Androgens in relationship to cardiovascular risk factors in the menopausal transition


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Key words: ANDROGENS, ABDOMINAL OBESITY, LIPOPROTEINS, INSULIN RESISTANCE, MENOPAUSAL TRANSITION

ABSTRACT

Objective To establish the relationship between androgens and cardiovascular disease (CVD) risk factors in the menopausal transition.

Methods A total of 124 women were divided into four groups: 29 premenopausal (PreM), 35 women in the menopausal transition still menstruating (MTM), 29 women in the menopausal transition with 3–6 months amenorrhea (MTA), and 31 postmenopausal women (PostM). Levels of triglycerides, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, glucose and insulin were assayed in all samples and waist circumference was measured. In a subgroup of 83 women (19 PreM, 21 MTM, 28 MTA and 15 PostM), levels of total testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS) and estradiol were determined. The free androgen index, Homeostasis Model Assessment (HOMA) index, Quantitative Insulin Sensitivity Check Index (QUICKI) and McAuley index, estradiol/total testosterone and triglyceride/HDL cholesterol ratios were calculated.

Results Androstenedione was higher in MTA vs. PostM women (p < 0.05); DHEAS was higher in PreM women vs. the other three groups (p < 0.05). Sex hormone binding globulin (SHBG) in MTM women was higher than in MTA women (p < 0.05); the free androgen index was lower in MTM women than in MTA and PostM women. SHBG and the free androgen index showed negative and positive correlations, respectively with waist circumference, insulin resistance and lipids. In a multiple regression analysis, considering waist circumference, neither free androgen index nor SHBG showed significant differences between groups. The waist circumference correlated only with SHBG (p = 0.022) and correlations between SHBG and insulin resistance markers continued to be significant, but relationships between SHBG and lipoproteins and all correlations found with free androgen index were lost.

Conclusions An increment in the androgenic milieu that correlates with abdominal fat, insulin resistance and atherogenic lipoproteins becomes evident after the menopausal transition and suggests that evaluation of cardiovascular disease risk in these women
Androgens and cardiovascular risk in the menopausal transition

INTRODUCTION
Changes in androgen levels during the menopausal transition are controversial. Although it is generally accepted that testosterone levels do not change significantly in this period of life, some authors have found a decrease in testosterone, androstenedione and sex hormone binding globulin (SHBG) 2 years around menopause. On the other hand, other investigators have found that the androgen mid-cycle peak is absent or diminished in perimenopausal women. More recently, Davison and colleagues found that levels of total testosterone, calculated free testosterone, dehydroepiandrosterone sulfate (DHEAS) and androstenedione declined steeply with age in a reference population. The decline of each hormone was greater in the earlier than the later decades, without variations in SHBG over the first five decades of adult life and minimal increments around 70 years old. Examination of serum androgen levels per year in women between 45 and 54 years old showed no independent effect of menopausal status on androgen levels. Although levels of DHEAS decrease with age, some authors report a transitory increment in some women, in relation to the last stages of the menopausal transition.

In relation to androgen levels in the postmenopause, some authors consider that this is a period of relative hyperandrogenism as a consequence of the greater decrease in estrogens in comparison with the decrease in androgens. In women, the increment in the androgenic status has been related to hyperinsulinemia, glucose intolerance and insulin resistance, as well as a high risk of cardiovascular disease (CVD) and type 2 diabetes. The alteration of the hormonal balance, with androgenic predominance, also contributes to an augmented visceral fat deposition, which in turn is associated with insulin resistance in postmenopausal women. As we demonstrated in previous studies, the deposition of abdominal fat is increased after the menopausal transition and is associated with alterations of the lipoprotein profile and carbohydrate metabolism. However, the relationship between these factors and the androgenic profile in the menopausal transition has been scarcely studied.

Androgens and SHBG have been associated with CVD risk factors in pre- and postmenopausal women. In the latter group, testosterone increment and SHBG diminution are associated with central adiposity, elevated triglycerides and diminished high density lipoprotein (HDL) cholesterol. More recently, SHBG and the free androgen index (FAI) have been associated with cardiovascular risk in pre- and perimenopausal women.

Considering the previous comments, the aim of this study is to establish the relationship between androgens and different risk factors such as an altered lipoprotein profile, android body fat distribution and insulin resistance, in a group of women in the menopausal transition.

METHODS
Patients
We studied 124 women divided into four groups, according to their menstrual bleeding stage: 29 premenopausal women (PreM) with regular menstrual cycles, from 26 to 40 years of age; 35 women in the menopausal transition with irregular menstrual cycles, all of them with oligomenorrhea, with cycles between 35 and 80 days, from 42 to 53 years old (MTM); 29 women in the menopausal transition with periods of 3–6 months amenorrhea, from 44 to 55 years of age (MTA); and 31 postmenopausal women, from 46 to 69 years old (PostM), with at least 1 year of spontaneous amenorrhea. The groups of women in menopausal transition and postmenopausal women were consecutively selected at the Climacteric Unit, Gynecology Division, University Clinical Hospital, Buenos Aires and the group of premenopausal women, included as a reference group, was recruited consecutively from patients that attended in the same division, for their routine health check. Women included in the study had to have an intact uterus, alcohol consumption lower than 10 g/day, be non-smokers, and have a varied diet. None of the women were included if they were pregnant or receiving hormonal replacement therapy, oral

should include androgens, considering that abdominal obesity is one of the main determinants of the relationship between androgenic parameters and cardiovascular risk factors.
contraceptives or any other drug modifying lipid metabolism in the previous 3 months. Women with diabetes, renal, hepatic or thyroid disorders, CVD, stroke, thromboembolic disease, as well as those whose weight had varied more than 5% in the previous 6 months, were also excluded. All subjects gave their informed consent and the protocol was approved by the Ethics Committee of the University Clinical Hospital of Buenos Aires. In a subgroup of 83 women (19 PreM, 21 MTM, 28 MTA and 15 PostM), in whom enough serum samples were available, androgenic profile and estradiol levels were also determined.

**Samples**

Blood samples were collected from women in the four groups by vein puncture, between 08:00 and 09:00, after 12 h fasting. In the PreM and MTM groups, blood samples were drawn in the follicular phase of the menstrual cycle (days 3–7) and at random in the other two groups. After serum was separated, disodium EDTA, 1.0 mg/ml serum, and 0.1 mg/ml sodium azide were added to inhibit lipoprotein peroxidative degradation and bacterial growth. Samples were kept at 4°C until their processing within 48 h. For hormonal determinations, serum was stored at −70°C until processing.

**Anthropometric parameters**

Waist circumference was measured at the level midway between the lateral lower rib margin and the superior anterior iliac crest, with the woman in a standing position, and always by the same investigator.

**General analytical methods**

Triglycerides and glucose were measured in a Hitachi 917 autoanalyzer by enzymatic methods (Roche Diagnostics, Mannheim, Germany). LDL and HDL cholesterol were determined by selective precipitation methods. Serum lipid measurement was conducted under good quality control (coefficient of variation (CV) routinely <3%). Insulin was measured through a radioimmunoassay (DPC, Diagnostic Products Corporation, LA, USA). The insulin method showed a CV lower than 10% across the whole concentration range and its analytical sensitivity was 1.2 μIU/ml. Insulin resistance was evaluated by the calculation of different proposed indices: Homeostasis Model Assessment (HOMA) index, as described by Matthews and colleagues, the Quantitative Insulin Sensitivity Check Index (QUICKI) and McAuley index. The triglyceride/HDL cholesterol ratio was also calculated as an indicator associated with insulin resistance.

Total testosterone was determined through radioimmunoassay (RIA) (DSL, Diagnostic System Laboratories, Inc., Texas, USA), analytical sensitivity 0.08 ng/ml and CV intra- and inter-assay lower than 10% in the whole range analyzed. Androstenedione was determined through a double-antibody RIA (DSL, Diagnostic System Laboratories), analytical sensitivity 0.02 ng/ml, CV intra-assay lower than 6.0% and CV inter-assay lower than 7.0% in the whole range analyzed. DHEAS was measured through RIA (DPC, Diagnostic Products Corporation), CV intra-assay lower than 5.3% and CV inter-assay lower than 11% in the whole range analyzed. SHBG determination was performed through a non-competitive chemoluminescent method (Immulite autoanalyzer, DPC, Diagnostic Products Corporation), with a CV intra-assay lower than 8% and a CV inter-assay lower than 13.5% in all the concentration range. Estradiol level was determined through RIA (E2 6 DPC, Los Angeles, USA), CV intra-assay lower than 8% in all the concentration range and the highest CV inter-assay observed of 15.3% for 40 pg/ml, and functional sensitivity of 20 pg/ml. The estradiol/testosterone ratio and free androgen index (FAI, testosterone/SHBG x 100) were also calculated, to complete the evaluation of androgenic status.

**Statistical analysis**

Results are expressed as mean ± standard deviation, except for the estradiol/testosterone ratio, which is expressed as the median (25–75th percentiles) because of its non-parametric distribution. Differences were considered significant at a p value <0.05. Mean differences among groups were performed by an analysis of variance (one-way ANOVA) or Kruskal–Wallis ANOVA as required, using Tukey or Dunn tests as post-hoc analysis. Additionally, correlations between variables were calculated using the Pearson or Spearman test for parametric or non-parametric variables, respectively. Multiple regression analysis was performed to assess the effect of abdominal obesity, evaluated through waist circumference, and menopausal status on FAI and SHBG levels, using step-up regression to build the model. Simple linear regressions were carried out on each of the selected variables; the result
accounting for the larger variation was chosen and kept as the first variable. Menopausal status was included in the model as a dummy variable. Statistical analysis was performed using GraphPad Prism 3.00 and SPSS 11.5 software.

RESULTS
Table 1 shows data on abdominal obesity evaluated by means of waist circumference, lipoprotein profile and insulin resistance markers in the four groups of women studied. Waist circumference was higher not only in PostM women but also in women in the menopausal transition, as compared with the PreM group (p = 0.0002). MTA and PostM women showed higher triglyceride and LDL cholesterol levels than PreM women. Moreover, triglyceride levels were also higher in the PostM women than in the menopausal transition women and LDL cholesterol was also significantly higher in the MTM group as compared to the PreM group. It is interesting to remark that HDL cholesterol levels did not show significant differences among groups.

Fasting glucose levels were higher in the PostM women vs. the PreM women and both groups of menopausal transition women, and there were no differences in fasting insulin levels and HOMA index between the groups. Only a slight decrease in the QUICKI was found in women in the menopausal transition and PostM women in relation to the PreM group, and the McAuley index was significantly higher in PreM women as compared to the other three groups. The triglyceride/HDL cholesterol ratio was higher in menopausal transition and PostM women as compared to PreM ones.

Table 2 shows the androgenic profile, the estradiol levels and the estradiol/testosterone ratio in the four groups studied. Total testosterone did not show significant differences between the groups; androstenedione levels were significantly higher in MTA vs. PostM women (p < 0.05). Correspondingly, DHEAS levels in PreM women were significantly higher than those of the other three groups (p < 0.05). Estradiol levels and the estradiol/testosterone ratio were statistically lower in the PostM group compared with the other three groups, without differences between these latter groups.

SHBG levels were significantly different among groups and post-hoc analysis revealed that concentrations in MTM women were higher than those in MTA women (p < 0.05), and with a tendency to be higher than PostM levels (p = 0.07). As a consequence of this SHBG increase, the FAI in the MTM group is significantly lower than the FAI in the MTA and PostM groups.

Table 1 Waist circumference, lipoprotein profile and insulin resistance markers in the four groups of women studied. Results are expressed as mean ± standard deviation

<table>
<thead>
<tr>
<th>Variable</th>
<th>PreM (n = 29)</th>
<th>MTM (n = 35)</th>
<th>MTA (n = 29)</th>
<th>PostM (n = 31)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 ± 5.6</td>
<td>47 ± 3.3*</td>
<td>49 ± 3.0*</td>
<td>55 ± 5.6*</td>
<td>0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.8 ± 12</td>
<td>88.0 ± 10.9*</td>
<td>90.6 ± 10.2*</td>
<td>88.1 ± 10.8*</td>
<td>0.0002</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.75 ± 0.28</td>
<td>1.19 ± 0.59</td>
<td>1.28 ± 0.71*</td>
<td>1.42 ± 0.50**</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.80 ± 0.61</td>
<td>3.48 ± 0.88*</td>
<td>3.67 ± 0.96*</td>
<td>4.00 ± 1.14*</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.47 ± 0.28</td>
<td>1.41 ± 0.33</td>
<td>1.54 ± 0.42</td>
<td>1.48 ± 0.34</td>
<td>0.49</td>
</tr>
<tr>
<td>Triglyceride/HDL cholesterol</td>
<td>0.53 ± 0.27</td>
<td>0.97 ± 0.67*</td>
<td>0.96 ± 0.77*</td>
<td>1.03 ± 0.52*</td>
<td>0.007</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>4.6 ± 1.3</td>
<td>4.8 ± 0.4</td>
<td>4.9 ± 0.4</td>
<td>5.2 ± 0.7**</td>
<td>0.04</td>
</tr>
<tr>
<td>Fasting insulin (µIU/ml)</td>
<td>9.3 ± 4.9</td>
<td>10.7 ± 5.4</td>
<td>13.3 ± 6.2</td>
<td>12.5 ± 9.2</td>
<td>0.09</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.03 ± 1.14</td>
<td>2.23 ± 1.34</td>
<td>2.92 ± 1.41</td>
<td>2.86 ± 2.39</td>
<td>0.09</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35 ± 0.02</td>
<td>0.34 ± 0.02</td>
<td>0.33 ± 0.02</td>
<td>0.34 ± 0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>McAuley index</td>
<td>8.7 ± 1.7</td>
<td>7.4 ± 1.6*</td>
<td>6.8 ± 1.4*</td>
<td>6.8 ± 1.4*</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*, Significantly different vs. PreM group; †, significantly different vs. MTM group; ‡, significantly different vs. MTA group.

PreM, premenopausal women; MTM, women in the menopausal transition with irregular menstrual cycles; MTA, women in the menopausal transition with 3–6 months of amenorrhea; PostM, postmenopausal women; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA, Homeostasis Model Assessment; QUICKI, Quantitative Insulin Sensitivity Check Index.
**Table 2**  Androgenic profile, estradiol levels and estradiol/testosterone ratio in the four groups of women studied. Results are expressed as mean ± standard deviation, except for estradiol and estradiol/testosterone ratio, which are expressed as median (25–75th percentiles).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PreM (n = 19)</th>
<th>MTM (n = 21)</th>
<th>MTA (n = 28)</th>
<th>PostM (n = 15)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (nmol/l)</td>
<td>1.25 ± 0.45</td>
<td>0.97 ± 0.35</td>
<td>1.14 ± 0.49</td>
<td>1.39 ± 0.55</td>
<td>0.072</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>5.24 ± 2.13</td>
<td>4.08 ± 1.40</td>
<td>5.13 ± 2.41</td>
<td>3.25 ± 1.95†</td>
<td>0.017</td>
</tr>
<tr>
<td>DHEAS (µmol/l)</td>
<td>3.50 ± 1.41</td>
<td>2.33 ± 0.94*</td>
<td>2.35 ± 1.44*</td>
<td>2.02 ± 1.15*</td>
<td>0.026</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>65.0 ± 19.0</td>
<td>75.3 ± 28.0</td>
<td>55.8 ± 26.0†</td>
<td>55.1 ± 21.3</td>
<td>0.032</td>
</tr>
<tr>
<td>Free androgen index</td>
<td>2.10 ± 0.94</td>
<td>1.50 ± 0.82</td>
<td>2.50 ± 1.58†</td>
<td>2.90 ± 1.87†</td>
<td>0.0087</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>162 (95–261)</td>
<td>110 (73–389)</td>
<td>95 (73–349)</td>
<td>73 (73–81)*††</td>
<td>0.01</td>
</tr>
<tr>
<td>Estradiol/testosterone</td>
<td>0.14 (0.09–0.30)</td>
<td>0.11 (0.08–0.34)</td>
<td>0.12 (0.06–0.29)</td>
<td>0.07 (0.04–0.09)*††</td>
<td>0.0053</td>
</tr>
</tbody>
</table>

*, Significantly different vs. PreM group; †, significantly different vs. MTM group; ††, significantly different vs. MTA group

PreM, premenopausal women; MTM, women in the menopausal transition with irregular menstrual cycles; MTA, women in the menopausal transition with 3–6 months of amenorrhea; PostM, postmenopausal women; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin

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We also studied whether there were any associations between androgens and insulin resistance markers, waist circumference and lipids and lipoproteins in the whole group of 83 women in whom all parameters were determined. Neither testosterone nor the estradiol/testosterone ratio correlated with any of the variables studied; however, SHBG and the FAI showed significant negative and positive correlations, respectively with waist circumference (Table 3 and Figure 1), insulin resistance markers and lipoprotein parameters (Table 3); the only exception was the correlation between the FAI and LDL cholesterol, which was non-significant. Regarding insulin resistance markers, the best correlation was found with the McAuley index (Figure 2).

Considering the significant correlation established between the FAI and SHBG with waist circumference, we performed a multiple regression analysis with this last parameter as the independent variable. When waist circumference was taken into account, neither FAI (β = −0.107, p = 0.440) nor SHBG (β = −0.094, p = 0.474) showed any significant differences between groups. Waist circumference showed a significant correlation only with SHBG (β = −0.307, p = 0.022). After adjusting by waist circumference, correlations between SHBG and markers of insulin resistance continued to be significant (p < 0.05 in all cases), but relationships between SHBG and lipoproteins were lost, as well as all correlations found with FAI.

**DISCUSSION**

In this study, we found that total testosterone, androstenedione and DHEAS levels did not show
significant changes throughout the menopausal transition. Taking into account that total testosterone levels were not different among the groups, we can assume that this androgen shows minimal changes through the lifespan of women, at least until 70 years old, the upper limit of age in postmenopausal women in this study. Our findings differ from those of Davison, who showed a decline of testosterone levels with age in women. We found a reduction in androstenedione levels in postmenopause, as would be expected in relation to the decrease in ovarian production of this hormone in this period of life. Moreover, DHEAS concentrations showed lower levels in women after the menopausal transition, corresponding with the reduction expected with aging.

Our results, as others have shown, show high variability of estrogen levels in the menopausal transition, which in turn can be responsible for the high SHBG concentrations found in the MTM group. Regarding the estradiol/testosterone ratio, we found that it was lower in the PostM group than in the other three groups, as could be expected due to the estrogen decrease in these women.

This study shows that, as the menopausal transition goes ahead, androgenic status increases in association with abdominal fat deposition, as confirmed by the significant negative correlation found between waist circumference and SHBG and the positive correlation between waist circumference and the FAI. It should be remarked that waist circumference is an interesting surrogate marker of abdominal obesity. Taking into account the association between SHBG and the FAI and waist circumference, a multiple regression analysis was performed. It revealed that abdominal obesity, more than menopausal status, was the main determinant of the differences found in androgenic profile.

All the insulin resistance markers studied correlated significantly with the androgenic parameters, SHBG and FAI. These results indicate that the gradual increment of the androgenic status could be implicated in the development of insulin resistance after the early stages of the menopausal transition, with its negative consequences for women in this period of life. However, when waist circumference was included in the model,
correlations with the FAI were lost, while, as expected, those with SHBG were maintained.

Regarding the lipid and lipoprotein profile, triglycerides, LDL cholesterol and the triglyceride/HDL cholesterol ratio were higher in the menopausal transition and postmenopausal women, in comparison with the premenopausal group; these parameters correlated negatively with SHBG and positively with FAI, showing that increased androgenicity goes in parallel with an unfavorable lipoprotein profile. When abdominal obesity was considered as an independent variable, all correlations were lost. In other words, the relation between SHBG and FAI with lipid parameters can be mainly attributed to visceral adipose tissue accumulation. Nevertheless, this fact shows that it is very difficult to evaluate the influence of two closely related variables such as abdominal obesity and the androgenic status.

Meanwhile, it has been reported that lower SHBG and higher FAI levels in postmenopausal women were associated with CVD events, but not independently of body mass index, hypertension, and diabetes. Others have reported a strong association between low SHBG levels and high FAI levels with the CVD risk factors, even after adjusting for body mass index. It is proposed that the relation between low SHBG levels and CVD risk should be mediated by the higher concentrations of active androgens, which in turn result from the decreased binding protein. However, it cannot be ruled out as an action of SHBG per se, considering previous findings about the function of this protein, as part of a transduction signal system mediating androgen and estrogen actions at the cell membrane level.

Our results are in agreement with others, in terms of SHBG and FAI correlations with insulin resistance markers, waist circumference and lipid parameters, but, in contrast, we observed that the associations between SHBG and the FAI with CVD risk factors were dependent on waist circumference. This finding was in accordance with Tchernof and colleagues, who evaluated abdominal obesity by computed tomography. It must be remarked that not all investigators used the same parameters to evaluate abdominal obesity.

The fact that we did not find significant correlations between markers of insulin resistance, waist circumference and lipoprotein parameters with the estradiol/testosterone ratio can be due to the high variability of estrogen levels found in the menopausal transition, as mentioned above.

More recently, SHBG and testosterone have shown associations with C-reactive protein, as an inflammation marker in postmenopausal women; this highlights the role of these hormonal parameters in the atherogenic process. It would be of interest to study the behavior of C-reactive protein and other inflammation markers in relationship to CVD risk factors in women in the menopausal transition.

Our study has some limitations such as the low number of patients, although it must be considered that we used strict inclusion criteria. On the other hand, it is well known that a prospective design is preferable to a cross-sectional one, such as the one we used. Although it was not possible to develop a longitudinal design, this study makes an interesting contribution to the knowledge about the relationship between hormonal and metabolic parameters in the menopausal transition.

In conclusion, this work contributes additional data about the increment in the androgenic milieu that becomes evident after the earlier stages of the menopausal transition and correlates with data on central fat deposition, insulin resistance markers and an atherogenic lipoprotein profile. Our results suggest that the evaluation of the risk of cardiovascular disease in women in this period of life should also include androgenic parameters, such as SHBG levels and the free androgen index, considering that abdominal obesity is one of the main determinants of the relationship between androgens and cardiovascular disease.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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