



The effect of weight loss on serum concentrations of FAS and tumour necrosis factor alpha in obese women

Wpływ zmniejszenia masy ciała na stężenie FAS oraz czynnika martwicy nowotworów w surowicy otyłych kobiet

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Abstract

Introduction: Apoptosis can influence both adipose tissue mass and its distribution. The suprafamily of tumour necrosis factor (TNF) receptors stimulate apoptosis. The aim of the study was to assess serum concentrations of tumour necrosis factor alpha (TNF- α), TNF soluble receptors (sTNFRs) and FAS in obese subjects and to examine the changes in these parameters after weight loss.

Material and methods: The study group consisted of 23 obese women without additional disease aged 36.6 ± 10.9 years. These were examined before and after three-month weight reduction treatment consisting of a diet of 1000 kcal/day and physical exercise. The control group comprised 17 lean healthy women aged 40.3 ± 5.5 years. Blood samples were taken in the morning after an overnight fast. Serum concentrations of TNF- α , sTNFRs and FAS were measured by enzyme linked immunosorbent assay (ELISA). Serum concentrations of insulin were measured by RIA. Serum concentrations of glucose, total cholesterol, HDL cholesterol and triglycerides were measured by an enzymatic procedure.

Results: The mean weight loss over the three-month treatment was 11.4 ± 3.1 kg. Following weight loss, serum TNF- α concentrations decreased significantly (7.3 ± 3.0 vs. 5.4 ± 1.6 pg/ml; $p < 0.005$) and concentrations of sTNFRs increased significantly (1222.6 ± 211.8 vs. 1325.6 ± 261.6 pg/ml; $p < 0.05$ and 1881.5 ± 337.2 vs. 2057.4 ± 358.7 pg/ml; $p < 0.05$ respectively). However, no changes in serum concentrations of FAS were observed after weight loss.

Conclusion: We observed increased serum concentrations of TNF- α but not of FAS in obese women. The concentrations of TNF decreased and those of sTNFRs increased after weight loss. However, the weight reduction therapy did not change serum concentrations of FAS. (Pol J Endocrinol 2008; 59 (1): 18–22)

Key words: obesity, FAS, TNF α , sTNFRs

Streszczenie

Wstęp: Proces apoptozy może wpływać zarówno na masę, jak i na rozmieszczenie tkanki tłuszczowej. Nadrodzina receptorów dla czynnika martwicy nowotworów (TNF, *tumour necrosis factor*) stymuluje apoptozę. Celem pracy była ocena stężeń TNF- α , rozpuszczalnych form receptorów dla TNF oraz FAS w surowicy otyłych kobiet oraz ich zmiany po zmniejszeniu masy ciała.

Materiał i metody: Grupę 23 otyłych kobiet bez chorób towarzyszących (średni wiek $36,6 \pm 10,9$ lat) poddano 3-miesięcznej kuracji odchudzającej składającej się z diety 1000 kcal oraz aktywności fizycznej. Grupę kontrolną stanowiło 17 zdrowych kobiet z prawidłową masą ciała (średnia wieku $40,3 \pm 5,5$ roku). Próbkę krwi pobierano na czczo w godzinach porannych. Stężenia TNF- α , receptorów dla TNF oraz FAS oznaczono metodą ELISA. Stężenie insuliny oznaczono przy użyciu metody RIA. Stężenia glukozy, cholesterolu całkowitego, cholesterolu frakcji HDL oraz triglicerydów oznaczono metodą enzymatyczną.

Wyniki: Średnie zmniejszenie masy ciała w trakcie 3-miesięcznej kuracji odchudzającej wyniosło $11,4 \pm 3,1$ kg. Stężenie TNF- α uległo obniżeniu ($7,3 \pm 3,0$ vs. $5,4 \pm 1,6$ pg/ml; $p < 0,005$), stężenie sTNFRs uległo podwyższeniu (odpowiednio: $1222,6 \pm 211,8$ vs. $1325,6 \pm 261,6$ pg/ml; $p < 0,05$ oraz $1881,5 \pm 337,2$ vs. $2057,4 \pm 358,7$ pg/ml; $p < 0,05$). Nie zaobserwowano zmian w stężeniu FAS po zmniejszeniu masy ciała.

Wnioski: U otyłych kobiet zaobserwowano wyższe stężenie TNF- α w osoczu w porównaniu z osobami z prawidłową masą ciała, nie zaobserwowano różnic w stężeniu FAS. Stężenie TNF uległo obniżeniu, a stężenie sTNFRs wzrosło po zmniejszeniu masy ciała. Redukcja masy ciała nie spowodowała zmian w stężeniu FAS. (Endokryol Pol 2008; 59 (1): 18–22)

Słowa kluczowe: otyłość, FAS, TNF- α , sTNFRs



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Introduction

Apoptosis can influence both adipose tissue mass and its distribution. The suprafamily of TNF receptors stimulate apoptosis.

Tumour necrosis factor α (TNF- α) is a proinflammatory cytokine produced by a wide range of cells, amongst which are fat cells [1]. The action of TNF- α includes modulation of lipid metabolism and insulin resistance, especially by defects in insulin-stimulated glucose disposal [2]. It decreases the activity of lipoprotein lipase, and increases hormone-sensitive lipase, thus preventing lipid accumulation [3]. TNF- α could be a local regulator of fat cell size, and its overproduction in adipocytes of obese animals may limit adipocyte size enlargement [4].

Tumour necrosis factor α acts by its two membrane and soluble receptors (sTNFRs). Of these, sTNFR1 dominates in the actions of TNF- α , such as apoptosis, cell differentiation and proliferation, cytotoxicity and insulin resistance, while sTNFR2 participates in the stimulation of cytokine production, cytotoxicity of T cells and insulin resistance [5].

FAS (CD95/APO-1) is a member of the tumour necrosis factor receptor family (TNFR) [6]. FAS is a 45-kDa type I membrane protein expressed constitutively in various tissues, such as the liver, lung, kidney, spleen, lymph nodes and ovary [7]. FAS and its specific ligand (FasL) have been implicated in the control of inflammation, response to infection, neoplasia, immune response and death of parenchymal cells in several organs [8, 9]. A defect of the FAS system can limit lymphocyte apoptosis and lead to autoimmunity and lymphoproliferation [10].

Many studies have shown that TNF- α and TNF receptors are expressed in fat cells and their production is increased in obesity [11, 12]. Our previous results [13, 14] and those of other studies [15, 16] have shown increased serum levels of TNF- α and sTNFRs in obese patients in comparison to lean subjects. However, it is still unknown how far obesity influences serum concentration of FAS. The aim of the study therefore was to assess serum concentrations of TNF- α , sTNFRs and FAS in obese subjects and to examine changes to the cytokine and its receptors after weight loss.

Material and methods

The study was carried out on 23 obese women weighing 97.5 ± 16.6 kg, aged 36.6 ± 10.9 years and with a body mass index (BMI) of 36.6 ± 5.6 kg/m². All subjects were diagnosed as having simple obesity with no concomitant diseases and without pharmacological treatment. The obese patients were stable in weight at the time of

enrolment and patients with a sudden loss or increase in weight were excluded from the study. Each had a history of obesity lasting for some years. All the patients had serum concentrations of glucose and insulin within the reference range.

The control group consisted of 17 apparently healthy women aged 40.3 ± 5.5 years, who were age-matched to the study subjects. Their weight and BMI were 60.4 ± 6.3 kg and 22.6 ± 1.8 kg/m² respectively.

The exclusion criteria included evidence of present or recent (during the preceding three months) infectious disease, fever or drug therapy.

The study was approved by the local committee for ethics. All the subjects had given their informed consent to the study.

All the obese patients participated in a three-month weight reduction programme with the following components:

- group instruction at two weekly intervals in behavioural and dietary methods of weight control;
- a 1000–1200 kcal/day balanced diet with 50–60% of carbohydrates, less than 30% of fat and 10–20% of protein;
- physical exercise (30–40 minutes every day of running, swimming or cycling).

The measurements were performed at the baseline (both study and control groups) and after the three-month programme (the study group only). Body weight and height were measured and BMI was calculated. Body composition was assessed by impedance analysis using the Bodystat analyser.

Determination of FAS, TNF- α and sTNFRs in the blood serum was carried out by enzyme-linked immunosorbent assay (ELISA). After an overnight fast 6–8 ml samples of venous blood were collected from each subject between 8 a.m. and 9 a.m. in the morning. Following clot formation, 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.

The Phoenix Pharmaceuticals Inc. kit was used for the FAS assay. The sensitivity of this was less than 20.0 pg/ml. The intra-assay and inter-assay coefficients of variation were $< 4.6\%$, and $< 2.9\%$ respectively.

Tumour necrosis factor- α and the soluble forms of both TNF- α receptors sTNFR1 and sTNFR2 were measured using a commercially available highly sensitive ELISA kit (Genzyme Diagnostics, Cambridge, USA).

The minimum detectable concentration 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 concentration of sTNFR1 is typically less than 3.0 pg/ml. The mean intra-assay coefficient of variance was 2.9%, range

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

Tabela I. Charakterystyka pacjentów i efekty kuracji odchudzającej

	Obese		Control
	Before	After	
Body mass (kg)	97.5 \pm 16.6	86.1 \pm 15.2 ***	60.4 \pm 6.3 ###
BMI [kg/m ²]	36.6 \pm 5.6	32.3 \pm 5.2 ***	22.6 \pm 1.8 ###
Fat-free mass (kg)	54.7 \pm 5.3	52.2 \pm 5.6	45.4 \pm 5.0 ###
Fat-free mass (%)	56.3 \pm 9.0	60.5 \pm 5.9 **	75.2 \pm 3.6 ###
Body fat (kg)	43.8 \pm 14.9	35.5 \pm 10.2 ***	15.0 \pm 3.2 ###
Body fat (%)	43.4 \pm 9.1	40.1 \pm 6.2 *	24.8 \pm 3.6 ###

* — $p < 0.05$; ** — $p < 0.005$; *** — $p < 0.0005$ obese before vs. after; ### — $p < 0.001$ obese before treatment vs. control

Table II. Plasma glucose, insulin and lipids

Tabela II. Stężenia glukozy, insuliny i lipidów w osoczu

	Obese		Control
	Before	After	
Glucose [mg/dl]	89.7 \pm 8.5	94.4 \pm 13.1	86.4 \pm 10.8
Insulin [μ IU/ml]	16.8 \pm 7.9	12.6 \pm 5.6*	7.8 \pm 3.4###
Total cholesterol [mg/dl]	201.0 \pm 34.5	198.6 \pm 31.6	205.1 \pm 25.2
HDL cholesterol [mg/dl]	51.9 \pm 9.0	55.7 \pm 8.2*	55.3 \pm 7.4#
LDL cholesterol [mg/dl]	130.0 \pm 35.2	129.76 \pm 30.1	134.0 \pm 28.3
Triglycerides [mg/dl]	96.6 \pm 36.3	89.0 \pm 26.1	91.9 \pm 45.4

* — $p < 0.005$ obese before vs. after; # — $p < 0.05$; ### — $p < 0.001$ obese before treatment vs. control

2.7–6.9%, and the mean inter-assay coefficient of variance was 3.7%, range 5.8–8.8%. The minimum detectable concentration 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%.

Plasma glucose, total cholesterol, HDL cholesterol and triglycerides were determined by an enzymatic procedure using a commercially available test kit (Cormay). LDL cholesterol was calculated using the Friedwald formula.

Insulin was determined by radioimmunoassay (DPC Diagnostic Products Corporation, Los Angeles, USA) with a lower limit of sensitivity of 1.2 μ IU/ml and intra-assay and inter-assay coefficients of variation of 5.2% and 5.8% respectively.

Statistical analysis

All text and table values are expressed as means \pm SD. The results were examined with the use of ANOVA with the Newman-Keuls correction. Wilcoxon rank-sum tests (for continuous and ordered variables) and Fisher's exact tests (for discrete variables) were used to compare baseline and post-follow-up clinical/laboratory charac-

teristics. Stepwise multivariate analysis was performed with serum levels of FAS, DFAS, TNF- α and DTNF- α as the dependent variables. A value of $p < 0.05$ was considered statistically significant.

Results

The characteristics of patients and the effects of treatment are presented in Table I.

Mean weight loss was 11.4 \pm 3.0 kg. BMI decreased from 36.6 \pm 5.6 at the baseline to 32.3 \pm 5.2 following treatment. Some significant differences in body composition were also found. The body weight reduction treatment led to a significant decrease in body fat (absolute and percentage $p < 0.005$ and $p < 0.05$ respectively), accompanied by an increase in fat-free mass percentage $p < 0.05$.

Serum concentrations of insulin decreased significantly and serum concentrations of HDL cholesterol increased significantly after weight loss ($p < 0.005$; $p < 0.005$ respectively) (Tab. II).

There were no differences between plasma FAS concentrations in obese patients and controls. In obe-

Table III. Serum concentrations of TNF- α , TNF receptors and FASTabela III. Stężenie TNF- α , receptorów TNF i FAS w surowicy

	Before	After	Control
TNF- α [pg/ml]	7.3 \pm 3.0	5.4 \pm 1.6**	2.3 \pm 0.5###
sTNFR1 [pg/ml]	1222.6 \pm 211.8	1325.6 \pm 261.6*	1144.0 \pm 102.0
sTNFR2 [pg/ml]	1881.5 \pm 337.2	2057.4 \pm 358.7*	1791.0 \pm 504.2
FAS [pg/ml]	8904.2 \pm 1777.2	8434.3 \pm 2122.6	8274.6 \pm 1459.9

* — $p < 0.05$; ** — $p < 0.005$ obese before vs. after; # — $p < 0.05$; ### — $p < 0.001$ obese before treatment vs. control

Table IV. Correlations between study parameters before treatment

Tabela IV. Korelacje między badanymi parametrami przed terapią

	Total cholesterol	LDL cholesterol	HDL cholesterol	Triglycerides	FAS	TNF
Age	0.47*	0.46*		0.44*		
Body mass	0.49*	0.52*				
BMI	0.56**	0.61*	-0.45*		0.43*	
FFM%	-0.52*	-0.51*			-0.43*	
FAS	0.62**	0.65***		0.43*		0.43*

* — $p < 0.05$; ** — $p < 0.01$

se subjects FAS levels did not change following weight loss (Tab. III).

The TNF- α determination showed significantly higher TNF levels in the obese subjects than in the controls ($p < 0.001$). Additionally, TNF- α levels decreased significantly after weight loss ($p < 0.005$) (Tab. III).

There were no differences between the obese patients and the controls in plasma sTNFRs concentrations (Tab. III). However, in the obese subjects sTNFRs levels increased significantly following weight loss ($p < 0.05$).

There was a positive correlation between BMI and FAS levels and between serum concentrations of FAS and TNF- α before treatment (Tab. IV).

No correlations were found between serum FAS, TNF- α and sTNFRs concentrations and age, body mass, body fat, glucose or insulin before and after weight reduction (Tab. V).

There were significant positive correlations between Δ body mass and Δ serum concentrations of insulin ($r = 0.43$; $p < 0.05$) and between Δ serum concentrations of insulin and Δ serum concentrations of sTNFR1 ($r = 0.49$; $p < 0.05$).

An analysis of the remaining regression coefficients did not reveal any significant differences.

Discussion

The present study evaluates baseline and post-weight loss concentrations of FAS and TNF- α , which seem to be po-

tent factors of apoptosis. As described above, FAS is a member of the tumour necrosis factor receptor family [6].

The TNF- α may exert a catabolic effect and could represent a form of local adipostat [4]. According to some studies, increased TNF- α production is restricted to adipose deposits [12]. Serum concentrations of some members of this family, such as TNF- α , sTNFR1 and sTNFR2 are increased in cases of obesity [13, 14], and it seems that this may be a counter-regulatory mechanism preventing further weight gain. Our recent studies [13, 17] revealed that weight reduction changes serum concentrations of TNF- α , sTNFR1 and sTNFR2. We observed that serum concentrations of TNF- α decreased and its soluble receptors increased after weight loss. However, there is so far a lack of data regarding serum concentrations of FAS in obesity and the influence of weight reduction on serum concentration of FAS.

We thus speculate that baseline FAS secretion in long-lasting obesity may represent a physiological adaptation to a positive energy balance. In support of our notion is the fact that the weight of our obese patients was stable at the time of enrolment, both patients with sudden weight loss and those with sudden weight gain having been excluded from the study. Further results of studies obtained during weight loss and weight gain will clarify the role of FAS in the development and prevention of obesity.

In the present study, as in our previous studies [12–14], we observed increased serum concentrations of

Table V. Correlations between study parameters after treatment

Tabela V. Korelacje między badanymi parametrami po terapii

	Total cholesterol	LDL cholesterol	Triglycerides	Glucose	STNFR2	FAS	TNF
Age	0.43*	0.48*	0.48*				
FFM%	-0.43*	-0.53*	-0.45*		0.48*		
sTNFR1	-0.45*	-0.44*	0.43*				0.43*
sTNFR2			-0.47*	-0.45*			

* — $p < 0.05$; ** — $p < 0.01$

TNF- α in obese subjects when compared to lean controls. However, our study no revealed increased serum concentration of FAS in obese women. Weight loss decreased serum concentration of TNF- α but not FAS. This is interesting because we observed a positive correlation between BMI and FAS levels and between serum concentrations of FAS and TNF- α before treatment. It seems that a decrease in serum concentrations of TNF- α after weight loss may be a result of both a decrease in fat deposit and an increase in serum concentrations of sTNFR1 and sTNFR2. This is in accordance with results obtained by Hotamisgli et al. [1], who reported a decrease in TNF- α mRNA expression in fat tissue after body weight reduction.

It seems that the cause of lack of change in the concentration of FAS after weight reduction may be its production by tissues other than adipose tissue and a mechanism of action of FAS in apoptosis other than the action of TNF- α . Thomas et al. [18] revealed that the C-terminal tails of TNF, the apoptosis-inducing ligand (TRAIL) and FAS receptors have opposing functions in FAS-associated death domain (FADD) recruitment and can regulate agonist-specific mechanisms of receptor activation. Further studies are necessary to clarify the role of FAS in apoptosis in obesity and the pathophysiology of obesity, because it seems that the decrease in serum concentrations of TNF- α and the lack of change in the serum concentration of FAS may be two independent mechanisms preventing further weight loss.

Conclusions

We observed increased serum concentrations of tumour necrosis factor alpha but not of FAS in obese women. The concentrations of tumour necrosis factor alpha decreased and tumour necrosis factor soluble receptors increased after weight loss. However, the we-

ight reduction therapy did not change serum concentration of FAS.

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