

Growth hormone secretion is impaired but not related to insulin sensitivity in non-obese patients with polycystic ovary syndrome

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BACKGROUND: The aim of the study was to elucidate the relationship between growth hormone (GH) secretion and insulin resistance in polycystic ovary syndrome (PCOS) patients. In order to exclude the influence of obesity on these parameters, only non-obese PCOS patients were studied. **METHODS:** Eleven PCOS patients and 11 controls with a body mass index (BMI) ≤ 25 kg/m² were studied. PCOS patients were studied on cycle day 14–15, controls on cycle day 5–9. GH secretion was determined by frequent sampling, from 20.00 h to 08.00 h. Insulin sensitivity was determined by a euglycaemic hyperinsulinaemic clamp and was expressed as the M-value. Insulin-like growth factor-1 (IGF-1) and IGF-binding protein-3 (IGFBP-3) levels were determined once. **RESULTS:** Pooled GH levels were significantly lower in PCOS patients than controls, as was GH pulse amplitude. The number of GH pulses was not different between PCOS patients and controls. The M-value was significantly lower in PCOS patients, although a wide overlap between patients and controls was present. IGF-1 and IGFBP-3 levels were not different between the groups. There was no correlation between the M-value and pooled GH or IGF-1 and IGFBP-3 levels. **CONCLUSION:** Non-obese patients with PCOS have impaired GH secretion and some but not all have impaired insulin sensitivity. These findings indicate that these patients may also be at risk for cardiovascular diseases and/or diabetes mellitus.

Key words: growth hormone/insulin/PCOS

Introduction

The polycystic ovary syndrome (PCOS) is a heterogeneous menstrual cycle disorder defined by oligo- or amenorrhoea or anovulation in combination with at least one of the following: hirsutism, hyperandrogenism, elevated LH levels in the presence of normal FSH levels or enlarged ovaries on ultrasound, as described by Adams *et al.* (1986). In Europe, obesity is found in 39% of PCOS patients (Balen *et al.*, 1995). It has been found that insulin resistance and impaired growth hormone (GH) secretion play a role in the pathogenesis of PCOS.

In several studies, the GH response during stimulation tests was found to be lower in PCOS patients when compared with normal controls (Acar and Kadanali, 1993; Lee *et al.*, 1993; Kaltas *et al.*, 1998). Others could not demonstrate such differences and concluded that blunted GH responses, as found in PCOS, are caused by obesity (Slowinska-Srzednicka *et al.*, 1992; Morales *et al.*, 1996). However, diminished GH responses were also found in non-obese PCOS patients when compared with non-obese controls (Piaditis *et al.*, 1995).

Data from studies in which spontaneous GH secretion was measured are also conflicting. It was found that GH levels in PCOS patients were higher (Prelevic *et al.*, 1992), lower (Kazer *et al.*, 1990) or not different (Morales *et al.*, 1996) when compared with controls, matched for body weight. The latter study is the only one in which the pulsatile pattern of GH was determined. The mean GH levels were not different, but the pulse amplitude was significantly higher in PCOS patients.

Although GH serum levels are found to be different, insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-binding protein-3 (IGFBP-3) levels do not differ between PCOS and controls (Homburg, 1998). Only recently it has been found that free IGF-1 levels are increased in PCOS patients (Van Dessel *et al.*, 1999).

After the studies of Dunaif *et al.* (1989), it became accepted that insulin resistance is present in obese, as well as non-obese PCOS patients. On the other hand, there are also data from which it is concluded that insulin resistance in PCOS is related predominantly to obesity (Ovesen *et al.*, 1993; Pasquali and Casimirri, 1993; Holte *et al.*, 1995). It is difficult to compare

the studies. The methods used are diverse, and overweight (but not obese) PCOS patients are often defined as being non-obese. In a study in which the prevalence of type 2 diabetes seems to be increased in PCOS patients, overweight patients accounted mostly for the impaired glucose tolerance in the so-called non-obese group (Legro *et al.*, 1999).

There seems to be a relationship between GH secretion and insulin resistance. Impaired GH secretion is found in non-obese type 2 diabetes patients (Giustina *et al.*, 1994; Franek *et al.*, 1997). In Laron-type dwarfism, it was found that impaired glucose tolerance was present during adulthood (Laron *et al.*, 1995). In adult GH-deficient patients, insulin sensitivity increased after GH treatment (Johannsson *et al.*, 1999). These findings suggest that GH levels influence insulin sensitivity.

As stated previously, disturbances have been found in both endocrine functions in PCOS patients. However, it still remains unclear whether the diminished GH response and the impaired insulin sensitivity are caused by obesity only or whether they are a result of the PCOS itself. Also it is not clear whether these disturbances are related to each other in PCOS patients.

Therefore, the first objective of the present study was to investigate whether disturbances of the somatotrophic axis and in insulin sensitivity are present in PCOS patients, by the two undisputedly best tests for these endocrine functions. Secondly, we wanted to clarify whether a relationship between these two endocrine functions is present in PCOS patients. To avoid the confounding effect of obesity on both GH secretion and insulin sensitivity, the investigation was carried out in non-obese PCOS patients only.

Materials and methods

Study design

The study was designed as a case-control study. GH secretion was tested during the night by frequent venous sampling. Subsequently insulin sensitivity was determined by the euglycaemic hyperinsulinaemic clamp technique.

Patients

Eleven non-obese PCOS patients defined by body mass index (BMI) ≤ 25 kg/m² were included. They all met criteria for the diagnosis according to the 2003 Rotterdam consensus conference on diagnosis of PCOS (Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004). The diagnosis is made when a patient has two out of the three following criteria: (i) oligomenorrhoea (intercycle interval >35 days); (ii) clinical and/or biochemical signs of hyperandrogenism; and (iii) polycystic ovaries determined with ultrasound. All patients in our study had oligomenorrhoea with clinical/biochemical hyperandrogenism. Nine patients had PCOS ovaria on ultrasound (Adams *et al.*, 1986) and all patients had elevated LH levels (>6.5 U/l) and normal FSH (10 U/l) determined at least 2 weeks after the beginning of a menstrual period and 3 weeks before the subsequent menstrual period (Van Hooff *et al.*, 1999). Patients were in good health, did not use any medication, and sex steroid treatment had been discontinued for at least 3 months. Thyroid function, prolactin and 24 h excretion of free cortisol were all within normal limits.

Table I. Patient data

	PCOS patients	Controls
<i>n</i>	11	11
Androstenedione (nmol/l)	8.3 (3.3–11)	4.6 (2.4–5.8) ^b
Testosterone (nmol/l)	1.71 (0.75–3.44)	0.88 (0.4–1.31) ^c
Estradiol (pmol/l)	112 (96–194)	159 (65–431) ^a
LH (IU/l)	9.5 (7–14)	3.9 (1.2–7) ^b
FSH (IU/l)	5.6 (3.8–6.8)	4.4 (2.6–7.1) ^a
Age (years)	27 (22–36)	25 (22–36) ^a
Weight (kg)	63 (50–75)	64.5 (56.5–82.0) ^a
BMI (kg/m ²)	23.8 (18.8–25.3)	22.1 (20.3–25.6) ^a
Waist ip ratio	0.77 (0.63–0.96)	0.76 (0.7–0.83) ^a
Non-obese body mass (%)	69.9 (62.6–82.8)	73 (66.6–76.9) ^a

Data are given as median and range.

^aNS; ^b*P* < 0.01; ^c*P* = 0.01.

Eleven controls were included. Inclusion was based on the existence of regular menstrual cycles (21–35 days), a BMI ≤ 25 kg/m² and absence of clinical/biochemical signs of hyperandrogenism. Ovarian ultrasound was not performed in this group. Controls were recruited among colleagues and medical students. Controls had not used oral contraceptives or other sex steroids for at least 3 months prior to the study. They were in good health and did not use any medication. Patient data are shown in Table I. One patient and one control who had a BMI of ≤ 25 kg/m² at inclusion appeared to have a slightly higher BMI on the day the tests were performed (one PCOS patient appeared to have a BMI of 25.3 kg/m² and one control appeared to have a BMI of 25.6 kg/m²).

The study was conducted under the rules of the Declaration of Helsinki and was subject to approval of the subcommittee for the ethics involving human subjects of the VU Medical Centre of Amsterdam. All patients gave informed consent.

Methods

Earlier studies addressing the same issue (Morales *et al.*, 1996) encountered the difficulty of differences in estradiol (E₂) levels between the study groups. It was our pertinent aim to minimize the possibility that such a difference would occur. We therefore chose to test the PCOS patients during a potentially optimal stable period with minimal influence of a previous cycle or a forthcoming ovulation (Van Hooff *et al.*, 1999). In the patient group, tests were performed on the 14th and 15th day after menstrual bleeding occurred (spontaneously, or induced by 100 mg of progesterone i.m.). Controls were tested on cycle day 5–9. By this procedure, we expected serum E₂ levels to be comparable between patients and controls.

GH sampling

Subjects arrived in the hospital at 18.00 h. BMI, waist-hip ratio and body composition including the lean body mass (LBM), measured by body impedance analysis, were determined. At 18.30 h, an i.v. catheter with heparin lock was inserted in a forearm vein of each arm. A sample was obtained for E₂, androstenedione, testosterone, LH, FSH, IGF-1 and IGFBP-3. Subjects received a standard hospital meal. At 20.00 h, venous sampling started for 12 h every 10 min. All patients fell asleep between 23.00 h and 01.00 h. Patients were only allowed to drink mineral water during the evening and were fasting from 24.00 h. Each sample was centrifuged, and 25 μ l of plasma of each sample was pooled, in order to determine mean GH levels during 12 h. All plasma samples were frozen and stored at -20°C for later assay. After a significant difference was established between the pooled GH levels of PCOS patients and controls, GH was determined every 20 min, in

order to elucidate whether differences in pulse frequency or amplitude were responsible for the difference as found in the pooled samples. For budgetary reasons, these pulse patterns were only determined in the first seven subjects of each group.

Euglycaemic hyperinsulinaemic clamp

Subsequently, a euglycaemic hyperinsulinaemic clamp was performed. After determination of fasting glucose levels at $t = -15$ and 0, an insulin infusion at 60 mU/kg/h was started at 08.30 h. With this concentration, it is known that the hepatic glucose production is completely suppressed (Ovesen *et al.*, 1993; Polderman *et al.*, 1994; Ferrannini and Mari, 1998) and the glucose disposal rate equals the amount of glucose infused. At 08.30 h (while GH sampling was continued), i.v. infusion of 3-[³H]glucose was started. This was used to measure total glucose turnover rate, to ensure that endogenous glucose production was suppressed completely. The concentration was 250 μ Ci in 100 ml. First a bolus of 20 ml was given, followed by continuous infusion of 7.2 ml/h. The total amount received by the subjects was 140 μ Ci.

The insulin solution was prepared by adding 20 IU of insulin (Velosulin[®], Novo Nordisk A/S, Bagsvaerd, Denmark) and 4.5 ml of 20% human albumin (Central Blood Transfusion Laboratory, Amsterdam, The Netherlands) to 45 ml of 0.9% sodium chloride. After the start of the insulin infusion, blood glucose levels were measured every 5 min with the use of a Yellow Springs Instruments Glucose Analyser (glucose oxidase method; Yellow Springs, OH). Glucose 20% was infused and the infusion rate was adjusted after glucose measurements in order to maintain a stable blood glucose level. Samples for insulin were taken every 15 min from $t = -15$ min until $t = 120$ min. Samples were centrifuged and serum was frozen and stored at -20°C for later assay. The glucose disposal rate, or the M-value, was determined as the amount of the glucose infused during the last 60 min of the clamp, expressed in $\mu\text{mol/kg LBM/min}$ (Blonk *et al.*, 1994).

Pulse detection method

The analysis of pulsatile GH secretion was carried out by the method developed by Lambalk *et al.* (1985), modified by Scheele *et al.* (1987).

Assays

Androstenedione was determined by radioimmunoassay (RIA) (Coat-A-Count, DPC, Los Angeles, CA). The intra-assay coefficient of variation (CV) was 6% at 3 nmol/l and 4% at 10 nmol/l. The inter-assay CV was 9% at 7 nmol/l and 8% at 28 nmol/l, with normal values 2–9 nmol/l. E₂ levels were determined by double antibody RIA (Sorin Biomedica, Saluggia, Italy). The intra-assay CV was 4% at 110 pmol/l and 5% at 1000 pmol/l. The inter-assay CV was 11% at 60 and 550 pmol/l. Testosterone was determined by RIA (Coat-A-Count). The intra-assay CV was 6% at 4 nmol/l and 10% at 3 nmol/l, with normal values <2.5 nmol/l in women. The inter-assay variation was 12% at the level of 2 nmol/L. GH was determined by immunoradiometric assay (IRMA) (colour), (Sorin Biomedica, Saluggia, Italy). The intra-assay CV was 4% at 4, 20 and 50 mU/l. The inter-assay CV was 16% at 1 mU/l and 9% at 15 mU/l. The lower level of detection was 1 mU/l. IGF-1 was determined by IRMA (extraction) (DSL, Webster, TX). The intra-assay CV was 4% at 10 nmol/l and 2% at 40 nmol/l. The inter-assay CV was 11% at 12 nmol/l and 9% at 31 nmol/l. IGFBP-3 levels were determined by IRMA (DSL). The intra-assay CV was 4% at 5 mg/l. The inter-assay variation was 12% at 2 mg/l and 7% at 4 mg/l. Plasma levels of LH and FSH were determined by immunofluorometric assays (Amerlite, Amersham, UK). Insulin levels were determined by IRMA (Biosource Diagnostics, Fleurus,

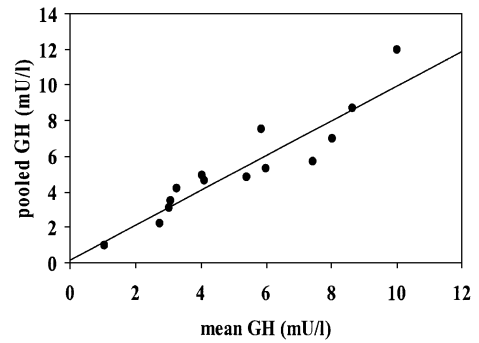


Figure 1. The correlation between pooled GH levels and mean GH levels as determined by the pulse programme ($n = 14$). $r = 0.93$, $P < 0.001$.

Table II. Hormonal levels

	PCOS patients	Controls
n	7	7
No. of GH pulses	3 (1–5)	2 (2–3) ^a
GH pulse amplitude (mU/l)	12.97 (1.63–37.84)	25.99 (17.22–36.14) ^b
n	11	11
Pooled GH levels (mU/l)	4.6 (1–8.1)	7.0 (2.2–12.0) ^b
IGF-1 (nmol/l)	23.9 (15.5–34)	24.2 (15.3–36.1) ^a
IGFBP-3 (mg/l)	3.7 (3.1–4.3)	4 (3.3–4.6) ^a
Fasting glucose (mmol/l)	4.6 (3.9–5.6)	4.2 (4.0–5.1) ^a
Fasting insulin (nmol/l)	43 (32–82)	42.5 (25–52) ^a

Data are given as median and range.

^aNS; ^b $P < 0.05$.

Belgium). The intra-assay CV was 5% at 40 pmol/l and 2% at 318 pmol/l. The inter-assay CV was 7% at 80 and 1200 pmol/l.

Statistics

The Wilcoxon rank sum test was used to determine differences between the groups. A P -value < 0.05 was considered to be significant. Pearson's correlation was used to determine correlations.

Results

Clinical data were comparable in both groups. LH, androstenedione and testosterone levels were higher in PCOS patients, and E₂ levels were not different between the two groups (Table I).

GH parameters

As shown in Figure 1, there was a strong correlation between the pooled GH levels and the mean levels as determined by the pulse programme ($r = 0.93$, $P < 0.001$).

The pooled GH levels were significantly lower in the PCOS patients than in controls; 4.6 (1–8.1) mU/l versus 7 (2.2–12) mU/l ($P < 0.05$) (data are given as median and range). The pulse amplitude was significantly lower in the PCOS patients; 12.97 (1.63–37.84) mU/l versus 25.99 (17.22–36.14) mU/l ($P < 0.05$). The pulse frequency, however, was not different in PCOS patients compared with controls: three (1–5) versus two (2–3) pulses per 12 h ($P = 0.14$). Data are shown in Table II. Figure 2 shows representative pulse patterns of each group.

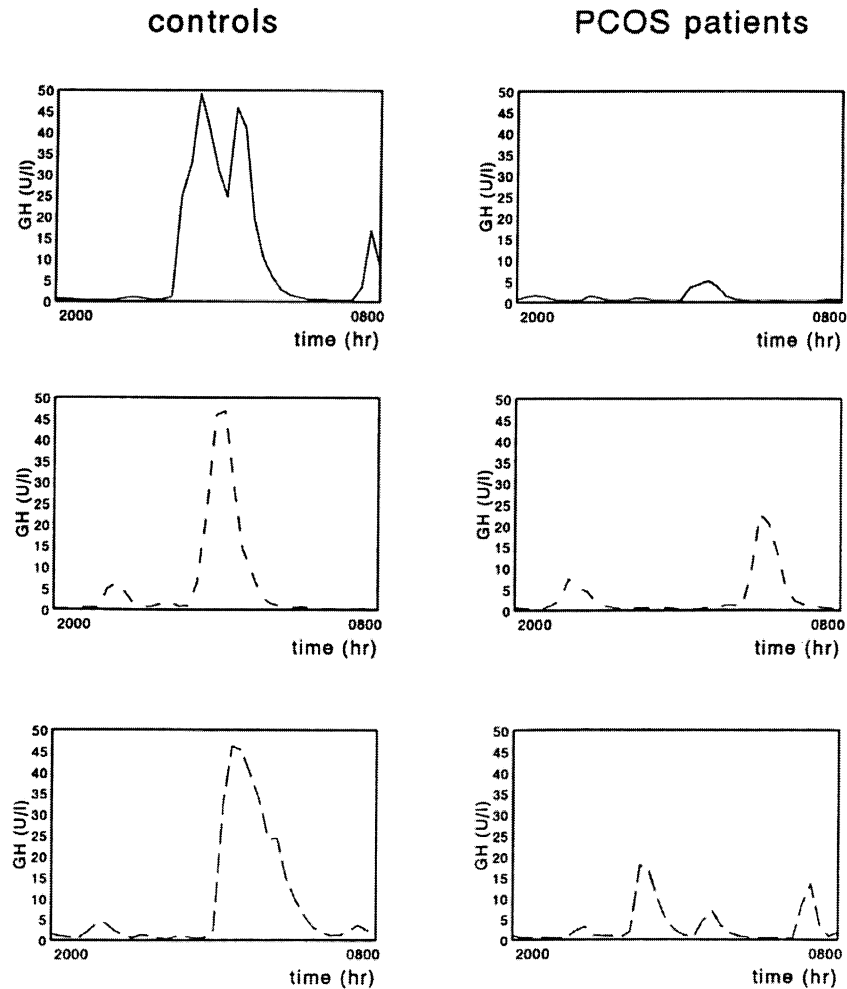


Figure 2. Representative pulse patterns of GH from three subjects of each group.

As shown in Table II, IGF-I and IGFBP-3 levels were not different between the groups.

Insulin sensitivity

As shown in Table II, there were no differences in fasting glucose and fasting insulin levels between the groups. During the clamp, the area under the curve (AUC) of plasma glucose levels and the AUC of serum insulin levels were not significantly different (Figure 3) between the PCOS and control groups. The M-value was significantly lower in PCOS patients compared with controls: 54.5 (27–74.3) $\mu\text{mol/kg LBM}/\text{min}$ versus 64.6 (47.7–120.9) $\mu\text{mol/kg LBM}/\text{min}$ ($P < 0.05$). As shown in Figure 4, a wide overlap exists between the M-value of PCOS patients and controls.

There was no correlation between the M-value and pooled GH levels or androstenedione levels. Neither was there a correlation between the M-value and IGF-1 or IGFBP-3 levels.

Discussion

The results of this study indicate that non-obese PCOS patients suffer from a certain degree of hypsomatotropism and some

non-obese PCOS patients are, to a certain extent, less insulin sensitive than non-obese controls.

In this study, ultrasound was not performed in the control group. Although others found that the presence of polycystic ovaries in non-hyperandrogenic women exhibits similar disturbances in the insulin and lipid profile compared with PCOS patients, this was especially found in the polycystic ovaries group with cycle irregularity (Norman *et al.*, 1995). Our control group had strict regular cycles, so the influence of polycystic ovaries would be negligible. This is also supported by the finding that polycystic ovaries were found in only 9% of women with strict regular cycles (Van Hooff *et al.*, 2000a). Only one woman in our control group would account for this.

Unfortunately, one control and one PCOS patient had a higher BMI on the day the tests were performed than at inclusion. However, neither was obese or overweight. If these two women were excluded, the results of the study were not affected.

With this study, it has become clear that disturbances in GH secretion in PCOS patients are not caused only by the often co-existing obesity. It has not become clear, either from our study or from other studies concerning GH secretion in PCOS patients, why PCOS patients have impaired GH secretion. The

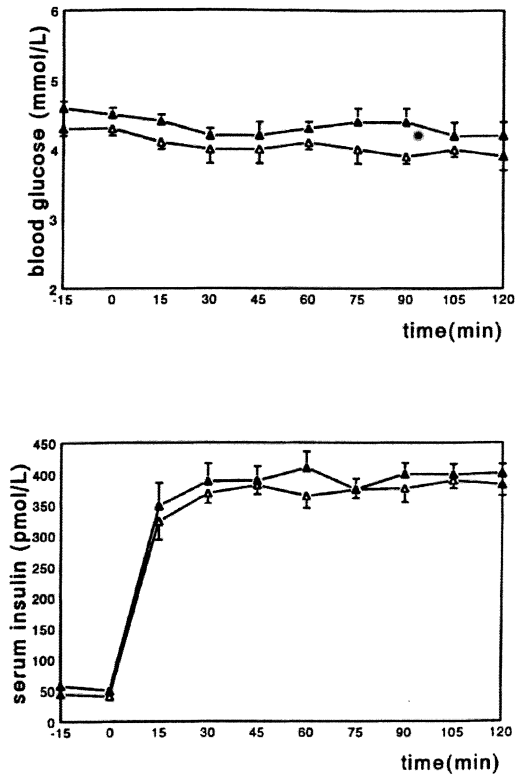


Figure 3. Insulin serum levels and blood glucose levels in PCOS patients (filled triangles) and controls (open triangles) during the clamp. The area under the curve (AUC) of insulin levels and the AUC of glucose levels were not significantly different between the two groups. Data are presented as mean \pm SEM.

lower pulse amplitude indicates a higher somatostatin tone, which also has been suggested by Lee *et al.* (1993). Some factors could be responsible for this higher somatostatin tone in PCOS patients. First, higher circulating free IGF-1 levels as found in PCOS patients by Van Dessel *et al.* (1999) could be a possible cause. Unfortunately, we only measured total IGF-1 and IGFBP-3, which were not different between the groups, as was also found by others (Homburg, 1998). The impaired GH secretion as found in PCOS patients probably is not severe enough to cause a decrease in IGF-1 or IGFBP-3 levels. Secondly, higher levels of free fatty acids (FFAs), which are found in PCOS patients (Holte *et al.*, 1994, 1995), are known to suppress GH secretion also at the hypothalamic level (Wilson and Foster, 1992), although Morin-Papunen *et al.* (2000) did not find a difference in FFA levels in non-obese patients. We did not take samples for FFA, because the lipid profile was not an objective in this study. Thirdly, lower E_2 levels could be responsible for lower GH levels (Ho *et al.*, 1996). Because of the timing of our tests, E_2 levels were not different between the groups on the day the tests were performed. This could explain the difference between our findings and the results of the study by Morales *et al.* (1996), in which no difference in mean GH levels, but higher GH pulse amplitudes were found in PCOS patients. In that study, E_2 levels were significantly higher in PCOS patients. These hypotheses might be the subject of further study.

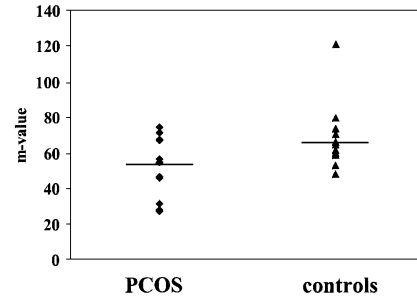


Figure 4. Insulin sensitivity in both groups expressed by the M-value in each group. The M-value (the glucose disposal rate) is the amount of glucose infused during the last 60 min of the clamp, expressed in $\mu\text{mol/kg LBM/min}$. The horizontal lines represent the median of each group, $P < 0.05$.

Several studies indicate that insulin resistance could be present in non-obese PCOS patients (Chang *et al.*, 1983; Dunaif *et al.*, 1989). However, others (Ovesen *et al.*, 1993; Holte *et al.*, 1995; Morin-Papunen *et al.*, 2000; Van Hooff *et al.*, 2000b; Elting *et al.*, 2001) could not confirm this. This may indicate that insulin resistance may not be a pertinent feature of the PCOS itself. Also, from our data, it is clear that a wide overlap is present in insulin sensitivity between non-obese PCOS patients and controls. One control patient was extremely sensitive and only two PCOS patients had M-values below the control group. Therefore, it is difficult to state that all PCOS patients suffer from insulin resistance. In the whole cohort of non-obese PCOS patients, a minor group might be present which suffer from insulin resistance. Easy tests should be developed to determine which patients are at risk and might benefit from treatment with metformin.

We were not able to show a relationship between impaired GH secretion and insulin sensitivity. However, impaired GH secretion can be accompanied by impaired insulin sensitivity, as found in some non-obese PCOS patients. These PCOS patients in particular are at risk for disease at an older age. These disturbances both lead to dyslipidaemia and increase the risks of cardiovascular diseases. Impaired insulin sensitivity might lead to non-insulin-dependent diabetes mellitus (NIDDM) (Legro *et al.*, 1999). However, because the cause of the impaired GH secretion is unknown and impaired insulin sensitivity is not seen in all patients, it is still not known which patients are at risk.

We conclude that (i) impaired GH secretion is present in non-obese PCOS patients; (ii) impaired insulin sensitivity is only present in a minor subgroup of non-obese PCOS patients; and (iii) there is no relationship between impaired GH secretion and insulin sensitivity in non-obese PCOS patients.

Further research should be directed to explore the disturbances in GH secretion and to define which PCOS patients are at risk for insulin resistance and might benefit from metformin.

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