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Insulin-like factor 3 — a new hormone related to polycystic ovary syndrome?

Insulinopodobny czynnik 3 — nowy hormon związany z zespołem policystycznych jajników?

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Abstract

Introduction: The aim of this study was to find a correlation between insulin-like factor 3 (INSL3) and androgens: androstenedione (A), free testosterone (fT), and total testosterone (T), in two groups of polycystic ovary syndrome (PCOS) women: those with a body mass index (BMI) lower than 25 kg/m^2 and those with a BMI higher than 25 kg/m^2. The association between INSL3 and other serum parameters: luteinising hormone (LH), follicle-stimulating hormone (FSH), dehydroepiandrosterone sulphate (DHEA-S), sex hormone binding globulin (SHBG) and glucose and insulin were also investigated.

Material and methods: The study group comprised 37 PCOS women aged 27 ± 4 years. The control group consisted of 34 healthy, premenopausal women (aged 24.2 ± 1.2) with regular menses and no signs of hyperandrogenism. There were 27 PCOS women of normal weight (BMI < 25 kg/m^2), and ten overweight individuals (BMI ≥ 25–30 kg/m^2). Correlations between level of INSL3 and LH, FSH, T, fT, A, DHEA-S, SHBG, metabolic tests, height, weight, and WHR (waist-to-hip ratio) were also investigated.

Results: PCOS women showed non-significantly higher levels of INSL3 compared to the healthy controls (64.6 ± 27.7 and 62.7 ± 20.0 ng/mL, respectively). However, we identified very strong correlations between INSL3 and androstenedione (r = 0.48, p = 0.0115), and free (r = 0.44, p = 0.0108) and total testosterone (r = 0.46, p = 0.0057) in the PCOS subgroup with a BMI of < 25 kg/m^2. There was no statistically significant correlation between INSL3 and LH in any subject of the PCOS group, nor between INSL3 and FSH, DHEA-S, glucose, basal insulin concentration or HOMA-IR.

Conclusions: We found a positive correlation between INSL3 and androgens in PCOS women, especially those with a BMI of < 25 kg/m^2. This may play a key role in PCOS pathophysiology. (**Endokrynol Pol 2012; 63 (5): 356–361**)

Key words: *polycystic ovary syndrome, insulin-like factor 3, luteinising hormone, hyperandrogenism*

Streszczenie

Wstęp: Celem pracy była analiza zależności między stężeniem insulinopodobnego czynnika 3 a stężeniami androgenów: androstendionu (A), wolnego testosteronu (fT) oraz całkowitego testosteronu (T) u kobiet z zespołem policystycznych jajników (PCOS), z uwzględnieniem wskaźnika masy ciała (BMI). Przedmiotem badania był także związek między INSL3 a hormonem luteotropowym (LH), folikulotropowym (FSH), siarczanem dehydroepiandrosteronu (DHEA-S), białkiem wiążącym hormony płciowe (SHBG) oraz stężeniami glukozy i insuliny.

Materiał i metody: Badaną grupę stanowiło 37 kobiet z PCOS, średnia wieku 27 ± 4 lat. Grupę kontrolną stanowiły 34 zdrowe kobiety (średnia wieku 24,2 ± 1,2 roku) z regularnymi miesiączkami, bez objawów hiperandrogenizacji. Kobiety z PCOS podzielono na grupy pod względem masy ciała; grupę z prawidłową masą ciała tworzyło 27 kobiet, natomiast z nadwagą i otyłością — 10 badanych. Analizie poddano także zależności między stężeniem INSL3 i stężeniami LH, FSH, T, fT, A, DHEA-S, SHBG, glukozy i insuliny oraz wskaźniki antropometryczne (wzrost, masa ciała, wskaźnik talia–biodra).

Wyniki: U kobiet z PCOS wykazano wyższe stężenie INSL3 w porównaniu z grupą kontrolną (64,6 ± 27,7 i 62,7 ± 20,0 ng/mL, odpowiednio). Wykazano silną zależność między stężeniem INSL3 a stężeniem androstendionu (r = 0,48; p = 0,0115), wolnego (r = 0,44; p = 0,0108) i całkowitego testosteronu (r = 0,46; p = 0,0057) w grupie kobiet z PCOS o prawidłowej masie ciała. Nie wykazano zależności między stężeniem INSL3 a stężeniami LH, FSH, DHEA-S, stężeniem glukozy, insuliny oraz wskaźnikiem insulinooporności HOMA.

Wnioski: Wykazano związek między stężeniem INSL3 a androgenemią u kobiet z PCOS, szczególnie silnie wyrażoną u kobiet z prawidłową masą ciała, co może być istotne w patofizjologii PCOS. **(Endokrynol Pol 2012; 63 (5): 356–361)**

Słowa kluczowe: *zespół policystycznych jajników, insulinopodobny czynnik 3, hormon luteotropowy, hiperandrogenizm*

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Introduction

The neohormone insulin-like factor 3 (INSL3) is a major secretors product of the testicular Leydig cells in the foetus and in adult men [1]. INSL3 expression is an early marker of testicular dysgenesis syndrome. In women, it is produced at a low level by the ovarian theca and luteal cells; however, circulating levels of INSL3 increase in women with polycystic ovary syndrome (PCOS) [2]. Female INSL3 knockout mice show impaired fertility connected to cycle length, suggesting an association between growing follicles and INSL3 expression in mouse ovaries, and they also show higher expression of INSL3 in the follicular phase than in the luteal phase [3, 4]. Recent results suggest an important role for luteinising hormone (LH) in stimulating INSL3 transcription in ovarian theca cells and testicular Leydig cells [5]. In addition, a significant correlation between serum concentrations of INSL3 and LH has been found in normal adult humans [6].

PCOS is the commonest endocrine disorder in women of reproductive age. Its prevalence varies from 2.2% to 26%, which could be due to several factors, one of the most important being the difference in diagnostic criteria used [7]. It is very important to emphasise that siblings of patients with PCOS are predisposed to the occurrence of hormonal abnormalities similar to those observed in PCOS and expressed by increased concentrations of androgens [8]. Ovaries from PCOS women are characterised not only by hyperplasia of the theca interna and cortical stroma, but also by an increased number of small antral follicles [9]. There is no dominant follicle development resulting in anovulation [10]. Moreover, some PCOS women exhibit increased LH serum levels [11]. Insulin resistance is also present in the majority of cases, with compensatory hyperinsulinaemia contributing to hyperandrogenism via the stimulation of ovarian androgen secretion and the inhibition of hepatic sex hormone-binding globulin production (SHBG). Adipose tissue dysfunction has been implicated as a contributor to the insulin resistance observed in PCOS. However, LH, endothelial dysfunction, insulin-like growth factor-1 (IGF-1), environmental and genetic factors also play important roles in PCOS development [12–14].

The aim of this study was to investigate any correlation between serum levels of INSL3 and androgens: androstenedione (A), free testosterone (fT) and total testosterone (T) in two groups of PCOS women: normal weight individuals and those with a body mass index (BMI) over 25 kg/m^2. In addition, we investigated the association between the level of INSL3 and other serum parameters: LH, follicle-stimulating hormone (FSH), dehydroepiandrosterone sulphate (DHEA-S), SHBG, and glucose and insulin.

Material and methods

The study population consisted of 37 women with PCOS, aged 18–35. Diagnosis of PCOS was established according to the Rotterdam criteria with the presence of at least two of the following diagnostic features after the exclusion of other aetiologies: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries shown by ultrasonography [15]. The ultrasound criteria used for the diagnosis of polycystic ovaries were the presence of at least 12 follicles in each ovary of 2–9 mm in diameter, and/or increased ovarian volume (> 10 mL). Hyperprolactinaemia, Cushing's syndrome, congenital adrenal hyperplasia and androgen secreting tumours were excluded based on clinical data and laboratory analysis. All women showed normal hepatic and renal function.

The control group consisted of 34 healthy, premenopausal women (aged 22–27) with regular menses and without clinical signs of hyperandrogenism (without hormonal contraception). The clinical characteristics of women with polycystic ovary syndrome and controls are set out in Table I.

All subjects had not received any medication for at least three months before the study, and none showed evidence of thyroid dysfunction, diabetes or internal diseases. All of the evaluations for the PCOS group were performed within the first ten days of menstrual cycle in cases of mild oligomenorrhea, or at random if they suffered severe oligo- or amenorrhea after excluding pregnancy by appropriate tests.

Standard anthropometric data, i.e. height, weight, and waist and hip circumferences (WHR, waist-to-hip ratio), were recorded for all PCOS and control women. According to their BMI, the women were classified into two categories: normal weight if the BMI was 18–25 kg/m^2, and overweight and obese if the BMI was ≥ 25–30 kg/m^2 and over.

The study group was also divided according to the LH concentration. One group included 27 women with normal LH value in serum and the other group consisted of ten women with elevated LH.

Blood pressure was measured according to the recommendations of the European Society of Hypertension [16]. Blood was collected at 8.00–8.30 am after an overnight fast. Serum samples were stored at –20°C.

Basal blood samples were taken for quantifying circulating hormones (LH, FSH, T, A), DHEA-S, SHBG, INSL3, and glucose and insulin. Free and bioactive testosterone was calculated using a Free&Bioavailable Testosterone calculator (http://www.issam.ch/freetesto.htm). Insulin resistance was assessed by the measurement of fasting insulin and the homeostasis model assessment (HOMA-IR) [17].

Table I. *Clinical characteristics of women with polycystic ovary syndrome and controls*
Tabela I. *Charakterystyka kliniczna kobiet z grupy z zespołem policystycznych jajników oraz z grupy kontrolnej*

	PCOS women ($\bar{x}$ ± SD)		Controls ($\bar{x}$ ± SD)	
	BMI < 25 kg/m², n = 27	BMI ≥ 25 kg/m², n = 10	BMI < 25 kg/m², n = 31	BMI ≥ 25 kg/m², n = 3
BMI [kg/m²]	21.63 ± 2.08	32.16 ± 6.6	20.37 ± 1.59	27.82 ± 2.71
WHR	0.78 ± 0.05	0.83 ± 0.05	0.78 ± 0.06	0.80 ± 0.06
Age	27.23 ± 4.71	27.00 ± 3.40	24.35 ± 1.40	23.00 ± 1.00

BMI — body mass index; WHR — waist-to-hip ratio; data is presented as mean (x) ± standard deviation (SD)

The LH, FSH, insulin, T, and SHBG serum concentrations were measured with electrochemiluminescence immunoassays (Roche Diagnostics, Germany) performed with the use of an Elecsys 2010 analyser (Hitachi, Japan). Androstenedione concentrations were measured with chemiluminescence immunoassays using the Immulite 2000 system (Siemens Healthcare Diagnostics Products Ltd, UK).

The serum level of INSL3 was measured with a commercial fluorescent ELISA kit (Phoenix Pharmaceutical, Belmont, CA, USA). The fluorometric version of this immunoassay was chosen because among commercial INSL3 assays the reported linear range (13–180 pg/mL) was most suitable for the measurement of this hormone in women (the linear ranges for EIA and RIA kits, according to the same manufacturer, were 90–1,500 pg/mL and 160–1,180 pg/mL, respectively).

Statistical analyses were performed using Statistics 9.0 for Windows. All results are expressed as the mean ± standard deviation. Statistical comparisons between the control and study groups were made using the ANOVA test. Spearman's correlation test was used to determine the relationship between analysed sets of values. Differences and correlations were considered to be statistically significant when $p < 0.05$.

Studies were performed in the Department of Internal Medicine and Endocrinology in the Public Central Teaching Hospital, Medical University of Warsaw. The protocol was approved by the local ethics committee and written informed consent was obtained from each subject.

Results

Previously obtained pathophysiological data prompted us to examine serum INSL3 levels in PCOS women with a normal range of LH and a BMI of < 25 kg/m², and to determine the relationships between LH and INSL3, and INSL3 and the degree of ovarian androgenism [5].

The PCOS women and controls were of similar age (27.0 ± 4.0 and 24.2 ± 1.0, respectively) and waist to hip ratio (0.8 ± 0.1 and 0.8 ± 0.1). We did not find any statistically significant difference in systolic and diastolic blood pressure between PCOS women and the controls (114.4 ± 10.9 mm Hg and 112 ± 8.6 mm Hg, 71.3 ± 9.1 mm Hg and 71.6 ± 2.9 mm Hg, respectively). The BMI was significantly higher in the PCOS group compared to the control (24.5 ± 6.0 *v.* 21.0 ± 2.7) ($p = 0.009$). The difference in androstenedione level between the two groups was also significant (12.6 ± 3.7 ng/mL *v.* 8.2 ± 1.5 ng/mL) ($p = 0.0001$). PCOS women showed slightly higher serum level of INSL3 compared to the control (64.6 ± 27.7 ng/mL and 62.7 ± 20.0 ng/mL, respectively) but the difference was not statistically significant ($p > 0.05$). However, there was a significant difference ($p < 0.001$) in the SHBG level between the PCOS group and the control (46.9 ± 29.3 nmol/L and 65.6 ± 29.4 nmol/L, respectively). When PCOS women were classified according to their BMI, higher concentrations of INSL3 were observed in the overweight and obesity subgroup; nevertheless, the differences were not significant (Fig. 1). Because of the small number of women

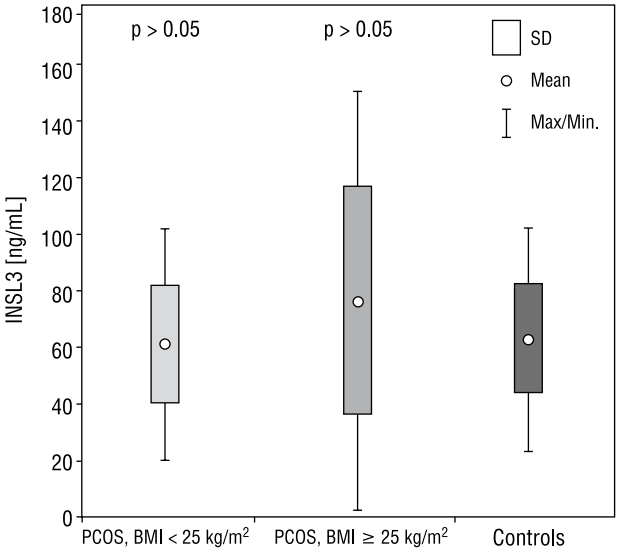

Figure 1. *INSL3 levels in women with polycystic ovary syndrome (PCOS) with body mass index (BMI) < 25 kg/m², with BMI ≥ 25 kg/m² and controls; SD — standard deviation*

Rycina 1. *Stężenia INSL3 u kobiet z zespołem policystycznych jajników (PCOS), u których wskaźnik masy ciała (BMI) wynosi ≥ 25 kg/m², > 25 kg/m², oraz w grupie kontrolnej*

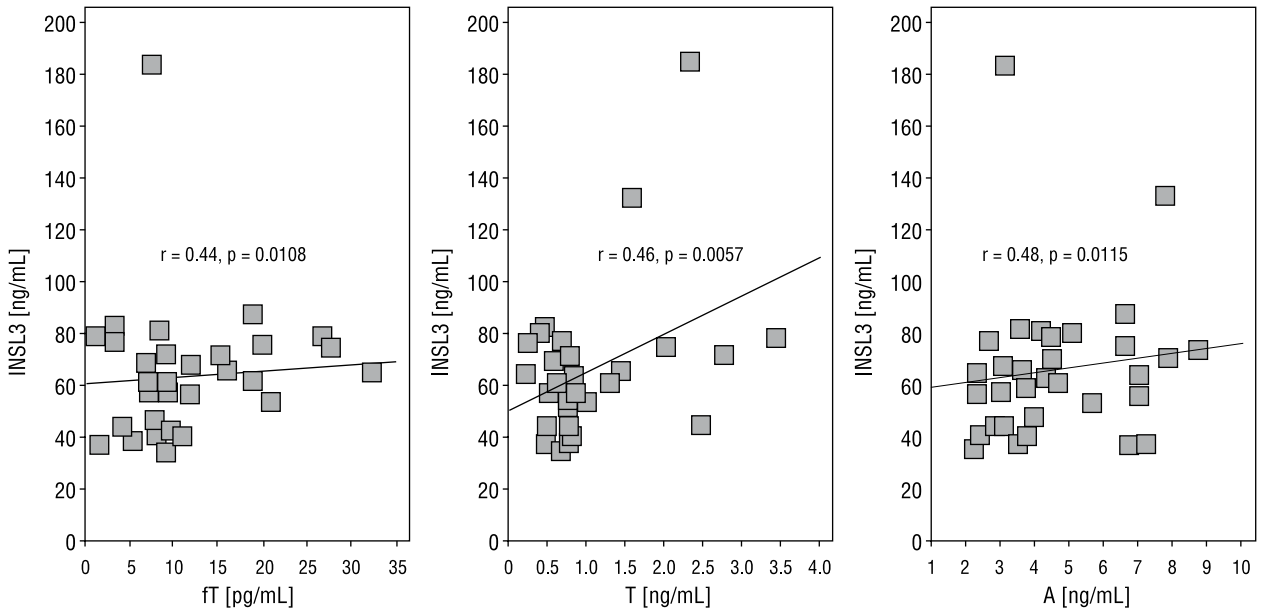

Figure 2. *Correlation between serum levels INSL3 and free testosterone (fT), total testosterone (T) and androstendione (A) in the subgroup with polycystic ovary syndrome with body mass index < 25 kg/m²*

Rycina 2. *Zależność między stężeniem INSL3 w osoczu i stężeniami wolnego testosteronu (fT), całkowitego testosteronu (T) i androstendionu (A) w podgrupie z zespołem policystycznych jajników i wskaźnikiem masy ciała < 25 kg/m²*

in the control group with BMI of ≥ 25 kg/m² (n = 3), we did not classify the group according to their BMI.

The correlation between INSL3 and androstenedione was evident in members of the PCOS subgroup with a BMI of < 25kg/m² (r = 0.48, p = 0.0115; Fig. 2). Such a correlation was not found in the PCOS obesity subgroup p > 0.05). A strong correlation between INSL3 and total and free testosterone (r = 0.46, p = 0.0057 and r = 0.44, p = 0.0108, respectively) was observed between PCOS women with a BMI of < 25kg/m² (Fig. 2). It is noteworthy that there was no correlation between INSL3 and androgens, or between INSL3 and LH levels, in the control group (p > 0.05).

No significant correlation was found between INSL3 and FSH, 17-OHP, DHEA-S, glucose, basal insulin concentration or HOMA-IR. The influence of body mass index on the endocrine parameters of PCOS women is shown in Table II. There was no statistically significant correlation between INSL3 and LH in either the entire PCOS group or in the two PCOS subgroups (Fig. 3). Also, we found no correlation between INSL3 and LH in non-obese PCOS women within the normal range.

Discussion

INSL3, a peptide almost exclusively of gonadal origin, is a valuable diagnostic tool in three areas of medicine: 1. It defines age-related hypoandrogenaemia [18, 19]. 2. Its level in amniotic fluid is an indicator of foetal development [20]. 3. Its concentration in PCOS women is a marker of androgenisation. In men, INSL-3 shows the Leydig cell function: in amniotic fluid it can be detected in a male foetus and its level reflects the time of the trans-abdominal phase of testicular descent. In a woman, its role requires further investigation.

The results of this study indicate that the circulating level of INSL3 is related to androgen status, especially in normal weight PCOS women. We identified a strong correlation between serum INSL3 and androstenedione in PCOS subjects with a BMI of < 25 kg/m² (r = 0.48, p = 0.0115). Moreover, such a correlation was absent in PCOS patients with obesity. Previous pathophysiological data prompted us to examine serum INSL3 levels in PCOS women with a normal range of LH and a BMI of < 25 kg/m², and to determine the relationships between LH and INSL3, and between INSL3 and the degree of ovarian androgenism. The lack of correlation between the levels of INSL3 and LH in normal weight women with levels of LH within the normal range still supports a cause-and-effect relationship.

Data from previous studies suggests that an increased LH level may be responsible for androgen production in the ovarian tissue through the regulatory action of INSL3. INSL3 is considered to be a hormone related to LH-dependent ovarian hyperandrogenism, especially in normal weight women. Increased levels of LH are typical for women with PCOS, particularly non-obese individuals with a BMI of < 25 kg/m². Gambineri et al. showed that circulating levels of INSL3 are significantly elevated in women with PCOS, and they found

Table II. *Influence of body mass index (BMI) on the endocrine parameters of polycystic ovary syndrome (PCOS) women*
Tabela II. *Wpływ wskaźnika masy ciała (BMI) na parametry endokrynologiczne kobiet z zespołem policystycznych jajników (PCOS)*

Parameters	PCOS		p value
	BMI <25 kg/m² (n = 27)	BMI ≥ 25 kg/m² (n = 10)	
Fasting insulin [mIU/mL]	6.1 ± 4.1	14.4 ± 10.4	> 0.05
HOMA-IR	1.2 ± 0.8	3.2 ± 2.5	> 0.05
LH [mIU/mL]	9.1 ± 5.9	9.6 ± 5.9	> 0.05
LH/FSH	1.4 ± 0.9	2.0 ± 1.1	> 0.05
T [ng/mL]	0.72 ± 0.36	0.6 ± 0.37	> 0.05
SHBG [nmol/L]	48.2 ± 27.7	43.6 ± 34.7	> 0.05
bioT [pg/mL]	270.5 ± 154.4	322.8 ± 264.1	> 0.05
bioT (%)	38.7 ± 11.9	44.4 ± 17.6	
fT ([pg/mL]	10.7 ± 6.1	13.1 ±10.5	> 0.05
fT (%)	1.5 ± 0.5	1.8 ± 0.7	
A [ng/mL]	4.5 ± 1.8	4.6 ± 2.0	> 0.05
INSL 3 [ng/mL]	60.7 ± 20.9	75.0 ± 40.5	> 0.5
DHEA-S [μg/dL]	314.3 ± 126.1	346.3 ± 143.2	> 0.05

HOMA-IR — homeostasis model assessment-insulin resistance; LH — luteinising hormone; FSH — follicle-stimulating hormone; DHEA-S — dehydroepiandrosterone sulphate; T — total testosterone; SHBG — sex hormone-binding globulin; bioT — bioactive testosterone; fT — free testosterone; A — androstenedione; INSL3 — insulin-like factor 3

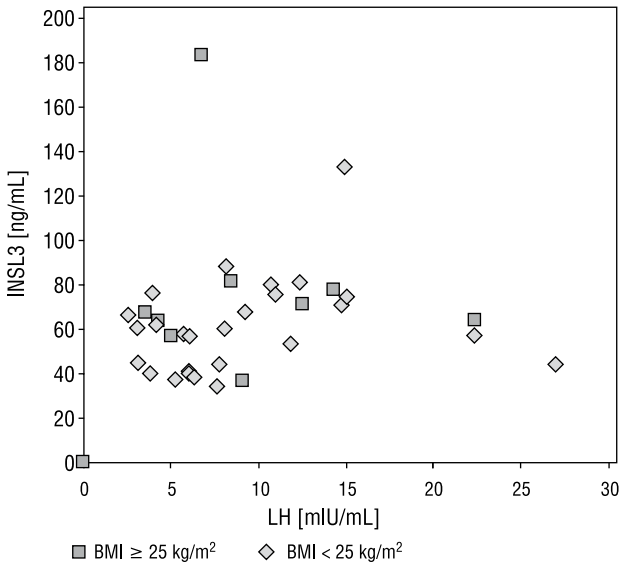

Figure 3. *Relationship between serum levels of INSL3 and luteinising hormone (LH) in the group with polycystic ovary syndrome divided according to body mass index (BMI)*

Rycina 3. *Związek między stężeniem INSL3 w osoczu i stężeniem hormonu luteinizującego (LH) w grupie z zespołem policystycznych jajników zależnie od wskaźnika masy ciała (BMI)*

a negative correlation between serum LH and body weight in PCOS women [2]. Overweight and obese women with PCOS are characterised by a significantly lower LH concentration in serum compared to normal weight PCOS women. The LH pulse amplitude and the LH response to GnRH tend to decrease with increasing BMI. It has also been demonstrated that PCOS women with a higher BMI show increased hyperandrogenism compared to PCOS individuals of normal weight [21]. LaZovic et al. concluded that BMI negatively contributes to LH concentration [22]. Lewandowski et al. [23] demonstrated a marked increase in GnRH-stimulated LH/FSH ratio in women with PCOS diagnosed according to the Rotterdam criteria, and suggested that assessment of GnRH-stimulated LH/FSH ratio might be potentially useful as a confirmatory test for the diagnosis of PCOS. Although we have not found a correlation between LH and INSL3 in non-obese PCOS women with LH in the normal range, this group showed a very strong correlation between INSL3 and androstenedione, and free and total testosterone.

In PCOS women with BMI of < 25kg/m² and high LH concentration, we found an elevated level of INSL3, but in the subgroup of patients with a normal LH level, the concentration of INSL3 varied within the normal range. These findings suggest a role for LH and INSL3 in the pathogenesis of PCOS. Insulin resistance is known to be more common in obese patients with PCOS [22, 24]. In the present study, both the fasting insulin level and HOMA-IR were higher in the obese

PCOS subgroup, although none of the increases were statistically significant. In women, INSL3 is expressed in the ovarian follicular theca cells and corpus luteum representing the ovarian equivalent of the Leydig cells [25, 26]. The INSL3 has also been detected in mouse [27], cow, and sheep ovaries [28, 29], and in the ovary of the marmoset monkey [30]. Further studies are necessary to find out whether INSL3 plays a role in the aetiology of PCOS or influences PCOS phenotype. It has previously been shown that PCOS women exhibit significantly elevated levels of INSL3 in peripheral serum. There is also a good correlation between INSL3 concentration and the number of ovarian follicles determined by ultrasonography [21].

One limitation of the present study is the small number of patients investigated. Expanded studies are required in order to collect more data on INSL3 levels in PCOS women and to determine whether this information might be used alone or with other elements in the diagnosis of ovarian hyperandrogenisation. As one of several parameters used in the diagnosis of PCOS, INSL3 may provide important supporting information on the pathophysiology of this disease.

Conclusions

The results of this study indicate that there is a positive correlation between androgen levels and INSL3 in PCOS women, especially those with BMI < 25 kg/m^2. Probably, this is a mechanism leading to hyperandrogenesis in PCOS women without overweight or obesity.

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