

# Persistence of hyperinsulinemia in polycystic ovary syndrome after ovarian suppression by gonadotropin-releasing hormone agonist

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To investigate the effect of long-term androgen suppression on insulin sensitivity, obese and non-obese women with the polycystic ovary syndrome and obese and non-obese ovulatory women were given an oral glucose tolerance test before and after treatment with a gonadotropin-releasing hormone agonist. The women with polycystic ovary syndrome showed higher basal luteinizing hormone and androgen levels than the ovulatory women. All women with the polycystic ovary syndrome responded non-diabetically to the glucose tolerance test. However, compared with controls, the obese women with the polycystic ovary syndrome showed a hyperinsulinemic response to the glucose tolerance test, indicating insulin resistance. During the 3-h glucose tolerance test there was no concomitant change in androgen levels in the hyperinsulinemic women with the polycystic ovary syndrome. The insulin response to an oral glucose tolerance test remained unchanged in all women, although a hypogonadotropic hypogonadal state was maintained for several weeks. This study therefore suggests that endogenous androgens do not play a role in sustaining insulin resistance in women with the polycystic ovary syndrome.

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The polycystic ovary syndrome is associated with hyperinsulinemia and hyperandrogenemia (1). Women with polycystic ovary syndrome may show insulin resistance independent of body weight, although obesity seems to have an additive deleterious effect on glucose homeostasis (2). Several studies have demonstrated a positive correlation between fasting insulin levels and androgen levels (3, 4), but the mechanisms underlying this relationship are still not elucidated. One hypothesis has been that hyperinsulinemia induces hyperandrogenemia as insulin administration has been shown to increase androgen levels *in vivo* (5) and to enhance ovarian theca cell androgen production *in vitro* (6). However, administration of androgens has been shown to increase insulin levels *in vivo* (7), while suppression of the effect of hyperandrogenemia in polycystic ovary syndrome with spironolactone may lead to reduced insulin levels (8). Decreasing the hyperandrogenemia by oophorectomy (9) or by employing a GnRH-agonist (10, 11) has not demonstrated changes in the hyperinsulinemic state in polycystic ovary syndrome. However, these studies have involved relatively few hyperandrogenic women.

We performed this study in obese and non-obese polycystic ovary syndrome women to determine whether a normalization of the hyperandrogenemia by long-term administration of a GnRH-agonist would affect insulin sensitivity.

## Materials and methods

Fifteen polycystic ovary syndrome women aged  $30.1 \pm 0.7$  years were selected for the study and grouped as non-obese (body mass index  $< 25$  kg/m<sup>2</sup>) or obese (body mass index  $> 25$  kg/m<sup>2</sup>). The diagnosis of polycystic ovary syndrome was made by the presence of polycystic ovaries on vaginal ultrasound examination (12) combined with three or more of the following criteria: oligo/amenorrhea, hirsutism, hyperandrogenemia, elevated LH levels or LH/FSH ratio  $> 2$ . Attenuated 21-hydroxylase deficiency, Cushing's syndrome, androgen secreting tumors and hyperprolactinemia were excluded by appropriate tests. None of the polycystic ovary syndrome women had acanthosis nigricans.

Eight non-obese and six obese women aged  $30.8 \pm 1.0$  years with regular 26 to 32-day ovulatory menstrual cycles constituted the control group. None of them had hirsutism or polycystic ovaries on ultrasound examination. Informed consent was obtained from each woman.

## Study design

The initial glucose tolerance tests were performed on cycle days 4–7; however, the polycystic ovary syndrome women with amenorrhea were studied at random. All women had an oral glucose tolerance test, receiving a

75 g glucose load at 08.00 following an overnight fasting of 10–12 h. Blood was sampled through an indwelling iv catheter at –15, 0, 30, 60, 120 and 180 min after the glucose ingestion. All women were then treated with the GnRH-agonist buserelin (Suprefact, Hoechst, Germany) 100 µg intranasally six times daily for six weeks. A second oral glucose tolerance test was then performed as described above.

In all samples of the oral glucose tolerance tests glucose and serum levels of insulin, testosterone (T), androstenedione (A) and dehydroepiandrosterone sulphate (DHEAS) were measured. In addition LH, FSH and estradiol (E<sub>2</sub>) were measured at time 0 of the oral glucose tolerance tests. Samples of the same patients before and after GnRH-agonist treatment were frozen at –20°C and assayed simultaneously.

### Assays

These were performed by routine methods. Plasma glucose was determined using glucose oxidase. Serum levels of LH and FSH were measured using DELFIA kits obtained from LKB Wallac (Finland). Serum levels of E<sub>2</sub>, T, A and DHEAS were measured by RIA as described earlier (13). Insulin was determined by RIA with kits from the Radio Chemical Center, Amersham (double antibody method) by the method described by Torjesen et al. (14). Between assay CVs for the individual analyses were: LH, 5–8%; FSH, 8%; E<sub>2</sub>, 8–10%; T, 5–10%; A, 8%; DHEAS, 5%; insulin, 6–9%. Normal serum ranges in our laboratory were as follows: LH, 1–12 IU/l; FSH, 1–12 IU/l; E<sub>2</sub>, 0.4–2.0 nmol/l; T, 0.3–2.8 nmol/l; A, 3–6.6 nmol/l; DHEAS, 2.0–8.3 µmol/l and insulin, <20 mU/l in the fasting state.

### Data analysis

The normal glycemic response to the oral glucose tolerance test was defined according to the criteria of the World Health Organization (15). The normal insulinemic response to the oral glucose tolerance test was considered by our laboratory standard procedure as a

maximal insulin level lower than 100 mU/l. Summed insulin responses during the oral glucose tolerance test were calculated using the formula: incremental area = (c + d)/2 + e + f – 3\*(a + b)/2 where a to f were the concentrations of insulin at –15, 0, 30, 60, 120 and 180 min respectively.

### Statistical analysis

All results are expressed as mean ± SEM. Differences in group locations were analyzed using the Mann-Whitney test. A p-value <0.05 was considered statistically significant.

## Results

### Subject characteristics

The ages of the groups did not differ statistically. The BMI was similar in both non-obese groups (polycystic ovary syndrome 21.6 ± 0.9 kg/m<sup>2</sup> vs controls 21.8 ± 0.6 kg/m<sup>2</sup>) and both obese groups (polycystic ovary syndrome 30.1 ± 0.7 kg/m<sup>2</sup> vs controls 28.6 ± 0.7 kg/m<sup>2</sup>), and remained so during the period of GnRH-agonist treatment.

### Endocrine features

At the beginning of the study the polycystic ovary syndrome women showed higher serum levels of LH, T and A than controls (Tables 1–2). GnRH-agonist treatment induced a hypogonadotropic state in all polycystic ovary syndrome women resulting in androgen levels decreasing into the normal follicular range, while E<sub>2</sub> levels decreased into postmenopausal levels (Tables 1–2). DHEAS levels, however, remained unchanged.

### Glucose and insulin responses

All women showed normal fasting glycemic values (Tables 1–2). Apart from one obese polycystic ovary syndrome woman who demonstrated latent diabetes, all

Table 1. Endocrine results (mean ± SEM) in non-obese (BMI < 25 kg/m<sup>2</sup>) polycystic ovary syndrome women and ovulatory women before and after six weeks treatment with a GnRH-agonist.

	Polycystic ovary syndrome		Ovulatory women	
	Before (N = 7)	After (N = 7)	Before (N = 8)	After (N = 8)
LH (IU/l)	15.9 ± 2.1*	1.2 ± 0.2	4.2 ± 0.5	1.4 ± 0.5
FSH (IU/l)	6.1 ± 0.4	2.7 ± 0.4	6.4 ± 0.3	2.8 ± 0.3
Testosterone (nmol/l)	2.9 ± 0.2*	1.2 ± 0.2	1.7 ± 0.2	1.4 ± 0.2
Androstenedione (nmol/l)	7.7 ± 1.0*	2.8 ± 0.4	4.7 ± 0.9	3.8 ± 0.7
Estradiol (nmol/l)	0.20 ± 0.02	0.08 ± 0.01	0.15 ± 0.04	0.06 ± 0.01
DHEAS (µmol/l)	6.3 ± 1.3	5.3 ± 1.1	5.3 ± 0.7	5.0 ± 0.6
Glucose (nmol/l)	4.8 ± 0.2	4.7 ± 0.1	5.4 ± 0.1	5.1 ± 0.1
Fasting insulin (mU/l)	8.8 ± 1.1	8.7 ± 0.9	10.1 ± 1.8	10.8 ± 1.3

\*P < 0.05 versus controls.

Table 2. Endocrine results (mean  $\pm$  SEM) in obese (BMI > 25 kg/m<sup>2</sup>) polycystic ovary syndrome women and ovulatory women before and after six weeks treatment with a GnRH-agonist.

	Polycystic ovary syndrome		Ovulatory women	
	Before (N=8)	After (N=8)	Before (N=6)	After (N=6)
LH (IU/l)	10.9 $\pm$ 1.6*	1.0 $\pm$ 0.3	3.2 $\pm$ 0.4	1.0 $\pm$ 0.2
FSH (IU/l)	4.7 $\pm$ 0.4	2.5 $\pm$ 0.3	6.0 $\pm$ 0.3	4.5 $\pm$ 0.7
Testosterone (nmol/l)	3.2 $\pm$ 0.2*	1.6 $\pm$ 0.1	2.0 $\pm$ 0.2	2.0 $\pm$ 0.3
Androstenedione (nmol/l)	8.0 $\pm$ 1.0	3.8 $\pm$ 0.5	7.5 $\pm$ 1.3	5.9 $\pm$ 1.6
Estradiol (nmol/l)	0.17 $\pm$ 0.02	0.09 $\pm$ 0.01	0.14 $\pm$ 0.03	0.06 $\pm$ 0.01
DHEAS ( $\mu$ mol/l)	6.5 $\pm$ 1.1	6.1 $\pm$ 0.9	4.9 $\pm$ 0.8	4.7 $\pm$ 0.3
Glucose (nmol/l)	5.4 $\pm$ 0.3	5.1 $\pm$ 0.2	5.2 $\pm$ 0.1	5.4 $\pm$ 0.1
Fasting insulin (mU/l)	25.0 $\pm$ 5.3*	24.1 $\pm$ 5.3	12.5 $\pm$ 2.8	16.7 $\pm$ 3.0

\* P < 0.05 versus controls.

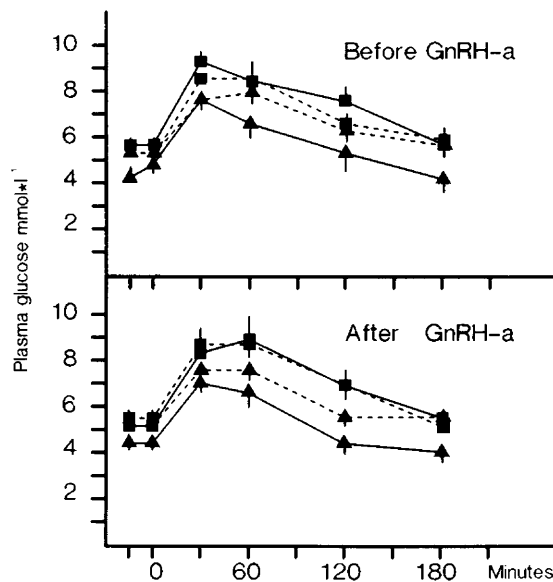


Fig. 1. Plasma glucose levels (mean  $\pm$  SEM) in response to an oral glucose tolerance test before and after six weeks of GnRH-agonist treatment (non-obese polycystic ovary syndrome  $\triangle$ — $\triangle$ , obese polycystic ovary syndrome  $\blacksquare$ — $\blacksquare$ , non-obese controls  $\triangle$ — $\triangle$ , obese controls  $\blacksquare$ — $\blacksquare$ ).

women showed a normal glycemic response to oral glucose tolerance tests (Fig. 1). GnRH-agonist treatment did not confer any significant change in the glycemic response to oral glucose tolerance test in either the polycystic ovary syndrome or the control groups (Fig. 1).

The obese polycystic ovary syndrome women showed a significantly higher fasting insulin level and insulin response to oral glucose tolerance test than the non-obese polycystic ovary syndrome women and the obese control women (Tables 1–2, Figs. 2–3). The non-obese polycystic ovary syndrome women as a group did not show fasting hyperinsulinemia or increased insulin responses to the oral glucose tolerance test (Table 1, Figs. 2–3). Hyperinsulinemic responses indicative of insulin resistance were recorded in 7/8 of the obese polycystic

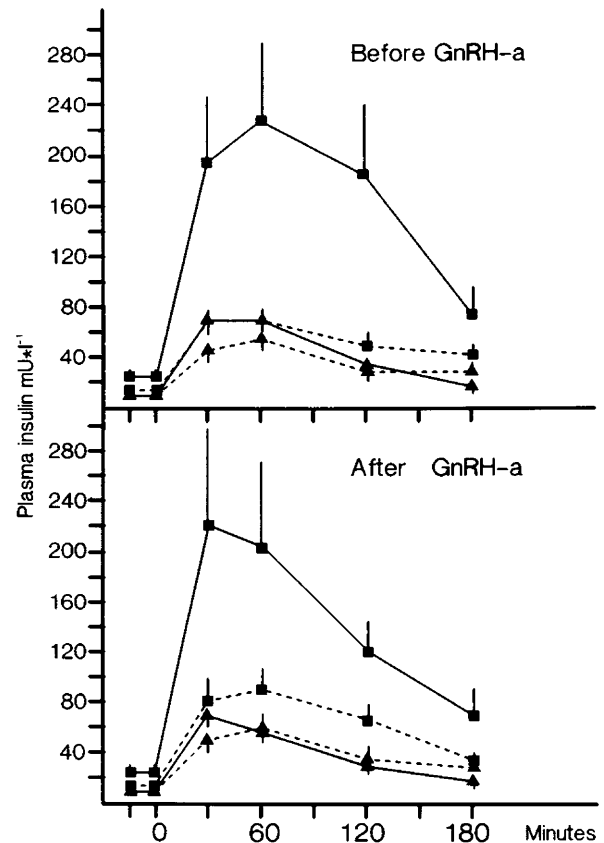


Fig. 2. Plasma insulin levels (mean  $\pm$  SEM) in response to an oral glucose tolerance test before and after six weeks of GnRH-agonist treatment (non-obese polycystic ovary syndrome  $\triangle$ — $\triangle$ , obese polycystic ovary syndrome  $\blacksquare$ — $\blacksquare$ , non-obese controls  $\triangle$ — $\triangle$ , obese controls  $\blacksquare$ — $\blacksquare$ ).

ovary syndrome women, in 1/7 of the non-obese polycystic ovary syndrome women, in 1/6 of the obese controls and in 1/8 of the non-obese controls.

Following GnRH-agonist treatment neither fasting insulin levels nor insulin responses to the oral glucose tolerance test changed significantly with regard to pretreatment values in any of the groups (Figs. 2–3).

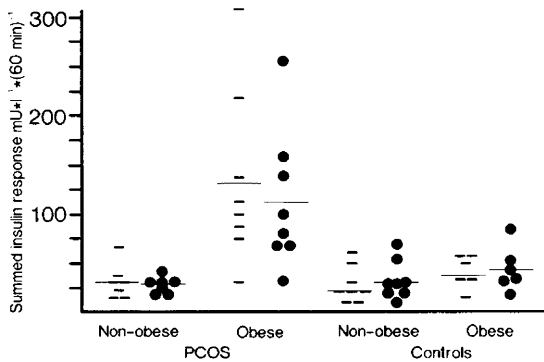


Fig. 3. Summed insulin response to an oral glucose tolerance test before and after six weeks of GnRH-agonist treatment (— before GnRH-a treatment, ● after GnRH-a treatment).

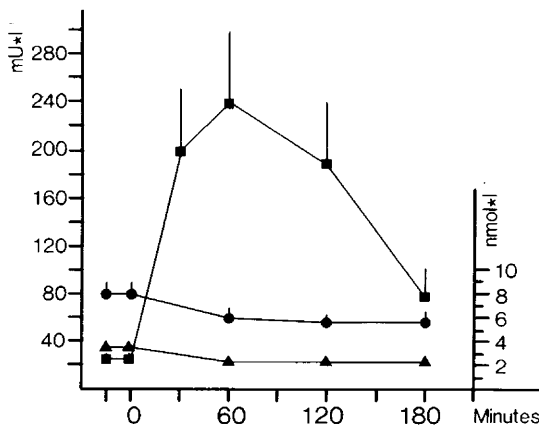


Fig. 4. Androgen levels (mean  $\pm$  SEM) during an oral glucose tolerance test in the hyperinsulinemic polycystic ovary syndrome women (insulin  $\blacksquare$ — $\blacksquare$ , testosterone  $\blacktriangle$ — $\blacktriangle$ , androstenedione  $\bullet$ — $\bullet$ ).

The ovarian androgen levels remained unchanged during the oral glucose tolerance test in all groups both before and following GnRH-agonist treatment. Even in the hyperinsulinemic polycystic ovary syndrome women the serum levels of testosterone and androstenedione remained unchanged during the oral glucose tolerance tests (Fig. 4).

## Discussion

The polycystic ovary syndrome is a heterogeneous condition often characterized by elevated LH levels, hyperandrogenemia and hyperinsulinemia (1, 16). Several studies have indicated the ovary as the primary site of excess androgen production (17, 18), although modulated by an intact hypothalamic/pituitary/ovarian feedback mechanism (16). Some women with polycystic ovary syndrome show insulin resistance (2, 4) and demonstrate a positive correlation between androgen levels and fasting insulin levels (3, 4). One line of theory

has suggested that hyperandrogenemia may induce insulin resistance and hyperinsulinemia. However, in our study there was no change in insulin response to the oral glucose tolerance test in either polycystic ovary syndrome women or controls in spite of long-term normalization of androgen levels. Our results and others (11, 19) suggest that endogenous androgens do not sustain insulin resistance in polycystic ovary syndrome women. The hyperinsulinemia reported following administration of androgens may therefore be a result of the synthetic androgens used in these studies (7, 20), as administration of natural androgens has not demonstrated alterations in insulin sensitivity (21). We cannot exclude the possibility that androgens produce irreversible changes in target tissue insulin action or changes in metabolic clearance of insulin leading to hyperinsulinemia (22). However, in the study by Dunaif et al. employing the glucose clamp technique, no variations in metabolic insulin clearance due to androgens could be detected in polycystic ovary syndrome women (11).

In our study both androstenedione and testosterone remained unaltered during the oral glucose tolerance test in both hyperinsulinemic and non-hyperinsulinemic polycystic ovary syndrome women (5, 19, 23). However, this may have been due to the relatively short sampling period of 3 h, as a recent report has shown that long-term elevations of serum insulin levels are needed to produce hyperandrogenemia (24). Although insulin may enhance ovarian androgen production in polycystic ovary syndrome, the exact mechanism on the cellular level remains undisclosed. Recently, both cross-reaction with the insulin growth factor I receptor on the ovarian theca cells (6) and insulin mediated changes in ovarian insulin growth factor-binding proteins have been introduced as mechanisms of insulin action (25).

This study also demonstrated that insulin resistance seems to be a heterogeneous feature of polycystic ovary syndrome, as some of the obese and most of the non-obese polycystic ovary syndrome women had an euinsulinemic response to the oral glucose tolerance test (18). Furthermore, the ability of GnRH-agonists to suppress hyperandrogenemia without altering insulin sensitivity establishes the dominant effect of gonadotropins in sustaining androgen excess even in hyperinsulinemic polycystic ovary syndrome women (13, 19). However, ameliorating the hyperinsulinemia by weight reduction or by administration of diazoxide has resulted in reduced androgen levels, thereby restoring ovulation and fertility in obese polycystic ovary syndrome women (26, 27). Weight reduction may therefore seem a main objective in advance of other medical therapies in obese polycystic ovary syndrome women (28).

In conclusion, we have demonstrated a hyperinsulinemic response to the oral glucose tolerance test in a subset of polycystic ovary syndrome women indicative of insulin resistance. Prolonged normalization of the hyperandrogenemia by a GnRH-agonist did not restore a normal insulinemic response to the oral glucose toler-

ance test, indicating that endogenous androgens do not play a role in sustaining insulin resistance in polycystic ovary syndrome women.

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