Obesity, body composition and metabolic disturbances in polycystic ovary syndrome

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BACKGROUND: We determined the impact of polycystic ovary syndrome (PCOS) and obesity on glucose and lipid metabolism and β-cell function in women with PCOS. METHODS: In 35 women with PCOS (17 lean, lean PCOS and 18 obese, obese PCOS) and 25 control women (9 lean, lean controls and 16 obese, obese controls), β-cell function was evaluated by the first-phase insulin response after intravenous glucose, acute insulin response to glucose (AIRg); insulin sensitivity, determined as insulin sensitivity index (ISI), was evaluated by the euglycemic hyperinsulinemic clamp. Indirect calorimetry was used for the assessment of glucose and lipid oxidation. Body composition was estimated by dual X-ray absorptiometry scan. RESULTS: When adjusted for obesity, PCOS was associated with higher 2-h glucose levels (P<0.05), higher trunk/periphery fat ratio (P<0.001), lower ISI (P<0.001), lower insulin-stimulated glucose oxidation (GOX 2) (P<0.05) and lower non-oxidative glucose metabolism (P<0.05), but a normal AIRg compared with control women. Lean women with PCOS had lower ISI (P<0.001), GOX-2 (P<0.05) and higher trunk/periphery fat ratio (P<0.05) than lean control women. In obese women with PCOS, ISI was reduced with 25% compared with obese control women, whereas trunk/peripheral fat ratio did not differ. AIRg was increased in obese groups compared with lean groups (P<0.05), but was, in all groups, appropriate for the ambient action of insulin. CONCLUSIONS: PCOS is associated with a low ISI, which in lean women with PCOS may partly be explained by higher trunk/peripheral fat ratio. AIRg was amplified by obesity, but was, in all groups, appropriate for prevailing insulin sensitivity, suggesting a normal β-cell adaptation.

Keywords: insulin sensitivity; calorimetry; central obesity; glucose; fat distribution

Background

Polycystic ovary syndrome (PCOS) is characterized by several hormonal and metabolic disturbances leading to the main phenotypic characteristics of the disease: anovulation and hyperandrogenemia. Insulin resistance (IR) and hyperinsulinemia are believed to play a central role in the pathogenesis of PCOS. This is documented in obese women with PCOS, although data are conflicting in lean women with PCOS (Dunaif et al., 1989; Omeara et al., 1993; Ovesen et al., 1993; Dunaif and Finegood, 1996; Mather et al., 2000; Vrbikova et al., 2004). Several steps in the glucose and insulin metabolism have been investigated, and it is debated whether IR is caused by a defect in insulin action (Ehrmann et al., 1995; Ciammelli et al., 1997; Dunaif et al., 2001; Corbould et al., 2005), a primary defect in β-cell function (Ehrmann et al., 1995), decreased hepatic clearance of insulin (Ciammelli et al., 1997), or a combination of all these factors. An intrinsic genetic defect in the post-receptor insulin signal transduction has been found in women with PCOS (Dunaif et al., 2001; Corbould et al., 2005). This may lead to decreased insulin action and a compensatory increased insulin secretion from the pancreatic β-cells. In regard to β-cell function, some investigators have shown a defective glucose-stimulated insulin secretion (Ehrmann et al., 1995; Dunaif and Finegood, 1996; Arslanian et al., 2001), indicating a primary defect in β-cell function. Others have found an increased insulin response (Holte et al., 1994a; Holte et al., 1995; Morin-Papunen et al., 2000), a possible compensatory mechanism to a peripheral defect in insulin action, and yet others have found unaffected acute insulin secretion (Gennarelli et al., 2005). Obesity, in particular, central obesity, plays a central role in the development of PCOS, and the majority of women with PCOS are overweight or obese (Gambineri et al., 2002). The mechanisms by which obesity influences the pathophysiology and clinical expression of PCOS are not completely understood, but obesity is, as an independent factor, associated
with IR (Reaven et al., 1983) and sex steroid disturbances (Pasquali et al., 2006), which may lead to an increased risk of menstrual irregularities and hyperandrogenemia. Obesity makes it difficult to interpret the role of genetic intrinsic defects in the etiology of PCOS (Grodstein et al., 1994; Rich-Edwards et al., 2002; Pasquali et al., 2006), and it is possible that different pathogenic factors account for the development of the PCOS-phenotype in lean and obese women. IR is associated with an increased risk of developing impaired glucose tolerance (IGT) or manifest type 2 diabetes, lipid disturbances and cardiovascular disease. Accordingly, an increased prevalence of IGT, type 2 diabetes and dyslipidemia has also been found in women with PCOS (Dahlgren et al., 1992; Wild et al., 2000; Ehrmann et al., 2005; Legro et al., 2005). The well-known obesity-associated disturbances in the glucose and insulin metabolism leading to IGT or type 2 diabetes may however be different from those in women with PCOS, in particular, lean women with PCOS.

The aim of the present study was to provide insight into the proposed disturbances in glucose, insulin and lipid metabolism, and to investigate the role of obesity and body composition in women with PCOS. We also examined the complex relationship between insulin sensitivity and β-cell function in lean and obese women with and without PCOS, which is critical for understanding the development of pre-diabetes or type 2 diabetes (Kahn, 2000).

Materials and Methods

There were 60 women, 35 women with PCOS, 17 lean [lean PCOS (LP)] [body mass index (BMI) ≤ 25] and 18 obese [obese PCOS (OP)] (BMI > 25) and 25 age- and weight-matched control women, 9 lean [lean controls (LC)] and 16 obese [obese controls (OC)], recruited by advertising in the local newspaper. All control women had regular menstrual cycles (<35 days) and androgen levels within the normal range. Women with other known chronic diseases and women who had used oral contraceptives or other drugs known to alter glucose and insulin metabolism, within the last 3 months, were excluded from the study. The study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the local ethics authorities. All subjects gave written informed consent before entering the study. A menstrual history and history of general health were obtained. The PCOS diagnosis was based on the Rotterdam Criteria (2004). Blood samples were taken for testing testosterone and sex hormone-binding globulin (SHBG), and other endocrinopathies were excluded by relevant testing. Because the majority of women with PCOS were an- or oligo- or amenorrheic, blood samples and tests were taken at random both in women with and without PCOS. Hirsutism was evaluated by the Ferrimen Gallwey score, and women with a score ≥ 8 were classified as having hirsutism (Ferriman et al., 1961). PCO-morphology was determined by transvaginal ultrasonography in all study subjects and categorized in accordance with the Rotterdam Criteria (2004).

Oral glucose tolerance test

After a 10-h overnight fast, a standard oral glucose tolerance test (OGTT) (75 g) was performed. Blood samples were drawn from the antecubital vein at 0 and 120 min for the measurement of plasma glucose concentrations. Patients with a 2-h plasma glucose concentration <7.8 mmol/l were categorized as having normal glucose tolerance, ≥7.8 and <11.1 mmol/l as IGT and ≥11.1 mmol/l as diabetes mellitus. All participants showed a fasting plasma glucose <7.0 mmol/l.

Intravenous glucose tolerance test and euglycemic hyperinsulinemic clamp

After an overnight fast of 10–12 h, all women reported to our laboratory between 8:00 and 8:30 a.m. Instructions were given to abstain from any strenuous physical activity 3 days before the assessment. Catheters were inserted in the right and left antecubital veins. The right catheter was used for blood sampling and the left for infusion. The right arm was kept in a heating box for obtaining arterialized venous blood. Fasting blood samples were taken for assessing serum glucose concentration, insulin, free fatty acids (FFA) and lipoproteins [total cholesterol, very low density (VLDL) and high density (HDL)]. Indirect calorimetry was performed for 30 min at base line and in the steady-state period of the clamp for the last 30 min, using a computerized canopy gas analyzer system (Deltatrac II Metabolic Monitor; Datex, Helsinki, Finland) to estimate non-protein glucose and lipid oxidation rates as described previously (Frayn, 1983). An intravenous glucose tolerance test (IVGTT) was initiated with a 1-min glucose infusion (0.3 g/kg body weight). Blood samples for assessing serum glucose and insulin were drawn at −4, −2, 0, 2, 4, 6, 8, 10, 15, 20 and 30 min. After 30 min, insulin (Actrapid: novo Nordisk A/S, Bagsværd, Denmark) infusion was started with a rate of 100 mU/m²/min followed by a stepwise decline in the infusion rate every third minute by 20 mU/min until an infusion rate of 40 mU/min was reached after 9 min. During the remaining 120 min, a constant infusion rate of 40 mU/min was maintained. Plasma glucose levels were measured every 5 min and kept at 5 mmol/l by adjusting glucose (200 g/l) infusion rate. Blood samples for assessing plasma insulin were taken at 10, 30, 60, 90 and 120 min; FFA was measured at 0 min in the base line fasting period and at 120 min during the steady-state period of the clamp; plasma urea was assessed at 0 and 30 min during the base line fasting period and at 90 and 120 min during the steady-state period of the clamp. Urine production during the assessment was measured and a urine sample was collected for analyzing urine-urea excretion. Serum and urine samples were sent to the laboratory for immediate analysis of blood and urine-urea. Blood samples for analyzing insulin and FFA were centrifuged immediately at 4°C, and plasma was stored at −80°C for later analysis. The combined test (IVGTT and euglycemic hyperinsulinemic clamp), also called the Botnia clamp, has been shown to provide reliable and independent measures of insulin sensitivity and β-cell function during the same test (Tripathy et al., 2003). Body composition was estimated by a whole-body DEXA scan that separately measured three of the principal compartments of the body (total bone mineral content, total lean body soft tissue mass and total fat mass. Furthermore, data on the regional distribution of the different body components were obtained for arms, legs, trunk (thorax+abdomen) and head.

Laboratory analysis

Plasma glucose was measured by Beckman glucose analyzer (Ramcon, Fullerton, CA, USA). Testosterone (T) and dihydrotestosterone (DHT) were measured by radioimmunoassay after ether extraction and subsequent celite chromatography. The intra- and inter-assay variations for testosterone were 8.2 and 13.8%, respectively, and for DHT, 9.1 and 11.0%, respectively. The detection limit for all analyses was 0.05 nmol/l. Sexual hormone-binding globulin (SHBG) was analyzed by a double-monoclonal immunofluorometric assay (AutoDelfia, Wallac, OY, Finland). Intra- and inter-assay variations were 5.2 and 7.5%, respectively. Free testosterone (FT) was estimated...
by a method described by Bartsch (Bartsch, 1980), based on the measurement of SHBG, total T and DHT and the use of the law of mass action, using the binding constant of T and DHT to SHBG, and including a calculation of T binding to albumin (Vermeulen, 1999). Threshold reference values for plasma total testosterone levels were 0.55–1.8 nmol/l, and 0.006–0.034 nmol/l for plasma FT levels. Women with either total and/or FT levels above the upper limit were categorized as having hyperandrogenemia. Insulin was analyzed by 1235 Auto DELPHIA automatic immunoassay system (Wallac, Oy, Turku, Finland), with a detection limit of 3 pmol/l. The intra- and inter analysis assay coefficients of variance were 4.5 and 7%, respectively. Total plasma cholesterol and triglycerides were determined by reflection photometry (Ortho-Clinical diagnostics kit, Raritan, NJ, USA).

Calculations
Glucose disposal rate (GDR) was determined during the steady-state period of the clamp and calculated as mg/kg fat-free mass (FFM)/min. The insulin sensitivity index (ISI) was calculated as (GDR)/(mean plasma insulin concentration during last 30 min of clamp), expressed in μg/glucose/kg FFM/min/(pmol/l insulin). In the fasting state, ISI was calculated by the homeostasis model assessment insulin resistance index (HOMA-IR): fasting insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5 (Matthews et al., 1985). Rates of glucose (GOX) and lipid oxidation (LIPOX) were calculated from measurements of O2 consumption, CO2 production and urinary nitrogen excretion as described by Frayn (Frayn, 1983). Δ-GOX was calculated as the difference between basal GOX (GOX-1) and insulin-stimulated GOX (GOX-2). Non-oxidative glucose metabolism (NOGM) during the steady-state period of the clamp was calculated as the difference between GDR and GOX. The acute insulin response to glucose (AIRg), during IVGTT, was calculated as the incremental ratio (NOGM) during the steady-state period of the clamp was calculated as the difference between basal GOX (GOX-1) and insulin-stimulated GOX (GOX-2). The acute insulin response to glucose (AIRg), during IVGTT, was calculated as the incremental ratio (NOGM) during the steady-state period of the clamp was calculated as the difference between basal GOX (GOX-1) and insulin-stimulated GOX (GOX-2). The acute insulin response to glucose (AIRg), during IVGTT, was calculated as the incremental ratio (NOGM) during the steady-state period of the clamp was calculated as the difference between basal GOX (GOX-1) and insulin-stimulated GOX (GOX-2).

Statistical analysis
Results are presented as mean ± SD or SEM. A two-way ANOVA was performed to determine the effects of PCOS and obesity and a possible interaction between PCOS and obesity on ISI and other variables. For comparison of subgroups, the Bonferroni method was used for adjusting P-values. A simple linear regression analysis was performed to investigate the role of androgens on body fat distribution (trunk/peripheral fat) and on insulin sensitivity (GDR, ISI and HOMA) and action (AIRg). Data on VLDL, HOMA-IR and DI did not follow a Gaussian distribution and were therefore log-transformed, and they were thereby approximated by the normal distribution. Levels of significance were set at 0.05%.

Results
In the total study population, women with and without PCOS were comparable regarding age and BMI (Table I). All control women had regular menstrual cycles, and in women with PCOS, nine (three lean and six obese) women had regular menstrual cycles, whereas the remaining had oligo- or amenorrhea. Of the 25 women with PCOS, 18 (8 lean and 10 obese) were hirsute. PCOS was associated with significantly higher total (P < 0.001) and FT (P < 0.001), higher 2-h glucose levels (P < 0.05) and lower SHBG (P < 0.05), independent of obesity. There was a negative synergistic effect of PCOS and obesity on SHBG (P < 0.05) (Table I). Women with PCOS also had significantly higher trunk/peripheral fat ratio (P < 0.001) independent of obesity (Table I).

In the weight-stratified groups, BMI did not differ between the two lean groups and the two obese groups (Table I). In the lean subgroups, testosterone levels (free and total) did not differ significantly between women with and without PCOS; however, levels of SHBG were lower in lean women with PCOS compared with lean control women (P < 0.05). Trunk/peripheral fat ratio was higher in lean women with PCOS compared with lean control women (P < 0.05) (Table I). In the obese subgroups, women with PCOS had significantly higher total (P < 0.05) and free (P < 0.01) testosterone levels compared with their weight-matched control group (Table I). The trunk/peripheral fat ratio did not differ between obese women with and without PCOS (Table I). Linear regression analysis showed a significant association between trunk/peripheral fat ratio and FT (P < 0.01) (Fig. 1a). The same regression was performed on

<p>| Table 1. Base line characteristics of women with and without PCOS. |
|-------------------|-----------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Total testosterone (nmol/l)</th>
<th>FT (pmol/l)</th>
<th>SHBG (nmol/l)</th>
<th>Fasting glucose (mmol/l)</th>
<th>2-h glucose (mmol/l)</th>
<th>Fasting insulin (pmol/l)</th>
<th>Trunk/peripheral fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (n = 9)</td>
<td>30 ± 4.1</td>
<td>22 ± 1.4</td>
<td>1.49 ± 0.3</td>
<td>15 ± 6</td>
<td>104 ± 33</td>
<td>5.5 ± 0.4</td>
<td>5.0 ± 1.0</td>
<td>24 ± 12</td>
<td>0.5 ± 0.08</td>
</tr>
<tr>
<td>LP (n = 17)</td>
<td>28 ± 4.7</td>
<td>23 ± 1.5</td>
<td>2.06 ± 0.8</td>
<td>34 ± 19</td>
<td>67 ± 27*</td>
<td>5.6 ± 0.3</td>
<td>6.0 ± 1.1</td>
<td>36 ± 18</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>OC (n = 16)</td>
<td>31 ± 5.4</td>
<td>34 ± 3.2</td>
<td>1.41 ± 0.4</td>
<td>24 ± 7</td>
<td>54 ± 21†††</td>
<td>5.8 ± 0.4</td>
<td>6.6 ± 1.2††</td>
<td>64 ± 31††</td>
<td>0.9 ± 0.1†††</td>
</tr>
<tr>
<td>OP (n = 18)</td>
<td>29 ± 3.9</td>
<td>33 ± 4.0</td>
<td>2.42 ± 0.8*</td>
<td>44 ± 22**</td>
<td>57 ± 39</td>
<td>6.0 ± 0.3</td>
<td>7.1 ± 1.1††</td>
<td>69 ± 39†††</td>
<td>1.0 ± 0.2††††</td>
</tr>
</tbody>
</table>

Two-way ANOVA

| PCOS             | * < 0.001       | * < 0.001       | NS               | NS               | NS               | NS               | NS               |
| Obesity          | NS              | NS              | NS               | NS               | NS               | NS               | NS               |
| Interaction      | NA              | NA              | NS               | NS               | NS               | NS               | NS               |

Values are given as mean ± SD. Student’s t-test. *= versus BMI-matched controls. †= obese controls vs. lean controls and obese PCOS vs. lean PCOS. †≡ = P < 0.05, **/†≡ = P < 0.01, ***/†≡ = P < 0.001. LC, lean controls; LP, lean PCOS; OC, obese controls; OP, obese PCOS; FT, free testosterone; NA, not applicable; NS, not significant.
Glucose tolerance and insulin sensitivity
In the total study population, all subjects had fasting plasma glucose <7.0 mmol/l. Seven obese women, four with PCOS and three control women, had IGT, but none had overt type 2 diabetes. There was no effect of PCOS on fasting glucose or insulin levels, after correction for obesity (Table I), but PCOS was associated with higher 2-h plasma glucose levels \( (P < 0.05) \) (Table I, Fig. 2a) and lower GDRs \( (P < 0.01) \) (Fig. 2b) and ISI \( (P < 0.001) \) (Fig. 2c) and higher HOMA-IR \( (P < 0.05) \) (Fig. 2d) independent of obesity (Table II). When adjusting for body fat distribution (trunk/peripheral fat), we found no differences in GDR \( (P = 0.2, \text{ lean subjects}) \); ISI \( (P = 0.05, \text{ lean subjects}) \); and HOMA \( (P = 0.6) \) between women with and without PCOS (data not shown).

In the subgroup analysis of the two lean groups, fasting glucose and insulin levels and 2-h glucose levels did not differ between women with and without PCOS, (Table I, Fig. 2a). GDR and ISI were lower in lean women with PCOS compared with lean control women \( (P < 0.05, P < 0.001) \), whereas HOMA-IR did not differ between the two groups (Table II, Fig. 2b–d). In the two obese groups, fasting glucose, insulin levels and 2-h glucose levels were similar (Table I, Fig. 2a). Insulin sensitivity, evaluated by ISI, was numerically reduced by \( \sim 25\% \) in the obese women with PCOS compared with obese control women \( (P = 0.06) \). However, the HOMA-IR was very similar in the two obese groups (Table II, Fig. 2d). ISI was lower in both obese groups compared with the two lean groups, \( (P < 0.001 \text{ LC vs. OC and } P < 0.05 \text{ LP vs. OP}) \) and there was a significant interaction between obesity and PCOS on ISI, \( (P < 0.05) \) indicating that women with PCOS have an added burden of IR related to adiposity (Table II, Fig. 2c). When adjusting for body fat distribution (trunk/peripheral fat) in the two lean groups and in the two obese groups, respectively, we found no differences in GDR \( (P = 0.3), \text{ ISI } (P = 0.05) \) and HOMA \( (P = 0.6) \) between women with and without PCOS (data not shown).

The DI \( (= \text{AIRg} \times \text{ISI}) \) was calculated to determine whether insulin secretion was appropriate for the prevailing action of insulin. In all subjects we found that PCOS had no effect on

\[ y = \frac{0.033x + 0.0044}{R^2 = 0.1291, p < 0.01} \]  
\[ y = -0.005x + 0.044 \]  
\[ y = -0.005x + 0.0441 \]  
\[ y = 0.004x + 0.024 \]  
\[ y = 3.06x + 0.023 \]  

Figure 1: Relationship between markers of insulin sensitivity and free testosterone.
Simple linear regression analyses on FT levels with trunk/peripheral fat ratio (t/p), the different measures of insulin sensitivity (GDR, ISI and HOMA-IR) and insulin secretion during an IIVGT, determined as AIRg.
AIRg and DI. In the subgroups, both AIRg and DI were similar in lean women with and without PCOS and in obese women with and without PCOS, but AIRg was significantly increased in obese women with PCOS compared with lean women with PCOS (P < 0.05), but was similar in lean and obese control women (Table II, Fig. 2e and f). Linear regression analysis

**Table II.** Measurements of insulin sensitivity and β-cell responsiveness in women with and without PCOS.

<table>
<thead>
<tr>
<th></th>
<th>GDR (mg kg FFM⁻¹ min⁻¹)</th>
<th>ISI (μg kg FFM⁻¹ min⁻¹ pmol l⁻¹)</th>
<th>HOMA-IR</th>
<th>AIRg (pmol l⁻¹ min⁻¹)</th>
<th>DI (ISI*AIRg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (n = 9)</td>
<td>13.3 ± 2.1</td>
<td>40 ± 16</td>
<td>0.8 ± 0.5</td>
<td>1698 ± 1286</td>
<td>68 ± 59</td>
</tr>
<tr>
<td>LP (n = 17)</td>
<td>10.4 ± 3*</td>
<td>24 ± 8**</td>
<td>1.3 ± 0.7</td>
<td>1879 ± 1371</td>
<td>46 ± 44</td>
</tr>
<tr>
<td>OC (n = 16)</td>
<td>8.1 ± 2.8***</td>
<td>20 ± 8†††</td>
<td>2.3 ± 1.6††</td>
<td>2654 ± 1089</td>
<td>52 ± 33</td>
</tr>
<tr>
<td>OP (n = 18)</td>
<td>6.9 ± 2.0†</td>
<td>15 ± 6†</td>
<td>2.6 ± 0.4††</td>
<td>3340 ± 1685†</td>
<td>46 ± 23</td>
</tr>
<tr>
<td>Two-way ANOVA</td>
<td></td>
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<tr>
<td>PCOS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Obesity</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD. Student’s t-test. * = versus BMI-matched controls; † = obese controls vs. lean controls and obese PCOS vs. lean PCOS. * / † = P < 0.05, ** / †† = P < 0.01, *** / ††† = P < 0.001. LC, lean controls; LP, lean PCOS; OC, obese controls; OP, obese PCOS; NS, not significant.
showed a significant association between AIRg and FT, $P < 0.05$ (Fig. 1e). The same regression analysis performed on AIRg and total testosterone showed no significance ($R^2 = 0.03$, NS) (data not shown).

**Glucose and lipid metabolism**

In the total study population, we found no independent effect of PCOS on basal oxidative glucose metabolism (GOX-1), or basal and insulin-stimulated lipid oxidation (LIPOX-1 and LIPOX-2). However, PCOS was associated with lower insulin-stimulated glucose oxidation (GOX-2) ($P < 0.05$) and non-oxidation (NOGM) ($P < 0.05$) and lower Δ-GOX ($P < 0.05$) independent of obesity (Table III, Fig. 3). Furthermore, we found a negative synergistic effect of PCOS and obesity on insulin-stimulated glucose oxidation (GOX-2) ($P < 0.05$) (Table III).

In the lean subgroups, GOX-1, NOGM, LIPOX-1 and LIPOX-2 were similar in lean women with and without PCOS, but insulin-stimulated glucose oxidation (GOX-2) was lower in lean women with PCOS compared with lean control women ($P < 0.05$) (Table III, Fig. 3). In the obese subgroups, all measures of glucose oxidation and non-oxidation and lipid oxidation did not differ between women with and without PCOS (Table III, Fig. 3).

**Triglycerides, lipoproteins and FFA**

PCOS was associated with increased levels of plasma cholesterol (total) independent of obesity ($P < 0.05$), and obesity alone was associated with increased levels of fasting cholesterol ($P < 0.05$), and decreased levels of HDL ($P < 0.05$), triglycerides and insulin-stimulated triglycerides ($P < 0.05$) (Table IV). We found a significant negative interaction of PCOS and obesity on total cholesterol ($P < 0.05$) and a significant positive interaction between PCOS and obesity on HDL ($P < 0.05$) (Table IV).

Triglycerides, lipoproteins and FFA did not differ between lean women with and without PCOS and between obese women with and without PCOS, but obese control women had higher levels of insulin-stimulated triglycerides ($P < 0.05$) and total cholesterol ($P < 0.05$) than lean control women, and obese women with PCOS had lower levels of HDL than lean women with PCOS ($P < 0.001$) (Table IV).

**Discussion**

The present study was performed to elucidate the intriguing relationship between PCOS and obesity. We have demonstrated that PCOS is associated with lower insulin sensitivity, calculated from clamp data and by HOMA-IR, which can be accounted for by a significant reduction of insulin-stimulated glucose oxidation and non-oxidation. In line with this, we also demonstrated a higher trunk/periphery fat ratio in women with PCOS compared with control women, after having adjusted for obesity. However, β-cell responsiveness, β-cell function, oxidative and non-oxidative lipid metabolism were not affected by the presence of PCOS alone. In the subgroup analysis, we found that lean women with PCOS had lower insulin sensitivity and a higher trunk to peripheral fat ratio than lean control women. Although genetic factors, i.e. defects in the insulin signal cascade in skeletal muscle, may be the primary explanation of the reduced insulin sensitivity in lean women with PCOS, it cannot be excluded that the phenotype trait of a higher trunk to peripheral fat ratio is a significant pathogenic factor in the development of PCOS.
Table IV. Triglycerides, total cholesterol and FFA in women with and without PCOS.

<table>
<thead>
<tr>
<th></th>
<th>TG 1 (mmol/l)</th>
<th>TG 2 (mmol/l)</th>
<th>Total cholesterol (mmol/l)</th>
<th>HDL (mmol/l)</th>
<th>VLDL (mmol/l)</th>
<th>FFA-1 (mmol/l)</th>
<th>FFA-2 (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC (n = 9)</td>
<td>0.8 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>3.7 ± 0.5</td>
<td>1.2 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.4</td>
<td>0.06 ± 0.08</td>
</tr>
<tr>
<td>LP (n = 17)</td>
<td>0.8 ± 0.4</td>
<td>0.6 ± 0.4</td>
<td>4.3 ± 1.0</td>
<td>1.4 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>OC (n = 16)</td>
<td>1.2 ± 0.6</td>
<td>1.0 ± 0.4†</td>
<td>4.7 ± 0.8†</td>
<td>1.1 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.06 ± 0.08</td>
</tr>
<tr>
<td>OP (n = 18)</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>4.3 ± 0.6</td>
<td>1.1 ± 0.2††</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.06 ± 0.06</td>
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<tr>
<td>Two-way ANOVA</td>
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<tr>
<td>PCOS</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Obesity</td>
<td>P &lt; 0.01†</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>NS</td>
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</table>

Values are given as mean ± SD. Student’s t-test. * = versus BMI-matched controls. † = obese controls vs. lean controls and obese PCOS vs. lean PCOS. */† = P < 0.05, **/†† = P < 0.01, ***/††† = P < 0.001. LC, lean controls; LP, lean PCOS; OC, obese controls; OP, obese PCOS. TG-1, basal triglycerides; TG-2, insulin-stimulated triglycerides; NS, not significant.

(Yildirim et al., 2003). Thus, an increased amount of truncal fat has been found to be an independent predictor of IR and may in this study account for some of the lower insulin action in lean women with PCOS (Holte et al., 1994b; Morin-Papunen et al., 2000; Puder et al., 2005; Lord et al., 2006; Yucel et al., 2006; Carmina et al., 2007). In line with this, we found no difference in insulin sensitivity evaluated by GDR, ISI and HOMA between women with and without PCOS after adjusting for fat distribution. Furthermore, we found significant associations between increased androgen levels, increased amounts of upper body fat and decreased insulin sensitivity. Hyperandrogenemia may therefore be an independent predictor of central obesity and IR, which has also been demonstrated in previous studies (Burghen et al., 1980; Douchi et al., 2001; Yucel et al., 2006).

In the obese subgroups, obese women with PCOS displayed a 25% reduction in insulin sensitivity compared with obese women without PCOS, when evaluated from clamp data (P = 0.06). The fat distribution did not differ between the two groups. Finally, statistical analysis showed that PCOS and obesity had a negative synergistic effect on insulin sensitivity, insulin-stimulated glucose oxidation and total plasma cholesterol levels.

AIRg was significantly increased in the obese groups compared with the lean groups. There were no differences in β-cell responsiveness between women with and without PCOS, even when considered in light of prevailing degree of insulin action by calculating DI, suggesting that insulin secretion was adequate to compensate for peripheral IR in both obesity and PCOS. Nevertheless, the 2-h plasma glucose level during the OGTT was higher in women with PCOS compared with the participants without PCOS, indicating a subtle β-cell dysfunction.

Our results are partly in line with the findings of other investigators who have evaluated the effect of obesity and PCOS on glucose, lipid and insulin metabolism (Dunaif and Finegood, 1996; Morales et al., 1996; Gambineri et al., 2002). Thus, in a previous study, Dunaif and Finegood (1996) described decreased insulin sensitivity in women with PCOS independent of obesity, although AIRg was increased only by obesity and not by PCOS, which is in accordance with the present study. They also found that DI was decreased in both PCOS and obese subjects, suggesting inadequate β-cell adaptation to the ambient insulin sensitivity. In our study, DI was not significantly different between the four groups, but a slight tendency to a lower DI was observed in the PCOS subjects. In another study, Morin-Papunen et al. (2000) found significant metabolic differences between obese women with and without PCOS, but not in lean women with and without PCOS. Several other investigators have evaluated insulin/glucose metabolism in PCOS women, and there seems to be little congruence in the results. These contradictions may partly be explained by the complexity and heterogenicity of the syndrome, but also by the use of different methods for the evaluation of the relevant metabolic parameters, different diagnostic criteria for PCOS, different BMI criteria and ethnicities. Dunaif and Finegood (1996) investigated insulin secretion in the context of insulin sensitivity by the use of the frequently sampled IVGTT and the minimal model. Morin-Papunen et al. (2000) used different diagnostic criteria for PCOS, different BMI criteria and different statistical methods for the evaluation of the effect of PCOS and obesity on glucose and insulin related metabolic variables. We used an independent measure of insulin secretion and insulin sensitivity by the use of IVGTT and a euglycemic hyperinsulinemic clamp. Furthermore we evaluated β-cell function in relation to the prevailing insulin sensitivity by calculating the DI. The first-phase insulin response has been shown to relate to insulin sensitivity in a hyperbolic manner, and it is therefore essential to adjust for the prevailing insulin sensitivity to achieve a correct interpretation of β-cell function. In the present study, insulin sensitivity was estimated by two independent methods. Thus, ISI was calculated from a euglycemic hyperinsulinemic clamp, and HOMA-IR was calculated from fasting plasma insulin and glucose levels. The HOMA-IR results indicate lower insulin sensitivity in women with PCOS compared with control women, whereas in the subgroup analysis, the HOMA-IR did not differ between the two lean groups or between the two obese groups with or without PCOS. The discrepancy between the results obtained from clamp data (ISI) and HOMA-IR may be of interest. ISI evaluates insulin sensitivity in the periphery tissue, primarily muscle, during high-insulin concentrations, whereas HOMA-IR is a measure of whole-body insulin sensitivity, including the liver, during lower fasting insulin levels. Taken together, our data support the central role of IR in the pathogenesis of PCOS, independent of body weight, and that obesity amplifies IR. Accordingly,
the metabolic disturbances seem to be more severe in obese women without PCOS than in lean women with PCOS.

Insulin plays a central role in the regulation of lipid oxidation, mainly by inhibiting the release of FFA from fatty tissue. In insulin-resistant subjects, this inhibition is compromised, leading to an increased concentration of FFA in the blood stream. Similarly, higher FFA concentrations and a defective suppression of the rate of lipid oxidation have been found during the hyperinsulinaemic clamp in PCOS subjects (Holte et al., 1995; Morin-Papunen et al., 2000), but we were not able to demonstrate any differences in lipid oxidation rates between women with and without PCOS, as would have been expected.

IR also increases the risk of type 2 diabetes (Haffner, 1996), and in PCOS, the risk of type 2 diabetes is increased 4–7-fold. In the present study, four participants in the group with PCOS and three control subjects had IGT. We have demonstrated that PCOS is associated with higher 2-h plasma glucose levels and lower insulin sensitivity assessed by both clamp and HOMA-IR, which are the first steps toward development of IGT or overt type 2 diabetes. In light of the results of previous epidemiological studies including women with PCOS, it seems reasonable to suspect that the age-induced changes in body composition, insulin action and β-cell function also will increase the future risk for cardiovascular diseases and type 2 diabetes in the present young population of women with PCOS (Legro et al., 2005). In the present study, we have been unable to demonstrate any differences in lipid metabolism between women with and without PCOS. This may partly be explained by the young age of our study group, but also by a relatively small study population, which is compensated for using gold standard and very accurate methods for the evaluation of the relevant metabolic variables.

We defined PCOS according to the Rotterdam Criteria (2004), and both the lean and obese subgroup of women with PCOS had higher serum androgen levels, and more frequently an anovulatory menstrual pattern and PCOS ovarian morphology. The criteria for PCOS suggested at the Rotterdam Meeting are questioned (Broekmans and Fauser, 2006) since they allow a number of different phenotypes of PCOS that may be caused by other pathophysiologic mechanisms. Simple obesity is often associated with several hormonal disturbances, and although all obese control women in our study had a regular menstrual cycles and androgen levels within the normal range, obesity can induce functional hyperandrogenism (Azziz, 1989; Pasquali et al., 2006), primarily due to an increased androgen production in fatty tissue, and also due to higher insulin levels inhibiting hepatic SHBG production. Elevated IGF and reduced IGF-BP may furthermore directly affect ovarian function. These women may resolve their hormonal disturbances by weight loss, whereas obese women who have developed PCOS, due to intrinsic defects in hypothalamic–pituitary–gonadal axis or in insulin action, may only improve, but not resolve their hormonal/metabolic disturbances by weight loss (Kiddy et al., 1992; Moran et al., 2003; Tolino et al., 2005).

We conclude that PCOS is associated with decreased insulin sensitivity independent of obesity. Furthermore, lean women with PCOS have lower insulin sensitivity and higher trunk/periphery fat ratios than lean control women. In obese women with PCOS, the reduction in insulin sensitivity was ~25% compared with obese women without PCOS, despite similar trunk/peripheral fat ratios. The obese groups displayed lower insulin sensitivity than the respective lean groups. β-Cell responsiveness is amplified in obese subjects, and women with PCOS have an increased compensatory insulin secretion to a given increment in insulin sensitivity. DI did not differ significantly between the groups, suggesting β-cell adaptation to the prevailing insulin sensitivity.

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