Adipocyte resistin mRNA levels are down-regulated by laparoscopic ovarian electrocautery in both obese and lean women with polycystic ovary syndrome

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BACKGROUND: The aim of this study was to investigate serum and adipocyte mRNA expression of resistin in lean and obese women with polycystic ovary syndrome (PCOS) before and 3 months after laparoscopic ovarian electrocautery (LOE). METHODS: Adipose tissue obtained from 12 women with PCOS (six obese and six lean, body mass index > 27 kg m\(^{-2}\) as threshold point) before and after LOE was analysed. Gene expression of resistin was measured by semi-quantitative RT–PCR. Ten lean, age-matched healthy women served as controls. RESULTS: Both lean and obese women with PCOS had significantly higher fasting and 2 h insulin and homeostasis model insulin resistance index (HOMAIR) values and lower fasting glucose-to-insulin ratios (\(G_0/I_0\)) than did the controls. The serum levels of glucose and insulin and HOMA IR were significantly decreased, and the \(G_0/I_0\) ratio was significantly increased 3 months after LOE. No difference was found in serum resistin levels between controls and either obese or lean women with PCOS before LOE, nor between PCOS patients before and after LOE. However, resistin mRNA expression levels in both lean and obese women with PCOS before LOE were significantly higher than that in controls and were decreased significantly after LOE back to control levels. CONCLUSION: Local resistin activity may be actively involved in the pathogenesis of PCOS. LOE reduces insulin resistance and down-regulates resistin mRNA expression in lean and obese women with PCOS.

Key words: adipocyte/insulin resistance/laparoscopic ovarian electrocauterization/polycystic ovary syndrome/resistin

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy characterized by oligo- or anovulation, hyperandrogenism and polycystic ovaries on ultrasonography (Rotterdam group, 2004). It affects 5–10% of women of reproductive age (Dunaif, 1997), and is frequently associated with insulin resistance accompanied by compensatory hyperinsulinaemia, offering an increased risk for the development of type 2 diabetes mellitus (Burghen et al., 1980). Obesity is present in ~16–80% of women with PCOS (Dunaif, 1992). However, several studies have shown that both obese and lean women with PCOS have significantly increased glucose and insulin levels during an oral glucose tolerance test (OGTT) compared with age- and weight-matched ovulatory hyperandrogenic and control women (Dunaif et al., 1991; Grulet et al., 1993).

Resistin, a novel 12.5 kDa cysteine-rich protein, also known as resistin-like molecule or ‘found in inflammatory zone’, is secreted by adipocytes (Steppan et al., 2001). Serum resistin levels are significantly increased in insulin-resistant mice and genetic or diet-induced obese mice (Steppan et al., 2001). In vivo administration of recombinant resistin to C57B1/6J mice causes insulin resistance, and intraperitoneal injection of diet-induced obese mice with antiresistin antibody significantly suppresses hyperglycaemia by improving insulin sensitivity (Steppan et al., 2001). In addition, resistin mRNA levels in obese mice are down-regulated by thiazolidinedione compounds, a new class of antidiabetic drugs with an insulin-sensitizing action (Steppan et al., 2001). These findings suggest that resistin serves as a hormonal link between obesity and insulin resistance. In Sprague–Dawley rats,
intra-arterial infusion of recombinant resistin for 5 h promotes
glucose intolerance by blocking the action of insulin in hepatic
glucose metabolism (Rajala et al., 2003). In human studies,
resistin gene expression can be detected in normal human adipocytes
(McTernan et al., 2002), and its levels are increased in
morbidly obese humans compared with lean control subjects
(Savage et al., 2001). Furthermore, in the adipocytes from
PCOS women, resistin mRNA levels are 2-fold higher than
that in controls (Seow et al., 2004). A genetic epidemiological
study of Caucasian families reported by Wang et al. (2002)
suggested that noncoding single nucleotide polymorphisms in
the resistin gene promoter region may alter the negative corre-
lation between the body mass index (BMI) and the insulin
sensitivity index in non-diabetic family members of type 2
diabetes. Thus, resistin may play an important role in modu-
lation of insulin sensitivity.

Laparoscopic ovarian electrocautery (LOE) is an alterna-
tive treatment for PCOS women who are clomiphene citrate-
resistant (Li et al., 1998). Ovarian wedge resection, the forerunner of LOE, was first introduced by Stein and Leventhal
(1935) for seven anovulatory women with PCOS, resulting in
resumption of menstruation and pregnancy. In addition, there
is a marked decrease in serum androgen levels, an increase in
FSH levels, a reduced amplitude of LH pulses, a decrease in
the LH/FSH ratio and a reduced ovarian volume following
LOE in women with PCOS (Campos et al., 1993; Amer et al.,
2002). Furthermore, several studies have reported that LOE
has a long-term effect (>6 years) in normalization of endocrine
abnormality in women with PCOS (Gjonnaess, 1998; Amer
et al., 2002). However, the mechanism of action of LOE is
unclear.

The purpose of this study was to evaluate the effect of LOE
on the expression of resistin in serum and adipocytes, using
semi-quantitative RT–PCR on samples obtained before and
after LOE in women with PCOS.

Materials and methods

Subjects

Twelve women (six obese and six lean: obesity defined as BMI
≥27 kg m⁻², lean as BMI < 27 kg m⁻²) who fulfilled the inclusion
criteria for PCOS discussed subsequently were enrolled in this
study. All were in good health and had not taken oral contraceptives
within the last 3 months. The protocol was reviewed and approved
by the Institutional Review Board of the Shin Kong Wu Ho-Su Mem-
orial Hospital. All patients entered this study only after informed
written consent was obtained.

PCOS was defined by clinical, laboratory and ultrasound criteria
according to the consensus criteria reported by the Rotterdam group
(2004). The clinical criterion included oligomenorrhea (menstrual
interval ≥6 weeks) or amenorrhea (no menstrual loss for ≥3
months) dating from menarche. None of the subjects had anovulation
nigricans. The biochemical criteria were an increased LH concen-
tration (≥6 mIU ml⁻¹), normal follicular range 1–6 mIU ml⁻¹),
a normal FSH concentration and elevated total serum testosterone
levels (≥0.8 ng ml⁻¹, normal range 0.06–0.80 ng ml⁻¹). The ultra-
sound criteria were enlarged ovaries with an increased stroma and
presence of 12 or more follicles in each ovary measuring 2–9 mm in
diameter and/or increased ovarian volume (>10 ml) (Balen et al.,
2003). Serum prolactin and thyroid hormone levels were checked in
all patients and were within the normal limits. Cushing’s syndrome
and androgenic tumours were excluded by appropriate testing. Conge-
nital adrenal hyperplasia was excluded by a morning serum
17-hydroxyprogesterone level of <2 ng ml⁻¹.

Ten healthy, lean, age-matched women served as controls. None
were hirsute, and all had normal regular cycling menstrual periods.
None were taking oral contraceptives. All had a normal appearance
of the ovaries on ultrasound and normal LH and FSH levels, and
none had elevated androgen levels.

Oral glucose tolerance test and homeostasis model insulin
resistance index

A 2 h OGTT with 75-g of glucose load was performed after an over-
night fast during the early follicular phase (days 3–7) on all women
before LOE, and 3 months after the LOE. Four blood samples were
collected at 0, 30, 60 and 120 min and the plasma stored at −20°C
until assayed for glucose and insulin. The fasting glucose to insulin
ratio (G0/I0) was measured as described previously (Legro et al.,
1999). The homeostasis model (HOMA) insulin resistance index
was calculated using the formula: fasting glucose (mg dl⁻¹) × fasting
insulin (μIU ml⁻¹)/405 (Matthews et al., 1985). A HOMAIR value of
3.8 or G0/I0 ratio ≤4.5 is suggestive of insulin resistance in PCOS
(Legro et al., 1999, Kaufman et al., 2002).

Hormonal profile

Blood was withdrawn for serum estradiol (E2), FSH, LH and testoster-
one measurements from the antecubital vein on the day of OGTT
before and after LOE. For women with amenorrhea, 75 mg of pro-
gerosterone was given i.m. to induce withdrawal bleeding, and then
the blood sample was collected on cycle day 3 or 4. Serum levels of
FSH, E2, testosterone and LH were measured by immunoassay
using Immulite® kits (Diagnostic Products Corporation, Los
Angeles, CA, USA). For FSH, the sensitivity was 0.1 mIU ml⁻¹ and
the intra- and inter-assay coefficients of variance (CV) 7.7 and
7.9%, respectively. For LH, the corresponding values were
0.1 mIU ml⁻¹, 6.5 and 7.1%, respectively; for E2, 15 pg ml⁻¹
(55 pmol l⁻¹), 6.3 and 6.4%, respectively; and for testosterone,
0.1 ng ml⁻¹, 4.0 and 5.6%, respectively.

Laparoscopic ovarian electrocautery

The procedure was performed in a lithotomy position using the
video-monitoring equipment. All the procedures were performed by
the first author (K.M.S) and an assistant doctor. Dextrose 5% water
500 cc was given intravenously before and during the operation. A
10-mm trocar was inserted in the umbilical position followed by one
5-mm trocar in the right- or left-sided lower quadrant lateral to the
inferior epigastric artery 6–8 cm oblique to the pubic rami. A pair
of grasping forceps was introduced through one of the 5-mm trocar
to grasp the utero-ovarian ligament and to lift the ovary away from
the bowel and ureter. A 2.5-mm hook monopolar diathermy needle
electrode was introduced through the third trocar. The cauterization
of the ovaries was performed using the Force 2 Valleylab® electrosur-
gical generator (Valleylab Inc., Boulder, CO, USA). A monopolar
couagulating current at 40 W power setting was used and a total of
10 punctures were made in each ovary to all the PCOS women.
The duration of each penetration was ~3 s. The bilateral ovaries were
cooled by irrigation with distilled water and check bleeding was
performed. All patients were discharged the same day without any
intraoperative complications.
Adipose tissue sampling
Adipose tissue weighing 5–6 g was obtained by laparoscopy on the day of LOE from the omental fat tissue of all obese and lean PCOS women before undergoing LOE. For control subjects, adipose tissue was obtained during laparoscopic examination for the reason of tubal infertility. Three months after the LOE, a second look operation by laparoscopy was performed only to all obese and lean women with PCOS. The peritoneal or periovarian adhesions were lysed and 5–6 g of adipose tissue was obtained and sent to the laboratory for adipocyte isolation.

Adipocyte cell isolation
The adipose tissue was transported to the laboratory in Krebs-Ringer bicarbonate (KRb) buffer (in mmol l⁻¹: NaCl 118, MgSO₄ 1.2, CaCl₂ 1.3, NaHCO₃ 2.5, pH adjusted to 7.4 at 37°C) within 30 min and adipocytes were isolated following the procedure described by Rodbell (1964) with minor modifications. The adipocyte tissue was finely minced, then disintegrated into cells by gentle shaking for 60 min at 37°C in KRb buffer containing 1 mmol l⁻¹ pyruvate, 1% bovine serum albumin (BSA) and 0.1% collagenase (10 ml g⁻¹ of tissue). The cell suspension was then filtered through a nylon mesh (400 μm l⁻¹) and centrifuged at 100 × g for 1 min at room temperature. The supernatant was harvested and washed three times with 50 ml of KRb buffer containing 1 mmol l⁻¹ pyruvate and 1% BSA. To count the cells and measure their size, an aliquot of diluted cell suspension was fixed overnight in collidine buffer containing 2% osmium tetroxide prior to microscopic photography. The mean cell volume and surface area were calculated from the known lipocrit of the cell suspension.

RNA extraction
Total RNA was extracted from the adipocytes isolated from each patient using a Tri Reagent kit (Molecular Research Center, Inc., USA). The integrity of the total RNA was examined by 1% agarose gel electrophoresis, and the RNA concentration was determined by UV absorbance at 260 nm (Genequant RNA/DNA calculator; Pharmacia, LKB Biochrom, UK).

Quantification of resistin mRNA
Levels of resistin and β-actin mRNA in adipocytes were assessed by RT–PCR. On the basis of the known cDNA sequences for resistin and β-actin, we designed the two pairs of primers listed in Table I. For RT, 5 μg of RNA template was incubated for 30 min at 37°C with 1 μl of RNase-free deoxyribonuclease (DNase) and 7 μl of H₂O, followed by incubation for 10 min at 70°C with 1 μl of 25 mmol l⁻¹ EDTA to inactivate the DNase, then 2 μl of 100 ng μl⁻¹ of oligo(dT) primer (Promega, USA) and 7.1 μl of H₂O were added and the sample was incubated for 10 min at 70°C. RT was then carried out for 50 min at 42°C in a total volume of 50 μl containing 1 × RT buffer, 10 mmol l⁻¹ diithiothreitol, 0.5 mmol l⁻¹ dNTP (HT Biotechnology Ltd), 16 IU of human placental ribonuclease inhibitor (HT Biotechnology Ltd) and 1 IU of Super RT reverse transcriptase (HT Biotechnology Ltd, UK). The RT mixtures were then heated for 3 min at 95°C to inactivate the reverse transcriptase. The resistin cDNA was amplified by touch-down PCR using a DNA Thermal Cycler (PTC-100; MJ Research, USA). For resistin expression, the PCR was performed in a total volume of 25 μl containing 2 μl of the cDNA template, 500 nmol l⁻¹ each of the sense and antisense primers, 0.625 IU of FailSafe Enzyme and a final concentration of 1 × FailSafe buffer D (both from Epicentre Technologies, USA). In a preliminary run, we found that a minimum of 30 PCR cycles was required to produce an optimal amount of nucleic acids for measuring on an agarose gel. As a reference for the quantitative gene expression, the housekeeping gene β-actin was simultaneously amplified under conditions identical to those for resistin PCR. For example, to determine the relative quantities of resistin mRNA, 5 μl each of resistin and β-actin PCR products amplified from the same RT template solution were combined and electrophoresed on a 2% agarose gel in 0.04 mol l⁻¹ Tris-acetate/0.001 mol l⁻¹ EDTA buffer for 40 min. After staining the gel with ethidium bromide (0.5 μg ml⁻¹) for 15 min, the resistin and β-actin cDNA bands were measured by densitometry, using Image Master VDS and Image Quant Analysis Software (Amersham Pharmacia Biotech, Hong Kong). The expression levels of resistin were normalized according to the β-actin levels.

Blood collection for resistin measurement
Blood samples (7 ml) were collected, before LOE and 3 months after the LOE, in Lavender Vacutainer tubes containing EDTA. The blood was then transferred to a centrifuge tube containing aprotinin (0.6 TIU ml⁻¹ of blood) and rocked several times to inhibit protease activity, then the sample was centrifuged at 1600 × g for 15 min at 4°C and the plasma stored at −70°C until assayed for resistin. The plasma sample was diluted 2-fold with enzymeimmunoassay (EIA) buffer to perform the EIA.

Resistin assay
Resistin was measured in a fasting blood sample, collected on the same day as the OGTT, using an EIA kit with a lower limit of sensitivity of 1.21 ng ml⁻¹ (range: 0–500 ng ml⁻¹) and intra- and inter-assay CV of <5 and 14%, respectively.

Ultrasound scanning
Patients underwent ultrasonographic scanning of bilateral ovaries prior to LOE and 3 months after surgery. Transvaginal sonography with Aloka SSD-5000 scanner (Aloka Ltd, Tokyo, Japan) equipped with a 5-MHz vaginal probe was used during this study period. The three diameters of the ovary were measured (longitudinal, antero-posterior and transverse). The ovarian volume was calculated using the formula of a prolate ellipsoid: 0.523 × length × width × thickness, according to the method of Sample et al. (1977). The mean volume of the right and left ovary was calculated for each subject.

Statistical analysis
Data are presented as mean ± SD. Statistical analysis was carried out using the student’s t-test or pair-t-test as appropriate. Computations were performed using the Statistical Package for Social Science (SPSS; Window version 12.0) software. In all cases, the threshold for significance was taken as P < 0.05.
Results

Clinical and endocrine metabolic characteristics

The clinical features and baseline hormonal and metabolic parameters for the control and lean women with PCOS before and 3 months after LOE are shown in Table II. As expected, lean women with PCOS had significantly higher LH, LH/FSH ratios and serum testosterone levels than did controls. Although fasting and 2 h glucose levels did not differ significantly, the fasting insulin and 2 h insulin post-75-g glucose load values were significantly higher in lean women with PCOS than in controls, consistent with the presence of compensatory hyperinsulinaemia. The HOMAIR was significantly higher in PCOS women than in controls (P = 0.006). Further, the G0/I0 ratio was significantly lower in women with PCOS before LOE compared with the controls (P = 0.006). Serum LH, LH/FSH ratios and testosterone levels were significantly lower in lean PCOS women 3 months after LOE. In addition, the fasting insulin, 2 h insulin, HOMAIR values and G0/I0 ratios were all significantly improved after LOE (P < 0.05) compared with before LOE.

The clinical features and baseline hormonal and metabolic parameters of obese women before and 3 months after LOE were also compared (Table II). Obese women with PCOS had significantly higher BMI and waist to hip ratio values than the controls. The fasting insulin and glucose and 2 h glucose levels did not differ significantly, the fasting and 2 h glucose levels did not differ significantly, the fasting insulin and glucose levels after a 75-g glucose load were significantly higher than controls. The fasting insulin and glucose and 2 h glucose levels did not differ significantly, the fasting and 2 h glucose levels did not differ significantly, the fasting insulin and glucose levels after a 75-g glucose load were significantly higher than controls. Although the fasting insulin and glucose results after a 75-g glucose load were significantly decreased compared with the levels before LOE. However, the insulin and glucose levels after LOE were still significantly higher than those of the controls. The obese women with PCOS had significantly increased levels of glucose and insulin (Figure 2) in a 75-g OGTT than did the controls. The insulin and glucose levels were significantly improved 3 months after LOE compared with before LOE. However, the insulin and glucose levels after LOE were still significantly higher than in the controls.

Serum resistin levels

No difference was found in serum resistin levels between either obese or lean women with PCOS before LOE and the controls, or between PCOS women before and after LOE (Table I).

Table II. Clinical and endocrine metabolic characteristics of lean or obese women with polycystic ovary syndrome (PCOS) and control subjects

<table>
<thead>
<tr>
<th>Metric</th>
<th>Controls (n = 10)</th>
<th>Lean PCOS (n = 6)</th>
<th>Obese PCOS (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>28.3 ± 3.3</td>
<td>32.2 ± 3.5 NS</td>
<td>32.3 ± 3.6 NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.7 ± 6</td>
<td>164 ± 7.5 NS</td>
<td>164 ± 7.3 NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.2 ± 14.9</td>
<td>57.1 ± 8.8 NS</td>
<td>56.6 ± 6.8 NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 14.9</td>
<td>21.1 ± 1.7 NS</td>
<td>21 ± 1.0 NS</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>92.5 ± 5.3</td>
<td>96.3 ± 4.1 NS</td>
<td>96.1 ± 3.9 NS</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>70.5 ± 7.2</td>
<td>74.7 ± 5.9 NS</td>
<td>75 ± 5.4 NS</td>
</tr>
<tr>
<td>WHR</td>
<td>0.76 ± 0.04</td>
<td>0.7 ± 0.03 NS</td>
<td>0.7 ± 0.02 NS</td>
</tr>
<tr>
<td>FSH (mIU ml⁻¹)</td>
<td>5.2 ± 1.7</td>
<td>5.0 ± 1.2 NS</td>
<td>6.7 ± 1.2 NS</td>
</tr>
<tr>
<td>LH (mIU ml⁻¹)</td>
<td>6.2 ± 3.8</td>
<td>15.6 ± 2.7 &lt;0.0001</td>
<td>6.7 ± 1.3 &lt;0.0001</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>1.2 ± 0.8</td>
<td>3.2 ± 0.7 &lt;0.0001</td>
<td>1.0 ± 0.3 &lt;0.0001</td>
</tr>
<tr>
<td>E2 (pg ml⁻¹)</td>
<td>46.3 ± 19.9</td>
<td>57.6 ± 28.3 NS</td>
<td>49 ± 25 NS</td>
</tr>
<tr>
<td>T (ng ml⁻¹)</td>
<td>0.38 ± 0.1</td>
<td>0.8 ± 0.3 0.002</td>
<td>0.4 ± 0.2 0.01</td>
</tr>
<tr>
<td>Fasting glucose (mg dl⁻¹)</td>
<td>86.1 ± 3.8</td>
<td>85 ± 5.4 NS</td>
<td>87.2 ± 3.9 NS</td>
</tr>
<tr>
<td>2 h glucose (mg dl⁻¹)</td>
<td>95 ± 24.9</td>
<td>102 ± 17.1 NS</td>
<td>93.5 ± 12.3 NS</td>
</tr>
<tr>
<td>Fasting insulin (mIU ml⁻¹)</td>
<td>4.8 ± 3.1</td>
<td>14.2 ± 1.2 0.002</td>
<td>8.1 ± 2.1 0.001</td>
</tr>
<tr>
<td>2 h insulin (mIU ml⁻¹)</td>
<td>14.4 ± 9.7</td>
<td>40.7 ± 9.0 0.005</td>
<td>23.2 ± 2.1 0.001</td>
</tr>
<tr>
<td>G0/I0 ratio</td>
<td>19.4 ± 9.6</td>
<td>5.9 ± 0.7 0.002</td>
<td>11.5 ± 3.7 0.005</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>1.03 ± 0.7</td>
<td>3.0 ± 0.3 0.006</td>
<td>1.8 ± 0.5 0.001</td>
</tr>
<tr>
<td>Resistin (ng ml⁻¹)</td>
<td>12.1 ± 0.2</td>
<td>12.2 ± 0.2 NS</td>
<td>11.9 ± 0.3 NS</td>
</tr>
<tr>
<td>Ovarian volume (ml)</td>
<td>7.8 ± 0.5</td>
<td>12.2 ± 0.2 0.001</td>
<td>8.8 ± 0.4 0.035</td>
</tr>
</tbody>
</table>

LOE, Laparoscopic ovarian electrocautery; BMI, body mass index; WHR, waist to hip ratio; FSH, follicle stimulating hormone; LH, luteinizing hormone; E2, estradiol; T, testosterone, HOMAIR, homeostasis model insulin index assessment; G0/I0 ratio, fasting glucose to fasting insulin ratio.
* Student’s t-test, lean-PCOS versus control.
** Paired t-test, lean-PCOS before LOE versus after LOE.
*** Student’s t-test, obese-PCOS versus control.
**** Paired t-test, obese-PCOS before LOE versus after LOE.
Mean ± SD.
No correlation was found between serum resistin and BMI, testosterone, fasting insulin levels or 2 h insulin levels in either obese and lean women with PCOS before or after LOE. In addition, serum resistin did not correlate with adipocyte resistin mRNA expression.

**Resistin mRNA expression**

In adipose tissue samples from both obese and lean women with PCOS, resistin mRNA expression before LOE was significantly higher than in samples from the controls and was decreased significantly after LOE back to control levels (Figure 3).

**Discussion**

LOE is an alternative treatment modality for PCOS women with anovulatory infertility after failure of clomiphene-citrate treatment (Li et al., 1998). The major advantages of LOE are the effect of producing high ovulation (61–100%) and pregnancy rates (37.5–87.5%) (Campo et al., 1993; Hironori et al., 2002). Furthermore, LOE also corrects the endocrine abnormalities associated with the syndrome, including a rapid and persistent fall of androgen levels and a reduction of LH/FSH ratios (Naether et al., 1994; Gjonnaess, 1998; Amer et al., 2002). In the present study, we further found that LOE had an excellent effect against insulin resistance, including improved glucose tolerance and reduced hyperinsulinaemia in obese and lean women with PCOS. This result differs from other studies (Tiitinen et al., 1993; Lemieux et al., 1999) in which LOE failed to correct hyperinsulinaemia, although it successfully restored ovulatory cycles (Lemieux et al., 1999), and decreased serum LH levels (Tiitinen et al., 1993). It is possible that the degree of hyperinsulinaemia in women with PCOS in the present study is worse than that in the studies of Tiitinen et al. (1993) and Lemieux et al. (1999). Four of the obese PCOS patients (66.7%) in this study had impaired glucose tolerance, and the HOMA IR of all the obese and lean women with PCOS was significantly higher than that of the controls. Saleh et al. (2001) reported that laparoscopic ovarian drilling was followed by a modest decrease in insulin and glucose levels in hyperinsulinaemic but not normoinsulinaemic women.

The exact mechanism of LOE in women of PCOS is still unknown. Two possible mechanisms of LOE in the improvement of ovulation and pregnancy in women of PCOS should be considered. The first is reduction of serum androgen levels after destruction of the androgen-producing stroma of the ovaries, and the second is improvement of hyperinsulinaemia. Evidence for these hypotheses comes from the observation that elevated circulating insulin levels may impede ovulation (Lobo et al., 1982; Nestler et al., 1998). Hyperandrogenaemia and insulin resistance have been implicated as independent features of PCOS (Franks, 1995). However hyperinsulinaemia enhances the risk of hyperandrogenaemia by increasing bioavailability of androgens. Increased insulin sensitivity or reduced androgen levels improves spontaneous ovulation and promotes fertility. For example, the administration of agents such as metformin or rosiglitazone which increases insulin sensitivity (Nestler et al., 1998), or Diane-35 which decreases LH and androgen

**Figure 1.** Basal glucose and insulin and responses after a 75-g oral glucose load in controls and lean women with Polycystic ovary syndrome (PCOS) before and after laparoscopic ovarian electracauterization (LOE). *P < 0.05, PCOS after LOE versus before LOE or control.

**Figure 2.** Basal glucose and insulin and responses after a 75-g oral glucose load in controls and obese women with PCOS before and after LOE. *P < 0.05, PCOS after LOE versus before LOE or control. Note the different scale to Figure 1.
levels (Hwang et al., 2004), induces ovulation and increasing the pregnancy rate. LOE could improve both hyperandrogenaemia and hyperinsulinaemia in obese and lean women with PCOS, thus stimulating ovulation and promoting fertility.

The role of resistin in insulin resistance in women with PCOS is still controversial. Our study (Seow et al., 2004) and another study (Lewandowski et al., 2005) have reported that serum resistin levels are no different between PCOS patients and normal controls. However, resistin mRNA is overexpressed in adipocytes from women with PCOS compared with controls (Seow et al., 2004). The resistin gene in adipocytes may be a local determining factor in the pathogenesis of PCOS (Seow et al., 2004). Interestingly, in the present study we found that adipocyte resistin mRNA expression was down-regulated to control levels in both obese and lean women with PCOS after LOE. Simultaneously, the serum glucose and insulin levels were significantly improved after LOE. However, serum resistin levels were not elevated in either obese or lean women with PCOS before or after LOE when compared to the controls. In addition, no correlation was observed between serum resistin levels and adipocyte mRNA expression of resistin before or after LOE. Moreover, resistin levels were not correlated with testosterone, or fasting or 2 h insulin levels before or after LOE. These findings imply that in women with PCOS, resistin may have a local paracrine, rather than a systemic, mode of action. It is well known that circulating levels do not always reflect the state of tissue hormonal circuits which, nonetheless, can be of substantial physiological and clinical significance such as that seen with angiotensin II (Frohlich, 2003). Heilbronn et al. (2004) reported that serum resistin levels were not different among nonobese, obese and obese diabetic subjects and were not significantly correlated to glucose disposal rates during a hyperinsulinaemic glucose clamp. Furthermore, Wu et al. (2004) reported that serum leptin levels did not significantly change after LOE in women with PCOS. We found that serum glucose and insulin levels did not return completely to normal and were still significantly higher than in the controls after LOE. This may be due to the local paracrine effect of resistin in adipose tissue and the short-term (3 months) nature of the study. We believe that serum insulin levels may continue to decrease to normal levels in a long-term follow-up. Thus, a further study should be performed due to the small sample size and short time period in the present study.

Testosterone has been found to be a possible regulator of resistin in the adipose tissue (Ling et al., 2001). Ling et al. (2001) showed that resistin mRNA is increased 2.6-fold in the adipose tissue of control male mice with elevated serum testosterone levels. Hyperandrogenism is a typical feature of PCOS, but the high level of serum androgen was rapidly reduced after LOE, possibly resulting in a profound down-regulation of resistin mRNA expression.

Resistin is a peptide hormone which is secreted mostly by adipocytes and which regulates insulin sensitivity. Several studies have reported that resistin mRNA expression is very low in isolated human adipocytes and does not correlate consistently with insulin resistance or obesity (Nagaev and Smith, 2001, Sentinelli et al., 2002). However, McTernan et al. (2002) also reported that resistin mRNA is more highly expressed in omental and abdominal s.c. white fat than in adipose tissue from the thigh or breast. We thus sampled adipose tissue from omentum of PCOS patients, and obtained isolated human adipocytes to exclude other sources of resistin, in an effort to increase the reliability of the study. Resistin mRNA is also found in human monocyte, and differentiation of monocytes into macrophage in vitro increases resistin mRNA expression (Savage et al., 2001; Nagaev and Smith, 2001).

We found that the ovarian volume was significantly reduced after LOE in both obese and lean women with PCOS. Tulandi et al. (1997) reported that 10–15 drillings in an ovary would
result in a total tissue destruction of 4–6 ml. The mechanism of destruction of the ovarian volume by LOE is still unclear. However, LOE might reduce the androgen levels in women with PCOS by destruction of the androgen-producing ovarian stroma or by drainage of the follicular fluid (Gjonnaess, 1998; Amer et al., 2002).

In conclusion, we suggest that LOE may reduce insulin resistance in both obese and lean women with PCOS by down-regulating local resistin expression in adipocytes. On the contrary, insulin sensitivity and other metabolic abnormalities of PCOS do not appear to play any pivotal role in the regulation of serum resistin levels in women with PCOS. Resistin seems to be actively involved in the pathogenesis of PCOS by a local paracrine action.

References
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