



Original paper

TNF system activity and plasma RBP4 and apelin-36 levels in obese and normal weight women

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Abstract

Objective: Tumour necrosis factor α (TNF- α), retinol-binding protein 4 (RBP4), and apelin-36 are adipokines with increased circulating levels in obesity, which are involved in the development of insulin resistance. It was shown that TNF- α stimulates synthesis of apelin-36 and inhibits RBP4 expression in adipocytes. The aim of the study was the assessment of relationships between TNF system activity and plasma RBP4 and apelin-36 levels in obese and normal weight women.

Material and methods: A total of 116 women (72 obese and 44 normal weight) were enrolled into the study. In addition to the anthropometric measurements and routine biochemical parameters, plasma TNF- α , sTNFRs, RBP4, and apelin-36, insulin levels were assessed by ELISA.

Results: In obese group plasma TNF- α , sTNFR1, RBP-4, and apelin-36 levels were significantly higher, while sTNFR2 levels were lower than in the normal-weight group (Me 4.4 vs. 2.3 pg/mL, $p < 0.05$; 1908 vs. 1285 pg/mL, $p < 0.001$; 27.6 vs. 14.7 ng/mL, $p < 0.001$; 1.6 vs. 1.2 ng/mL, $p < 0.05$, and 2361 vs. 2574 pg/mL, $p < 0.05$, respectively). Multivariate regression analysis revealed that plasma RBP4 levels were proportional to age ($\beta = 0.01$), and plasma TNF- α ($\beta = 0.26$) and sTNFR1 ($\beta = 0.39$) levels, and inversely proportional to sTNFR2 levels ($\beta = -0.55$) independently of body mass index (BMI) and waist circumference, while apelin-36 was proportional to BMI ($\beta = 0.01$) and insulin levels ($\beta = 0.19$) and inversely proportional to age ($\beta = -0.01$), independently of waist circumference and TNF system activity.

Conclusions: Plasma RBP4, but not apelin-36 level, is associated with TNF system activity.

Key words: apelin, RBP4, TNF system, nutritional status.

Introduction

Excessive visceral fat accumulation is associated with macrophage infiltration of adipose tissue, activation of lipolysis, hormonal function disturbances, and insulin resistance development [1]. It has been shown that both macrophages and adipocytes are a source of tumour necrosis factor α (TNF- α), and its production and secretion increase proportionally to adipocytes volumes [2]. It is

well known that circulating TNF- α levels are increased in obese subjects [3,4] and decreased after weight loss [5,6]. Additionally, our previously published results revealed that circulating TNF- α levels increase in the early stage of visceral obesity development [7] sequent weight gain cause a slight influence of systemic microinflammation and seems not to lose the benefit achieved with weight loss [8]. However, it has been suggested that, in terms of TNF- α , we should rather talk about the TNF system in-



cluding the membrane (TNFI and TNFRII) and soluble (sTNFR1 and sTNFR2) receptors [9-11]. TNFRI activation stimulates apoptosis, cell proliferation and growth, and inflammation, while TNFRII activation stimulates growth of thymocytes, cytotoxicity of T lymphocytes, production of other proinflammatory cytokines, insulin resistance development, and regulated binding of TNF- α to sTNFR1 [12]. Soluble TNF receptors are generated by proteolytic splitting of the extracellular part of the membrane receptors, and the process is stimulated by TNF- α [9]. sTNFR1 probably plays a key role in insulin resistance development [13]. As was mentioned above, inflammation in adipose tissue and increased TNF- α secretion is associated with adipokines release disturbances. The results of experimental studies revealed that TNF- α inhibits expression of mRNA retinol-binding protein 4 (RBP4) in adipocytes [14,15], while Erikstrup *et al.* [16] found that TNF- α may play a role in ectopic fat accumulation in muscles stimulated by RBP4. Additionally, it has been shown that in subjects diagnosed with type 2 diabetes and coronary artery disease, circulating RBP4 levels were associated with TNF- α levels, but not with insulin resistance [17]. Increased circulating RBP4 levels were described in obese subjects [18-21]. It is interesting that both TNF- α and RBP4 participate in insulin resistance development by similar mechanisms, including suppression of tyro-

sine autophosphorylation in type 1 substrate of insulin receptor, inhibition of inositol phosphorylate kinase 3 signalling, and GLUT4 expression in adipocytes, as well as stimulation of gluconeogenesis pathways enzymes in hepatocytes [22,23]. Apelin is another adipokine with increased circulating levels in obesity. Its synthesis is stimulated by insulin or glucose levels [24]. In turn, apelin inhibits insulin release [25]. It has also been shown that growth hormone and TNF- α stimulates apelin mRNA expression in adipocytes [26,27]. However, there is a lack of studies assessing associations between TNF system activity and circulating RBP4 and apelin levels.

Therefore, the aim of this study was the assessment of the relationships between TNF system activity and plasma RBP4 and apelin-36 levels in obese and normal-weight women.

Material and methods

The cross-sectional study involved 116 women (44 normal weight and 72 obese) with stable body mass during the last three-month period. The obese women were not diagnosed with any complications of obesity or other chronic diseases. The normal-weight women were apparently healthy. The exclusion criteria included: evidence of present or recent (last three months) infectious disease, cigarette smoking, and any medication.

The study was conducted after obtaining of informed consent from each participant. The study protocol was approved by the Bioethical Committee of the Medical University of Silesia.

Normal weight was defined according to World Health Organisation (WHO) criteria [28] as body mass index (BMI) from 18.5 to 24.9 kg/m² and obesity as ≥ 30.0 kg/m². The characteristics of the analysed groups are listed in Table 1.

Anthropometric measurements (body mass, height, and waist circumference) were performed, and the BMI was calculated according to the standard formula. Body composition was assessed by the bioimpedance method using a Bodystat 1500 (Douglas, Isle of Man).

Samples of venous blood (15 mL) were withdrawn between 8.00 and 9.00 a.m., after an overnight fast (16 h). The blood samples were collected according to recommendations of the kits' manufacturers. Serum and plasma samples were frozen and stored at -70°C .

Laboratory procedures

Plasma glucose and lipids were estimated by colorimetric methods using commercially available test kits (Roche, Switzerland). Serum insulin concentration was determined by enzyme-linked immunosorbent assay (ELISA) (DRG Instruments GmbH, Marburg, Germany) with a lower limit of sensitivity of 1.76 uU/mL and in-

Table 1. Study groups' characteristics

Parameter	Obese (n = 72)	Normal weight (n = 44)	p
Age (years)	38.1 \pm 11.6	30.3 \pm 11.0	< 0.001
Body mass (kg)	95.3 \pm 16.8	60.4 \pm 6.9	< 0.001
BMI (kg/m ²)	35.4 \pm 6.0	22.6 \pm 2.2	< 0.001
Body fat (kg)	48.0 \pm 13.4	19.2 \pm 4.6	< 0.001
Body fat (%)	47.1 \pm 7.1	31.4 \pm 5.0	< 0.001
Waist circumference (cm)	106.4 \pm 14.4	73.0 \pm 9.1	< 0.001
Total cholesterol (mg/dL)	197.1 \pm 36.9	184.4 \pm 43.0	NS
LDL-cholesterol (mg/dL)	120.5 \pm 35.5	106.7 \pm 41.1	NS
HDL-cholesterol (mg/dL)	54.5 \pm 12.5	60.3 \pm 15.0	< 0.05
Triglycerides (mg/dL)	101.0/59.0	65.0/35.0	< 0.001
Glucose (mmol/L)	5.1 \pm 0.6	4.8 \pm 0.6	< 0.01
Insulin (μ U/mL)	8.5/7.3	6.7/3.8	< 0.01
HOMA-IR	1.7/1.8	1.5/1.0	< 0.01
TNF- α (pg/mL)	4.4/4.1	2.3/4.1	< 0.05
sTNFR1 (pg/mL)	1909/1163	1285/472	< 0.001
sTNFR2 (pg/mL)	2361/637	2574/1259	< 0.05
RBP4 (ng/mL)	27.6/20.4	14.7/14.2	< 0.001
Apelin-36 (ng/mL)	1.6/1.1	1.2/0.9	< 0.05

tra- and inter-assay coefficients of variations of 2.2% and 4.4%, respectively. HOMA-IR index was calculated with the standard formula: $\text{HOMA-IR} = \text{fasting concentration of insulin (uU/mL)} \times \text{fasting concentration of glucose (mmol/L)} / 22.5$.

ELISA method was also used for measurements of plasma TNF- α and sTNFRs (R&D System, Minnesota, MN, USA), RBP4, and apelin-36 (Phoenix Pharmaceuticals, Burlingame, USA) levels with the lower limit of sensitivity of 0.18 pg/mL, 0.77 pg/mL, 0.6 pg/mL, 2.17 ng/mL, and 0.11 ng/mL, respectively, and intra- and inter-assay coefficients of variations were 14.4% and 18.7% for TNF- α , 3.6% and 2.6% for sTNFR1, 3.7% and 3.5% for sTNFR2, 5.0% and < 14.0% for RBP4 and 5.0-10.0% and < 15.0% for apelin-36.

Statistical analysis

Statistical analysis was performed using STATISTICA 9.0 PL (StatSoft, Cracow, Poland) software and the R software environment. There were no missing data in the database. The results are presented as mean values \pm standard deviation and as median and interquartile range for the data deviating from the normal distribution percentages for the data in nominal and ordinal scale. Distribution of variables was evaluated by the D'Agostino-Pearson test. Homogeneity of variances was assessed by the Levene test. Quantitative variables were compared with two-way multivariate analysis of variance with Duncan test post-hoc. The assessment of associations between variables was done with the multivariate linear regression and the backward stepwise procedure. Outliers were identified based on Cook's distance values. The Cook-Weisberg test was used to test the residuals for heteroskedasticity. Model calculation was performed, including evaluation of multicollinearity, which was assessed with the variance inflation factor (VIF). The VIF should not exceed more than 5. Goodness of fit of the obtained model was assessed with the F test and determination coefficient R^2 . All the results were considered as statistically significant with a p value of < 0.05.

Results

The serum concentrations of cholesterol and low-density lipoprotein (LDL) cholesterol were similar in the study and control groups. Serum triglyceride levels were significantly higher and high-density lipoprotein (HDL) cholesterol levels lower in obese than in normal-weight women (Table 1).

Serum glucose and insulin levels as well as HOMA-IR values were significantly higher in the study group than in the control group (Table 1).

Plasma TNF- α , sTNFR1, RBP4, and apelin-36 levels were significantly higher and sTNFR2 lower in obese

women than in normal-weight women (Table 1). According to the results of ROC analysis, obese women were characterised by plasma TNF- α levels ≥ 2.60 pg/mL (with 70.5% sensitivity and 59.5% specificity), sTNFR1 levels ≥ 1335 pg/mL (with 70.3% sensitivity and 54.8% specificity), sTNFR2 levels ≤ 2447 pg/mL (with 61.9% sensitivity and 58.1% specificity), RBP4 levels ≥ 18.7 ng/mL (with 68.9% sensitivity and 66.7% specificity), and apelin-36 levels ≥ 1.41 ng/mL (with 63.5% sensitivity and 61.9% specificity).

Correlation between anthropometric parameters and study adipokines

Plasma TNF- α levels correlated positively with body mass, BMI, and waist circumference ($R = 0.19$, $p < 0.05$; $R = 0.22$, $p < 0.05$ and $R = 0.29$, $p < 0.01$, respectively). There were also positive correlations between sTNFR1 and body mass, BMI, and waist circumference ($R = 0.25$, $p < 0.01$; $R = 0.27$, $p < 0.01$ and $R = 0.32$, $p < 0.001$, respectively). Plasma RBP4 levels were also proportional to body mass, BMI, body fat mass, and waist circumference ($R = 0.35$, $p < 0.001$; $R = 0.36$, $p < 0.001$; $R = 0.24$, $p < 0.01$ and $R = 0.47$, $p < 0.001$, respectively). We also observed positive associations between apelin-36 levels and body mass, and body fat mass ($R = 0.19$, $p < 0.05$ and $R = 0.30$, $p < 0.01$, respectively). There were negative correlations between sTNFR2 levels and body mass, BMI, body fat mass, and waist circumference ($R = -0.26$, $p < 0.01$; $R = -0.23$, $p < 0.05$; $R = -0.21$, $p < 0.05$ and $R = -0.29$, $p < 0.01$, respectively).

Additionally, there was a positive correlation between age and TNF- α , sTNFR1, and RBP4 levels ($R = 0.63$, $p < 0.001$; $R = 0.37$, $p < 0.001$ and $R = 0.60$, $p < 0.001$, respectively) and negative with sTNFR2 and apelin-36 levels ($R = -0.30$, $p < 0.01$ and $R = -0.27$, $p < 0.01$, respectively) in all study groups.

Correlation between lipid, glucose, and insulin concentrations as well as HOMA-IR values and study adipokines

Serum cholesterol and LDL cholesterol concentrations were proportional to TNF- α and RBP4 levels ($R = 0.43$, $p < 0.001$; $R = 0.50$, $p < 0.001$ and $R = 0.40$, $p < 0.001$; $R = 0.45$, $p < 0.001$, respectively) and inversely proportional to sTNFR2 ($R = -0.37$, $p < 0.001$ and $R = 0.31$, $p < 0.001$, respectively). There were no associations between HDL cholesterol and the study adipokine levels.

There was a positive correlation between serum triglycerides concentrations and TNF- α , sTNFR1, and RBP4 levels ($R = 0.36$, $p < 0.001$; $R = 0.30$, $p < 0.01$ and $R = 0.39$, $p < 0.001$, respectively).

Serum glucose concentrations were proportional to TNF- α , sTNFR1, and RBP4 levels ($R = 0.35$, $p < 0.001$;

$R = 0.26, p < 0.01$ and $R = 0.48, p < 0.001$, respectively) and inversely proportional to sTNFR2 ($R = -0.21, p < 0.05$), while insulin levels were associated with apelin-36 levels only ($R = 0.19, p < 0.05$). There was no correlation between HOMA-IR values and the studied adipokines.

Correlation between the studied adipokines

Plasma RBP4 levels were proportional to TNF- α and sTNFR1 levels ($R = 0.61, p < 0.001$; $R = 0.46, p < 0.001$, respectively) and inversely proportional to sTNFR2 ($R = -0.41, p < 0.001$). Plasma apelin-36 levels were negatively associated with TNF- α levels only ($R = -0.34, p < 0.001$).

Multiple regression analyses

The multivariate regression analysis revealed that plasma RBP4 level was proportional to age ($\beta = 0.01, p < 0.001$), plasma TNF- α ($\beta = 0.26, p < 0.001$), and sTNFR1 ($\beta = 0.39, p < 0.001$) levels and inversely proportional to sTNFR2 level ($\beta = -0.55, p < 0.001$), regardless of the BMI and waist circumference, while apelin-36 was proportional to BMI ($\beta = 0.01, p < 0.05$) and insulin level ($\beta = 0.19, p < 0.05$) and inversely proportional to age ($\beta = -0.01, p < 0.001$), regardless of the waist circumference and TNF system activity.

Discussion

In accordance with the results of previously published studies [3,4,18,19,29-35], plasma TNF- α , sTNFR1, RBP4, and apelin-36 levels are significantly higher in obese than in normal weight women. However, contrary to earlier studies, circulating sTNFR2 levels were lower in obese than in normal-weight women [13,36,37]. Moreover, we observed that circulating TNF- α , sTNFR1, RBP4, and apelin-36 levels were proportional to nutritional status, but visceral obesity was associated with higher TNF- α , sTNFR1, and RBP4 but not with apelin-36 levels, while the sTNFR2 levels were inversely proportional to nutritional status and visceral obesity. We suggest that the decrease of circulating sTNFR2 in obese subjects is a contraregulatory mechanism for the increased TNF- α and sTNFR1 levels. This hypothesis is confirmed by our previously published results which showed that sTNFR2 levels increased after weight loss [8]. In contrast to this data are our results showing increases of both sTNFR1 and sTNFR2 levels during the development of the early stage of visceral obesity in the five-year follow-up [7]. Further studies are necessary to verify our hypothesis because of the poor understanding of the role of sTNFRs in the development of obesity complications.

This study is the first to assess the cut-off points of study adipokines characteristic for obesity. However, further studies in large homogenous group are necessary to prove this observation. Moreover, the gender, age, and race as well as visceral fat depot-related variability for these cut-off points may preclude their generalisation. Their establishment seems to be valid in terms of potential practical use of adipokine evaluation.

It has been shown that both TNF- α and RBP4 participated in insulin resistance development [13,22,23]. However, we did not observe associations between these adipokine circulating levels and insulin resistance assessed on the basis HOMA-IR values, while, contrary to some studies [2,38], serum glucose concentrations were proportional to TNF- α , sTNFR1 but in accordance with other studies [18,39-41] regarding RBP4 levels. Moreover, according to previously published results [24,42], our study showed that the circulating insulin concentration is proportional to apelin-36 level.

Additionally, we observed an association between circulating TNF- α and RBP4 but not apelin-36 levels with serum total cholesterol, LDL cholesterol, and triglycerides concentrations. These results are in accordance with experimental and clinical studies [43-48]. Moreover, serum cholesterol and LDL cholesterol concentrations were inversely proportional to sTNFR2. Our results indicate that a decrease of circulating sTNFR2 levels in obesity is the contraregulatory mechanism partially preventing the negative metabolic action of TNF- α and sTNFR1. However, further studies, especially experimental ones, are necessary to clarify this hypothesis.

The novelty of our study is that plasma RBP4 levels are proportional to TNF- α and sTNFR1 and inversely proportional to sTNFR2, independently of nutritional status. This observation was confirmed by the results of multiple regression analysis. An additional factor explaining RBP4 level variability was age, regardless of the BMI and waist circumference. However, apelin-36 levels were proportional to BMI and insulin levels and inversely proportional to age, regardless of the waist circumference and TNF system activity. Our findings are in accordance with results that showed that RBP4 mRNA expression in adipocytes is proportional to circulating TNF- α levels [15], and a clinical study in subjects diagnosed with type 2 diabetes [17]. These results are in contrast to experimental studies that showed that TNF- α inhibits RBP4 mRNA expression in adipocytes and hepatocytes [14,16,49]. Therefore, it seems that TNF system activity changes are the factor participating in glucose and lipid metabolism disturbances directly and indirectly by stimulating the RBP4 release, while the impact on apelin-36 affects both nutritional status and insulin levels but not TNF system activity. These results are in part contrary to previously published studies, which showed that plasma apelin levels are proportional

to circulating TNF- α in subjects diagnosed with type 2 diabetes [42], and experimental studies that revealed that mRNA apelin in adipocytes and its circulating levels increases after TNF- α infusion in mice [50]. However, in accordance with our results in 3T3-L1 adipocyte cultures, TNF- α did not influence the apelin mRNA expression [26]. Additionally, our study showed that both nutritional status and insulin levels are factors influencing circulating apelin levels. Thus, we suggest that increased apelin levels are the effect of a contraregulatory mechanism preventing insulin resistance development. This hypothesis is confirmed by the experimental study showing that apelin stimulates insulin signalling and increased glucose uptake [51]. Moreover, the lack of TNF system activity on circulating apelin-36 levels suggests that this adipokine release is independent of adipose tissue inflammation and the mechanism is secondary to impaired glucose up-take mediated by TNF system activity and RBP4 changes. This hypothesis is confirmed by the results of a study showing that weight reduction and insulin sensitivity improvement is associated with decreased apelin levels [33].

The limitation of our study is the size of study subgroups. Moreover, the distribution of body fat and its visceral deposits were not directly assessed using DEXA or a CT scanner. Additionally, only apelin-36 isoform was measured.

Conclusions

Plasma RBP4 but not apelin-36 levels are associated with TNF system activity.

Disclosure

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The authors report no conflict of interest.

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