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Insulin sensitivity, insulin secretion, and metabolic and hormonal parameters in healthy women and women with polycystic ovarian syndrome

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To study the contributions of body mass, body fat distribution and family history of type 2 diabetes mellitus to hyperinsulinaemia, insulin secretion and resistance in polycystic ovarian syndrome (PCOS), 17 lean (LC) and 17 obese (OC) healthy control subjects, and 15 lean (LPCOS) and 28 obese (OPCOS) women with PCOS were investigated. Waist:hip ratio (WHR), serum concentrations of sex steroids, glucose and insulin during a 75 g oral glucose tolerance test (OGTT), and insulin and C-peptide early phase secretion, and insulin sensitivity index using a euglycaemic hyperinsulinaemic clamp were assessed. The PCOS subjects had a higher mean WHR than the controls. A trend towards hyperinsulinaemia and impairment of insulin sensitivity (including the rates of both glucose oxidation and non-oxidation) was observed in LPCOS subjects, but only in OPCOS subjects were these changes significant. Early phase insulin secretion but not the early phase C-peptide secretion was increased in PCOS subjects compared to controls, suggesting that peripheral hyperinsulinaemia in PCOS women was mainly due to the observed lowered hepatic insulin extraction and insulin resistance in skeletal muscle. Moreover, the presence of a family history of type 2 diabetes did not affect early phase insulin or C-peptide secretion in the PCOS group. These results confirm and strengthen earlier contentions, that insulin resistance is a characteristic defect in PCOS and is worsened particularly by abdominal obesity.

Key words: early phase secretion of insulin/insulin sensitivity/polycystic ovarian syndrome

Introduction

Hyperinsulinaemia and insulin resistance are a well-known feature in polycystic ovarian syndrome (PCOS). Whether hyperinsulinaemia in PCOS is primarily due to a defect in insulin action (Dunaif *et al*., 1989), to increased insulin secretion (Holte *et al*., 1994a), to decreased hepatic clearance of insulin (Ciampelli *et al*., 1997), or to an interaction between all these disorders is, however, not clear. Furthermore, the contribution of body mass and body fat distribution in insulin resistance has been a major question for a long time (Dunaif *et al*., 1992; Holte *et al*., 1995), and results regarding lean polycystic ovarian syndrome (LPCOS) subjects in particular are controversial (Chang *et al*., 1983; Dunaif *et al*., 1989; Ovesen *et al*., 1993; Holte *et al*., 1994a; Morales *et al*., 1996).

Up to 20% of women with PCOS screened by means of an oral glucose tolerance test (OGTT) show impaired glucose tolerance (Dunaif *et al*., 1987; Ehrmann *et al*., 1999) suggesting that this subgroup of patients is at risk of developing type 2 diabetes mellitus. The results of previous studies have suggested that the impairment of insulin action in PCOS could differ from that seen in type 2 diabetes mellitus or in obese women without typical features of PCOS. In at least some PCOS women, an intrinsic biochemical defect in insulin receptor transduction could be the cause of insulin resistance (Ciaraldi *et al*., 1992; Dunaif *et al*., 1995). In addition, women with PCOS present a more atherogenic lipid profile with elevations of serum triglycerides (TG) and reductions of serum high density lipoprotein (HDL) cholesterol concentrations and are at a greater risk of cardiovascular diseases compared with age-matched control subjects (Dahlgren *et al*., 1992). These clustering risk factors associated with PCOS emphasize the importance of studies to distinguish between metabolic and hormonal alterations which are related to PCOS or to obesity *per se*.

The present study was designed to assess the contributions of body weight and PCOS to glucose tolerance, insulin resistance, basal and insulin-stimulated glucose metabolism, e.g. the rates of glucose oxidation and non-oxidation, and insulin secretion in lean and obese women with PCOS compared with healthy women. Special attention was also paid to family history of type 2 diabetes mellitus, and to ovarian and adrenal steroid secretion.

Materials and methods

Subjects

Twenty-eight obese PCOS [OPCOS, body mass index (BMI) >27 kg/m^2, mean age 30.1 ± 0.9 (SE) years, range 21–39] and 15 lean PCOS (LPCOS, BMI ≤27 kg/m^2, mean age 28.9 ± 1.2 years, range 18–36) subjects were referred to the Reproductive Endocrinology Unit, University Hospital of Oulu, and participated in the study. Obesity was defined by a threshold value of 27 kg/m^2, above which insulin sensitivity decreases significantly (Campbell and Gerich, 1990). Control women were contacted through an advertisement in a local newspaper and recruited after a phone conversation. The control subjects were 17 healthy lean (LC, BMI

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⩽27 kg/m^2, mean age 37.1 ± 0.8 years, range 31–40) and 17 obese (OC, BMI >27 kg/m^2, mean age 35.1 ± 1.2 years, range 21–40) women with normal menstrual cyclicity (27–34 days) and normal ovaries as observed in transvaginal ultrasonography. They did not use any medication including oral contraceptive pills. Some of the subjects had participated in other studies on glucose metabolism, and their data were analysed retrospectively.

Criteria for PCOS were as defined by Homburg (Homburg, 1996). All patients had polycystic ovaries shown by transvaginal ultrasonography (eight or more subcapsular follicles of 3–8 mm diameter in one plane in one ovary and increased stroma) and at least one of the following symptoms: oligomenorrhoea or amenorrhoea, clinical manifestations of hyperandrogenism such as hirsutism scored according to Ferriman and Gallwey (Ferriman and Gallwey, 1961), acne and/or elevated serum testosterone concentrations (>2.7 nmol/l). Diabetics, smokers, alcohol users and those using sex hormones or other medication known to affect lipoprotein metabolism during the 2 months preceding the study were excluded. Late onset adrenal hyperplasia in PCOS subjects was excluded on the basis of a normal serum 17-hydroxyprogesterone concentration (17-OHP <9 nmol/l).

Study protocol

Clinical parameters and ultrasonography

All subjects were evaluated between cycle days 1 and 7 after spontaneous or progestin-induced menstruation (amenorrhoeic women). The aim of using progestin in these subjects was to avoid examinations (ultrasonography and hormone assays) during a spontaneous luteal phase. Dydrogesterone was used (10 mg/day for 10 days) which has only negligible effect on insulin sensitivity (Crook *et al*., 1997). Furthermore, to assure minimal progestin effect the examinations were performed at least 7 days after the last progestin pill. Blood pressure was measured after a 20 min rest in a sitting position. Diastolic blood pressure was measured as Korotkoff phase V. Waist and hip circumferences were measured to the nearest centimetre with a soft tape at the narrowest part of the torso and at the widest part of the gluteal region.

Transvaginal ultrasonography (General Electric RT-X200, Milwaukee, WI, USA with a 6.5 MHz probe) was carried out to measure ovarian volumes and the number of follicles. Volume determinations were carried out using the formula for the volume of an ellipsoid: 0.523×length×width×thickness (Robert *et al*., 1995).

A family history of type 2 diabetes mellitus was recorded if subjects reported type 2 diabetes mellitus in first degree relatives.

OGTT

After an overnight fast of 10–12 h, all subjects underwent an OGTT (a load of 75 g glucose in 300 dl water) in order to evaluate the degree of glucose tolerance and the β cell response to the OGTT. Venous blood samples for blood glucose, serum insulin and serum C-peptide assays were drawn at 0, 15, 30, 60 and 120 min. Glucose tolerance was defined according to the new American Diabetes Association (ADA) criteria of 1997: diabetes mellitus (DM): 0 min ⩾6.1 and/or at 120 min ⩾10 mmol/l; impaired glucose tolerance (IGT): 0 min <6.1 and at 120 min 6.7–10.0 mmol/l; impaired fasting glycaemia (IFG): 0 min ⩾5.6 and <6.1, at 120 min <6.7 mmol/l; normal glucose tolerance: 0 min <5.6 and at 120 min <6.7 mmol/l (Anonymous, 1997).

Early phase insulin secretion (insulinogenic index) was calculated as a ratio of the increment of serum insulin 30 min after the oral glucose load to blood glucose concentration 30 min after the glucose load [(30 min insulin-fasting insulin)/30 min glucose] (Wareham *et al*., 1995). Insulinogenic index has previously been shown to correlate strongly with first phase insulin response following i.v. GTT (r = 0.88) (Kosaka *et al*., 1996). Early phase C-peptide secretion was calculated consequently [(30 min C-peptide – fasting C-peptide)/30 min glucose]. The incremental insulin (AUC_{ins}) and glucose (AUG_{gluc}) areas under the curve were calculated by the trapezoidal method. Fasting serum C-peptide/fasting serum insulin ratio was calculated as an index of the hepatic insulin extraction in the fasting state (Shuster *et al*., 1988).

Euglycaemic hyperinsulinaemic clamp

The euglycaemic hyperinsulinaemic clamp technique was used for assessment of insulin sensitivity (DeFronzo *et al*., 1979). A priming dose of insulin infusion (Actrapid 100 IU/ml; Novo Nordisk, Gentoffe, Denmark) was administered during the initial 10 min to raise serum insulin acutely to the desired level, where it was maintained by continuous insulin infusion of 80 mIU/m^2 body surface area per min. Blood glucose was clamped at 5 mmol/l for the next 180 min by adjusting the rate of 20% glucose infusion according to blood glucose measurements performed every 5 min using a photometric assay (HemoCue AB, Ängelholm, Sweden). The mean coefficients of variation of blood glucose during the last 60 min of the clamp were <4% in all four groups of the study. The M-value (amount of glucose infused i.e. whole body glucose disposal, μmol/kg/min) was calculated as the mean value for each 20 min interval during the last 60 min of the clamp. The insulin sensitivity index (M/I) was calculated by dividing the M-value by the mean steady state insulin concentration during the last 60 min of the clamp [glucose μmol/l×100/kg of body weight (BW)/min/insulin (mIU/l)]. Blood samples for assay of serum insulin and free fatty acids (FFA) were drawn at 0, 120, 140, 160 and 180 min.

Calorimetry

Indirect calorimetry was performed with a computerized flow-through canopy gas analyser system (Deltatrac^R, TM Datex, Helsinki, Finland) in connection with the euglycaemic clamp, as previously described (Laakso *et al*., 1988). This device has a precision of 2.5% for O_2 consumption and 1.0% for CO_2 production. On the day of the experiment, gas exchange (O_2 consumption and CO_2 production) was measured after a 12 h fast before and during the last 30 min of the clamp. The values obtained during the first 10 min of both time periods were discarded, and the mean value for the remaining 20 min of data was used for calculation. Protein, glucose and lipid oxidation were calculated according to Ferrannini (Ferrannini, 1988). Protein oxidation was calculated on the basis of the urinary non-protein nitrogen excretion rate (Ferrannini, 1988). The fraction of carbohydrate non-oxidation during the euglycaemic clamp was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the glucose infusion rate (determined by the euglycaemic clamp) and both values were adjusted for the prevailing insulin concentrations during the clamp by dividing the values by the mean steady state insulin concentration during the last 60 min of the clamp (glucose oxidation index and non-oxidation index respectively).

Assays

The concentrations of sex hormone-binding globulin (SHBG), LH and FSH were analysed by fluoroimmunoassays (Wallac Ltd, Turku, Finland), and radioimmunoassays were used for dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S), androstenedione, 17-hydroxyprogesterone (17-OHP), C-peptide (Diagnostic Products Corporation, Los Angeles, CA, USA), cortisol (Orion Diagnostica, Oulunsalo, Finland), leptin (Linco Research Inc., St Charles, MO, USA) and insulin (Pharmacia Diagnostics, Uppsala, Sweden), following the instructions of the manufacturers. Concentrations of human serum insulin-like growth factor-binding protein-1 (IGFBP-1) were determined by immunoenzymometric assay using commercial reagents (Medix Biochemica, Kauniainen, Finland) and

Table I. Clinical and biochemical parameters in the lean and obese control and polycystic ovarian syndrome (PCOS) subjects

	Lean subjects			Obese subjects			Reference range
	Controls (n = 17)	PCOS (n = 15)	Significance	Controls (n = 17)	PCOS (n = 28)	Significance	
BMI (kg/m^2)	22.9 ± 0.3	22.7 ± 0.5	NS[b]	31.8 ± 1.15	34.5 ± 1.0	NS[c]	
Waist (cm)	75.3 ± 0.9	77.7 ± 1.8	NS[b]	95.8 ± 2.8	105.2 ± 2.2	0.01[a,b]	
Hip (cm)	96.9 ± 1.1	96.0 ± 1.5	NS[b]	108.8 ± 4.8	122.0 ± 2.5	0.03[a,c]	
WHR	0.78 ± 0.01	0.81 ± 0.01	0.002[b]	0.83 ± 0.01	0.86 ± 0.01	0.04[a,b]	
Hirsutism score	0.5 ± 0.3	6.4 ± 0.7	0.03[c]	2.6 ± 0.4	8.4 ± 1.0	<0.001[c]	<7
Mean ovarian volume (cm^3)	6.0 ± 1.2	7.6 ± 0.9	0.004[a,b]	3.9 ± 0.6	7.13 ± 0.4	<0.001[b]	
Fasting glucose (mmol/l)	4.8 ± 0.1	4.7 ± 0.1	NS[b]	4.99 ± 0.08	5.06 ± 0.06	NS [b]	3.3–5.6
Fasting insulin (mIU/l)	7.3 ± 0.4	8.7 ± 0.8	NS[a,b]	10.2 ± 1.2	16.3 ± 1.5	NS[a,b]	5–20
Fasting C-peptide (nmol/l)	0.3 ± 0.05	0.3 ± 0.05	NS[a,c]	0.47 ± 0.06	0.60 ± 0.06	NS[a,c]	0.2–1.0
Hepatic extraction (nmol×10^{-2}/mIU)	4.6 ± 0.6	3.9 ± 0.5	NS[b]	4.9 ± 0.4	4.1 ± 0.3	NS (0.1)[c]	

Data are shown as means ± SE.
[a]After correction for the impact of age.
[b]*t*-Test and [c]Mann–Whitney test.
BMI = body mass index; WHR = waist:hip ratio; NS = not significant.

testosterone by automated chemiluminescence system (Ciba-Corning ACS-180, Medfield, MA, USA). The free androgen index (FAI) was calculated according to the equation: (T×100)/SHBG. Blood glucose was determined by a glucose dehydrogenase method (Granutest 250; Diagnostica Merck, Darmstadt, Germany) and serum FFA concentrations were measured by an enzymatic colorimetric method (Wako NEFA C test kit; Wako Chemicals GmbH, Neuss, Germany). The intra- and inter-assay coefficients of variation were 1.3 and 5.1% for SHBG respectively, 4.9 and 6.5% for LH, 3.8 and 4.3% for FSH, 6.5 and 7.9% for DHEA, 5.3 and 7.0% for DHEA-S, 5.0 and 8.6% for androstenedione, 5.0 and 5.4% for 17-OHP, 4.0 and 5.6% for testosterone, 4.0 and 4.3% for cortisol, 5.3 and 7.2% for C-peptide, 5.3 and 7.6% for insulin, 3.4 and 7.4% for IGFBP-1, 5.0 and 6.0% for leptin, 1.5 and 2.3% for blood glucose and 3.8 and 5.5% for FFA.

Statistical analysis

Student's two-tailed *t*-test was used for comparison of normally distributed variables, with or without log transformation. The Mann–Whitney *U*-test was used for variables with a persisting skewed distribution after log transformation. A linear regression method was used to identify the influence of age on variables in the control and PCOS groups. If the level of significance was <0.05, covariance analysis was carried out to evaluate the impact of this variable on the results.

Results

Clinical parameters

Lean subjects

The clinical characteristics of LC and LPCOS subjects are shown in Table I. The WHR (P = 0.002), hirsutism score (P = 0.03) and mean ovarian volume (P = 0.004) were significantly greater in the LPCOS than in the LC subjects (Table I).

Obese subjects

The waist (P = 0.01), hip (P = 0.03), WHR (P = 0.04), hirsutism score ($P < 0.001$), and mean ovarian volume ($P <$ 0.001) were significantly higher in the OPCOS than in the OC subjects (Table I).

Glucose tolerance, insulin secretion, insulin sensitivity, serum free fatty acids and lipid oxidation

Lean subjects

In the LPCOS group, two patients had IGT (2/15, 13.3%), while in the LC group one subject had IFG (1/17, 5.9%) and three had IGT (3/17, 17.6%). None of these results was significantly different between the two groups. There were no significant differences between LPCOS and LC subjects concerning blood glucose, serum insulin and C-peptide concentrations (Table I), AUC_{gluc} (LPCOS: 11.6 ± 0.7 versus LC: 11.6 ± 0.4 mmol/l/h). AUC_{ins} during OGTT tended to be slightly higher in LPCOS than in LC subjects (89.5 ± 20.3 versus LC: 66.2 ± 6.5 mIU/l/h, not significant, Figure 1).

There was no difference in early phase insulin secretion and early phase C-peptide secretion during OGTT (Figure 2), and in hepatic extraction of insulin between the two groups (Table I).

During the euglycaemic clamp, the M/I tended to be lower in LPCOS subjects compared with the LC subjects (LPCOS: 41.1 ± 3.7 versus LC: 48.2 ± 2.4 µmol/kg/min/mIU/l, not significant, Figure 3), including both glucose oxidation (LPCOS: 12.0 ± 1.2 versus LC: 14.4 ± 1.2 µmol/kg/min/mIU/l, not significant) and glucose non-oxidation indexes (LPCOS: 29.1 ± 2.7 versus LC: 33.9 ± 1.9 µmol/kg/min/mIU/l, not significant, Figure 3).

No significant difference was observed between the two groups as regards fasting and insulin-mediated serum FFA concentrations and rates of lipid oxidation (Table II).

In the LPCOS subjects, a significant negative correlation was observed between WHR and M/I value (r = –0.64, P = 0.01), and a significant positive correlation was observed between WHR and serum 2 h insulin concentrations (r = 0.57, P = 0.03), and WHR and AUC_{ins} (r = 0.53, P = 0.04).

Obese subjects

One OPCOS woman had IFG (1/28, 3.6%) and nine had IGT (10/28, 36%) while two OC subjects had IGT (2/17, 11.7%). The frequency of IGT was significantly higher in the OPCOS

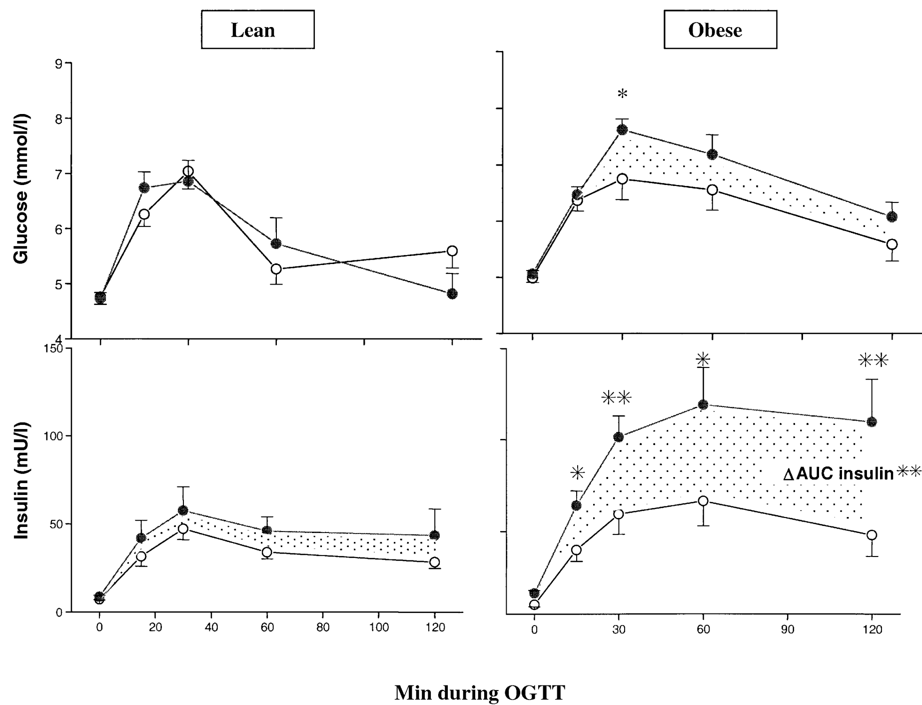

Figure 1. Glucose and insulin concentrations (mean ± SE) during oral glucose tolerance test (OGTT) in the subjects of the study (controls, ○; polycystic ovarian syndrome, ●). *$P < 0.05$, **$P < 0.01$ compared with obese control.

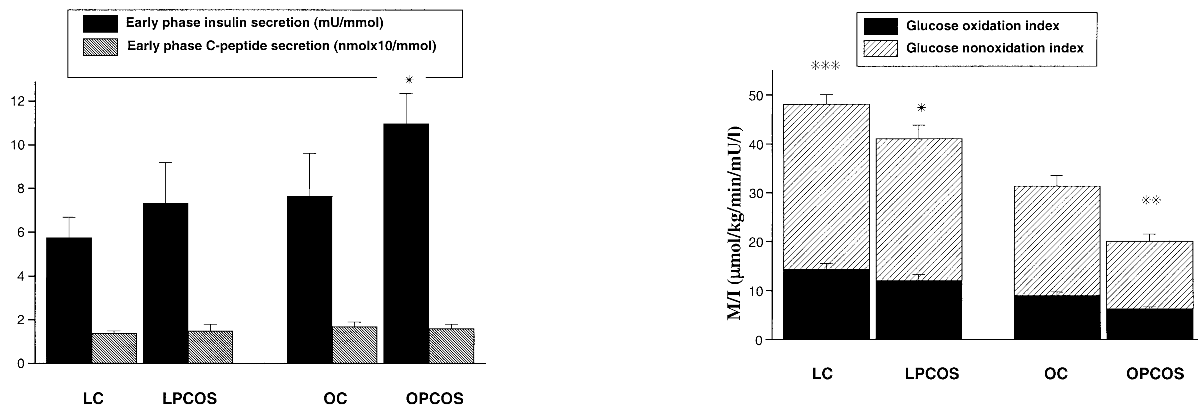

Figure 2. Early phase secretions of insulin and C-peptide in lean and obese subjects. LC = lean control; LPCOS = lean polycystic ovarian syndrome; OC = obese control; OPCOS = obese PCOS. *$P = 0.015$ compared with OC.

Figure 3. Mean (± SE) insulin sensitivity index (M/I), glucose oxidation (black section) and non-oxidation (hatched section) indices expressed as µmol/kg/min/mIU/l during the euglycaemic hyperinsulinaemic clamp. *Not significant (M/I) compared with LC (glucose oxidation index: not significant; glucose non-oxidation index: not significant) and $P < 0.001$ (M/I) compared to OPCOS (both glucose oxidation and non-oxidation indices: $P < 0.001$). **$P = 0.04$ (M/I) compared with OC (glucose oxidation index: $P = 0.002$; glucose non-oxidation index: $P = 0.05$). ***$P < 0.001$ (M/I) compared with OC (both glucose oxidation and non-oxidation indices: $P < 0.001$). For calculation of M/I see Materials and methods.

compared to the OC group ($P < 0.001$, χ^2-test). Except for a higher glucose concentration at 30 min in the OPCOS group ($P = 0.02$), no significant differences were observed between the OPCOS and OC groups concerning the blood glucose concentrations during the OGTT (Table I). The AUC_{gluc} tended to be higher in OPCOS (OPCOS: 13.5 ± 0.4 versus OC: 12.4 ± 0.5 mmol/l/h, not significant, Figure 1). All age corrected serum insulin concentrations (Table I, Figure1) and AUC_{ins} (OPCOS: 200.2 ± 31.1 versus OC: 107.4 ± 20.8 mIU/l/h, $P = 0.003$, Figure 1) during the OGTT were significantly higher (except serum fasting insulin) in OPCOS than in OC subjects. Fasting serum concentrations of C-peptide tended to be higher (not significant) in the OPCOS group, but the difference disappeared after correction for age (Table I).

Early phase insulin secretion was significantly greater in OPCOS than in OC subjects ($P = 0.015$, Figure 2). However, early phase secretion of C-peptide did not differ between OPCOS and OC or between all PCOS and controls (Figure 2). The hepatic extraction of insulin was slightly but not significantly decreased in the OPCOS group (Table I).

The M/I during euglycaemic hyperinsulinaemic clamp was significantly lower in the OPCOS group than in the OC group (OPCOS: 20.5 ± 1.5 versus OC: 31.6 ± 2.7 µmol/kg/min/mIU/l, $P = 0.04$, Figure 3), with significant decreases of both

Table II. Serum free fatty acid (FFA) concentrations and lipid oxidation in the fasting state and during the euglycaemic clamp in the lean and obese control and polycystic ovarian syndrome (PCOS) subjects

	Lean subjects			Obese subjects		
	Controls (n = 17)	PCOS (n = 15)	Significance	Controls (n = 17)	PCOS (n = 28)	Significance
Fasting FFA (mmol/l)	0.46 ± 0.22	0.48 ± 0.21	NS[b]	0.55 ± 0.23	0.61 ± 0.19	NS[a,c]
Fasting lipid oxidation (mg/kg/min)	1.03 ± 0.35	0.89 ± 0.32	NS[b]	0.91 ± 0.16	0.86 ± 0.25	NS[b]
Clamp FFA (mmol/l)	0.00235 ± 0.006	0.007 ± 0.018	NS[c]	0.0123 ± 0.024	0.019 ± 0.015	NS (0.2)[a,c]
Clamp lipidoxidation (mg/kg/min)	0.41 ± 0.31	0.44 ± 0.21	NS[b]	0.41 ± 0.19	0.55 ± 0.20	0.03[b]

Data are shown as means ± SE.
[a]After correction for the impact of age.
[b]*t*-Test and [c]Mann–Whitney test.

Table III. Endocrine parameters in the lean and obese control and polycystic ovarian syndrome (PCOS) subjects

	Lean subjects			Obese subjects			Reference range
	Controls (n = 17)	PCOS (n = 15)	Significance (n = 17)	Controls (n = 28)	PCOS	Significance	
LH (IU/l)	4.7 ± 0.4	7.5 ± 0.7	0.002[a,b]	5.3 ± 0.8	7.02 ± 0.5	0.003[b]	2–10
FSH (IU/l)	6.2 ± 0.4	5.1 ± 0.5	0.03[c]	7.9 ± 1.4	6.4 ± 0.5	NS[c]	2–12
Testosterone (nmol/l)	1.3 ± 0.2	2.0 ± 0.2	0.01[b]	1.3 ± 0.1	2.3 ± 0.2	<0.001[b]	0.4–2.7
SHBG (nmol/l)	60.5 ± 5.7	43.0 ± 4.3	0.02[b]	51.0 ± 6.7	30.8 ± 2.4	0.005[b]	20–140
FAI	2.6 ± 0.5	5.3 ± 0.6	0.001[b]	3.5 ± 0.7	8.9 ± 1.0	<0.001[b]	1.4–7.3
IGFBP-1 (μg/l)	5.6 ± 0.9	4.8 ± 0.5	NS[a,b]	3.8 ± 0.4	2.5 ± 0.3	NS[a,b]	0.8–13
Leptin (ng/l)	13.4 ± 1.5	12.4 ± 2.2	NS[b]	28.4 ± 3.7	34.5 ± 2.1	NS[b]	
Cortisol (μmol/l)	0.41 ± 0.03	0.45 ± 0.03	NS[a,b]	0.4 ± 0.03	0.4 ± 0.02	NS[a,b]	0.15–0.65
DHEA (nmol/l)	19.1 ± 2.7	33.2 ± 4.1	0.007[a,b]	17.9 ± 2.1	32.3 ± 3.4	0.001[a,b]	1.7–36
DHEA-S (μmol/l)	5.2 ± 0.6	5.8 ± 0.7	NS[a,b]	4.4 ± 0.4	7.0 ± 0.8	0.01[a,b]	1.0–14
Androstenedione (nmol/l)	7.2 ± 0.8	12.8 ± 1.1	0.04[a,b]	6.9 ± 0.6	13.9 ± 0.9	<0.001[a,b]	0.7–16

Data are shown as means ± SE.
[a]After correction for the impact of age.
[b]*t*-Test and [c]Mann–Whitney test.
SHBG = sex hormone binding globulin; FAI = free androgen index; IGFBP-1 = insulin-like growth factor binding protein-1; DHEA = dehydroepiandrosterone; DHEA-S = dehydroepiandrosterone sulphate.

glucose oxidation (6.3 ± 0.4 versus 9.0 ± 0.8 μmol/kg/min/mIU/l, P = 0.002) and glucose non-oxidation indexes (13.9 ± 1.4 versus 22.5 ± 2.1 μmol/kg/min/mIU/l, P = 0.05, Figure 3).

The insulin-stimulated rates of lipid oxidation were significantly increased in OPCOS compared to OC subjects (P = 0.02). Serum fasting FFA concentrations and insulin-stimulated serum FFA concentrations were slightly but not significantly increased in OPCOS (Table II).

Endocrine parameters

Lean subjects

The serum concentrations of LH (P = 0.002) were significantly higher and those of FSH significantly lower (P = 0.03) in LPCOS than in LC subjects (Table III). The significant difference in FSH was thought to be due to chance and of no clinical significance.

Serum SHBG concentrations were significantly lower (P = 0.02) and the serum testosterone concentrations (P = 0.01) and the FAI (P = 0.001) significantly higher in the LPCOS group. Significantly higher serum concentrations of DHEA (P = 0.007) and androstenedione (P = 0.04) were observed in the LPCOS subjects, but serum DHEA-S and cortisol concentrations did not differ between these two groups (Table III).

Obese subjects

The serum concentrations of LH (P = 0.003) and testosterone (P < 0.001), and the FAI (P < 0.001), were significantly higher and the concentrations of SHBG lower (P = 0.005) in OPCOS than in OC subjects. OPCOS subjects had significantly higher serum concentrations of DHEA (P = 0.001), DHEA-S (P = 0.01) and androstenedione (P < 0.001, Table III).

A significant negative correlation was observed between serum testosterone concentrations and M-value (P = 0.03, r = –0.41) and a significant positive correlation between serum

testosterone and respectively serum fasting insulin ($P = 0.02$, $r = 0.44$), serum 2 h insulin ($P = 0.01$, $r = 0.47$), and AUC_{ins} ($P = 0.02$, $r = 0.45$).

Effect of BMI on metabolic parameters

LC versus OC subjects

In the OGTT, the 1 h glucose concentrations ($P = 0.009$) and the 1 h insulin concentrations ($P = 0.03$) were significantly higher in the OC than in the LC group, but serum fasting C-peptide and all other blood glucose and insulin concentrations during the OGTT did not differ. The M/I was significantly lower in OC than in LC subjects ($P < 0.001$, Figure 3, Table I), and this was the case for both glucose oxidation and non-oxidation indexes (Figure 3). No differences were observed in early phase secretion of insulin, early phase secretion of C-peptide (Figure 2) or hepatic insulin clearance (Table I) between these two groups.

Serum concentrations of leptin were significantly higher in OC compared with LC subjects ($P < 0.001$, Table III).

LPCOS versus OPCOS subjects

All the blood glucose and serum insulin concentrations, AUC_{gluc} ($P = 0.009$) and AUC_{ins} ($P < 0.001$) during the OGTT were significantly higher in the OPCOS than in the LPCOS group (Table I, Figure 1). The concentration of fasting C-peptide was significantly higher (Table I) and the M/I significantly lower in the OPCOS than in the LPCOS subjects ($P < 0.001$), including glucose oxidation and non-oxidation (Table I, Figure 3). The early phase secretion of insulin was significantly higher in the OPCOS than in the LPCOS subjects ($P = 0.04$), but no difference was seen in the early phase secretion of C-peptide (Figure 2). No difference was observed in the hepatic clearance of insulin between the two groups (Table I).

As expected, serum concentrations of leptin were significantly higher in OPCOS compared with LPCOS subjects ($P < 0.001$, Table III).

The insulin sensitivity index decreased significantly with weight over the entire range of BMI in both control and PCOS subjects, the difference between the two groups becoming significant with increasing obesity (Figure 3).

Effects of family history of type 2 diabetes mellitus on glucose metabolism

To study the possible influence of a hereditary predisposition to a glucose metabolism disorder/type 2 diabetes mellitus, the OPCOS and LPCOS subjects were grouped into two categories: those with a known family history of type 2 diabetes mellitus in first degree relatives (OPCOS FH+, $n = 11$, 39.3% and LPCOS FH+, $n = 4$, 26.7%) and those without any relatives affected by type 2 diabetes mellitus (OPCOS FH–, $n = 17$, 60.7% and LPCOS FH–, $n = 11$, 73.3%).

Because of the low number of LPCOS FH+ subjects, it was not possible to seek for significant differences versus LPCOS FH– subjects. Early phase insulin secretion (OPCOS FH+: 10.7 ± 1.6 versus OPCOS FH–: 11.1 ± 2.1 mIU/mmol) or early phase C-peptide secretion (OPCOS FH+: 0.14 ± 0.02 versus OPCOS FH–: 0.16 ± 0.03 nmol/mmol) did not differ between the two groups. No differences were observed between OPCOS FH+ and OPCOS FH– subjects as regards M/I (OPCOS FH+: 22.4 ± 2.9 versus OPCOS FH–: 19.3 ± 01.6 μmol/kg/min/mIU), including glucose oxidation and non-oxidation indexes. No significant differences in endocrine parameters were observed between the two groups. Similar results were observed when all PCOS FH+ subjects were compared with all PCOS FH– subjects.

Discussion

The present results suggest that insulin resistance in PCOS women is, at least partly, related to obesity and fat distribution and not entirely to PCOS itself. Although LPCOS women showed a trend towards impairment of insulin sensitivity, the findings of this study were not as striking as reported in previous studies, which have shown a significant decrease of insulin sensitivity in LPCOS subjects (Dunaif *et al*., 1989; Morales *et al*., 1996). The current results are in part in line with those of studies conducted in Europe (Ovesen *et al*., 1993; Holte *et al*., 1994a,b; Acien *et al*., 1999), suggesting regional differences or heterogeneity and a complex aetiology of this syndrome.

The role of insulin secretion in the pathogenesis of PCOS has been a particular matter of debate. In a normal β cell, insulin is secreted in response to a glucose stimulus in a biphasic mode, with an early burst (early phase), followed by progressively increasing insulin secretion (second phase) as long as the hyperglycaemic stimulus is present (DeFronzo *et al*., 1979). However, a defect in β cells leading to an exaggerated insulin response and subsequent chronic hyperinsulinaemia may lead to secondary impaired insulin sensitivity through down-regulation of both insulin receptor (Insel *et al*., 1980; Baker *et al*., 1984) and post-receptor events (Mandarino *et al*., 1984; Nankervis *et al*., 1985). Similarly, defective early phase insulin secretion resulting in delayed second phase hyperinsulinaemia may produce secondary insulin resistance. Holte *et al*. have shown that women with PCOS have exaggerated early insulin responses in relation to insulin resistance during i.v. GTT (Holte *et al*., 1994a,b, 1995). On the other hand, other investigators have reported defective early phase insulin secretion and delayed hyperinsulinaemia in women with PCOS (Ehrmann *et al*., 1995). In this study, in addition to the observed insulin resistance and hyperinsulinaemia, increased early phase insulin secretion (the first 30 min) in the OGTT in OPCOS subjects and a similar trend in LPCOS subjects was found, which is in line with previous results (Holte *et al*., 1994a,b). In order to study further whether the observed increase in the early phase insulin response was truly due to enhanced insulin secretion or alternatively due to reduced hepatic insulin extraction, early phase C-peptide secretion was also measured. C-peptide accurately reflects the β cell secretory capacity (Faber *et al*., 1978) since its hepatic extraction, unlike that of insulin, is negligible (Polonsky *et al*., 1983; Waldhausl *et al*., 1986). Early phase C-peptide secretion, however, did not differ between PCOS and control subjects, suggesting that peripheral hyperinsulinaemia in OPCOS women is mainly due to lowered hepatic insulin extraction

and to insulin resistance in skeletal muscles. On the other hand, Peiris *et al*. (1989) have shown that in non-obese hyperandrogenic women hepatic insulin extraction is normal. In the present study, the hepatic insulin extraction in LPCOS subjects remained probably efficient enough to avoid a significant increase of serum insulin concentrations during OGTT.

The decreased insulin sensitivity in OPCOS women and a similar tendency in lean subjects were accounted for by impaired rate of both glucose oxidation and non-oxidation. These results are in line with those of Dunaif *et al*. (1995), showing that the defect in insulin sensitivity in PCOS may lie in excessive serine phosphorylation of the insulin receptor, which, in turn, inhibits the proximal intracellular insulin signalling cascade, leading to impaired glucose oxidation and glucose non-oxidation. The impairment in insulin sensitivity in the current study was most profound in OPCOS women, suggesting that obesity in PCOS contributes to insulin resistance in a synergetic manner. Indeed, the insulin resistance seen in normal obesity is due to a decrease of both glucose oxidation and glucose non-oxidation (Ferrannini *et al*., 1983) and is mainly explained by the fact that excessive amounts of free fatty acids, used as substrates for lipid oxidation, compete with glucose in the muscle cells as a source of energy (Randle *et al*., 1963). Similarly, a tendency towards higher FFA concentrations and defective suppression of rate of lipid oxidation were found during the hyperinsulinaemic clamp in OPCOS subjects, which together with hyperandrogenism, has been associated with abdominal obesity and insulin resistance (Bringer *et al*., 1993; Holte *et al*., 1995).

These observations allow some speculations concerning the pathogenesis of PCOS. Firstly, there is evidence that serine phosphorylation also modulates the activity of the key regulatory enzyme of androgen biosynthesis, *P*450-c17α (Zhang *et al*., 1995), suggesting that a single defect might induce insulin resistance and hyperandrogenism. These defects could operate with each other, leading to manifestations of PCOS such as abdominal obesity, which *per se* is strongly associated with insulin resistance (Bringer *et al*., 1993; Holte *et al*., 1994a,b, 1995). Note that the PCOS women in this study had a higher mean WHR than the control women, partly explaining the differences in insulin sensitivity. Secondly, environmental/genetic factors modulating body fat mass or fat distribution may lead to insulin resistance, which, together with a defect in steroidogenesis, might lead to PCOS. Particularly, hyperinsulinaemia (either primary or secondary to insulin resistance) could interfere with the ovulation mechanisms through insulin–IGF-I interactions in the ovary (Robinson *et al*., 1993), and lead to anovulation, unfavourable oestrogen/progesterone balance and further to the accumulation of abdominal fat (Rebuffe-Scrive *et al*., 1989). Furthermore, hyperandrogenism may cause insulin resistance (Polderman *et al*., 1994) by increasing the number of less-insulin-sensitive type II b skeletal muscle fibres (Holmang *et al*., 1990) and contribute to the development of abdominal obesity (Björntorp, 1997). In the present study, serum insulin concentrations and insulin sensitivity index were significantly correlated to testosterone concentrations in OPCOS and to WHR in LPCOS women, confirming the hypothesis of an association between abdominal obesity, hyperandrogenism and hyperinsulinaemia in these subjects.

The serum concentrations of SHBG were significantly lower in both OPCOS and LPCOS subjects compared to their respective controls. The inhibiting effect of insulin on SHBG synthesis in the hepatic cells could explain the lower SHBG concentrations observed in OPCOS (Plymate *et al*., 1988). However, as LPCOS subjects were only minimally hyperinsulinaemic, a direct inhibiting effect of androgens on hepatic SHBG synthesis/secretion cannot be excluded.

Since PCOS obviously is a heterogeneous disorder with many environmental/acquired and genetic factors modulating its phenotype, the effect of a family history of type 2 diabetes on insulin secretion and insulin sensitivity was studied. A family history of type 2 diabetes was frequent among PCOS women, suggesting that a gene defect involved in type 2 diabetes could also be operative in PCOS. In contrast to earlier reports (Ehrmann *et al*., 1995), no difference in early phase insulin secretion between PCOS women with and without a family history of type 2 diabetes was found. This result is somewhat surprising, and could be explained, at least partly, by the small number of subjects in these subanalyses, but it could also suggest that the β cell function in PCOS is heterogeneous in nature.

An interesting finding in the current study was the significantly higher serum concentrations of DHEA and DHEA-S in OPCOS subjects and DHEA in LPCOS subjects compared to controls, and this observation was not related to obesity. Adrenal steroid secretion decreases with age (Gray *et al*., 1991). Thus, the age difference between the PCOS subjects and the controls may have affected the results, but the significance remained after correction for age. Most studies have shown hypersecretion of adrenal hormones in PCOS subjects compared with healthy women, but the mechanism of this hypersecretion is not well understood. Possible explanations include hypersensitivity of the adrenal gland to adrenocorticotrophic hormone (ACTH) stimulation, conjugated hypersecretion from the ovary and the adrenals (McKenna and Cunningham, 1995), dysregulation of steroid synthesis enzymes such as 11β-hydroxysteroid dehydrogenase (11β-HSD) (Rodin *et al*., 1994), and hyperstimulation of cytochrome *P*450c-17α activity in the adrenal glands as a result of hyperinsulinaemia in these patients (Moghetti *et al*., 1996). The role of 11β-HSD type 1 in the aetiology of adrenal hyperandrogenism is of particular interest. A recent study has shown that the activity of this enzyme is decreased by abdominal obesity (Steward *et al*., 1999), partly explaining the higher serum DHEA and DHEA-S concentrations seen in LPCOS subjects in the present study. As insulin has also been shown to inhibit 11β-HSD expression *in vitro* (Hammami and Siiteri, 1991), the observed hyperinsulinaemia of PCOS subjects could also lead to higher serum concentrations of some adrenal steroids, particularly in OPCOS subjects. In line with these data, catheterization of the ovarian and adrenal veins in hyperinsulinaemic hyperandrogenic patients has shown strong positive correlations between serum insulin and ovarian and adrenal androgen concentrations (Martikainen *et al*., 1996). These studies and the present results suggest that insulin

may have a regulatory role in both ovarian and adrenal endocrine function.

In conclusion, a marked impairment of insulin sensitivity in OPCOS subjects, including a decrease of rates of both glucose oxidation and non-oxidation, was observed. There was also a tendency towards decreased insulin sensitivity in LPCOS subjects, but only in obese women did these changes become statistically significant, suggesting that obesity, and particularly abdominal obesity, is an important contributor to the development of insulin resistance in PCOS. Whether the hyperinsulinaemia of these patients is secondary to a primary impairment of insulin action, to primarily increased abdominal obesity, or to an initial defect in β-cell function, could not be solved by this study and needs further investigation.

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