11,13,23] This is important, as it has been shown that T levels fluctuate during women's life span.[28,29] Hence, we lack clear evidence about whether HA, particularly during reproductive age, is a risk factor for developing CVDs in women.

In the present study, a large, prospective, longitudinal, population-based birth cohort of 5889 women was used to investigate the association of serum levels of T (measured by LC-MS/MS), as a simple hormonal diagnostic tool, with BP and hypertension at ages 31 and 46, and with the development of CVDs by age 53.

Methods

Study population

The study population was derived from the Northern Finland Birth Cohort 1966 (NFBC1966), which is a large, prospective, longitudinal, population-based birth cohort comprising all individuals expected to be born in 1966 in the 2 northernmost provinces of Finland (Oulu and Lapland).[30] During that year, 5889 females were born alive, after which data about these women have been collected (at age 1, 14, 31, and 46) and linked to multiple other data sources, including national registers. The follow-up protocol has been previously described in detail.[31,32] The present study utilized cohort data collected at ages 31 and 46 combined with data from national registers. The flow chart of the study is presented in Figure 1.

Measurements

In the clinical examinations, after 15 min of rest, brachial systolic and diastolic BP were measured, at 1-min intervals, twice on the right arm of the seated participants (women aged 31) and 3 times on the right arm of the seated participants (women aged 46). For these measurements, a manual mercury sphygmomanometer was used for women aged 31, and an automated, oscillometric BP device with an appropriately sized cuff (Omron Digital Automatic Blood Pressure Monitor Model M10-IT, Omron, Kyoto, Japan) was used for women aged 46. The averages of systolic and diastolic BP were calculated. Information about medication, doses, and reasons for use was also gathered from the postal questionnaires sent to women of both ages. Hypertension was defined as BP ≥ 140/90 mmHg[33] and/or self-

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reported use of 1 or more of the following antihypertensive medications according to Anatomical Therapeutic Chemical (ATC) Classification: C02, C03, C04, C07, C08, or C09. Spironolactone is a diuretic that can also be used for the treatment of hirsutism or alopecia. Among subjects included in the analysis, no women aged 31 reported using spironolactone. One woman, aged 46, who reported using spironolactone for an indication other than hypertension was excluded.

Preselected major CVD events leading to a specialized healthcare referral (angina pectoris [AP], acute myocardial infarction [AMI], transitory cerebral ischaemia [TIA], stroke, heart failure) were identified based on The International Classification of Diseases, Revisions 8-, 9-, and 10-codes from hospital discharges, hospital outpatient clinics, and basic healthcare registers, with data covering years 1972 to 2018, 1998 to 2018, and 2011 to 2019, respectively. Cardiovascular mortality data were retrieved from the mortality register of Statistics Finland, which contains information about the cause of death and time of death for every deceased individual in Finland. Cardio- and cerebro-vascular events were categorized as AP and/or AMI and as TIA and/or stroke. In addition, individual CVD events (AP, AMI, heart failure, or stroke) and/or CVD mortality were combined to form 1 major composite end point.

Body mass index (BMI) was calculated as the ratio of weight (kg) and height squared (m^2). Body mass index values were obtained from the clinical examination and were supplemented with self-reported BMI values if clinically measured BMI was not available ($n = 40$). Waist circumference (WC) was measured halfway between the iliac crest and the lowest rib during exhalation and was rounded to the nearest 0.5 cm in the examination template.

Information about lifestyle habits was gathered from the postal questionnaires. Smoking habit was classified as never a smoker, former smoker for >6 months, former smoker for <6 months, and current smoker. Consumption of alcohol was classified as no use, light use (<150 g/week), moderate use (150-210 g/week), and heavy use (>210 g/week). Physical activity at age 31 was classified according to the frequency of weekly light exercise (once or less, 2 to 3 times, 4 to 6 times per week or daily). Education was classified as basic, secondary, and tertiary.

Laboratory methods

The serum levels of total T (using LC/MS equipment [Agilent Technologies, Wilmington, DE]) and sex hormone-binding globulin (SHBG) were measured as previously described.[32] Free androgen index (FAI) was calculated using the equation $100 \times$ T (nmol/L)/SHBG (nmol/L).

Definition of PCOS

Women with PCOS ($n = 268$) were identified according to the presence of oligo-amenorrhea (OA) and hirsutism (H) at age 31 or previous diagnostic of PCOS by a practitioner, as previously described, using information gathered from postal questionnaires at ages 31 and 46.[34] The validity of this questionnaire in distinguishing PCOS cases has already been shown in our previous studies of the same cohort.[35-38]

Statistical analysis

Continuous data were presented as means ± SDs for normal distributions and medians for skewed distributions (25th percentile; 75th percentile). The differences between continuous baseline parameters were analysed using the ANOVA and Kruskal-Wallis test, when appropriate. Categorical data were analysed using cross-tabulation and Pearson's χ^2 test.

A linear regression model was used to investigate the association of T, SHBG, and FAI with systolic and diastolic BP, and the results were reported as beta coefficients (βs) with 95% CIs. For linear regression analysis, skewed distributions for T, SHBG, and FAI were ln-transformed to achieve normality.

A logistic regression model was used to investigate the association of quartiles of T, SHBG, and FAI with hypertension, and the results were reported as odd ratios with 95% CIs.

The Cox proportional-hazards model was used to examine the association of T, SHBG, and FAI with CVD events, and the results were reported as hazard ratios (HRs) with 95% CIs. The results were further adjusted with 2 models to estimate the effect of possible confounding factors. Thus, Model I was adjusted for baseline BMI, and Model II for combined oral contraceptives (COCs), lifestyle habits (smoking, alcohol consumption, and physical activity), and education. Because COCs had effects on both the dependent variables (T, SHBG, and FAI) and the outcome variables (BP and CVD), they fulfilled the criteria for a confounding factor. We therefore adjusted the results for COC use rather than excluding women who were using COCs. The proportional-hazards assumption for each model was checked using the Schoenfeld residuals test. A P-value of <.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 22.0 (SPSS Inc., 1989, 2013, IBM Corp.) and R version 4.0.3 (R Core Team, 2014, Vienna, Austria).

Sensitivity analysis

We also performed analyses in which women with PCOS were excluded, to allow us to separate the effect of PCOS and hyperandrogenaemia per se, as PCOS is the most common cause of hyperandrogenaemia.

Ethical approval

The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern Ostrobothnia Hospital District approved the study (decision number 94/2011 and 12/2003). All subjects received both written and oral information about the study and gave their written consent to participate.

Results

Baseline characteristics in women in quartiles of testosterone (T)

Body mass index increased with increasing T quartiles ($P < .001$), as well the prevalence of PCOS ($P < .001$). As expected, use of COC decreased with increasing T quartiles ($P < .001$). Women in the highest T quartile were more often current smokers ($P < .001$) and heavy alcohol users (P-value .01) than women in the other T quartiles. Waist circumference ($P = .19$), weight gain between age 31 and 46 ($P = .07$), and education level ($P = .39$) did not differ significantly between T quartiles (Table 1).

Cross-sectional analysis of association between T, SHBG, and FAI levels, and BP levels and hypertension at age 31

Systolic and diastolic BP levels, as well as the prevalence of measured BP ≥ 140/90 mmHg and use of antihypertensive medication, increased with increasing T quartiles (respective

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Table 1. Baseline characteristics in quartiles of T levels at age 31 in the women of the study population.

Characteristic	At age 31				*P*-value
	Q1 (*n* = 329-680)	Q2 (*n* = 397-678)	Q3 (*n* = 404-684)	Q4 (*n* = 422-833)	
T (nmol/L)	0.59 [0.49; 0.67]	0.85 [0.80; 0.92]	1.12 [1.05; 1.19]	1.56 [1.40; 1.81]	<.001
SHBG (nmol/L)	40.54 [29.07; 54.89]	43.73 [32.92; 57.14]	44.80 [33.76; 62.16]	49.37 [34.98; 66.65]	<.001
FAI	1.44 [1.01; 1.93]	1.99 [1.48; 2.62]	2.49 [1.82; 3.35]	3.26 [2.41; 4.88]	<.001
BMI (kg/m²)	22.73 [20.83; 25.43]	22.92 [21.03; 25.41]	23.44 [21.31; 26.33]	23.50 [21.16; 27.01]	<.001
ΔBMI* (kg/m²)	2.34 ± 2.84	2.82 ± 3.24	2.83 ± 3.43	2.65 ± 3.43	.07
WC (cm)	76.00 [70.50; 84.00]	75.50 [70.50; 82.00]	76.90 [70.50; 84.50]	77.00 [70.50; 85.50]	.19
Systolic BP (mmHg)	117.00 [111.00; 125.00]	117.00 [110.00; 126.00]	119.00 [112.00; 128.00]	120.00 [113.00; 130.00]	<.001
Diastolic BP (mmHg)	73.00 [67.50; 81.00]	74.00 [67.00; 80.00]	75.00 [69.00; 82.00]	76.00 [69.00; 84.00]	<.001
Use of antihypertensive medication % (*n*)	1.1 (7/660)	1.4 (9/660)	2.1 (14/661)	3.0 (20/660)	.04
Measured BP ≥ 140/90 mmHg % (*n*)	23.0 (149/649)	23.6 (153/647)	28.2 (182/646)	32.9 (209/636)	<.001
Use of COC % (*n*)	31.4 (207/660)	22.7 (150/660)	21.2 (140/661)	20.6 (136/660)	<.001
Smoking % (*n*)					<.001
Never	47.7 (305/640)	39.1 (253/647)	38.8 (252/649)	33.5 (217/648)	
Former > 6 months	25.0 (160/640)	23.6 (153/647)	21.6 (140/649)	19.6 (127/648)	
Former < 6 months	10.2 (65/640)	12.8 (83/647)	13.7 (89/649)	12.2 (79/648)	
Current	17.2 (110/640)	24.4 (158/647)	25.9 (168/649)	34.7 (225/648)	
Alcohol consumption % (*n*)					.01
Not use	10.6 (67/633)	9.9 (64/646)	7.8 (50/645)	10.6 (68/639)	
Light use	87.0 (551/633)	86.1 (556/646)	86.8 (560/645)	82.5 (527/639)	
Moderate use	1.4 (9/633)	2.0 (13/646)	2.9 (19/645)	3.1 (20/639)	
Heavy use	0.9 (6/633)	2.0 (13/646)	2.5 (16/645)	3.8 (24/639)	
Frequency of light physical activity % (*n*)					.02
Once a week or less	26.0 (167/642)	21.2 (136/642)	25.4 (162/639)	29.0 (186/641)	
2-3 times a week	23.4 (150/642)	27.4 (176/642)	23.0 (147/639)	26.1 (167/641)	
4-5 times a week	15.6 (100/642)	18.2 (117/642)	14.9 (95/639)	13.3 (85/641)	
Daily	35.0 (225/642)	33.2 (213/642)	36.8 (235/639)	31.7 (203/641)	
Education % (*n*)					.39
Basic	8.1 (53/653)	8.0 (53/660)	8.5 (56/659)	9.8 (64/655)	
Secondary	73.0 (477/653)	75.9 (501/660)	71.9 (474/659)	74.4 (487/655)	
Tertiary	18.8 (123/653)	16.1 (106/660)	19.6 (129/659)	15.9 (104/655)	
Presence of PCOS % (*n*)	7.0 (23/329)	7.6 (30/397)	9.4 (38/404)	21.3 (90/422)	<.001
Prevalence of CVD by age 53% (*n*)					
AP/AMI	1.2 (8/680)	0.7 (5/678)	2.0 (14/684)	2.2 (18/833)	.04
TIA/Stroke	3.4 (23/680)	3.7 (25/678)	2.6 (18/684)	4.2 (35/833)	.57
CVD event/CVD death	3.4 (23/680)	3.2 (22/678)	2.9 (20/684)	4.3 (36/833)	.34

The results are reported as medians (25th percentile; 75th percentile), as means ± SD or as percentages (*n*). The differences between quartiles of the continuous data were analysed using a Kruskal-Wallis test or ANOVA, when appropriate. The differences between the categorized data were analysed using a Pearson's χ^2 test. *ΔBMI = BMI at age 46 − BMI at age 31.
Abbreviations: T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; BMI, body mass index; WC, waist circumference; BP, blood pressure; COC, combined oral contraceptives; PCOS, polycystic ovary syndrome; CVD, cardiovascular disease; AP, angina pectoris; AMI, acute myocardial infarction; TIA, transitory cerebral ischaemia.

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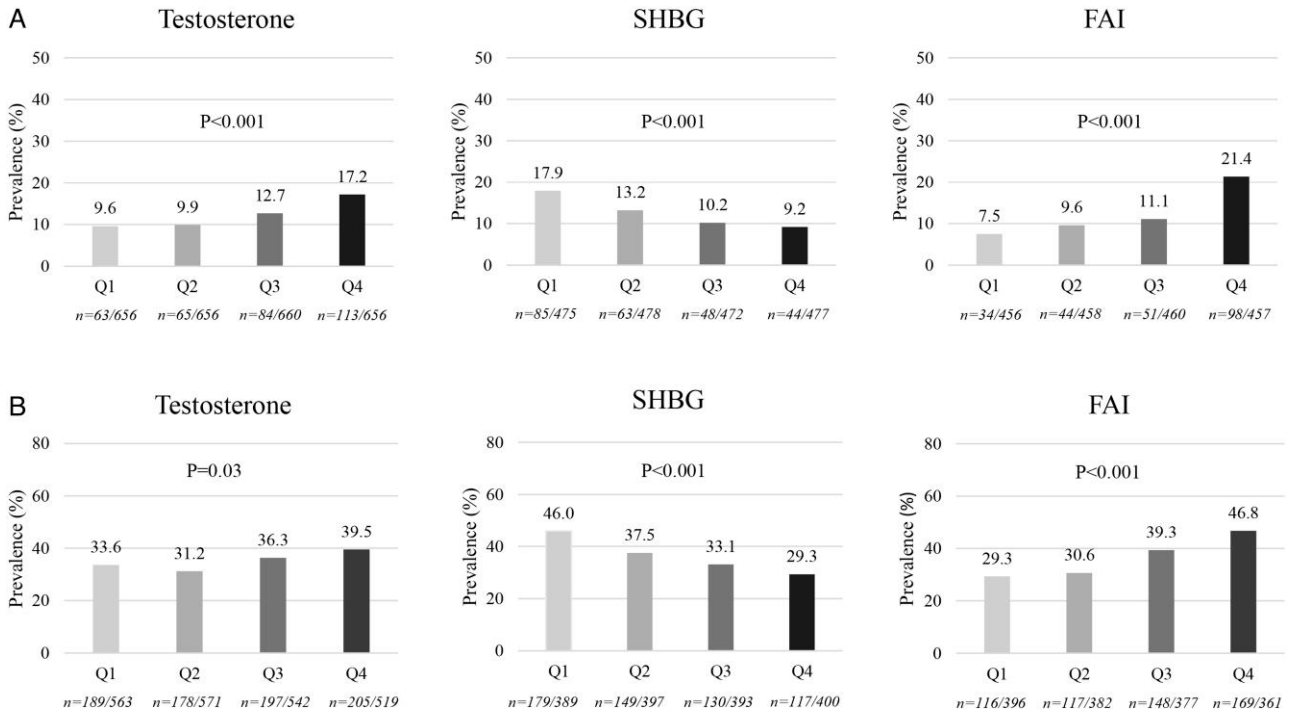


Figure 2. Prevalence of hypertension at age 31 in the cross-sectional study setting (A) and at age 46 in the longitudinal study setting (B) in quartiles of T, SHBG, and FAI at age 31.

Table 2. Association of levels of T, SHBG, and FAI at age 31 with systolic and diastolic BP at ages 31 and 46.

	At age 46							
	Systolic BP		Diastolic BP		Systolic BP		Diastolic BP	
Parameter	β	95% CI	β	95% CI	β	95% CI	β	95% CI
T								
Unadjusted	**2.71**	**1.67-3.77**	**2.46**	**1.55-3.37**	**2.38**	**0.76-4.01**	**2.12**	**1.01-3.20**
Model I	**2.00**	**0.99-3.01**	**1.86**	**0.98-2.73**	**1.63**	**0.03-3.23**	**1.45**	**0.40-2.52**
Model II	**2.80**	**1.75-3.86**	**2.46**	**1.54-3.38**	**2.12**	**0.45-3.79**	**1.65**	**0.54-2.77**
SHBG								
Unadjusted	**−3.75**	**−4.89-−2.61**	**−3.79**	**−4.76-−2.81**	**−3.49**	**−5.29-−1.69**	−3.47	−4.68-2.27
Model I	−0.08	−1.31-1.16	−0.64	−1.69-0.42	−0.16	−2.16-1.83	−0.88	−2.20-0.45
Model II	−0.33	−1.63-0.97	**−0.93**	**−2.04-−0.18**	−1.21	−3.34-0.91	**−1.50**	**−2.91-−0.09**
FAI								
Unadjusted	**4.26**	**3.31-5.20**	**4.14**	**3.33-4.95**	**3.34**	**1.81-4.86**	**3.31**	**2.29-4.33**
Model I	**1.78**	**0.76-2.79**	**2.01**	**1.14-2.88**	1.17	−0.50-2.84	**1.51**	**0.40-2.62**
Model II	**2.25**	**1.18-3.32**	**2.56**	**1.64-3.48**	**2.16**	**0.39-3.93**	**2.03**	**0.86-3.21**

Model I: adjusted for BMI. Model II: adjusted for BMI, use of COCs, smoking, alcohol consumption, physical activity, and education. Statistically significant results bolded.
Abbreviations: T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; BP, blood pressure; BMI, body mass index; COC, combined oral contraceptives.

P-values: <.001, < .001, < .001, .04) (Table 1). Prevalence of hypertension at age 31 increased with increasing quartiles of T ($P < .001$) and FAI ($P < .001$) and decreased with increasing quartiles of SHBG ($P < .001$) at age 31 (Figure 2A).

Levels of T and FAI were positively associated with systolic and diastolic BP levels at age 31 in the unadjusted and adjusted linear regression models. SHBG was inversely associated with BP levels in the unadjusted, but not in the adjusted, models (Table 2).

The highest quartiles of T and FAI were both significantly and positively associated with hypertension at age 31 in the unadjusted and adjusted logistic regression models. Quartiles of SHBG were inversely associated with hypertension in the unadjusted, but not in the adjusted, logistic regression models (Table 3).

Longitudinal analysis between T, *SHBG*, and FAI levels at age 31, and BP levels and hypertension at age 46

Increasing quartiles of T ($P = .03$) and FAI ($P < .001$) and decreasing quartiles of SHBG ($P < .001$) at age 31 were associated with an increased prevalence of hypertension at age 46 (Figure 2B).

Levels of T and FAI at age 31 were positively associated with systolic and diastolic BP levels at age 46 in the unadjusted

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Table 3. Association of quartiles of T, SHBG, and FAI at age 31 with hypertension at age 31.

T				
	Q1 (*n* = 609-656)	**Q2 (*n* = 616-656)**	**Q3 (*n* = 620-660)**	**Q4 (*n* = 611-656)**
Unadjusted	1.00	1.04 (0.72-1.49)	1.37 (0.97-1.94)	**1.96 (1.41-2.72)**
Model I	1.00	1.02 (0.70-1.47)	1.21 (0.85-1.73)	**1.72 (1.23-2.42)**
Model II	1.00	1.10 (0.75-1.62)	1.26 (0.87-1.84)	**1.81 (1.26-2.60)**
SHBG				
	Q1 (*n* = 451-475)	**Q2 (*n* = 440-478)**	**Q3 (*n* = 447-472)**	**Q4 (*n* = 443-477)**
Unadjusted	1.00	**0.70 (0.49-0.99)**	**0.52 (0.36-0.76)**	**0.47 (0.32-0.69)**
Model I	1.00	1.08 (0.73-1.58)	0.99 (0.65-1.53)	0.96 (0.62-1.50)
Model II	1.00	1.09 (0.74-1.62)	0.94 (0.60-1.46)	0.90 (0.57-1.42)
FAI				
	Q1 (*n* = 422-456)	**Q2 (*n* = 435-458)**	**Q3 (*n* = 433-460)**	**Q4 (*n* = 426-457)**
Unadjusted	1.00	1.32 (0.83-2.12)	1.55 (0.98-2.44)	**3.39 (2.24-5.13)**
Model I	1.00	1.21 (0.76-1.94)	1.17 (0.73-1.86)	**1.93 (1.23-3.04)**
Model II	1.00	1.36 (0.83-2.24)	1.28 (0.78-2.10)	**2.07 (1.27-3.39)**

Model I: adjusted for BMI. Model II: adjusted for BMI, use of COCs, smoking, consumption of alcohol, physical activity, and education. Statistically significant results bolded.
Abbreviations: T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; BMI, body mass index; COC, combined oral contraceptives.

Table 4. Association of quartiles of T, SHBG, and FAI at age 31 with hypertension at age 46.

T				
	Q1 *n* = 522-563	**Q2 *n* = 536-571**	**Q3 *n* = 509-542**	**Q4 *n* = 486-519**
Unadjusted	1.00	0.90 (0.70-1.15)	1.13 (0.88-1.45)	**1.29 (1.01-1.66)**
Model I	1.00	0.87 (0.68-1.13)	1.03 (0.80-1.33)	1.15 (0.89-1.49)
Model II	1.00	0.89 (0.68-1.16)	1.01 (0.77-1.32)	1.17 (0.89-1.54)
SHBG				
	***n* = 370-389**	***n* = 366-397**	***n* = 373-393**	***n* = 372-400**
Unadjusted	1.00	**0.71 (0.53-0.94)**	**0.58 (0.43-0.78)**	**0.49 (0.36-0.65)**
Model I	1.00	0.99 (0.73-1.34)	0.98 (0.71-1.36)	0.87 (0.63-1.21)
Model II	1.00	1.04 (0.76-1.43)	0.99 (0.71-1.40)	0.89 (0.63-1.27)
FAI				
	***n* = 368-396**	***n* = 363-382**	***n* = 357-377**	***n* = 336-361**
Unadjusted	1.00	1.07 (0.78-1.45)	**1.56 (1.16-2.10)**	**2.13 (1.58-2.87)**
Model I	1.00	0.98 (0.72-1.34)	1.22 (0.89-1.67)	1.29 (0.92-1.79)
Model II	1.00	1.02 (0.73-1.41)	1.19 (0.86-1.66)	1.28 (0.90-1.83)

Model I: adjusted for BMI. Model II: adjusted for BMI, use of COCs, smoking, consumption of alcohol, physical activity, and education. Statistically significant results bolded.
Abbreviations: T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; BMI, body mass index; COC, combined oral contraceptives.

and adjusted linear regression models (Table 2). The highest quartiles of T and FAI at age 31 were significantly and positively associated, and the highest quartile of SHBG was inversely associated with hypertension at age 46, in the unadjusted logistic regression models, but all significances were lost in the adjusted models (Table 4).

Risk of individual CVD events and composite CVD events and mortality

During the 22-year follow-up (by age 53 years, 116 479 person-years in total), 74 women developed AP and/or AMI, 150 women developed TIA or stroke, and 160 women had suffered at least 1 prespecified cardio- and/or cerebrovascular event (AP, AMI, heart failure, stroke) or had died from a CVD. Prevalence of AP/AMI by age 53 increased from 1.2% in the lowest T quartile to 2.2% in the highest T quartile ($P = .02$).

Serum levels of T at age 31 were not associated with AP and/or AMI, TIA, and/or stroke, or with combined cardio- and cerebro-vascular events and CVD mortality by age 53 in the Cox regression analysis. SHBG was inversely associated, and FAI positively associated, with an increased risk of AP and/or AMI and combined cardio- and cerebro-vascular events

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Table 5. Association of levels of T, SHBG, and FAI with risk of incident of AP/AMI, TIA/stroke, and CVD event/CVD death.

	AP/AMI		TIA/Stroke		CVD Event/CVD Death	
Parameter	HR	95% CI	HR	95% CI	HR	95% CI
T						
Unadjusted	0.46	0.78-3.34	1.04	0.65-1.66	1.15	0.72-1.84
Model I	1.41	0.68-2.95	1.01	0.63-1.61	1.03	0.64-1.65
Model II	2.00	0.88-4.56	1.15	0.69-1.91	1.13	0.67-1.91
SHBG						
Unadjusted	**0.44**	**0.20-0.96**	0.68	0.42-1.12	**0.57**	**0.35-0.94**
Model I	0.57	0.23-1.39	0.90	0.52-1.54	0.89	0.52-1.54
Model II	0.55	0.21-1.45	0.95	0.54-1.70	0.95	0.53-1.69
FAI						
Unadjusted	**2.02**	**1.06-3.85**	1.06	0.87-2.00	**1.54**	**1.02-2.33**
Model I	1.63	0.79-3.38	1.05	0.67-1.65	1.06	0.68-1.65
Model II	2.10	0.95-4.68	1.10	0.68-1.80	1.06	0.66-1.72

Model I: adjusted for BMI. Model II: adjusted for BMI, use of COCs, smoking, consumption of alcohol, physical activity, and education. Statistically significant results bolded.
Abbreviations: T, testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index; BMI, body mass index; COC, combined oral contraceptives; AP, angina pectoris; AMI, acute myocardial infarction; TIA, transitory cerebral ischaemia; CVD, cardiovascular disease.

and CVD mortality in the unadjusted models. However, the significance of both associations was lost after adjusting for BMI (Table 5).

Exclusion of women with PCOS

After excluding women with PCOS, cross-sectional analyses remained substantially unchanged. However, in the longitudinal analyses, the levels of T and FAI at age 31 were positively associated with systolic and diastolic BP at age 46 only in the unadjusted linear regression models. After BMI adjustment, a significant association remained only between the highest FAI quartiles and hypertension. Regarding CVD events, a positive association remained only between FAI and combined cardio/cerebrovascular events by age 53 in the unadjusted Cox regression analyses (Supplementary material).

Discussion

In this large, population-based cohort study with a 22-year follow-up, significant independent positive associations were shown between serum T levels and FAI at age 31 with systolic and diastolic BP at both age 31 and 46. Also, the highest quartiles of T and FAI were independently and positively associated with hypertension at age 31. Lastly, higher FAI at age 31 was associated with an increased risk of AP and/or AMI and combined cardio- and cerebro-vascular events and CVD mortality, but this association vanished after adjustment for BMI.

The present study provides novel information concerning the risk of hypertension and CVDs among premenopausal hyperandrogenic women, as previous studies primarily focused on women with PCOS, postmenopausal women, or women of diverse ages. T levels fluctuate throughout a woman's life span, decreasing steadily during reproductive age, reaching a nadir at age 60, and then increasing back to a premenopausal level after the age of 70,[28,29] and so results of previous studies that did not stratify for age may have been confounded by this. Further, prior studies that investigated CVD risk among premenopausal hyperandrogenic women did not focus solely on HA, as they were conducted mostly on women with PCOS, leaving the cardiovascular impact of HA among women on the population level unknown.

Studies investigating the potential mechanisms underlying the relationship between androgens and elevated BP in women have shown that in dihydrotestosterone (DHT)-treated female mice, an increase in BP may be mediated through the sympathetic nervous system and via the activation of the melanocortin-4-receptor.[39] DHT-treated female mice have also shown an increased expression of 20-hydroxyeicosatetraenoic acid (HETE-20) in kidney microvessels as well as increased expression of angiotensinogen[40] and angiotensin-converting enzyme.[41] Results of clinical studies, however, remain conflicting. To our knowledge, only 1 other study used LC-MS/MS[10] to investigate the association of T levels with BP and hypertension among non-selected premenopausal women. This population-based study with a 5-year follow-up included 777 premenopausal and 651 postmenopausal women. The results showed a positive association of T levels with BP among the whole population and, more specifically, in postmenopausal, but not premenopausal, women.[10] Another cohort study that employed LC-MS/MS reported a positive relationship between total T and systolic BP as well as hypertension among 2560 women aged 20-80 years, although it did not present the results separately according to menopause status.[13] Conversely, a recent cohort study of 3626 women aged 18-79 years using LC-MS/MS did not find an association between T and BP or hypertension.[16] Our results partially align with these previous data, as the association of T levels and FAI with BP levels and hypertension at age 31 was consistently strong. We speculate that such discrepancies may be due to age-related fluctuations in T levels. Furthermore, postmenopausal women in the cohort study by Ziemens et al.[10] had a higher BMI, possibly predisposing them to elevated BP compared to premenopausal women.

Only a few studies have investigated the association of premenopausal T levels with CVDs and CVD mortality in women in the context of HA alone.[17,19,21] A recent population-based cohort study of 264 282 women aged 40-69 years and with a 12.5-year follow-up reported that the highest quartiles of FAI were positively, independently associated with a risk of AMI among premenopausal women compared to women in the lowest quartile.[17] In contrast, in one population-based study of 2129 mainly premenopausal women with a mean age of 49.0 years and a 10.9-year follow-up using LC-MS/MS detected no longitudinal relationship between T and CVDs or CVD mortality. Of note, a positive association between free

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T and FAI and CVDs at baseline in the age-adjusted model was found.[19] Again, these previous results are partly in line with our own, as we found a positive association of FAI with AP and/or AMI as well as with cardio- and cerebro-vascular events during the 22-year follow-up in the unadjusted model—an association that disappeared after adjustment for BMI. Of note, the relatively young age of our population at the end of the follow-up period (53 years) may have limited the possible association of HA with CVD events. Also, as the incidence of CVD events in women increases after menopause, the number of CVD events in the present study can be expected to increase during upcoming decades.

Another important issue to consider in the relationship between sex-steroids' concentrations and CVD risk in women. Oestrogens and especially oestradiol have been shown to exert a potential cardioprotective effects during premenopause. This oestrogen protection hypothesis has been built on previous clinical observations showing that the incidence of CVD in women delays that of men by 10 years.[42] Additionally, surgically induced menopause and premature natural menopause are associated with an increased CVD risk.[43-45] During the past decades several potential, oestrogen-driven cardioprotective mechanisms have been recognized. Oestrogens have been shown to improve mitochondrial function and to reduce reactive oxygen species,[46,47] to improve extracellular matrix remodelling,[48,49] and to modulate vasorelaxation, vasoconstriction and endothelial function.[50] During the menopausal years women undergo a major hormonal transition when ovarian sex-steroid production declines, including both androgens and oestrogens with a relatively more dramatic decrease of oestradiol's production with a shift of the sex-hormone profile towards a more androgenic pattern. This change is speculated to partially predispose women to CVD after menopause[18] highlighting the importance of following the women of the present cohort for longer after the menopausal years.

Interestingly, one large cohort study recently demonstrated that BP trajectories increase more rapidly in women than in men, beginning as early as the third decade.[51] A growing body of evidence shows that elevated BP levels are especially harmful for young women, increasing their BP-mediated risk of acute coronary syndrome in mid-life compared to men.[52-55] Further, even a modest increase in BP of 1.5 to 2 mmHg has been shown to have a marked impact on CVD risk at the population level.[56] In this context, the independent positive association of HA with BP levels and hypertension in young women in their early 30s is of great relevance.

Overall, both previous[10,13,14,18] and present results suggest that premenopausal HA might increase the risk for hypertension and CVD events in women. Of note, previous studies have shown that HA associates also with other significant CVD risk factors including obesity, insulin resistance and type 2 diabetes mellitus[32,57-59] and that HA exacerbates these unfavourable metabolic conditions.[59,60] If future studies confirm the association between HA, hypertension and later life CVD, more attention should be paid to CVD risk profile in premenopausal women with HA, including regular screening for hypertension, dyslipidaemia, and abnormal glucose metabolism. Weight control should already be strongly recommended in this group of women. The recent International Guidelines for the diagnosis and treatment of PCOS state that PCOS is associated with major adverse cardiovascular events independently of the diagnostic criteria used,[61-63] but whether the presence of HA increases the CVD risks linked to PCOS is not highlighted.[61] Therefore, whether the measurement of T or other androgens would improve CVD risk assessment in women with PCOS remains to be clarified.

Our study has several strengths, including its homogenous study population, high participation and response rate, and low dropout rate. We also used the gold standard method, LC-MS/MS, to measure T levels.[27] Additionally, we evaluated the possible confounding effect of PCOS. Several longitudinal associations lost their significance after this exclusion, suggesting that, in addition to elevated BMI, PCOS is an important contributing factor to metabolic risks of HA in women. Importantly, BP was measured at both ages 31 and 46. The diagnosis of CVD events was based on hospital discharge and outpatient registers, which have been shown to have very high coverage and accuracy (88% to 98%) for vascular diseases in Finland.[64]

However, this study has important limitations. First, we used only serum T as a marker of HA, even though other androgens, such as adrenal androgens, play a role in the evaluation of androgenicity in women. In addition, we lacked information on the phase of the menstrual cycle in which blood samples were drawn. We also had no data on the timing of menopause. However, we do not expect that many of the participants were menopausal, as the mean age of menopause in Finland is 51 years.[65] The study population's homogeneity can be considered as a limitation regarding generalisation of the results to other populations. On the other hand, the homogeneity regarding ethnicity as well as the similarity of age is indisputably the greatest strengths of the present study. The present cohort study included women born in 1966, whose BMI was lower than seen nowadays in most populations. This is an important limitation of the present study regarding its generalisability to other populations, as body composition and BMI related thresholds for obesity-related health risks differ between different ethnic groups. These results should therefore be replicated in population including women of different ethnicity and BMIs. Further, it is well known that diet is an important CVD risk modifying factor, but we could not adjust our study results with dietary information. It is also important to note that some of our data is self-reported, which includes a potential source of bias. However, most of the present study important data is clinically measured (levels of T, blood pressure) or drawn from national registers (diagnostic codes for major adverse cardiovascular events). Lastly, the number of CVD events was low, which likely reduced the power of the study for detecting significant associations.

Conclusion

The present study showed a positive association of premenopausal HA with both higher BP as well as diagnosis of hypertension. Furthermore, HA at age 31, together with BMI, was positively associated with adverse CVD events and CVD mortality in the 22-year follow-up, although the low number of CVD cases at this early age may have underestimated the association. Considering our results, women with HA at premenopausal age, especially those who are overweight or obese, may be at elevated risk of hypertension as well as of adverse CVD events in mid-life, and weight control should be emphasised for these women. A longer follow-up of this cohort with postmenopausal data is needed to clarify long-term CVD risks, increased CVD mortality, and their potential relationship to premenopausal HA. Also, future research of the impact of HA on CVD in women in diverse populations is needed.

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Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Authors' contributions

Katri Tuorila (Conceptualization [supporting], Formal analysis [lead], Investigation [equal], Methodology [supporting], Project administration [equal], Visualization [lead], Writing—original draft [lead]), Meri-Maija Ollila (Conceptualization [lead], Investigation [equal], Methodology [lead], Project administration [equal], Supervision [lead], Visualization [supporting], Writing—original draft [supporting]), Elisa Hurskainen (Formal analysis [supporting], Writing—original draft [supporting]), Juha Tapanainen (Writing—original draft [supporting]), Stephen Franks (Writing—original draft [supporting]), Terhi Piltonen (Conceptualization [supporting], Writing—original draft [supporting]), Kari Kaikkonen (Conceptualization [supporting], Methodology [supporting], Writing—original draft [supporting]), and Laure Morin-Papunen (Conceptualization [lead], Funding acquisition [lead], Investigation [equal], Methodology [lead], Project administration [lead], Supervision [lead], Visualization [supporting], Writing—original draft [supporting])

Data availability

NFBC data are available from the University of Oulu, Infrastructure for Population Studies. Permission to use the data can be applied for research purposes via an electronic material request portal. In the use of data, we follow the EU general data protection regulation (679/2016) and the Finnish Data Protection Act. The use of personal data is based on a cohort participant's written informed consent in their latest follow-up study, which may cause limitations to its use. Please, contact the NFBC project center (NFBCprojectcenter(at)oulu.fi) and visit the cohort website (www.oulu.fi/nfbc) for more information.

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