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Increased free androgen index is associated with hypertension in premenopausal women

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ABSTRACT

Objective: Increased testosterone and decreased sex hormone-binding globulin (SHBG) are associated with a number of adverse cardiovascular risk factors in postmenopausal women. The aim of this population-based study of women aged 25 to 50 was to assess the relationship between free androgen index (FAI) and cardiovascular risk factors in premenopausal women. Methods: A population-based survey of 396 premenopausal women with no hormonal treatment was undertaken as part of the Northern Sweden MONICA study. The study involved questionnaires, anthropometry and assays of testosterone and SHBG. Results: Increased FAI was associated with a number of cardiovascular risk factors in premenopausal women but this relationship was strongly affected by body mass index (BMI). After adjustment for age and BMI, FAI was significantly associated with increased systolic and diastolic blood pressures. Conclusion: Hyperandrogenism is associated with increased blood pressure and these findings emphasize the need to assess cardiovascular risk factors in women with hyperandrogenism of all ages.

Keywords: Free Androgen Index; Blood Pressure

1. INTRODUCTION

Testosterone is carried in peripheral blood bound to sex hormone binding globulin, with approximately 1% - 2% of testosterone free and the remaining testosterone loosely bound to albumin [1]. The free androgen index (FAI), *i.e*. the ratio between testosterone and sex hormone binding globulin (SHBG), may be used as an approximation of biologically available androgens in women, and longitudinal studies in aging women indicate that SHBG levels decrease with increasing age, thus resulting in successively increasing amounts of biologically available androgen in healthy women [2].

Increased testosterone and decreased sex hormone-binding globulin (SHBG) have been strongly associated with a number of adverse cardiovascular disease (CVD) risk factors in postmenopausal women, such as central adiposity, decreased high-density lipoprotein (HDL) cholesterol levels and increased systolic and diastolic blood pressures [3-6]. A nested case-control study of postmenopausal women also suggested that higher free androgen index was associated with CVD events such as first occurrence of nonfatal myocardial infarction, coronary revascularization, nonfatal stroke, coronary disease, or stroke death, although this association was not independent of body mass index and other cardiovascular risk factors [7]. Besides a possible role of androgen hormones and SHBG in cardiovascular disease, low levels of SHBG have consistently been linked to higher rates of diabetes [8-9] and might contribute to a more adverse cardiovascular risk profile in women with diabetes [10-11].

Sex hormone levels may also be associated with CVD risk factors in pre- and perimenopausal women, although fewer studies have been conducted in this age-group [12-14]. In younger women most interest has instead been devoted to patients with polycystic ovary syndrome (PCOS), which is a hyperandrogenic population with numerous risk factors for later development of CVD [15]. However, in contrast to healthy women, we have previously shown that testosterone levels gradually decrease with increasing age in PCOS women [16], although levels remain elevated in comparison with age-matched controls [17].

Outside the PCOS patient population, no population-based studies have been conducted in premenopausal women concerning the association between free androgen index and cardiovascular risk factors. Thus, the purpose of this population-based study of premenopausal

women aged 25 to 50 was to assess whether free androgen index is associated with risk factors for cardiovascular disease, even in younger women.

2. MATERIALS AND METHODS

2.1. Patients

This study used data from the Northern Sweden component of the WHO MONICA study, originally designed to evaluate risk factors for cardiovascular disease and type 2 diabetes. Briefly, information was collected during a population-based survey during January to April 2004 [18]. Subjects were randomly selected from population registers, stratified for age (25 years - 74 years) and gender, in the two most northern counties of Sweden (target population 312,000). For the entire MONICA study 1250 men and 1250 women were invited, but for the purpose of this study, only women below age 50 years were considered. Details of sampling and selection appear elsewhere and participation rate was 78% [18]. A total of 500 women 50 years or younger were eligible for this study. Exclusion criteria were postmenopausal status (more than 12 months since last menses), ongoing pregnancy, and use of hormone replacement therapy or combined oral contraceptives during the last year.

The Research Ethics Committee of Umeå University and the National Computer Data Inspection Board approved the Northern Sweden MONICA study. A specific approval for this sub-study was also obtained from The Research Ethics Committee of Umeå University. Participants gave written consent.

2.2. Study Procedures

Subjects were weighed on an electronic scale. The subjects wore light clothes and no shoes and weight was measured to the nearest 0.2 kg. Height without shoes was measured to the nearest centimeter. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). To record waist circumference, measurement was performed midway between the lower rib margin and iliac crest to the nearest 0.0 cm or 0.5 cm. The blood pressure was measured twice in every person after a five-minute rest in a sitting position with Hawksley's random zero sphygmomanometer. The mean value of the two measurements was used.

Blood samples were drawn after at least a 4-h fast (in approximately 65% of subjects blood samples were drawn after an overnight fast).

The participants were asked to complete questionnaires including items about smoking habits, sociodemographic data and previously known cardiovascular disease. The questionnaire also included a number of questions for assessment of reproductive status including menstrual pattern, last menstrual period, use of oral contraceptives and hormone replacement therapy during the past year.

The highest attained educational level was classified as primary school (up to 9 years of school), secondary school (10 years - 12 years of school) and university studies. Regular smokers smoked at least one cigarette a day; all other subjects were considered as nonsmokers. Participants were classified as physically active if reporting physical activity more than two hours per week; all other subjects were considered not physically active. A positive family history of diabetes, stroke and cardiovascular disease was only reported when occurring in first-degree relatives. A history of gestational diabetes was only assessed in parous subjects. Hypertension and elevated serum cholesterol were considered prevalent in women on medication for these reasons.

2.3. Assays

Total cholesterol was determined by a dry chemistry method (Vitros 950; Kodak Echtachem, Rochester, NY, USA). The measurement of total cholesterol is accredited by the national accreditation body, SWEDAC, with coefficients of variation of 3.6% at 3.91 $mmol \cdot L^{-1}$ and 3.1% at 6.66 $mmol \cdot L^{-1}$.

An oral glucose tolerance test (OGTT; 75 g glucose) was performed in a random sub-sample (approximately 65%) of the non-diabetic participants after an overnight fast**.** A venous blood sample was taken immediately before the glucose load and after two hours. Glucose levels were analyzed without delay on a HemoCue Glucose 201 device (HemoCue AB, Angelholm, Sweden) and converted to plasma glucose levels by a correction factor of 1.11. According to previous validation of this procedure, the adjusted data for glucose showed a high correlation to plasma glucose simultaneously measured by the hexokinase method in a sub-sample [18]. SHBG and testosterone were analyzed on a Modular E170 (Roche Diagnostics, Mannheim, Germany). The total coefficients of variation of the instrument for the analytes were 1.5% at 43 nmol/L for SHBG and 6.8% at 3.9 nmol/L for testosterone. Free androgen index was calculated as testosterone (nmol/L)/SHBG (nmol/L) × 100.

Physical and biochemical parameters were analyzed using partial correlation test with adjustment for age and BMI and with logarithmic FAI. The women were grouped into quartiles depending on level of FAI. Dichotomized and continuous variables were compared between groups using linear or logistic regression analysis and adjusted for age and BMI. The software package SPSS (version 12.0) was used for statistical analyses.

3. RESULTS

Fifteen postmenopausal women, 53 women on combined oral contraceptives, 16 women on hormone replacement

therapy, 10 pregnant women and 20 women lacking a blood sample were excluded. Hence, 396 women 50 years or younger were included in the study.

Sociodemographic, anthropometric and reproductive data are presented in **Table 1** and given according to quartiles of FAI. Women in the highest quartile, *i.e*. with the highest level of free testosterone (or the lowest level of SHBG), were significantly older and had higher BMI than those in the lower three quartiles. BMI was 4.5 kg/m^2 higher in the fourth FAI quartile compared to the lowest. Compared to the lowest FAI quartile, women with the highest FAI had lower educational level and were more often smokers. Otherwise, women in the different FAI quartiles did not differ regarding use of snuff or physical activity.

Hypertension was significantly more common among women in the highest FAI quartile (**Table 2**). In the lowest FAI quartile was hypertension prevalent in 19.2% of the subjects while 35.4% of women in the highest quartile had hypertension.

Table 1. Physical characteristics, sociodemographic and reproductive data in women according to free androgen index quartiles.

			FAI lowest 0.13 - 1.10 (n = 99)	FAI 1.11 - 1.84 (n = 99)	FAI 1.85 - 3.07 (n = 99)	FAI highest 3.08 - 68.9 (n = 99)
Age (years ± SD)			39.3 ± 7.2	38.5 ± 6.7	37.3 ± 7.3	41.0 ± 5.5[a]
Parity (mean ± SD)			2.2 ± 1.0	2.4 ± 1.2	2.0 ± 0.9	2.4 ± 0.8
BMI			24.2 ± 3.6	24.6 ± 4.6	26.1 ± 4.6[c]	28.4 ± 6.2[b]
Waist, cm			80.2 ± 10.0	80.3 ± 11.3	84.7 ± 12.6	90.9 ± 14.3[a]
Marital status	Living single	OR (95% CI)	1	1.40 (0.72 - 2.72)	0.70 (0.33 - 1.46)	0.88 (0.42 - 1.85)
		Cases, n	20 (20.2%)	24 (24.5 %)	13 (13.1%)	18 (18.2%)
Educational level	< university level	OR (95% CI)	1	1.10 (0.62 - 1.93)	0.87 (0.49 - 1.53)	1.98 (1.05 - 3.75)[d]
		Cases, n	59 (59.6%)	63 (63.6%)	57 (57.6%)	73 (74.5%)
Smoking	Smokers	OR (95% CI)	1	1.62 (0.77 - 3.41)	3.50 (1.74 - 7.04)[d]	2.38 (1.13 - 5.02)[d]
		Cases, n	13 (13.1%)	21 (21.4%)	35 (35.4%)	26 (26.5%)
Snuff	Snuff users	OR (95% CI)	1	1.43 (0.62 - 3.31)	1.66 (0.72 - 3.83)	1.51 (0.62 - 3.70)
		Cases, n	10 (10.2%)	16 (16.3%)	14 (14.3%)	14 (14.1%)
Physically active	Not active	OR (95% CI)	1	0.63 (0.29 - 1.38)	1.20 (0.59 - 2.43)	1.04 (0.50 - 2.18)
		Cases, n	18 (18.2%)	12 (12.1%)	23 (23.2%)	23 (23.2%)

[a]$p < 0.05$ - 0.01 compared to the two intermediate FAI quartiles, one-way ANOVA with post hoc Tukey Honestly Significance test. [b]$p < 0.05$ - 0.001 compared to all other FAI quartiles, one-way ANOVA with post hoc Tukey Honestly Significance test. [c]$p < 0.05$ compared to the lowest FAI quartile, one-way ANOVA with post hoc Tukey Honestly Significance test. [d]$p < 0.05$ - 0.01 compared to the lowest FAI quartile, multivariate logistic regression adjusted for age and body mass index.

Table 2. Data on diabetes, cardiovascular disease and family history in women according to free androgen index quartiles.

		FAI lowest 0.13 - 1.10 (n = 99)	FAI 1.11 - 1.84 (n = 99)	FAI 1.85 - 3.07 (n = 99)	FAI highest 3.08 - 68.9 (n = 99)
Diabetes	OR (95 % CI)	1	1.12 (0.07 - 18.70)	n.c.	4.62 (0.49 - 43.22)
	cases, n (%)	1 (1.0%)	1 (1.0%)	0	5 (5.1%)
Hypertension	OR (95 % CI)	1	0.55 (0.24 - 1.22)	0.98 (0.47 - 2.03)	2.00 (1.01 - 4.00)[a]
	cases, n (%)	19 (19.2%)	11 (11.1%)	19 (19.2%)	35 (35.4%)
Elevated serum cholesterol	OR (95 % CI)	1	1.50 (0.40 - 5.66)	1.26 (0.32 - 5.00)	2.14 (0.63 - 7.28)
	cases, n (%)	4 (4.0%)	6 (6.1%)	5 (5.1%)	12 (12.1%)
Previous gestational diabetes	OR (95 % CI)	1	1.65 (0.57 - 4.74)	0.68 (0.20 - 2.35)	2.30 (0.81 - 6.56)
	cases, n (%)	6 (6.1%)	10 (10.1%)	5 (5.1%)	15 (15.2%)
Family history of diabetes	OR (95 % CI)	1	1.12 (0.58 - 2.40)	1.00 (0.46 - 2.21)	1.86 (0.90 - 3.83)
	cases, n (%)	16 (16.2%)	17 (17.2%)	15 (15.2%)	29 (29.3%)
Family history of hypertension	OR (95 % CI)	1	1.04 (0.58 - 1.86)	1.55 (0.85 - 2.81)	1.11 (0.61 - 2.04)
	cases, n (%)	48 (48.5%)	47 (47.5%)	55 (55.6%)	56 (56.6%)
Family history of myocardial infarction	OR (95 % CI)	1	0.68 (0.23 - 2.01)	1.00 (0.36 - 2.76)	0.51 (0.16 - 1.64)
	cases, n (%)	9 (9.1%)	6 (6.1%)	8 (8.1%)	5 (5.1%)
Family history of stroke	OR (95 % CI)	1	0.84 (0.25 - 2.89)	0.16 (0.02 - 1.42)	0.41 (0.09 - 1.80)
	cases, n (%)	6 (6.1%)	5 (5.1%)	1 (1.0%)	3 (3.0%)

[a]$p < 0.01$ compared to all other FAI quartiles, multivariate logistic regression adjusted for age and body mass index.

Among women within the highest FAI quartile cholesterol, waist circumference, and blood pressure were significantly higher than in the three groups with lower FAI. However, after adjustment for age and BMI, only systolic and diastolic blood pressures were significantly higher (**Table 3**). Blood pressure was 9/7 mm Hg higher in the highest FAI quartiles than in the lowest quartile. Similarly, free androgen index was significantly and positively correlated with systolic and diastolic blood pressures (**Table 4**).

4. DISCUSSION

The main finding of the present study was that young and premenopausal women within the highest quartile of free androgen levels had several risk factors for cardiovascular disease. However, when adjusted for age and BMI the only associations that remained were the ones between high FAI and high systolic and diastolic blood pressures, respectively. This finding is in line with previous studies in pre- and perimenopausal [14,19] as well as postmenopausal women [3-6]. In perimenopausal women free androgen index was positively and independently associated with a number of cardiovascular risk factors such as total cholesterol, low-density lipoprotein cholesterol, lipoprotein (a), insulin, plasminogen activator inhibitor-1, tissue plasminogen activator and high sensitive c-reactive protein, systolic and diastolic blood pressures [14].

In our cohort of women across different age groups, women with the highest free androgen index were found to be significantly older than the remaining women. This finding is in line with previous longitudinal studies indicating that increased BMI over time is closely related to lowering of SHBG levels [20]. However, a recent longitudinal study in healthy women suggested that, not only did SHBG levels decline with increasing age, testosterone levels also displayed a gradual increase from the age of 40 and onwards [2].

We were also able to confirm prior findings of an association between free androgen levels and increased systolic and diastolic blood pressures, although the majority of these studies were conducted in older, postmenopausal women [3-6,12-14,19]. In addition, the prevalence of manifest hypertension was approximately 35% among women with the highest FAI. Notably, in our previous longitudinal study of 40-year old PCOS women, systolic and diastolic did not differ from controls following adjustment for BMI, and manifest hypertension was only prevalent in approximately 16% of PCOS women [21].

The free androgen index is mainly driven by the SHBG levels and the best known function of SHBG is to regulate the availability of biologically active free testosterone and estradiol and their metabolic clearance rate [22]. SHBG production is stimulated by estradiol and inhibited by androgens indicating that a high level of SHBG would temper the unfavorable effects of free androgens on blood pressure. Besides its contribution to free androgen levels, low levels of SHBG have consistently been linked to insulin resistance [23], higher rates of diabetes [8-9,23] and might contribute to a more adverse cardiovascular risk profile in women with diabetes [10-11]. Testosterone on its own may also contribute to the increased blood pressure found in our women within the highest FAI quartile. Previous animal studies have indicated a prohypertensive effect of androgens which may be mediated through increased vascular tone via upregulation of thromboxane A2 expression, norepinephrine, angiotensin II and endothelin-1 synthesis [24]. Other means by which androgens may influence blood pressure also include effects on the renin-angiotensin-aldosterone system [24].

Our findings also indicate a clear association between obesity and increased waist circumference, as a measure of central adiposity, and elevated free androgen levels. This finding is in line with a number of studies [3-6, 12-14,19,25] and is further evidenced by studies suggesting that weight loss, whether induced by bariatric surgery, anti-obesity agents, or life-style interventions, will

Table 3. Physical and biochemical measures in women according to free androgen index quartiles.

	FAI lowest 0.13 - 1.10 (n = 99)	FAI 1.11 - 1.84 (n = 99)	FAI 1.85 - 3.07 (n = 99)	FAI highest 3.08 - 68.9 (n = 99)
Fasting glucose, mmol/l	5.1 ± 0.5	5.2 ± 0.5	5.1 ± 0.6	5.5 ± 1.6
2-hour glucose, OGTT, mmol/l	5.3 ± 1.3	5.5 ±1 .0	5.5 ± 1.6	5.9 ± 2.2
Total chole-sterol, mmol/l	5.1 ± 1.1	4.9 ± 0.9	5.2 ± 1.0	5.5 ± 1.1
SBP, mmHg	115 ± 15	112 ± 11	114 ± 11	124 ± 19 [a]
DBP, mmHg	72 ± 9	71 ± 8	71 ± 8	79 ± 13 [b]

[a]$p < 0.01$, [b]$p < 0.001$ compared to all other FAI quartiles, ANCOVA adjusted for age and body mass index. For the glucose variable and for the 2 hour glucose variable, data was missing in 161 (40.7%) women and in 212 (53.5%) women, respectively. For all other variables, data was missing in 0.3% - 1.0% of women. OGTT, oral glucose tolerance test, SBP systolic blood pressure, DBP diastolic blood pressure.

Table 4. Partial correlations between free androgen index and physical or biochemical parameters, adjusted for age and body mass index.

	Fasting glucose	2 hour glucose	Cholesterol	WC	SBP	DBP
Free androgen index	–0.018	0.119	0.052	–0.08	0.213[a]	0.262[b]

[a]$p < 0.01$, [b]$p < 0.001$. For the glucose variable, data was missing in 161 (40.7%) women. For all other variables, data was missing in 0.3% - 1.0% of women. SBP systolic blood pressure, DBP diastolic blood pressure, WC waist circumference.

reduce androgen levels [25-26].

The major limitation of this study is the relatively small sample size, which might raise questions whether this study was adequately powered for some of the analyses. However, the study has pointed out that increased FAI is associated with a number of cardiovascular risk factors but these relationships seem to be strongly affected by BMI. Free androgen index was significantly and independently associated with increased systolic and diastolic blood pressures, emphasizing the need to assess cardiovascular risk factors in women with hyperandrogenism across all ages.

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