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Wpływ obniżenia masy ciała na stężenie w surowicy tlenku azotu, TNF-α i rozpuszczalnych receptorów TNF-α

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Streszczenie

Wstęp: Celem prezentowanej pracy była ocena wpływu obniżenia masy ciała na stężenie w surowicy tlenku azotu (NO, *nitric oxide*) i czynnika martwicy nowotworów α (TNF-α, *tumor necrosis factor* α) oraz zbadanie czy istnieje związek między aktywnością układu TNF a stężeniem w surowicy NO po zmniejszeniu masy ciała.

Materiał i metody: Badaną grupę stanowiły 43 otyłe kobiety (wiek 41,8 ± 11,9 lat, masa ciała 95,2 ± 15,0 kg, wskaźnik masy ciała [BMI, *body mass index*] 36,5 ± 4,6 kg/m^2). Badane poddano 3-miesięcznej kompleksowej grupowej kuracji odchudzającej. Zalecono im dietę 1000–1200 kcal i regularną aktywność fizyczną. Nie stosowano leczenia farmakologicznego. Przed i po kuracji oznaczono stężenie w surowicy metabolitów tlenku azotu, TNF-α i jego rozpuszczalnych receptorów (sTNFR1, sTNFR2) za pomocą metody *Enzyme-Linked ImmunoSorbent Assay* (ELISA), insuliny przy użyciu metody radioimmunologicznej (RIA, *Radioimmunoassay*), a stężenie glukozy, cholesterolu, cholesterolu frakcji HDL i triglicerydów — metodą enzymatyczną. Skład ciała oceniono za pomocą metody bioimpedancji przy użyciu aparatu *Bodystat*.

Wyniki: Średni ubytek masy ciała wynosił 8,3 ± 4,3 kg. Po obniżeniu masy ciała stężenie w surowicy TNF-α obniżyło się istotnie ($p < 0,000$), a obu receptorów sTNFR1 i sTNFR2 istotnie się podwyższyło ($p < 0,000$). Nie obserwowano natomiast zmian stężenia NO po zmniejszeniu masy ciała. Przeprowadzono analizę regresji wieloczynnikowej ze zmiennymi zależnymi, takimi jak ΔTNF-α, ΔsTNFR1, ΔsTNFR2 i ΔNO. Zaobserwowano istotne korelacje między ΔNO a wyjściowym stężeniem w surowicy TNF-α, sTNFR1 i sTNFR2.

Wnioski: Po obniżeniu masy ciała zaobserwowano zmniejszenie stężenia w surowicy TNF-α i wzrost stężenia obu receptorów TNF, nie stwierdziliśmy, natomiast zmian stężenia w surowicy NO. Wydaje się, że zmiany aktywności układu TNF mogą być mechanizmem kontrregulacyjnym, który hamuje dalszą redukcję masy ciała. Związku między zmianami aktywności układu TNF a stężeniem w surowicy NO po redukcji masy ciała nie wykazano.

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Słowa kluczowe: *tlenek azotu, TNF-α, receptory TNF, redukcja masy ciała*

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The effect of weight loss on serum concentrations of nitric oxide, TNF-α and soluble TNF-α receptors

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Abstract

Introduction: The aims of the present study were to evaluate the effect of weight-loss treatment on serum concentrations of NO and TNF-α and to examine whether there is an association between TNF-system activity and serum concentrations of NO after weight loss.

Material and methods: The study group involved 43 obese women (aged 41.8 ± 11.9 years, weight 95.2 ± 15.0 kg, BMI 36.5 ± 4.6 kg/m^2). The women were subjected to three-month complex weight-loss treatment. Patients were advised to keep to a 1000–1200 kcal diet and to exercise regularly. Pharmacological treatment was not administered. Serum concentrations of nitric oxide metabolites, TNF-α and its soluble receptors (sTNFR1, sTNFR2) were measured by ELISA kits; insulin was measured by RIA and glucose, cholesterol, HDL cholesterol and triglicerydes by an enzymatic procedure before and after weight loss. Body composition was determined by impedance analysis using Bodystat.

Results: The mean weight loss during treatment was 8.3 ± ± 4.3 kg. The serum concentrations of TNF-a decreased significantly ($p < 0.000$) and both receptors sTNFR1 and sTNFR2 increased significantly ($p < 0.000$) after weight loss. No significant changes in serum concentrations of NO were observed after weight loss. A multiple regression analysis was performed using ΔTNF-α, ΔsTNFR1, ΔTNFR2 and ΔNO as dependent variables. A significant correlation was observed between ΔNO and initial plasma concentrations of TNF-α, sTNFR1 and sTNFR2.

Conclusions: This study demonstrates a decrease in serum TNF-α concentration as well as an increase in plasma concentration of both TNF receptors but does not show any change in serum concentrations of NO after weight-loss treatment in obese women. It seems that changes in TNF-system activity may be a counter-regulating mechanism, which inhibits further body mass loss. We did not observe any association between changes in TNF-system activity and serum concentrations of NO after weight loss.

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Key words: *nitric oxide, TNF-α, soluble TNF receptors, weight loss*

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Introduction

Obesity is a chronic and widespread disease. Nevertheless, the mechanism of its pathogenesis and regulation of weight reduction in the process of weight-loss treatment is still unknown. It has been shown that weight loss becomes proportionally smaller in consecutive weeks of treatment. The mechanism responsible for organism defence against the loss of reserve material has yet to be discovered. Recently some authors have put particular emphasis on genetic predisposition to this disease [1–3]. Similarly, the development of studies focusing on cellular transmission and cytokines appears promising in explanation of the pathomechanism responsible for weight gain and loss.

It has been shown in a number of studies that adipose tissue alone generates many substances which may participate in the regulation of its content in the organism [4, 5].

The results of recent *in vivo* and *in vitro* studies have revealed that one of these substances is nitric oxide (NO), which is shown to exert an inhibiting effect on lipolysis stimulated by catecholamines [6, 7].

It is known that both iNOS (inducible nitric oxide synthase) and eNOS (endothelial nitric oxide synthase) participate in the production of NO in adipose tissue; increased values of both of these substances were found in isolated cells of adipose tissue in obese subjects when compared to a lean control group [8]. Increased serum concentrations of NO in overweight and obese subjects have also been shown in the recent studies conducted by Choi et al. [9] and by our results [10].

Tumor necrosis factor (TNF-α) is another substance produced by the adipose tissue. There have been stu-

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

Tabela I
Charakterystyka pacjentów i efekty kuracji odchudzającej

	Before	After	Δ
Weight [kg]	95.2 ± 15.1	87.0 ± 13.7***	–8.2 ± 4.3
BMI [kg/m^2]	36.5 ± 4.6	33.4 ± 4.6***	–3.1 ± 1.7
Fat-free mass [kg]	53.0 ± 7.5	50.8 ± 6.0*	–2.2 ± 6.2
Fat-free mass (%)	56.0 ± 8.3	58.9 ± 5.8*	2.9 ± 6.6
Body fat [kg]	41.5 ± 12.1	35.4 ± 9.0***	–6.1 ± 6.4
Body fat (%)	43.4 ± 7.9	40.7 ± 5.7*	–2.7 ± 6.1

*$p < 0.05$; ***$p < 0.0001$

dies suggesting that TNF-α participates in the development of insulin resistance and may be responsible for glucose and lipid metabolism disorders associated with obesity and type 2 diabetes [11, 12].

Experimental studies performed on cultures of human adipose cells have shown that TNF-α may prevent the development of adipose cells and that it impairs their metabolic functions. One of the mechanisms which inhibit lipogenesis probably arises from the strong insulin-inhibiting activity in human adipocytes of TNF-α and the decreased activity of lipoprotein lipase induced by TNF-α [13, 14]. Our previous studies have also revealed increased serum concentrations of TNF-α in obese subjects [15, 16].

The determination of circulating cytokines is complicated by their relative instability in biological fluids and the presence of soluble inhibitors may interfere with their measurement. TNF is rapidly cleared from the circulation and is frequently extremely low or undetectable. The endogenous formation of TNF leads to the shedding of sTNFRs (soluble receptors of tumour necrosis factor). The increase in sTNFRs parallels or exceeds TNF-α production and therefore measurement of their concentrations may better reflect TNF-system activity [17].

There have been studies showing increased expression of TNF receptors in adipose tissue and increased serum concentrations of soluble TNF receptors in obesity [18]. A few studies have evaluated the effect of weight loss on serum concentrations of TNF-α and its soluble receptors. In these studies a decrease in serum concentrations of TNF-α and an increase in serum concentrations of sTNFR1 and sTNFR2 after weight loss have been observed [16, 18]. However, there have been no studies concerning the influence of weight-loss treatment on changes in TNF-system activity and serum concentrations of NO.

The aims of the present study, therefore, were to evaluate the effect of weight-loss treatment on serum concentrations of NO and TNF-α and to examine whether there is an association between TNF-system activity and serum concentrations of NO after weight loss.

Material and methods

The study was carried out in a group of 43 obese women aged 41.8 ± 11.9 years, weighing 95.2 ± 15.0 kg, and with a BMI of 36.5 ± 4.6 kg/m^2. Patient characteristics and the effect of weight-loss treatment are presented in Table I.

All the individuals were diagnosed as having simple obesity without additional diseases, patients with evidence of acute or chronic inflammatory diseases having been excluded.

The women were subjected to a three-month complex weight-loss treatment, during which they were seen by a physician, a dietician, a psychologist and a physical therapist every two weeks. 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 minutes three times a week). Pharmacological treatment was not administered.

The study was conducted after obtaining informed consent from all the subjects. The study was approved by the local Ethics Committee.

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

In order to avoid the effect of diet on serum concentrations of metabolite NO, the subjects were given a list of foods potentially rich in nitrates and were requested to abstain from these for two days before sample collection. Specifically, herbal or black teas, beer, wine, cured meat, fish and cheese were excluded from the diet.

The blood samples were collected after an overnight fast before and after the weight-loss treatment.

Plasma glucose, cholesterol, HDL cholesterol and triglicerydes were determined by an enzymatic procedure using a commercially available test kit (Cormay). LDL cholesterol was calculated using the Friedewald formula. Insulin was determined by radioimmunoassay (DPC Diagnostic Products Corporation, Los Angeles, USA).

The blood serum concentration of NO metabolites was measured using the commercially available highly sensitive ELISA kits (Genzyme Diagnostics, Cambridge, USA). The transient and volatile nature of NO makes it unsuitable for most convenient detection methods. However, since most of the NO is oxidised to nitrite (NO_2^-) and nitrate (NO_3^-), the concentration of these anions was used as a quantitative measure of NO production. After the conversion of NO_3^- to NO_2^-, the spectrophotometric measurement of NO_2^- was accomplished by using the Griess Reaction (1. $NO + O_2^- \rightarrow ONO_2^- + H^+ \rightarrow NO3^- + H^+$, 2. $2NO + O_2 \rightarrow N_2O_4 \xrightarrow{H2O} NO_2^- + NO_3^- + 2H^+$, 3. $NO + NO_2^- \rightarrow N_2O_3 \xrightarrow{H_2O} 2NO_2^- + 2H^+$). The conversion of NO into nitrate and nitrite by these reactions varies from system to system. The interaction of NO in a system is measured by the determination of total nitrate and nitrite concentrations in the sample.

R&D Systems' Total Nitric Oxide Assay involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of total nitrite is then determined as a coloured azo-dye product of the Griess Reaction that absorbs visible light at 540 nm. The sensitivity of the Total Nitric Oxide Assay is typically less than 1.35 mmol/L. The mean intra-assay coefficient of variance was 3.1%, ranging from 1.2 to 5.3%, and the mean inter-assay coefficient of variance was 4.1%, in the range 3.3–7.0%.

TNF-α and soluble forms of both TNF-a receptors (sTNFR1, and sTNFR2) were measured using the commercially available highly sensitive ELISA kits (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%, ranging from 8.7 to 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%, ranging from 2.7 to 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%, ranging from 1.6 to 2.5% and the mean inter-assay coefficient of variance was 3.5%, range 3.5–5.1%.

Data were analysed using the t-test and correlation analysis was performed using Pearson's and multiple regression analyses. Values of $p < 0.05$ were considered to be statistically significant.

Results

The mean weight loss during treatment was 8.3 ± 4.3 kg and BMI decreased from 36.5 ± 4.6 at the baseline to 33.4 ± 4.6 after treatment. The effect of the weight-loss treatment is presented in Table I.

Serum NO, TNF-α, sTNFR1 and sTNFR2 concentrations

No significant changes were observed in serum concentrations of NO after weight loss. Serum concentrations of TNF-α decreased significantly after weight loss. The most pronounced differences during weight loss were observed in the soluble TNF receptors. Both receptors sTNFR1 and sTNFR2 increased significantly ($p < 0.0001$) (Table II).

Serum insulin, glucose and lipid concentrations

Plasma concentrations of HDL cholesterol decreased ($p < 0.05$) and plasma concentrations of glucose increased ($p < 0.005$); no significant changes were observed in total cholesterol, LDL cholesterol, triglycerides and insulin (Table III).

Table II
Plasma lipids, glucose and insulin

Tabela II
Stężenie w surowicy lipidów, glukozy i insuliny

	Before	After weight loss
Total cholesterol [mg/dl]	207.2 ± 33.7	206.3 ± 33.8
HDL cholesterol [mg/dl]	53.9 ± 10.6	51.1 ± 8.1*
LDL cholesterol [mg/dl]	132.1 ± 34.0	131.6 ± 33.0
Triglycerides [mg/dl]	106.5 ± 46.5	98.0 ± 39.1
Glucose [mg/dl]	89.9 ± 10.6	95.4 ± 13.9**
Insulin [μIU/ml]	17.0 ± 8.0	14.8 ± 8.7

*$p < 0.05$; **$p < 0.005$

Table III
Serum concentrations of NO, TNF-α and TNF soluble receptors

Tabela III
Stężenie w surowicy NO, TNF-α i rozpuszczalnych receptorów TNF

	Before	After weight loss
NO [μmol/l]	33.5 ± 10.1	35.6 ± 13.5
TNF-α [pg/ml]	6.9 ± 2.4	5.4 ± 1.6***
sTNF R1 [pg/ml]	1237.2 ± 225.7	1432.8 ± 304.9***
sTNF R2 [pg/ml]	1782.7 ± 417.4	2063.0 ± 340.6***

***$p < 0.0001$

Correlations between all study parameters

Significant positive linear correlations were found between ΔNO and ΔBMI (r = 0.33; p = 0.03) and ΔTNF-α and Δ of adipose tissue mass (kg) (r = 0.34; p = 0.03) and ΔsTNFR1 and Δ of body mass (r = 0.32; p = 0.04).

There was also a significant negative correlation between ΔTNF-α and Δ glucose (r = –0.31; p = 0.04). No correlation was observed between ΔNO and ΔTNF-α.

No correlation was found between serum NO concentrations and age, BMI, body mass, lipid levels and serum TNF-α, sTNFR1 and sTNFR2 concentrations before and after treatment. No correlations were found either between serum TNF-α and age, BMI, body mass, lipid levels and serum TNF-α, sTNFR1 and sTNFR2 concentrations before and after treatment. We did not observe any association between serum concentrations of NO and TNF-α, sTNFR1 and sTNFR2 and insulin.

A multiple regression analysis was performed using ΔTNF-α , ΔsTNFR1, ΔsTNFR2 and ΔNO as dependent variables. Models were fitted to estimate the role of age, BMI, weight and body fat. Other models with ΔNO as a dependent variable were fitted to estimate the role of serum concentrations of insulin, TNF-α and soluble TNF receptors.

ΔTNF-α was significantly associated with a reduction in adipose tissue mass (kg), ΔsTNFR1 with loss of weight and ΔNO with a reduction in BMI (Table IV). ΔNO was also significantly correlated with initial plasma concentrations of TNF-α, sTNFR1 and sTNFR2. Analysis of the remaining parameters revealed no significant correlation.

Discussion

We observed a significant decrease in serum concentrations of TNF-α after weight-loss treatment. This result is in accordance with the findings of Kern et al. [19], which show a decreased expression of TNF-α m RNA and TNF-α protein levels in the adipose tissue of obese humans after weight loss. A decrease in circulating TNF-α after weight-loss treatment was also described by Brunn et al. [20] and in our previous studies [15, 16]. On the other hand, results contradictory to ours have also been reported. Hauner et al. [18] showed no alteration in plasma TNF-α concentrations in nine subjects after weight-loss treatment over one year.

Moreover, decreased serum concentrations of TNF-α correlate positively with decreased body fat mass, an association which is confirmed by the findings of multiple regression analysis. This result is in accordance with the study performed by Winkler et al. [21], which revealed both the expression of the TNF-α protein in fat deposits and the correlation of serum TNF-α with adipocyte cell volume.

It is known that TNF-α participates in obesity-related insulin resistance. Adipose tissue insulin resistance could be explained as a mechanism for preventing further adipocyte lipid accumulation. We observed no correlation between serum concentrations of insulin and TNF-α before and after treatment. Similar data concerning serum TNF-α concentrations and insulin sensitivity were obtained in our previous studies in which insulin-sensitive and insulin-resistant obese subjects were compared [22] and the effect of weight loss on these parameters was assessed in insulin-sensitive and insulin-resistant obese subjects [23].

Paradoxically, we observed a significant increase in serum concentrations of glucose after weight loss and negative associations between Δ glucose and ΔTNF-α. This observation is difficult to account for and requires further study.

It was shown that obese subjects express more TNFR2 mRNA in adipose tissue and more soluble TNFR2 in the circulation than a lean control group. TNFR1 expression and protein levels were similar in these subjects [24, 25]. However, in our recent study we found no differences between serum concentrations of sTNFR1 and sTNFR2 in overweight, obese and lean women [10].

In the present study, as in the previous one [16], serum concentrations of soluble TNF receptors significantly increased after weight-loss treatment. We also observed a significant positive correlation between an

Table IV
Regression analysis with ΔTNF-α, ΔsTNFR1 and ΔNO as dependent variables

Tabela IV
Analiza regresji z ΔTNF-α, ΔsTNFR1 i ΔNO jako zmiennymi zależnymi

	Age (years)	ΔBMI	Δ Body fat [kg]	Δ Body mass [kg]
ΔTNF-α	–0.007		0.116*	
ΔsTNFR1	–0.999			–19.415*
ΔNO	–0.12	2.061*		

*p < 0.05

increased concentration of sTNFR1 and decreased body mass, a correlation that was confirmed by multiple regression analysis.

The question of whether the decrease in serum concentrations of TNF-α after the body-fat reducing treatment is only a result of a decrease in its synthesis in adipose tissue or whether it could also be caused by increased serum concentrations of soluble TNF receptors is a matter of interest and merits further study.

The result of increased TNF-α binding by its soluble receptors besides the blocking of its binding by cell surface receptors 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 is that they could be serving as a reservoir of bio-active TNF, which prolongs the TNF-α activity [17].

In our previous study the value of serum concentrations of NO in obese women was significantly higher when compared to controls [10]. In the present paper no significant changes in serum concentrations of NO were observed after weight-loss treatment. We also observed a positive association between Δ serum concentration of NO and ΔBMI ($r = 0.32$; $p = 0.03$), which was also demonstrated in multiple regression analysis (Table IV).

However, the findings obtained by Choi et al. [9] show a positive correlation between increased concentrations of NO and BMI in both male and female adolescents. In our [10] previous study we also observed a positive significant correlation between serum concentrations of NO and BMI. It therefore seems that the source of increased serum concentrations of NO after weight-loss treatment may be tissues other than adipose tissue.

Because of a lack in the available literature both of data concerning the activity level of NO synthases in adipose tissue after weight-loss treatment and of studies assessing the influence of such treatment on serum concentrations of NO, further investigation of this question is required.

Conclusions

1. This study demonstrates a decrease in serum TNF-α concentration as well as an increase in plasma concentration of both TNF receptors but does not show a change in serum concentrations of NO after weight-loss treatment in obese women. It appears that changes in TNF-system activity may be a counter-regulating mechanism which inhibits further loss of body mass.
2. We observed no association between changes in TNF-system activity and serum concentrations of NO after weight loss.

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