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The effect of weight loss on inflammation in obese women with polycystic ovary syndrome

Wpływ redukcji masy ciała na proces zapalny u otyłych kobiet z zespołem policystycznych jajników

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Abstract

Introduction: The aim of the present study was to evaluate the effect of modest weight reduction on serum concentrations of tumour necrosis factor α (TNF-α), TNF soluble receptors (sTNFRs) and interleukin-6 (IL-6) in obese women with polycystic ovary syndrome (PCOS).
Material and methods: The study group consisted of 15 obese women with PCOS (mean age 28.5 ± 7.7 years). Serum concentrations of TNF-α, sTNFRs and IL-6, insulin, FSH, LH, DHEAS, androstendione, total and free testosterone, cortisol, 17OH-progesterone, oestradiol and sex hormone binding globulin (SHBG), glucose, total cholesterol, HDL cholesterol and triglycerides were measured before treatment and after 10% weight loss.
All patients were advised to follow a 1000–1200 kcal diet with a limited intake of simple carbohydrate and animal fats and to exercise regularly (30 min, 3 times a week).
Body composition was measured by bioimpedance. Serum concentrations of TNF-α, sTNFRs and IL-6 were determined by enzyme linked immunosorbent assay (ELISA). Plasma insulin, FSH, LH, DHEAS, androstendione, total and free testosterone, cortisol, 17OH-progesterone, oestradiol and SHBG were measured by a commercial RIA. Blood glucose, total cholesterol, HDL cholesterol and triglycerides were measured by an enzymatic procedure.
Results: We observed no differences in serum concentrations of TNF-α, sTNFRs or IL-6 after treatment.
Conclusions: It seems that more than a modest weight reduction is necessary to obtain a decrease in serum concentrations of proinflammatory cytokines and an improvement in ovarian function in obese women with polycystic ovary syndrome.
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Key words: *proinflammatory cytokines, weight reduction, polycystic ovary syndrome*

Streszczenie

Wstęp: Celem prezentowanej pracy była ocena wpływu umiarkowanej redukcji masy ciała na stężenia czynnika martwicy nowotworu α (TNF-α, *tumour necrosis factor* α) rozpuszczalnych receptorów dla TNF w osoczu otyłych kobiet z zespołem policystycznych jajników.
Materiał i metody: Piętnaście otyłych kobiet (średnia wieku 28,5 ± 7,7 roku) z zespołem policystycznych jajników poddano kuracji odchudzającej składającej się z diety 1000 kcal oraz aktywności fizycznej. Oceniano stężenie czynnika martwicy nowotworów, rozpuszczalnych receptorów dla TNF oraz IL-6, insuliny, FSH, LH, DHEAS, androstendionu, całkowitego oraz wolnego testosteronu, kortyzolu, 17OH-progesteronu, estradiolu, SHBG, glukozy, cholesterolu całkowitego, cholesterolu frakcji HDL oraz triglicerydów przed rozpoczęciem kuracji odchudzającej oraz po 10-procentowym zmniejszeniu masy ciała. Pacjentkom zalecono przestrzeganie diety 1000–1200 kcal z ograniczeniem spożycia węglowodanów prostych oraz tłuszczów zwierzęcych oraz regularną aktywność fizyczną (co najmniej 30 min, 3 razy w tygodniu).
Skład ciała oznaczono przy użyciu metody bioimpedancji. Stężenia TNF-α, receptorów dla TNF oraz IL-6 w surowicy oznaczono metodą ELISA. Stężenie insuliny, FSH, LH, DHEAS, androstendionu, całkowitego i wolnego testosteronu, kortyzolu, 17OH-progesteronu, estradiolu oraz SHBG oznaczono przy użyciu metody RIA. Stężenia glukozy, cholesterolu całkowitego, cholesterolu frakcji HDL oraz triglicerydów oznaczono metodą enzymatyczną.
Wyniki: Nie zaobserwowano różnic w stężeniu TNF-α, rozpuszczalnych receptorów dla TNFR oraz IL-6 przed rozpoczęciem kuracji oraz po redukcji masy ciała.
Wnioski: Wydaje się, że umiarkowana redukcja masy ciała jest niewystarczająca do uzyskania zmniejszenia stężeń cytokin prozapalnych u otyłych kobiet z zespołem policystycznych jajników oraz do poprawy czynności jajników. **(Endokrynol Pol 2008; 59 (1): 13–17)**

Słowa kluczowe: *cytokiny prozapalne, redukcja masy ciała, zespół policystycznych jajników*

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Introduction

Obesity is a very common clinical feature in women affected by polycystic ovary syndrome (PCOS); approximately 50% of PCOS women are obese and their history of weight gain frequently precedes the onset of oligomenorrhea and hyperandrogenism [1].

It is well-known that hyperinsulinaemia and insulin resistance play an important part in the pathogenesis of PCOS [2]. It has been shown in a number of studies that the adipose tissue alone generates many substances which may participate in the development of insulin resistance, including free fatty acids, tumour necrosis factor α (TNF-α), interleukin-6 (IL-6), leptin and resistin [3].

Our previous studies have revealed increased serum concentrations of proinflammatory cytokines such as TNF-α and IL-6 in obese subjects without additional disease [4, 5, 6]. However, we observed no difference between PCOS and obese women without additional disease in plasma TNF-α concentrations, but plasma concentrations of both TNF soluble receptors (sTNFRs) sTNFR1 and sTNFR2 were significantly increased and serum IL-6 concentrations were significantly decreased in women without additional diseases and in women with PCOS [7].

So far few studies have been carried out to evaluate the effect of weight loss on serum concentrations of TNF-α, sTNFRs and IL-6. In those that have been published a decrease in serum concentrations of TNF-α and IL-6 and an increase in serum concentrations of sTNFR1 and sTNFR2 were observed after weight loss [4–6].

Recent studies have revealed an improvement in ovarian function after weight reduction in obese women with PCOS [8, 9]. However, no studies have been conducted to investigate the influence of weight loss treatment on changes in TNF system activity and serum concentrations of IL-6 in obese women with PCOS. The aims of the present study, therefore, were to evaluate the effect of modest weight loss on serum concentrations of TNF-α, sTNFRs and IL-6 and to examine whether there is any association between these cytokines, changes in their concentrations and serum concentrations of androgens after weight loss.

Materials and methods

The study group consisted of 15 obese women with PCOS (aged 28.5 ± 7.7 years). The diagnosis of PCOS was based on both clinical symptoms, primarily oligomenorrhea and hirsutism, and laboratory findings, including serum androgen levels (DHEAS, androstendione, testosterone, and free testosterone) which were above the upper limit of the norm for the respective assay.

Table I. *Patient characteristics and the effect of weight-reducing treatment*

Tabela I. *Charakterystyka pacjentów i efekty kuracji odchudzającej*

n = 15	Before	After
Weight (kg)	96.6 ± 17.0	84.7 ± 14.5 ***
BMI [kg/m^2]	36.1 ± 6.6	31.6 ± 5.8 ***
Fat-free mass (kg)	53.8 ± 8.1	49.1 ± 4.7 *
Fat-free mass (%)	56.4 ± 6.7	58.9 ± 6.4
Body fat (kg)	42.6 ± 12.5	35.5 ± 11.2 ***
Body fat (%)	43.5 ± 6.7	41.1 ± 6.4

* — $p < 0.01$; *** — $p < 0.00001$

The patients were included in the study directly after hormonal and ultrasonographic diagnosis of PCOS.

In all the subjects included in the study Cushing's syndrome, thyroid dysfunction, androgen-secreting tumour and enzyme deficiency (of 21-hydroxylase in particular) were excluded. All patients were free of other diseases and were not undergoing pharmacological treatment. Other exclusion criteria included evidence of present or recent (during the preceding three months) infectious disease, fever, smoking or drug therapy. The characteristics of the study group are summarised in Table I.

The women underwent weight loss treatment. They were advised to follow a 1000–1200 kcal diet with a limited intake of simple carbohydrate and animal fats and to exercise regularly (30 min, 3 times a week). Pharmacological treatment was not administered.

The study was only conducted after informed consent had been obtained from all the subjects. The study was approved by the local committee for ethics.

Before and after treatment body weight and height were measured and body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in metres. Body composition was determined by impedance analysis using a Bodystat analyser.

All the women were studied within 10 days of the onset of menstruation. Blood samples were taken in the morning subsequent to an overnight fast. The samples were centrifuged (1000 g) at room temperature for 10 minutes. The serum obtained was drawn into plastic vials, and stored at –80°C until the time of the assay.

Assays

Blood glucose, total cholesterol, HDL cholesterol and triglycerides were measured by an enzymatic procedure (Diagnostic Products Corporation USA). LDL cholesterol was calculated with the Friedewald formula. Plasma insulin was measured by a commercial RIA (Diagnostic Pro-

ducts Corporation, USA) with a lower limit of sensitivity of 1.2 mIU/ml and intra-assay and inter-assay coefficients of variations of 5.2% and 5.8% respectively.

Insulin resistance was assessed on the basis of fasting serum concentrations of glucose and insulin, the HOMA index being calculated by the formula HOMA = fasting serum concentration of insulin (mIU/ml) × × fasting serum concentration of glucose (mmol/l)/22.5. The normal range according to HOMA is < 2.77.

Serum FSH and LH were determined by RIA (Orion Diagnostics, Finland) with a lower limit of sensitivity of 0.1 IU/l and 0.07 IU/l respectively. The respective intra-assay and inter-assay coefficients of variation were 2.0 and 4.2 for FSH and 2.6 and 4.4 for LH.

DHEAS, androstendione, free testosterone, cortisol, 17OH-progesterone and sex hormone binding globulin (SHBG) were assayed by RIA (Diagnostic Products Corporation, USA) with lower detectable concentrations of 0.03 mmol/l; 0.1 nmol/l; 0.5 pmol/l; 5.5 nmol/l; 0.2 nmol/l; 3 nmol/l respectively.

The respective inter-assay and intra-assay coefficients of variation were 4.7% and 8.3% for DHEAS, 4.2% and 7.6% for androstendione, 11.6% and 11.6% for free testosterone, 4.3% and 5.2% for cortisol, 5.6% and 8.0% for 17OH-progesterone and 2.7% and 10.2% for SHBG.

Oestradiol (E2), total testosterone, and progesterone were determine by RIA (Orion Diagnostics, Finland) with a lower sensitivity of 20.0 pmol/l, 0.1 nmol/l and ≤ 0.3 nmol/l respectively. The respective inter-assay and intra-assay coefficients of variation were 5.8% and 6.5% for E2, 5.3% and 5.4% for total testosterone and 4.3% and 5.0% for progesterone.

Of the proinflammatory cytokines, TNF-α, sTNFR1 and sTNFR2 and IL-6 were determined by enzyme linked immunosorbent assay (ELISA) (Genzyme Diagnostics, Cambridge, USA, R&D Systems); IL-6 was also assayed by ELISA (Diagnostic Products Corporation, USA).

The minimum detectable dose of TNF-α is typically less than 0.18 pg/ml. The mean intra-assay coefficient of variance was 14.4%, range 8.7–14.8%, and the mean inter-assay coefficient of variance was 18.7%, range 16.1––22.6%. The minimum detectable dose of sTNFR1 is typically less than 3.0 pg/ml. The mean intra-assay coefficient of variance was 2.9%, range 2.7–6.9% and the mean inter-assay coefficient of variance was 3.7%, range 5.8––8.8%. The minimum detectable dose of sTNFR2 is typically less than 1.0 pg/ml. The mean intra-assay coefficient of variance was 2.5%, range 1.6–2.5% and the mean inter-assay coefficient of variance was 3.5%, range 3.5––5.1%. The minimum detectable dose of IL-6 is typically less than 1.0 pg/ml. The mean intra-assay coefficient of variance was 10% and the mean inter-assay coefficient of variance was < 10%.

Table II. *Plasma lipids, glucose, insulin and HOMA*

Tabela II. *Stężenia lipidów, glukozy i insuliny w osoczu oraz wskaźnik HOMA*

	Before	After weight loss
Total cholesterol [mmol/l]	5.2 ± 1.0	4.9 ± 0.7
HDL cholesterol [mmol/l]	1.3 ± 0.3	1.2 ± 0.3
LDL cholesterol [mmol/l]	3.4 ± 1.1	3.2 ± 0.7
Triglycerides [mmol/l]	1.4 ± 0.7	1.1 ± 0.4*
Glucose [mmol/l]	5.3 ± 0.7	5.1 ± 0.7
Insulin [μIU/ml]	13.4 ± 8.6	17.1 ± 8.7
HOMA	3.3 ± 2.5	4.0 ± 2.1

* — $p < 0.05$

Statistics

Statistical analyses were performed with STATISTICA software. All text and table values were expressed as means ± SD. P values less than 0.05 were considered to be statistically significant. The non-parametric Mann Whitney U test was used to analyse differences between the study group and controls. Spearman's correlation coefficients were used to test the correlation between variables. We used the Bonferroni correction for the multiple comparisons when four serum markers were tested in the same set of patients.

Results

The mean weight loss during treatment was 11.9 ± 4.5 kg (12.2 ± 3.6%). The effect of the weight loss treatment is presented in Table I.

There were no differences between serum concentrations of insulin, glucose, HOMA index and lipids before and after treatment. These data are shown in Table II.

No significant changes of serum FSH, LH, LH/FSH ratio, total and free testosterone, androstendione and 17OH-progesterone were observed after weight loss (Table III), nor did we observe differences in concentrations of plasma TNF-α, sTNFRs sTNFR1 and sTNFR2 or IL-6 after weight reduction (Table IV).

Correlations between the anthropometric parameters and the remaining parameters before treatment

We observed significant positive linear correlations between serum concentration of IL-6 and body mass ($r = 0.55$; $p < 0.05$), BMI ($r = 0.63$; $p < 0.05$), body fat in kilograms ($r = 0.67$; $p < 0.01$) and body fat percentage ($r = 0.77$; $p < 0.005$).

Significant positive linear correlations were also shown between serum concentrations of sTNFR1 and

Table III. *Serum concentrations of hormones*
Tabela III. *Stężenia hormonów w surowicy*

	Before	After
FSH [IU/l]	6.3 ± 2.3	5.8 ± 2.8
LH [IU/l]	7.7 ± 5.8	9.3 ± 7.6
DHEAS [μg/dl]	525.7 ± 168.1	244. 3 ± 125.4
Androstendione [ng/ml]	4.6 ± 2.0	5.4 ± 2.4
Total testosterone [nmol/l]	2.4 ± 0.9	2.0 ± 0.8
Free testosterone [pg/ml]	4.1 ± 2.2	4.0 ± 2.1
Cortisol [μg/dl]	19.5 ± 10.4	17.9 ± 7.7
Oestradiol [pmol/l]	330.5 ± 320.2	452.6 ± 482.1
17OH-progesterone [ng/ml]	2.2 ± 1.8	2.0 ± 1.3
SHBG [nmol/l]	30.2 ± 17.1	33.2 ± 17.1

Table IV. *Serum concentrations of TNF-a, TNF receptors and IL-6*
Tabela IV. *Stężenie TNF-α, receptorów TNF i IL-6 w surowicy*

	Before	After
TNF-α [pg/ml]	6.6 ± 3.0	6.1 ± 3.6
sTNFR1 [pg/ml]	2209.7 ± 979.7	2043.7 ± 631.4
sTNFR2 [pg/ml]	2415.7 ± 692.8	2001.8 ± 793.2
IL-6 [pg/ml]	6.0 ± 2.0	4.7 ± 2.1

body mass ($r = 0.58$; $p < 0.05$), BMI ($r = 0.58$; $p < 0.05$) and body fat in kilograms ($r = 0.62$; $p < 0.02$).

We also observed a negative linear correlation between serum concentrations of DHEAS and body mass ($r = –0.53$; $p < 0.05$).

Correlations between anthropometric parameters and the remaining parameters after treatment

Significant positive linear correlations wee shown between serum concentrations of TNF-α and body mass ($r = 0.58$; $p < 0.05$) and between serum concentrations of insulin and body mass ($r = 0.58$; $p < 0.05$) and BMI ($r = 0.54$; $p < 0.05$).

Correlations between serum cytokines levels and the remaining parameters before treatment

A positive linear correlation was found between serum TNF-α and TNFR2 ($r = 0.60$; $p < 0.05$).

Correlations between serum cytokine levels and the remaining parameters after treatment

Positive linear correlations were found between concentrations of serum TNFR1 and LH ($r = 0.66$; $p = 0.01$) and between serum IL-6 and estuarial concentrations ($r = 0.64$; $p = 0.04$).

Discussion

Some recent studies have revealed that in obese women with PCOS weight loss improves insulin sensitivity, decreases serum concentrations of androgens and increases serum concentration of SHBG [8, 10]. In the present study in 15 obese subjects with PCOS we obtained a mean weight reduction of 12.2 ± 3.6%. However, favourable changes in hormonal parameters were not observed. This agrees with the results obtained by Moran et al. [11], which showed that only 44% of obese subjects with PCOS saw an improvement in the regularity of periods after weight reduction. However, Dixon and O'Brien [12] reported an increase in SHBG and a decrease in testosterone only one year after gastric by-pass and a weight reduction of 43%. On the basis of these results it seems that more than modest weight reduction is necessary to obtain an improvement in the hormonal profile of obese women with PCOS.

Our recent results and those of other authors [7, 13] have revealed that PCOS is not *per se* associated with increased chronic inflammation. However, as pointed out above, there have so far been no studies concerning the influence of weight loss treatment on inflammation in obese women with PCOS.

An interesting and novel finding of the present study is that, in contrast to the results described above [4–6], we did not observe changes in serum concentrations of TNF-α after weight reduction. Therefore it seems that in obese women with PCOS adipose tissue is not the most important source of TNF-α and that there are other mechanisms regulating serum concentrations of TNF-α in this disease. Winkler et al. [14] revealed expression of the TNF-α protein in both subcutaneous and visceral fat deposits and a correlation of serum TNF-α with adipocyte cell volume in obese subjects without additional disease.

Our recent study revealed increased serum concentrations of sTNFRs in obese women with PCOS when compared to obese women with no additional disease [7]. Previously we had also observed significantly increased serum concentrations of sTNFRs after weight loss [4]. However, the present study did not reveal changes in serum concentrations of sTNFR1 and sTNFR2 after weight loss in PCOS women. This may be one of the causes of the lack of decrease in serum concentrations of TNF-α. Besides blocking TNF binding by cell surface receptors, sTNFRs may stabilise and even enhance the effects of TNF-α by slowing down the dissociation rate from a trimeric to an inactive monomeric structure. The second property of soluble receptors makes it possible that they serve as a reservoir of bio-active TNF which prolongs the TNF-α activity [15]. However, further studies are required to establish the causes of the lack of

change in serum concentrations of sTNFRs after weight reduction.

In the present paper serum concentration of IL-6 decreased, albeit not significantly, after weight loss. It therefore seems that this cytokine is most dependent on fat deposits in PCOS too. This is in accordance with the results of our earlier study [6] and with the correlations observed in the present study, namely the significant positive linear correlations between serum concentration of IL-6 and body mass, BMI, body fat in kilograms and body fat percentage.

In summary, this is a preliminary study to examine the effect of weight reduction on serum TNF system activity and serum concentrations of IL-6. Moreover, the results obtained differ from those of studies involving women with simple obesity but without additional disease. Further studies are therefore necessary to clarify the role of proinflammatory cytokines in PCOS and the mechanisms regulating their serum concentrations.

Conclusions

It seems that more than a modest weight reduction is necessary to obtain a decrease in serum concentrations of proinflammatory cytokines and an improvement in ovarian function in obese women with polycystic ovary syndrome.

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