Insulin-induced capillary recruitment is impaired in both lean and obese women with PCOS


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Background: Insulin resistance, i.e. impaired insulin-mediated glucose uptake (IMGU), is a major risk factor for type 2 diabetes in women with polycystic ovary syndrome (PCOS). Insulin-induced capillary recruitment (IICR) is considered a significant determinant of IMGU. We investigated whether IICR is a determinant of IMGU in obese and lean women with and without PCOS.

Methods: The study included 36 women with PCOS (20 lean, BMI 21.9 ± 2.3 kg/m² and 16 obese, BMI 35.9 ± 6.0 kg/m²) and 27 age-matched healthy controls (14 lean, BMI 22.2 ± 1.8 kg/m² and 13 obese, BMI 40.5 ± 7.0 kg/m²). IICR was evaluated by capillary microscopy during an isoglycemic-hyperinsulinemic clamp. IMGU was expressed as M/I value.

Results: The M/I value was significantly lower in obese PCOS women compared with obese controls [0.5 (0.2–1.1) versus 0.8 (0.3–1.4) (mg kg⁻¹ min⁻¹ pmol l⁻¹) × 100, P < 0.01], whereas the small difference between lean PCOS and lean control women was non-significant [1.5 (0.5–2.6) versus 1.7 (1.0–3.7) (mg kg⁻¹ min⁻¹ pmol l⁻¹) × 100, P = 0.17]. Hyperinsulinemia increased capillary recruitment in lean controls (53.5 ± 20.3 versus 64.9 ± 27.4 n/mm², P < 0.05), but not in either PCOS group nor in obese controls. IICR and androgens were a determinant of M/I value only in lean women with or without PCOS.

Conclusions: PCOS per se is associated with impaired IICR. Obese women with PCOS, in part independent of obesity, demonstrated a profound insulin resistance, whereas the difference between lean PCOS women and healthy controls was small and statistically non-significant. IICR was a determinant of IMGU in lean, but not in obese, women regardless of the presence of PCOS.

Key words: insulin resistance / capillary recruitment / obesity / PCOS

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder of unknown etiology characterized by hyperandrogenism, anovulatory infertility, and, frequently, profound insulin resistance in premenopausal women (Ehrmann, 2005). Insulin resistance, i.e. decreased insulin-mediated glucose uptake (IMGU), is characterized by the diminished ability of insulin to initiate intracellular signaling and is considered a prelude to type 2 diabetes in women with PCOS (Ehrmann, 2005; Palomba et al., 2009). The primary targets of insulin are skeletal muscle, adipose tissue and the liver. Impaired insulin signaling in these tissues reduces glucose uptake and promotes a metabolic syndrome that is characterized by elevated levels of insulin, inappropriate synthesis of glucose and dyslipidemia (Saltiel et al., 2001). However, insulin receptors and insulin signaling are not exclusively restricted to metabolically active tissue and can be observed in most cell types including vascular cells.

Recently, it has become clear that vascular tissue, particularly the endothelial cell, is an important physiological target for insulin and a significant regulator of overall insulin-stimulated glucose uptake (Serne et al., 2007; Barrett et al., 2009). Insulin promotes its own access to muscle interstitium by increasing blood flow and by recruiting capillaries to expand the endothelial transporting surface available for nutrient exchange. Using capillary microscopy, we have reported that, in healthy individuals, systemic hyperinsulinemia increases the number of perfused capillaries (Serne et al., 2002). In contrast,
blunted capillary recruitment is associated with metabolic insulin resistance seen in obesity (de Jongh et al., 2004b), elevated plasma free fatty acids (FFA) concentrations (de Jongh et al., 2004a) and the metabolic syndrome (Serne et al., 2007). These observations are particularly interesting as they suggest that altered microvascular responses may be an early dysfunction in individuals at risk of diabetes. Women with PCOS are at increased risk of diabetes, but it is unknown whether PCOS is associated with an impaired action of insulin to increase capillary density. In our earlier reports, we demonstrated that obesity is a major determinant of insulin resistance in PCOS and that insulin resistance in obesity is strongly related to impaired insulin-induced capillary recruitment (IICR) (de Jongh et al., 2004b; Ketel et al., 2008). The present study was designed to test the hypothesis that PCOS women compared with control women display impaired IICR and if so, to determine whether this abnormality is worse in obese women and related to IMGU.

Materials and Methods

Subjects

Four groups of subjects were recruited: 20 lean (BMI < 25 kg/m²) women with PCOS, 14 lean women without PCOS, 16 obese (BMI > 30 kg/m²) women with PCOS and 13 obese women without PCOS. All cases had the diagnosis of PCOS according to the European/American society (2004). All women with PCOS had oligo- or amenorrhea and polycystic ovarian morphology on transvaginal ultrasound; 13 obese women and 16 lean women also had hyperandrogenism estimated by clinical and/or laboratory measurements, and therefore fulfilled all three Rotterdam criteria. Women without PCOS were as a group matched for age and weight with the PCOS groups. All were healthy as judged by medical history, were non-diabetic (Report of the expert committee, 2003) and normotensive (<140/90 mmHg) as determined by triplicate office blood pressure measurement. All women without PCOS had a normal ovarian morphology on ultrasound, had a regular cycle and no clinical or laboratory features of PCOS. All participants were Caucasian and non-smokers, and had not used any medication or oral contraceptives for the last 3 months. Exclusion criteria for all subjects included abnormal thyroid-stimulating hormone (TSH), prolactin, 17 hydroxyprogesterone concentrations (de Jongh et al., 2004a) and the metabolic syndrome (Serne et al., 2007). These observations are particularly interesting as they suggest that altered microvascular responses may be an early dysfunction in individuals at risk of diabetes. Women with PCOS are at increased risk of diabetes, but it is unknown whether PCOS is associated with an impaired action of insulin to increase capillary density. In our earlier reports, we demonstrated that obesity is a major determinant of insulin resistance in PCOS and that insulin resistance in obesity is strongly related to impaired insulin-induced capillary recruitment (IICR) (de Jongh et al., 2004b; Ketel et al., 2008). The present study was designed to test the hypothesis that PCOS women compared with control women display impaired IICR and if so, to determine whether this abnormality is worse in obese women and related to IMGU.

Insulin-induced capillary recruitment is impaired in PCOS

Skin microvascular measurements

Nailfold capillary studies were performed as described previously (Serne et al., 2001; de Jongh et al., 2004b) before and during a hyperinsulinemic isoglycemic clamp (Fig. 1). Briefly, nailfold capillaries in the dorsal skin of the fourth finger of the left hand were visualized by a capillary microscope. A visual field of 1 mm² was recorded before and after 4 min of arterial occlusion with a digital cuff. Capillaries at baseline and directly after release of the cuff were counted for, respectively, 15 and 30 s. Baseline capillary density was defined as the number of continuously erythrocyte-perfused capillaries per square millimeter of nailfold skin. Other capillaries can be seen to be intermittently perfused, and these may represent an important functional reserve. Post-occlusive reactive hyperemia after 4 min of arterial occlusion was used to assess this functional reserve (capillary recruitment). Capillary recruitment was expressed as percentage change from baseline. IICR was defined as the percentage change from baseline during hyperinsulinemia. The procedures were then repeated using a visual field adjacent to the first visual field and the mean of both measurements was used for analyses. The same operator performed the measurements for each patient in order to avoid inter-observer variability. The operator and an observer (I.J.G.K. and L.V.) counting the capillaries were blinded for the characteristics of the subjects and test results. The day-to-day coefficient of variation of post-occlusive capillary recruitment was 11.4 ± 7.7%, as determined in nine individuals on two separate days. The intra-observer variability between I.J.G.K. and L.V. of counting the percentage increase was 13.4 ± 8.1%, as determined in 16 individuals. The day-to-day coefficient of variation of peak density during venous congestion was 3.3 ± 3.0% as determined in nine individuals on two separate days. The intra-observer variability between I.J.G.K. and L.V. of this procedure was 6.9 ± 5.7%, as determined in 16 individuals.

Study design

All individuals underwent the study protocol as shown in Fig. 1. All participants came to the clinic after a 10-h overnight fast. To confirm that the short-term non-specific changes in microcirculatory function are small (de Jongh et al., 2004b), an additional a time- and volume-control experiment was performed in 8 lean and 10 obese women with PCOS in a fashion identical to the clamp experiment with the infusion of the same amounts of fluid (0.65% saline) and microvascular measurements at the same time intervals, but without insulin or glucose infusion. All measurements were conducted in a quiet, temperature-controlled room (T = 23.4 ± 0.4°C), after a 10-h fast, with the subjects in the supine position. Subjects were asked to refrain from drinking alcohol for a period of 24 h before each study day and to perform no strenuous exercise for a period of 48 h before each study day. Baseline measurements were obtained after allowing 30 min of rest and acclimatization after the insertion of two polytetrafluoroethylene catheters (Venflon, Viggo, Gotenborg, Sweden). During the tests, skin temperature was monitored and all subjects were studied at the same time of the day.

Insulin-mediated glucose uptake

IMGU was determined with the hyperinsulinemic, isoglycemic clamp technique as described previously (Ferrannini et al., 1998), with glucose concentrations clamped at fasting level and an insulin infusion rate of 40 mU m⁻² min⁻¹. Mean fasting glucose concentrations were determined from glucose concentrations measured three times between t = −30 and t = 0 min (Fig. 1). Normoglycemia was maintained by adjusting the rate of a 20% D-glucose infusion based on plasma glucose measurements performed at 5–10 min intervals. Whole body glucose uptake (M) was calculated from the glucose infusion rates during the last 60 min of hyperinsulinemia. M was expressed per kg body weight per unit of plasma insulin concentration (M/I), thereby correcting for differences in steady-state insulin concentrations. For convenience, the M/I ratio was multiplied by 100. Mean insulin concentrations during the hyperinsulinemic clamp were determined from steady-state insulin levels measured.
Blood pressure
Ambulatory monitoring (SpaceLabs 90207, Redmond, WA, USA) was used to obtain 24-h recordings of blood pressure and heart rate, as described previously (Serri et al., 1999, 2001). During study days, blood pressure and heart rate were determined (Colin Press-Mate BP-8800, Colin, Komaki City, Japan). The first blood pressure reading was discarded and the mean of the second and third consecutive blood pressure and heart rate readings during each period were used for further analyses.

Laboratory data
Blood was collected before and during insulin infusion (Fig. 1). All serum samples were immediately centrifuged at 4°C and stored at −80°C. A radioimmunoassay was used to measure levels of androstenedione (DSL, Webster, TX, USA) and DHEA-S (DPC, Los Angeles, CA, USA). Testosterone and 17OH-progesterone levels were measured with a competitive immunoassay (DRG Instruments, Marburg, Germany). The sex hormone-binding globulin (SHBG) level was measured by using an immunometric assay (Immulite 2500, Los Angeles, CA, USA). The free androgen index (FAI) was calculated as the total testosterone divided by the sex SHBG level × 100. A competitive immunoassay with luminescence was used to measure progesterone (Abbott Laboratories Diagnostic Division Abbott Park, IL, USA). An immunometric assay (Delfia, Wallac, Turku, Finland) was used to measure LH and FSH in serum. E2 levels were measured using a radio immunoassay with double antibody (Diasorin, Saluggia, Italy).

Insulin was measured by using an immunometric assay with luminescence (Bayer Diagnostics, Mijdrecht, Netherlands). The inter-assay coefficient of variation was below 5% and the intra-assay coefficient of variation was below 10%. Glucose was measured with the hexokinase method (Roche Diagnostics, Mannheim, Germany). The inter- and intra-assay coefficients of variation were both below 2%. TSH, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were measured with an enzymatic colorimetric assay (Roche Diagnostics). The low-density lipoprotein (LDL)-cholesterol level was calculated according to the Friedewald formula. FFAs were measured with an enzymatic colorimetric test (NEFA-C, WAKO chemicals, Neuss, Germany). All laboratory assays were performed in the clinical chemistry/endocrine laboratory of the VU University Medical Center.

Statistical analyses
All analyses were performed with SPSS 15.0 (Chicago, IL, USA). The distribution of variables was tested for normality. Data are expressed as mean ± standard deviation, or as median (range), when appropriate. To examine the differences in anthropometric and laboratory properties between normal-weight PCOS, obese PCOS and group-matched women without PCOS, we used one-way analysis of variance with post hoc analyses (Bonferroni). We used analysis of covariance (ANCOVA) to compare capillary density and insulin sensitivity between obese versus lean and PCOS versus controls. The latter analysis was adjusted for differences in BMI, because PCOS women were less obese compared with controls and BMI was inversely associated with both capillary density and insulin sensitivity in both groups (data not shown). Within subjects, a paired Student’s t-test was used to compare capillary recruitment before and during insulin infusion.

A linear regression analysis was used to analyze the associations between IICR and androgens on the one hand and IMGU on the other. We tested for interaction to see whether these associations differed between lean and obese women. P < 0.05 was considered statistically significant, except for the interaction analyses, where we used P < 0.10.

Results
Characteristics of the study population
By design, obese women had higher BMI, waist circumference and waist-to-hip ratio measurements compared with lean women (Table I). Women with PCOS had a higher androstenedione, free testosterone (i.e. FAI) and LH levels when compared with those without PCOS. In general, obese women, regardless of the presence or absence of PCOS, had a less favorable cardiovascular disease risk factor profile (i.e. higher systolic blood pressure, triglycerides and lower HDL cholesterol) (Table I).

Capillary recruitment before insulin infusion
There were no differences in baseline capillary density between lean and obese PCOS and their controls before insulin infusion (Table II). Also, capillary recruitment, expressed as increase and percentage increase, was not different among the four groups.

Insulin-induced capillary recruitment
Baseline capillary density did not change during insulin infusion and was similar in PCOS and control women. Hyperinsulinemia increased capillary recruitment in lean women without PCOS (53.5 ± 20.3 versus 64.9 ± 27.4 n/mm²; P < 0.05), but not in obese controls nor in both PCOS groups (Table II). PCOS women had a significantly lower percentage increase in capillary recruitment when compared with controls.

Figure 1 Design of study: Microcirculation indicates microvascular measurements; X, blood sample, BP, blood pressure measurements and IV, insertion of intravenous catheters. Glucose concentration on t = 0 and insulin concentration on t = 0 and the mean of the four blood, samples during insulin infusion were determined.
Insulin-induced capillary recruitment is impaired in PCOS

**Table I** Profiles of anthropometrics, lipids, hemodynamics, endocrinology and glucose metabolism of lean and obese women with PCOS and weight, age-matched controls.

<table>
<thead>
<tr>
<th>SI units</th>
<th>Lean Controls (n = 14)</th>
<th>PCOS (n = 20)</th>
<th>Obese Controls (n = 13)</th>
<th>PCOS (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Years</td>
<td>27.3 ± 5.1</td>
<td>27.9 ± 4.3</td>
<td>28.6 ± 5.3</td>
<td>30.3 ± 4.4</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>22.2 ± 1.8</td>
<td>21.9 ± 2.3</td>
<td>40.5 ± 7.0*****</td>
<td>35.9 ± 6.0***</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.76 ± 0.03</td>
<td>0.78 ± 0.05</td>
<td>0.80 ± 0.05</td>
<td>0.84 ± 0.05*****</td>
</tr>
<tr>
<td>Waist Cm</td>
<td>74.1 ± 3.8</td>
<td>74.9 ± 5.8</td>
<td>105.7 ± 12.1******</td>
<td>107.1 ± 12.3****</td>
</tr>
<tr>
<td>Serum cholesterol mmol/l</td>
<td>4.0 ± 0.6</td>
<td>4.2 ± 0.9</td>
<td>4.1 ± 0.7</td>
<td>4.7 ± 1.1</td>
</tr>
<tr>
<td>Serum LDL cholesterol mmol/l</td>
<td>2.1 ± 0.5</td>
<td>2.3 ± 0.6</td>
<td>2.5 ± 0.5</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>Serum HDL cholesterol mmol/l</td>
<td>1.6 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>1.2 ± 0.3******</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Serum triglycerides mmol/l</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.4</td>
<td>1.2 ± 0.5*****</td>
</tr>
<tr>
<td>Plasma FFAs mmol/l</td>
<td>0.60 ± 0.27</td>
<td>0.45 ± 0.16</td>
<td>0.63 ± 0.14</td>
<td>0.65 ± 0.17 ***</td>
</tr>
<tr>
<td>Serum glucose pmol/l</td>
<td>29.0 ± 7.4</td>
<td>33.2 ± 16.8</td>
<td>60.8 ± 38.9</td>
<td>89.6 ± 47.3****</td>
</tr>
<tr>
<td>24-h systolic pressure MmHg</td>
<td>112 ± 7</td>
<td>112 ± 8</td>
<td>130 ± 14*****</td>
<td>121 ± 8***</td>
</tr>
<tr>
<td>24-h diastolic pressure MmHg</td>
<td>68 ± 7</td>
<td>70 ± 10</td>
<td>73 ± 12</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>24-h heart rate Bpm</td>
<td>70 ± 6</td>
<td>68 ± 10</td>
<td>75 ± 6</td>
<td>77 ± 9***</td>
</tr>
<tr>
<td>Androstenedione nmol/l</td>
<td>4.7 ± 1.2</td>
<td>6.9 ± 1.9**</td>
<td>4.9 ± 1.7</td>
<td>7.6 ± 2.5**</td>
</tr>
<tr>
<td>Total testosterone nmol/l</td>
<td>1.2 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.4</td>
<td>1.9 ± 0.5****</td>
</tr>
<tr>
<td>Free androgen index %</td>
<td>2.8 ± 2.0</td>
<td>3.1 ± 1.4</td>
<td>6.0 ± 3.6*****</td>
<td>8.9 ± 3.7***</td>
</tr>
<tr>
<td>17 OH-progestrone nmol/l</td>
<td>2.1 ± 0.5</td>
<td>2.7 ± 1.0</td>
<td>2.1 ± 0.9</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>DHEA-S μmol/l</td>
<td>4.4 ± 1.5</td>
<td>5.2 ± 2.2</td>
<td>6.4 ± 2.1</td>
<td>6.4 ± 3.5</td>
</tr>
<tr>
<td>LH U/l</td>
<td>4.7 ± 2.3</td>
<td>8.0 ± 3.9**</td>
<td>3.7 ± 0.9</td>
<td>7.0 ± 2.6**</td>
</tr>
<tr>
<td>FSH U/l</td>
<td>6.0 ± 1.8</td>
<td>4.7 ± 0.9*</td>
<td>5.4 ± 0.9</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>Estradiol (E₂) pmol/l</td>
<td>141.1 ± 101.7</td>
<td>186.5 ± 169.7</td>
<td>126.4 ± 61.4</td>
<td>133.5 ± 38.7</td>
</tr>
<tr>
<td>Prolactin U/l</td>
<td>0.19 ± 0.06</td>
<td>0.17 ± 0.07</td>
<td>0.19 ± 0.06</td>
<td>0.22 ± 0.18</td>
</tr>
</tbody>
</table>

Mean ± standard deviation, median (range)
BMI, body mass index; FFA, free fatty acids; DHEA-S, dehydroepiandrosterone sulfate; LH, luteinized hormone; FSH, follicle-stimulating hormone.

*P < 0.05
**P < 0.01 PCOS versus corresponding control group.
***P < 0.001
****P < 0.001 obese PCOS versus lean PCOS.
*****P < 0.001 obese versus lean controls.

(P = 0.03, Table II, Fig. 2). There was no interaction between obesity and PCOS regarding IICR (P-value for interaction = 0.51).

**Insulin-mediated glucose uptake**

IMGU (mean ± 95% confidence interval) was significantly lower in the pooled group of obese women compared with lean women [0.5 (0.4–0.6) versus 1.5 (1.3–1.8) (mg kg⁻¹ min⁻¹ pmol l⁻¹) × 100, P < 0.01]. After adjustment for differences in BMI, IMGU was also significantly lower in the pooled group of PCOS women compared with controls [0.8 (0.7–0.9) versus 1.2 (1.0–1.4) (mg kg⁻¹ min⁻¹ pmol l⁻¹) × 100, P < 0.01]. Because we could not exclude interaction of PCOS and obesity regarding IMGU (P-value for interaction = 0.15) (Ketel et al., 2008), we also performed a one-way ANCOVA with adjustment for BMI to compare the separate groups of PCOS women with their appropriate controls. This analysis demonstrated that obese women with PCOS had a significantly lower IMGU (median ± range) compared with obese control women [0.5 (0.2–1.1) versus 0.8 (0.3–1.4) (mg kg⁻¹ min⁻¹ pmol l⁻¹) × 100, P < 0.01], whereas the small difference between lean PCOS and control women was statistically non-significant [1.5 (0.5–2.6) versus 1.7 (1.0–3.7) (mg kg⁻¹ min⁻¹ pmol l⁻¹) × 100, P = 0.17] (Fig. 2, Table II).

**Associations between IICR, androstenedione and IMGU**

Interaction analysis demonstrated that the associations between IICR and androstenedione on the one hand, and IMGU on the other hand were similar in PCOS women versus control women (P-value for interaction = 0.82 and = 0.68, respectively). However, these associations were significantly different in lean versus obese women, with or without PCOS (P-value for interaction <0.01 and <0.05, respectively). Therefore, associations with IMGU were analyzed in lean and obese women separately. Both IICR and androstenedione were significantly associated with IMGU in lean, but not obese, women with or...
without PCOS (Fig. 3). Similar conclusions were reached when statistical analyses were performed with the use of testosterone or the FAI instead of androstenedione (data not shown).

**Discussion**

In the present study, we investigated whether IICR is impaired and contributes to IMGU in lean and obese women with PCOS. We provide evidence that both lean and obese women with PCOS are characterized by impaired IICR. Only obese women with PCOS, in part independent of obesity, demonstrated a profound impairment of IMGU. In contrast, although displaying a large variation in IMGU, lean women with PCOS did not have obvious metabolic insulin resistance (Ketel et al., 2008). Subsequent multiple linear regression analysis demonstrated that IICR is a determinant of glucose uptake in lean, but not in obese, women with or without PCOS. Furthermore, our data provide correlative evidence that hyperandrogenism in PCOS may contribute to insulin resistance.

Studies over two decades have considered metabolic insulin resistance to be an integral pathogenic feature of PCOS (Chang et al., 1983; Corbould et al., 2005; Svendsen et al., 2008). Whereas metabolic insulin resistance is consistently demonstrated in obese women with
PCOS, data are conflicting in lean women with PCOS (Vrbikova et al., 2004; Ketel et al., 2008; Svendsen et al., 2008). The present study demonstrates that obese women with PCOS have independent of obesity, an impaired IMGU (Ketel et al., 2008). This finding is compatible with studies that suggest insulin resistance in skeletal muscle of obese PCOS women involve both PCOS-specific and obesity-related defects in insulin signaling (Kelley et al., 1996; Glintborg et al., 2008). However, our data are not entirely conclusive about the presence of a reduced IMGU in the lean PCOS women. On average, lean women with PCOS do not have frank metabolic insulin resistance but ~30% of these women displayed a reduced IMGU in magnitude comparable to the obese groups [i.e. M/I < 1.0 (mg kg$^{-1}$ min$^{-1}$ pmol l$^{-1}$) × 100], whereas this was the case in none of the lean control women (Figs 2 and 3). This may imply that the lean PCOS group is heterogeneous concerning glucose uptake and that a sub-group of lean PCOS women is indeed insulin resistant. In support of such a hypothesis, studies that demonstrated a higher trunk/peripheral fat ratio in lean PCOS women could also demonstrate a reduced IMGU (Svendsen et al., 2008). Studies that found comparable visceral fat accumulation in lean women with and without PCOS or adjusted for small differences in truncal-abdominal subcutaneous fat distribution did not demonstrate metabolic insulin resistance in lean PCOS (Holte et al., 1994; Ketel et al., 2008). Nevertheless, overall most studies suggest that obesity has a larger impact on IMGU than PCOS per se (Holte et al., 1994; Vrbikova et al., 2004; Ketel et al., 2008; Svendsen et al., 2008).

Figure 3 Correlations between IMGU, androstenedione and IICR (% increase) in lean and obese women with PCOS and their controls. *P < 0.05 for interaction lean vs. obese; **P < 0.01 for interaction lean vs. obese; r = partial correlation, corrected for PCOS.
A key and novel finding of the present study was that both lean and obese PCOS women are characterized by impaired IICR. The finding of IICR in healthy lean controls, which is impaired in obese subjects confirms earlier findings (Serne et al., 2002; de Jongh et al., 2004b; Clerk et al., 2006;jonk et al., 2010). Impaired IICR has been stated to impede insulin delivery to skeletal muscle interstitium and thereby contributes significantly to impaired IMGU and insulin resistance (Baron et al., 2000; Serne et al., 2007; Barrett et al., 2009). In accordance with this statement and with earlier studies (de Jongh et al., 2004b), the results showed that impaired IICR is paralleled by reduced IMGU in obese women with and without PCOS. In apparent contradiction, however, IICR appeared to be a determinant of glucose uptake only in lean but not in obese women with and without PCOS. This finding is in line with known capillary/tissue glucose exchange principles, which suggest that IICR becomes more rate limiting for glucose uptake in situations in which tissue permeability to glucose is high (Baron et al., 2000). In contrast, when tissue permeability is relatively low, one would not expect capillary recruitment to have as great a modulating effect on glucose uptake. In the latter situation, cellular permeability of glucose would be rate limiting for overall glucose uptake. Insulin-resistant states, such as obesity and obese PCOS, characterized by defects in insulin signaling impairing glucose transport (Kelley et al., 1996; Corbould et al., 2005), therefore, would not be expected to exhibit capillary recruitment-limited glucose exchange. This may, in part, explain why the use of vasodilators to increase limb blood flow has generally been ineffective in increasing muscle glucose uptake in insulin-resistant individuals (Barrett et al., 2009). Nevertheless, it should be appreciated that these cross-sectional findings do by no means negate an important role of impaired IICR in the pathogenesis of insulin resistance. Experimental studies suggest that insulin resistance develops in the vasculature well before impaired insulin action is detected in muscle, liver, or adipose tissue, which raises the possibility that the early onset of vascular dysfunction could play a key role in the subsequent development of muscle insulin resistance (Kim et al., 2008). Moreover, in lean PCOS women or in previously insulin-resistant subjects in whom insulin action has been ameliorated, perfusion would be predicted to become relatively more rate limiting. Therefore, it follows logically that complete normalization of insulin action requires a physiologic vascular response to insulin. Interestingly, the normalization of muscle insulin signaling in PCOS despite less than full normalization of insulin-stimulated glucose uptake during treatment with thiazolidinediones (Glintborg et al., 2008; Hojlund et al., 2008) might just indicate such a mechanism. Both lean and obese women with PCOS exhibited elevated androgen levels. A previous study reported an inverse association between androgen levels and insulin-stimulated glucose uptake in women with hyperandrogenism (Moghetti et al., 1996). In addition, elevated androgen levels have also been found to be associated with impaired endothelium-dependent vasodilatation of resistance arteries in obese women with PCOS (Paradisi et al., 2001). Moreover, experimental studies in rats have demonstrated that androgen treatment was associated with a decrease in muscle capillary density which was paralleled by decreased IMGU (Holmang et al., 1990). These results raise the possibility that androgens may influence IICR and subsequent glucose uptake. In the present study, androstenedione was significantly and inversely associated with IMGU and tended to be associated with IICR only in lean PCOS women, but not obese PCOS women. Clearly, further studies are needed to define the precise mechanisms by which androgens impair insulin action on glucose metabolism.

This study has several limitations. First, it was cross-sectional and therefore any causal inference on the link between microcirculatory function and metabolic insulin resistance should be made with caution. Second, although muscle tissue is the main peripheral site of IMGU and vascular resistance, we studied skin and not muscle. However, the cutaneous microcirculation is considered a representative vascular bed to examine the mechanisms of generalized microvascular dysfunction (Holowatz et al., 2008; IJzerman et al., 2008). The human skin is the only site available in humans to directly and non-invasively assess capillary density and recruitment of capillaries. Moreover, the effects of obesity and FFAs on insulin-mediated microvascular recruitment in muscle (Clerk et al., 2006; Liu et al., 2009) can be reproduced in human skin (de Jongh et al., 2004a,b), suggesting that the vascular responses observed in skin reflect those in muscle. Third, the small sample size may have led to type 2 errors, especially because microvascular measurements have a relatively large variability (Ketel et al., 2008, 2009), so that small differences may have been missed. Fourth, because of the smaller range of M/I values in obese women when compared with lean women with or without PCOS, the lack of correlations with IICR and androstenedione in obese women as plotted in Fig. 3 might be partly due to insufficient statistical power.

To summarize, we demonstrate that PCOS per se is associated with impaired IICR. A profound reduction in insulin-mediated glucose could only be demonstrated in obese women with PCOS, whereas lean PCOS women, although displaying a large variation in IMGU, did not have obvious metabolic insulin resistance. Our data indicate that IICR is a determinant of glucose uptake in lean PCOS women, but not in obese PCOS women. The role for hyperandrogenism in impaired insulin-mediated glucose metabolism warrants further studies.

**Authors’ roles**

I.J.G.K., E.H.S., R.H., C.D.A.S. and C.B.L. contributed to the study design. I.J.G.K., T.I.M.K. and L.V. collected the data and I.J.G.K., J.W.T. and E.H.S. performed the analyses. I.J.G.K., E.H.S. and R.G.I. wrote the first draft of the manuscript. All authors participated in the reporting stage and have seen and approved the final version of the manuscript.

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