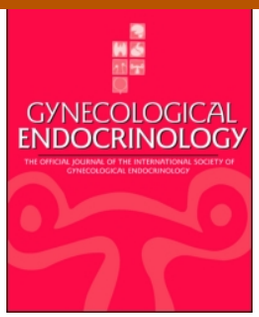

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ORIGINAL ARTICLE

Testosterone is associated with insulin resistance index independently of adiposity in women with polycystic ovary syndrome

Kari Luotola[a,b], Terhi T Piltonen[c], Johanna Puurunen[c], Laure C Morin-Papunen[c] and Juha S Tapanainen[a,c]

[a]Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland; [b]Heart and Lung Center, Cardiology, Helsinki University Hospital, Helsinki, Finland; [c]Department of Obstetrics and Gynecology, Oulu University Hospital, University of Oulu and Medical Research Center and PEDEGO Research Unit (Research Unit for Pediatrics, Dermatology, Clinical Genetics, Obstetrics and Gynecology), Oulu, Finland

ABSTRACT

Objective: To study the associations between androgens, glucose homeostasis, inflammation and statin treatment in women with polycystic ovary syndrome (PCOS).

Design and methods: Oral glucose tolerance tests, androgens, hs-CRP and interleukin-1 receptor antagonist (IL-1Ra) were analyzed at baseline and after 6 months of atorvastatin (20 mg/d) or placebo treatment in 27 women with PCOS.

Results: Testosterone associated with insulin resistance measured with $ISI_{Matsuda}$ independently of BMI, age and SHBG concentrations and the full model, including IL-1Ra, hs-CRP and HDL-C, also showed independency of BMI and waist circumference ($p \leq .042$). Free androgen index (FAI) associated with $ISI_{Matsuda}$ independently of adiposity ($p \leq .025$) but in the full model with waist circumference the association was insignificant. $ISI_{Matsuda}$ decreased with testosterone >1.2 nmol/l compared with lower levels at baseline ($p = .043$) and at six months ($p = .003$). Accordingly, 30-minute insulin levels were increased with moderately elevated testosterone independently of adiposity ($p \leq .046$). Increased fasting glucose and AUC insulin associated with statin treatment independently of adiposity and the associations attenuated after adjusting for testosterone.

Conclusions: Moderately elevated testosterone concentrations together with obesity-related inflammatory factors modify glucose homeostasis by increasing insulin resistance and early insulin secretion.

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Insulin secretion; insulin sensitivity; inflammation; polycystic ovary syndrome; adiposity; androgens

Introduction

Glucose metabolism disorders are more common in hyperandrogenic women with polycystic ovary syndrome (PCOS) compared with the nonhyperandrogenic phenotype (polycystic ovaries in ultrasonography and the presence of chronic oligo-anovulation) [1]. During early adulthood, women with PCOS show a propensity to experience excessive weight gain and dyslipidemia [2], and later, in life, they also present with other cardiovascular risk factors, such as hypertension and metabolic syndrome [3,4]. Therefore, medical therapy of hyperlipidemia with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins) has been suggested in the prevention of cardiovascular disease in women with PCOS [5]. Moreover, as statins have been proposed to inhibit androgen synthesis [6,7], their use might have therapeutically significant advantages. Recently we found in a prospective, placebo-controlled study that despite substantial benefits of statins in improving lipid profiles, treatment with atorvastatin for six months in women with PCOS was followed by only a marginal change in androgen synthesis and resulted in the impairment of glucose homeostasis and reduced insulin sensitivity [8]. Furthermore, statins seem to increase the risk of development of type-2 diabetes (T2DM) [9].

Increased inflammatory activity is common in women with PCOS [1]. The (pro-inflammatory) interleukin-1 (IL-1) ligands are known to be important mediators of metabolic disturbances and the natural anti-inflammatory IL-1 receptor antagonist (IL-1Ra) increases with adiposity and has protective effects on insulin secretion in pancreatic β-cells [10]. IL-1Ra reflects inflammatory activity and appears to be a useful parameter that has been reported to be associated with glucose tolerance and disposal in women with PCOS [11]. Furthermore, IL-1Ra has been shown to independently predict the development of T2DM [12,13]. In the present study, we hypothesized that androgen excess and increased inflammation decrease insulin sensitivity and that androgens may have interactions with inflammatory factors in adipose tissue.

Subjects and methods

Subjects

Our randomized, double-blind, placebo-controlled follow-up study involved 38 women (aged 29–50 years) with PCOS and without a history of impaired glucose tolerance (IGT) or T2DM. The detailed study design of this complementary study has been previously reported [8]. The subjects used either atorvastatin (Pfizer Inc., 20 mg/day) or placebo for 6 months (ClinicalTrials.gov NCT01072097). Twenty-eight women completed the study

CONTACT Kari Luotola kari.luotola@helsinki.fi Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Haartmaninkatu 8, PO Box 700, FI-00290 Helsinki, Finland

Supplemental data for this article can be accessed here.

protocol ($n = 15$ in the atorvastatin group; $n = 13$ in the placebo group) and 27 subjects aged 29–47 years were included in the analyses. The diagnosis of PCOS was based on the Rotterdam consensus criteria [1] except for the diagnosis of polycystic ovaries in ultrasonography, where more stringent criteria were used [14]. Biochemical or clinical hyperandrogenism was present in 48% of the subjects. Oral contraceptives and other medication affecting glucose or steroid metabolism were interrupted at least two months before the study. The study protocols were approved by the Ethics Committee of Oulu University Hospital. All subjects signed a written informed consent document.

Glucose tolerance tests

Two-hour oral glucose tolerance tests (OGTTs) were performed in 27 PCOS cases, and at baseline, four women were found to have IGT or T2DM based on the results. The degree of insulin sensitivity was measured by using HOMA-IR or the Matsuda index ($ISI_{Matsuda}$) [15]. Incremental area under the curve (AUC) values were calculated in OGTTs. Insulin sensitivity was also assessed by using the intravenous glucose tolerance test (Si_{IVGTT}) [16].

Assays

Serum samples were analyzed for testosterone by using Agilent triple-quadrupole 6410 liquid chromatography-mass spectrometry equipment with an electrospray ionization source operating in positive-ion mode (Agilent Technologies), with a sensitivity of 0.03 nmol/l. Multiple reaction monitoring was used to quantify testosterone by using trideuterated testosterone (d3-testosterone) with the following transitions: mass to charge ratios 289.2 to 97 and 289.2 to 109 for testosterone and 292.2 to 97 and 292.2 to 109 for d3-testosterone. Serum concentrations of androstenedione, dehydroepiandrosterone sulfate (DHEAS) and sex-hormone-binding globulin (SHBG) were analyzed by chemiluminometric immunoassays, with sensitivities of 1.0 nmol/l for androstenedione, 0.08 µmol/l for DHEAS and 0.02 nmol/l for SHBG (Immulite 2000; Siemens Healthcare Diagnostics). The free androgen index (FAI) was calculated by using the following equation: testosterone (nmol/l) × 100/SHBG (nmol/l). Levels of high-density lipoprotein cholesterol (HDL-C) (Advia 1800; Siemens Healthcare diagnostics), C-reactive protein (hs-CRP) (BN ProSpec; Siemens Healthcare diagnostics) and IL-1Ra (Quantikine® ELISA kits R&D Systems) were measured at baseline and at six months.

Statistical methods

Correlation analyses were performed by using Pearson's coefficients. Linear regression analysis was used to study androgen levels and glucose homeostasis at baseline and to study associations with glucose homeostasis after six months of follow-up. Levels of IL-1Ra and glucose homeostasis parameters were analyzed by using independent *t*-test. Statistical significance was set at $p < .05$. Statistical analyses were performed by using IBM SPSS Statistics Version 22 software.

Results

Associations between androgens and glucose homeostasis parameters

Demographic data on the women with PCOS are shown in Supplemental Table. Testosterone levels at baseline correlated significantly with insulin secretion during OGTTs (30-min insulin: $r = 0.421$, $p = .029$; 1-h insulin: $r = 0.421$, $p = .029$ and AUC insulin: $r = 0.412$, $p = .033$). Accordingly, insulin secretion correlated with FAI values (30-min insulin: $r = 0.671$, $p < .001$; 1-h insulin: $r = 0.633$, $p < .001$ and AUC insulin: $r = 0.653$, $p < .001$) and with SHBG (30-min insulin: $r = -0.422$, $p = .028$; 1-h insulin: $r = -0.406$, $p = .036$ and AUC insulin: $r = -0.419$, $p = .029$). FAI and SHBG levels correlated significantly also with other OGTT-derived parameters (data not shown). Concentrations of testosterone, androstenedione and DHEAS showed non-significant correlations with OGTT glucose and C-peptide levels.

At baseline the FAI correlated negatively with insulin sensitivity measured by the way of $ISI_{Matsuda}$ ($r = -0.585$, $p = .001$), HOMA-IR ($r = 0.582$, $p = .001$) and Si_{IVGTT} ($r = -0.446$, $p = .020$) in women with PCOS. Similarly, SHBG levels correlated with $ISI_{Matsuda}$ ($r = 0.572$, $p = .002$) and HOMA-IR ($r = -0.488$, $p = .010$). $ISI_{Matsuda}$ correlated negatively with HOMA-IR ($r = -0.756$, $p < .001$) and positively with Si_{IVGTT} ($r = 0.717$, $p < .001$). HOMA-IR correlated negatively with Si_{IVGTT} ($r = -0.605$, $p = .001$).

$ISI_{Matsuda}$ decreased with increasing serum testosterone and FAI levels independently of BMI at baseline in women with PCOS (Table 1(a)). Multivariate analysis including all covariates (age, BMI, testosterone, SHBG, IL-1Ra, hs-CRP and HDL-C) showed associations between $ISI_{Matsuda}$ and testosterone ($p = .031$) and FAI ($p = .025$) and were significant in full-model analysis (testosterone: $r^2 = 0.705$ and FAI: $r^2 = 0.688$) (Table 1(a)). When using waist circumference as a covariate in the full model, $ISI_{Matsuda}$ decreased with increasing testosterone levels ($p = .040$). $ISI_{Matsuda}$ decreased with increasing FAI ($p = .020$) independently of waist circumference and age, and a nonsignificant association was seen in the full model ($p = .054$).

Increased OGTT 30-min insulin levels were associated with increased testosterone levels ($p = .046$) and FAI values ($p = .028$) in the full model including BMI and in the model including age, BMI and/or SHBG (Table 1(b)). Adjusting for waist circumference, age and SHBG showed that the increased 30-min insulin levels were associated positively with testosterone levels ($p = .043$). The FAI was associated with 30-min insulin independently of age and waist circumference ($p = .028$) and showed nonsignificant association in the full model ($p = .054$).

The mean serum testosterone concentration in women with PCOS at the beginning of the study was 1.19 nmol/l. The 10 subjects with levels above this value displayed decreased insulin sensitivity ($ISI_{Matsuda}$ values) when compared with those with testosterone levels below the mean (mean [SD]: 4.50 [2.68] vs. 7.20 [3.43], $p = .043$, respectively). Accordingly, OGTT 30-min insulin levels were increased in subjects with higher testosterone levels (115.7 [74.1] mU/l vs. 48.9 [35.0] mU/l, $p = .021$). After 6 months' follow-up, insulin sensitivity ($ISI_{Matsuda}$) was still decreased in the 10 subjects with testosterone above 1.19 nmol/l compared with the levels in 17 subjects with testosterone under this limit (3.10 [2.10] vs. 7.80 [5.21], $p = .003$) and, accordingly, 30-min insulin levels were increased (146.7 [88.6] mU/l vs. 70.3 [55.2] mU/l, $p = 0.010$).

Association of IL-1Ra levels with FAI and SHBG

IL-1Ra correlated with FAI values ($r = 0.415$, $p = .032$) and SHBG concentrations ($r = -0.454$, $p = .017$) at baseline and after adjusting for age the correlations remained significant (FAI: $\beta = 0.535$, $p = .018$ and SHBG: $\beta = -0.453$, $p = .020$). IL-1Ra levels in women with PCOS undergoing atorvastatin treatment

Table 1. Multivariate analysis of baseline insulin resistance (IR) indices (a) and OGTT insulin levels (b) in women with PCOS. The models were adjusted for age, BMI, sex hormone-binding globulin (SHBG) and testosterone, and also for the free androgen index (FAI) together with age and BMI.

a

β-coefficient (*with p value*) for IR index

IR index	BMI	SHBG	testosterone	FAI	r^2
$ISI_{Matsuda}$	−0.589, −0.529 *(0.001, 0.002)*	0.221 *(0.16)*	−0.332 *(0.042)*	−0.449 *(0.016)*	0.662, 0.660
HOMA-IR	0.870, 0.769 *(≤0.001)*	0.020 *(0.87)*	0.073 *(0.56)*	0.143 *(0.31)*	0.783, 0.789
Si_{IVGTT}	−0.598, −0.450 *(0.007, 0.030)*	−0.036 *(0.86)*	−0.266 *(0.20)*	−0.316 *(0.18)*	0.412, 0.414

IVGTT: intravenous glucose tolerance test.

b

β-coefficient (*with p value*) for insulin levels

OGTT insulin	BMI	SHBG	testosterone	FAI	r^2
fasting insulin	0.874, 0.798 *(≤0.001)*	0.021 *(0.86)*	0.076 *(0.53)*	0.147 *(0.28)*	0.794, 0.800
30-min insulin	0.624, 0.449 *(≤0.009)*	−0.041 *(0.81)*	0.371 *(0.038)*	0.481 *(0.015)*	0.595, 0.619
1-h insulin	0.695, 0.569 *(≤0.001)*	0.016 *(0.92)*	0.303 *(0.069)*	0.300 *(0.11)*	0.641, 0.625
2-h insulin	0.763, 0.644 *(≤0.001)*	0.061 *(0.69)*	0.182 *(0.25)*	0.210 *(0.24)*	0.659, 0.656
AUC insulin	0.721, 0.584 *(≤0.001)*	0.017 *(0.91)*	0.289 *(0.070)*	0.321 *(0.074)*	0.668, 0.664

Table 2a. Associations of 1-h glucose level (a) and AUC insulin (b) in women with PCOS at 6 months.

β-coefficient (*with p value*) for 1-h glucose

Model	Atorvastatin	Age	IL-1Ra	Waist	FAI	SHBG	HDL-C	CRP	r^2
1	−0.120 *(0.39)*	0.150 *(0.29)*	0.512 *(0.013)*	0.305 *(0.12)*					0.634
2	−0.035 *(0.85)*	0.255 *(0.22)*	0.489 *(0.021)*	0.244 *(0.25)*	0.186 *(0.48)*				0.643
3	0.093 *(0.51)*	0.163 *(0.25)*	0.487 *(0.021)*	0.238 *(0.24)*		−0.184 *(0.26)*			0.657
4	−0.109 *(0.44)*	0.093 *(0.54)*	0.592 *(0.010)*	0.330 *(0.097)*			0.176 *(0.34)*		0.650
5	−0.122 *(0.40)*	0.150 *(0.30)*	0.514 *(0.016)*	0.309 *(0.14)*				−0.013 *(0.93)*	0.634

Model 1 was adjusted for atorvastatin therapy/placebo, age, IL-1 receptor antagonist (IL-1Ra) and waist circumference. Models from 2 to 5 were further adjusted for FAI, SHBG, HDL-C and CRP.

Table 2b.

β-coefficient (*with p value*) for AUC insulin

Model	Atorvastatin	Age	IL-1Ra	Waist	FAI	SHBG	HDL-C	CRP	r^2
1	0.294 *(0.021)*	−0.145 *(0.24)*	0.379 *(0.031)*	0.409 *(0.020)*					0.727
2	0.235 *(0.15)*	−0.72 *(0.68)*	0.363 *(0.044)*	0.367 *(0.055)*	0.130 *(0.57)*				0.731
3	0.288 *(0.029)*	−0.143 *(0.26)*	0.372 *(0.040)*	0.394 *(0.035)*		−0.040 *(0.78)*			0.728
4	0.30 *(0.021)*	−0.111 *(0.41)*	0.331 *(0.082)*	0.393 *(0.028)*			−0.105 *(0.51)*		0.733
5	0.270 *(0.033)*	−0.147 *(0.22)*	0.361 *(0.038)*	0.354 *(0.045)*				0.164 *(0.21)*	0.748

(402.4 [273.2] pg/ml) were comparable with the placebo group (280.2 [190.6] pg/ml, $p = .19$) at baseline. Accordingly, at six months, IL-1Ra levels in atorvastatin group (430.4 [375.9] pg/ml) were comparable with the placebo group (242.8 [103.7] pg/ml, $p = .093$).

Glucose homeostasis at 6 months

Increased baseline IL-1Ra levels were associated positively with 1-h glucose levels at six months in women with PCOS independently of age, waist circumference and intervention (atorvastatin or placebo), as shown in Table 2(a). Levels of IL-1Ra were also associated with 1-h glucose levels at six months in the full model that included intervention, age, waist circumference, testosterone, SHBG, HDL-C and hs-CRP ($p = .018$) but was nonsignificant after adjusting for BMI.

Atorvastatin treatment, IL-1Ra and waist circumference were associated with increased AUC insulin values (Table 2(b)). After adjusting for testosterone ($p = .140$) or FAI the association between atorvastatin treatment and AUC insulin became non-significant. Atorvastatin treatment was also associated with increased fasting glucose independently of BMI and age, but the association disappeared after adjustment for testosterone (Table 3). Atorvastatin treatment was associated positively with fasting glucose after adjustment for waist circumference and age ($p = .014$) and adjusting further for testosterone showed attenuation of the significance ($p = .040$). Models with atorvastatin, testosterone, SHBG, age and adiposity (BMI or waist circumference) and the full models showed that baseline testosterone levels were solely associated positively with testosterone levels at six months ($p < .001$).

Discussion

The present study shows evidence of an association between testosterone levels and FAI and decreased insulin sensitivity index values, independently of BMI and waist circumference in women with PCOS. Statin treatment was positively associated with fasting glucose and AUC insulin values independently of adiposity, and the associations attenuated after adjusting for testosterone.

Increased early-phase insulin secretion has previously been reported to reflect increased functional capacity of β-cells

Table 3. Associations of fasting glucose levels in women with PCOS at 6 months.

β-coefficient (*with p value*) for fasting glucose

Model	Atorvastatin	Age	BMI	FAI	SHBG	Testosterone	DHEAS	Androstenedione	r^2
1	0.396 *(0.037)*	0.194 *(0.27)*	0.263 *(0.15)*						0.345
2	0.330 *(0.15)*	0.281 *(0.26)*	0.208 *(0.33)*	0.153 *(0.62)*					0.352
3	0.363 *(0.051)*	0.207 *(0.23)*	0.104 *(0.62)*		−0.294 *(0.16)*				0.402
4	0.374 *(0.11)*	0.222 *(0.38)*	0.265 *(0.16)*			0.043 *(0.87)*			0.345
5	0.392 *(0.040)*	0.147 *(0.42)*	0.307 *(0.11)*				−0.161 *(0.39)*		0.366
6	0.379 *(0.070)*	0.219 *(0.30)*	0.255 *(0.18)*					0.050 *(0.82)*	0.346

Model 1 includes atorvastatin therapy/placebo, age and BMI. Models from 2 to 6 were further adjusted for FAI, SHBG, testosterone, DHEAS and androstenedione.

together with decreased glucose disposal in obese women with PCOS (17). In the present study, increased 30-min insulin levels in OGTTs were associated with testosterone levels independently of adiposity, suggesting that androgens may alter insulin sensitivity. This was also supported by the observation of a decreased insulin sensitivity index and increased 30-min insulin levels in subjects with high (>1.2 nmol/l) versus low (<1.2 nmol/l) testosterone levels. The relatively low sample size and number of variables in the full model limit to draw detailed presumptions about the role of inflammatory factors. Adipose tissue plays a central role in subclinical inflammation, which has been observed in cases of PCOS [1,11] and is associated with abdominal obesity and metabolic disturbances [18]. The findings of the present study are further supported by the results of a recent study in which adipocyte morphology, increased inflammation and large waist circumference were associated with insulin resistance in women with PCOS [19]. We also found that increased levels of IL-1Ra, a natural anti-inflammatory factor reflecting inflammatory activity, correlated with increased FAI values independently of age in women with PCOS and predicted 1-h glucose levels at six months independently of abdominal adiposity. These results further strengthen the roles of androgens and inflammatory factors in insulin resistance and thereby long-term health consequences in women with PCOS.

Atorvastatin treatment was associated positively with fasting glucose levels and AUC insulin at six months independently of adiposity, and the association weakened after adjusting for serum testosterone levels. This suggests a possible role of androgens in altered glucose homeostasis during statin therapy. However, the mechanisms remain unclear, as in one of our previous studies only DHEAS levels decreased [8] and androgen metabolism may be affected by the interaction of glucose, insulin and immune factors during atorvastatin treatment. However, some other short-term studies have revealed decreased testosterone levels during atorvastatin treatment [20–26] and it has been suggested that the effect of statins on androgen and glucose metabolism is time-, dose- and drug-dependent. In line with this, in the present study, women with testosterone levels >1.2 nmol/l exhibited greater early insulin responses and decreased insulin sensitivity index values compared with women with testosterone levels <1.2 nmol/l, suggesting that even moderately increased testosterone levels (within the normal range) may alter insulin sensitivity.

Although testosterone levels were associated with insulin sensitivity when assessed by means of $ISI_{Matsuda}$, there were no significant associations with HOMA-IR or Si_{IVGTT}. This may be explained by the notion that insulin sensitivity indices reflect the situation in different body compartments, that is, whole body, hepatic and peripheral tissues [15]. $ISI_{Matsuda}$ is based on OGTTs and better reflects whole-body insulin sensitivity, whereas HOMA-IR reflects hepatic and Si_{IVGTT} peripheral tissues. The results of earlier studies involving the hyperinsulinemic euglycemic clamp technique are in line with our results, showing associations between androgens and insulin resistance measured by $ISI_{Matsuda}$ [27,28]. Moreover, the present study showed that the FAI robustly correlated with all indices of insulin resistance in unadjusted analyses. Multivariate analysis of Si_{IVGTT} showed substantially lower r^2 values compared with analyzes of $ISI_{Matsuda}$ and HOMA-IR in women with PCOS as regards the association between testosterone levels and insulin sensitivity. It is therefore possible that Si_{IVGTT} captures different components of changes in insulin sensitivity compared with $ISI_{Matsuda}$ and HOMA-IR, as recently suggested [29]. However, in our study, $ISI_{Matsuda}$ correlated almost identically with HOMA-IR and Si_{IVGTT} in unadjusted analyses.

The present study shows that increased testosterone levels and FAI values in women with PCOS are associated with decreased insulin sensitivity index values and increased early insulin secretion, independently of adiposity. Moreover, hyperandrogenemia assessed by way of the FAI correlated positively with IL-1Ra levels, underlining the role of androgens and inflammatory factors in glucose homeostasis. This study shows evidence that moderately elevated testosterone levels, within the normal range, may alter insulin sensitivity in women with PCOS.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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