# ADIPOKINES' VARIATIONS DUE TO A LOWER CALORIC INTAKE IN ENDOCRINE OBESE PATIENTS ASSOCIATING THE METABOLIC SYNDROME

SABINA OROS[1], OLGA IANAŞ[2], LIVIU LERESCU[3], AURORA SĂLĂGEANU[3], MIHAELA GIURCĂNEANU[2], SUZANA VLĂDOIU[2] and CONSTANTIN DUMITRACHE[1,2]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, [2]"C.I. Parhon" National Institute of Endocrinology, Bucharest, [3]"I. Cantacuzino" National Institute for Research-Development in Microbiology and Immunology, Bucharest

Adipokines and cytokines have modified values in the metabolic syndrome (MetSyn), without knowing if they are the cause or the effect of insulin resistance. Multiple studies evaluating adipokines and cytokines in endocrinopathies associating MetSyn presented controversial results.

The aim of this study was to evaluate adiponectin, leptin, resistin, CRP and TNFα in obese subjects with different endocrine diseases that associate also insulin resistance before and after a low calorie diet.

*Subjects and methods:* This study enrolled 59 subjects (male and female), that were clinically evaluated. Blood samples were taken in fasting conditions in order to determine the biochemical profile, adipokine and cytokine levels, before and after a 3 months diet. Insulin resistance was appreciated using HOMA index. The MetSyn was diagnosed according to International Diabetes Federation's definition.

Results were statistically analyzed using SPSS program, version 15.

*Results:* Subjects were divided into 2 groups-37 subjects with MetSyn (the study group), and 22 subjects- control group (age matching controls).

Compared mean values for both groups during the first visit, revealed statistically significant differences regarding waist circumference (p=0.009), triglycerides (p<0.001), HDL-cholesterol (p<0.001), diastolic blood pressure (p=0.012), leptin (p=0.005), insulin (p=0.049), glycaemia (p=0.013), HOMA (p=0.033) and TNF α (p=0.026), significant differences kept for triglycerides (p=0.03) and glycaemia (p=0.033) after 3 months diet.

Adiponectin values were low, leptin, CRP and TNFα levels were high and resistin concentrations were normal in both groups, without any differences regarding the associated endocrine disease. Although adiponectin levels were lower and CRP higher in the MetSyn group, these results did not reach any statistical significance.

Adipokines and cytokines values were lower after 3 months diet, but with higher adiponectin and resistin values in the MetSyn group. Weight loss determined a slightly improvement in insulin sensibility.

*Conclusions*: Obese subjects with different endocrine diseases that associate MetSyn have low levels of adiponectin, high leptin, CRP and TNF α values and normal resistin concentrations. Insulin sensibility improved after weight loss. Adipokines'variations cannot be the cause of insulin sensibility improvement, being more the result of visceral adiposity reduction.

*Key words:* Adiponectin; Leptin; Resistin; CRP, TNF α; Insulin resistance; MetSyn, Obesity.

## INTRODUCTION

MetSyn is a complex clinical entity with multifactor ethiopathogeny involving mechanisms that affect the whole endocrine system that led to renaming it the neuroendocrine- metabolic syndrome[1].

Adipose tissue is a metabolic active organ, synthesizing peptid hormones and inflammatory proteins involved in insulin resistance.

Adiponectin is considered a marker of the MetSyn, having anti-inflammatory and anti-atherogenic roles, important in preventing insulin resistance and promoting insulin sensitivity.

Several studies suggest insulin involved in controlling adiponectin levels and insulin resistance causative for adiponectin variations.

Obese adults have lower adiponectin levels compared with normal weight adults, negatively correlated with waist circumference. Adiponectin raises due to weight loss, especially when visceral fat mass is lost, supporting the fact that adiponectin can be considered the main insulin resistance predictor[2–4].

Low adiponectin levels are correlated with high apolipoprotein B concentrations, hepatic and muscular insulin resistance, high TNFα, CRP, PAI1 and other adipocytokines.

Leptin controls food ingestion and energy expenditure, neuroendocrine and immune function, insulin resistance and secretion and lipids' metabolism. High levels of leptin appear in insulin resistance situations, probably due to a leptin resistance. Low levels can appear in lipodistrophy that associate also insulin resistance. High mARN levels for leptin, IL6, TNF α and low for adiponectin are described in excessive visceral fat storages. Subcutaneous adiposity has also high leptin levels that correlate with PAI1, IL6 and CD68.

Studies involving resistin are controversial; some reveal high levels, other low and some do not consider resistin being involved in insulin resistance. Resistin is certain involved in cardiovascular diseases because of its inflammatory effects[8], stimulation of adhesion molecules, chemokine expression and endothelin release[9].

Low levels of adiponectin, high concentrations of leptin, CRP, TNF α, resistin and interleukins contribute to the pro-inflammatory state in MetSyn[9].

There are no differences regarding TNF α expression in visceral and subcutaneous fat masses. Muscle and adipose tissue express high levels of TNF α that correlate positive with the obesity grade[10, 11]. TNF α induces insulin resistance either directly, acting on insulin sensitive cells or indirectly on cells producing IL. TNFα correlates negatively with LPL activity, reduced GLUT4 expression and augments the activity of hormone sensitive lipase.

High CRP levels have been pointed out in multiple studies as being associated with high cardiovascular risk.

Subjects with preexisting ischemic cardiac disease, that associate MetSyn have a higher cardiovascular risk compared with insulin sensitive individuals.

CRP activates the complement, stimulates cytokines synthesis and monocytes chemoattraction, raises PAI1 levels and activity, macrophages LDL-cholesterol inclusion, the expression of angiotensin receptors and lowers NO levels. High CRP levels can directly induce endothelial dysfunction through raising adhesion molecule expression and indirectly through monocyte chemoattractant protein secretion.

Obese people associating MetSyn that loose weight improve their insulin resistance. Adiponectin levels raise, leptin levels are getting lower and resistin levels may be unmodified after weight loss.

If adipokines and cytokines have been intensively studied in obesity, little is known in endocrinopathies.

In cortisol excess, adiponectin levels seam to be low and leptin levels high, without any significant difference compared with obese subjects that have no cortisol excess.

Several studies described resistin levels to be either high in hyperthyroidism[12], or low[13], while adiponectin levels correlate with fT4 levels[14].

In hypothyroidism, adiponectin levels are low and leptin levels are high[15]. Low levels of adiponectin might be responsible for blood flow reduction in adipose tissue and muscles, explaining low glucose uptake[16].

In PCOS, a study showed low adiponectin levels, high resistin levels and unmodified leptin concentrations[4]. Other studies demonstrated that women with leptin deficiency associate PCOS, hyperandrogenism and amenorrhea.

Are adipokines and cytokines imbalance in obesity and endocrine diseases the cause or the consequence of insulin resistance?

The aim of the study was to evaluate adipokines and cytokines in obese endocrine patients with MetSyn compared with matched controls.

## SUBJECTS AND METHODS

Obese volunteers (male and female), older than 18 years, with different endocrinopathies were enrolled and divided, according to IDF definition of MetSyn into two groups – group 1 (with MetSyn, study group) and group 2 (controls).

### Exclusion criteria

Weight over 200 kg, pregnancy, subjects enrolled in other studies, weight losing medication or bariatric surgery, subjects that withdrawn themselves from the study or were excluded because of not respecting the study protocol were the exclusion criteria.

### Subjects

59 subjects were included- 37 with MetSyn (37 females) and 22 controls (16 females and 6 males) and fallowed the same low caloric diet (1000kcal/day) and 30 minutes exercise every day.

21 subjects form the MetSyn group and 11 controls returned for the second visit. Significant weight loss was present in 14 subjects from the MetSyn group and 7 controls.

Subjects were physically evaluated and the CRF was completed after reading the written consent, getting the explanation of the study (protocol, conditions, data confidentiality and study benefits).

Twelve subjects (32.43%) with MetSyn were with hypothyroidism, 10.81% (4 subjects) associated Type II Diabetes Mellitus and equal parts-5.4% (2 subjects) were known with Basedow Graves Disease, PCOS, prolactinoma, just one case with Cushing disease the rest being obese subjects without any associated endocrinopathies. The control group was mostly made up of obesity (72.8%) without any associated endocrinopathy, 13.6% of PCOS and 13.6% of multinodular goiter with hypothyroidism.

Fasting blood samples were taken the same day or the next day, in order to evaluate glycaemia, cholesterol, HDL-cholesterol, triglycerides in the C.I. Parhon National Endocrinology Biochemistry Laboratory. Next formula was used to appreciate insulin resistance:

HOMA-IR= [insulinemia ($\mu$UI/mL) $\times$ glycaemia (mg/dL)]/ 405.

Adiponectin, leptin and resistin levels were dosed using R&D commercial kits based on the Fluorokine Map technique in Cantacuzino National Institute, Bucharest. Insulin was also dosed in this institute using commercial Mercodia kits.

CRP and TNF$\alpha$ were dosed using commercial kits-Promokine and R&D in C.I.Parhon National Institute of Endocrinology Polypeptide and Steroid Laboratory, Bucharest.

Normal ranges were for adiponectin: 0.8–21.42 $\mu$g/ml, 2.205–11.149 $\mu$g/L in men and 3.87–77.27 $\mu$g/L in women for leptin and 6.39–26.4 ng/ml for resitin.

Normal CRP values were 0.0101–0.0268 $\mu$g/ml and 0.4–26.4 ng/ml for TNF$\alpha$.

SPSS version 15 program was used for the statistical evaluation of the results. Results are expressed as mean values ± standard deviation.

### RESULTS

The study group was characterized by a mean age of 48 year, with a BMI of 37.207±5.28 cm, uniform distributed (Fig. 1), a mean waist circumference of 112.49±13.6 cm, a hip circumference of 121.14± 12.32 cm, a waist hip ratio (WHR) of 0.92±0.06, triglycerides of 171.39±75.01 mg/dl, total cholesterol 209.03± 55.27mg/dl, HDL-cholesterol of 44.28±9.69 mg/dl, glycaemia of 115.73±46.27mg/dl, insulinemia of 18.77±14.24 mU/L and HOMA index of 5.24± 4.64 DS.
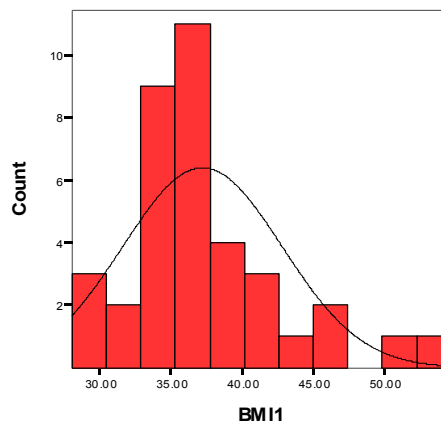


Fig. 1. BMI distribution in MetSyn group.

Mean values for systolic and dyastolic blood pressures were 132.43±21.39 mmHg, and 80.54±12.23mmHg. Mean values for adiponectin were 0.53±0.55 $\mu$g/ml, 92.35±43.65 $\mu$g/L for leptin, 9.11±3.83 ng/ml for resistin, 7.6±5.47 $\mu$g/ml for CRP and 5.51±5.79 pg/ml for TNF$\alpha$.

Adiponectin levels were low, leptin, CRP and TNF$\alpha$ high and resistin normal indifferent of the associated endocrine disease.

The control group was different from the MetSyn group regarding BMI (p=0.05)–34.24±5.28 kg/m$^2$, waist circumference–102.50±13.62 cm (p=0.009), waist hip ratio–0.85±0.06 (p=0.001), triglycerides–85.77±31.98 mg/dl (p<0.001), HDL-cholesterol–55.87±11.97 mg/ml (p<0.001), diastolic blood pressure–72.5±10.2 mmHg (p=0.012) and systolic blood pressure–117.5± 15.71 mmHg (p=0.006), heart rate–82.29± 10.53 bpm (p=0.03), leptin–60.24±26.84 $\mu$g/L (p=0.005) (Fig.2) and insulin-13.60±4.28 mU/L (p=0.049), glycaemia–99.03±9.01 mg/ml (p=0.013), HOMA-IR-3.03±0.89 (p=0.033) (Fig.3) and TNF$\alpha$.–1.7±2.903 (p=0.026) (Fig.4).
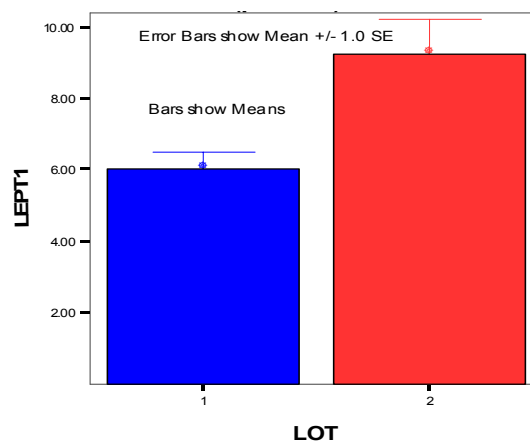


Fig. 2. Leptin mean values in controls (column 1) *vs.* MetSyn group (column 2) visit 1 (p=0.001).

Although adiponectin and resistin values were lower and CRP higher in the study group, there were no significant differences between the two groups.
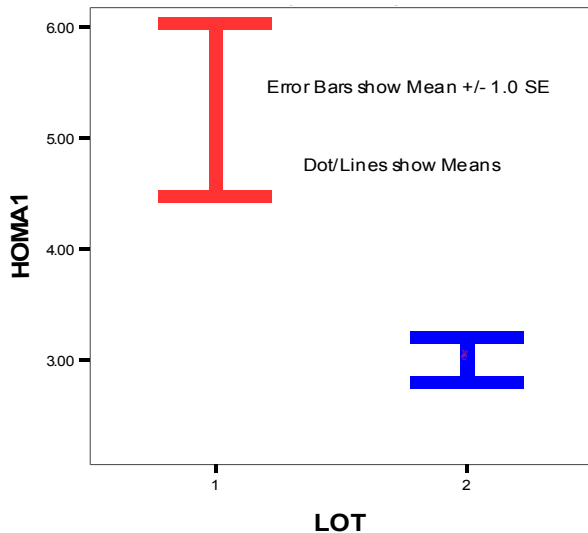


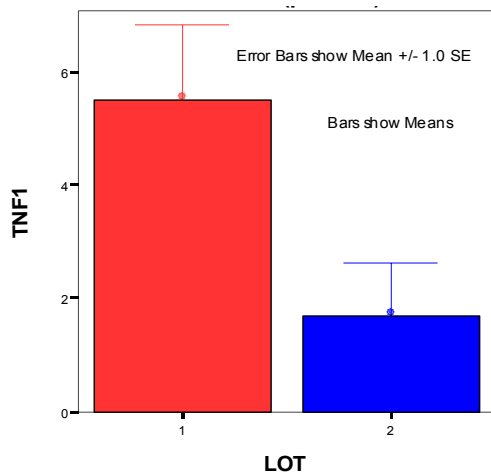Fig. 3. HOMA-IR mean values in MetSun group (column 1) *vs*. controls (column 2) visit 1 (p=0.033).



Fig. 4. Mean TNF alpha values in MetSun group (column 1) *vs*. controls (column 2) visit 1 (p=0.026).

Analysing mean values during visit 2 demonstrated differences regarding triglycerides (p=0.03) and glycaemia (p=0.033). Adipokines and cytokines values were even lower after weight loss, but with slightly higher adiponectin and resistin concentrations and lower CRP, TNFα and leptin levels in the MetSyn group. In this group total cholesterol, blood pressure, glycaemia, heart rate, leptin, insulinemia and HOMA index showed improvement.

In order to prove the efficiency of the diet, variables were compared as pairs (visit 1-visit 2). In the MetSyn group, weight (p=0.002), BMI

(p=0.001), waist circumference (p=0.002), hip circumference (p=0.001), adiponectin (p=0.012), resistin (p<0.001) and CRP (p<0.001) were statistically significant different for a p<0.01; HDL-cholesterol (p=0.01) and glycaemia (p=0.022) for a p<0.05.

The control group showed also statistically significant differences for weight (p=0.002), BMI (p=0.003), waist circumferences (p=0.025), hip circumference (p=0.012), cholesterol (p=0.034), HDL-cholesterol (p=0.004), adiponectin (p=0.011), leptin (p=0.005), resistin (p=0.004), CRP (p=0.05) and TNFα (p=0.04).

Pearson's correlations in the study group during visit 1, showed positive correlations between BMI and waist circumference (p< 0.001), hip circumference (p< 0.001), CRP (p< 0.001) (Fig. 5), positive for waist circumference with hip circumference (p< 0.001) and CRP (p=0.017).



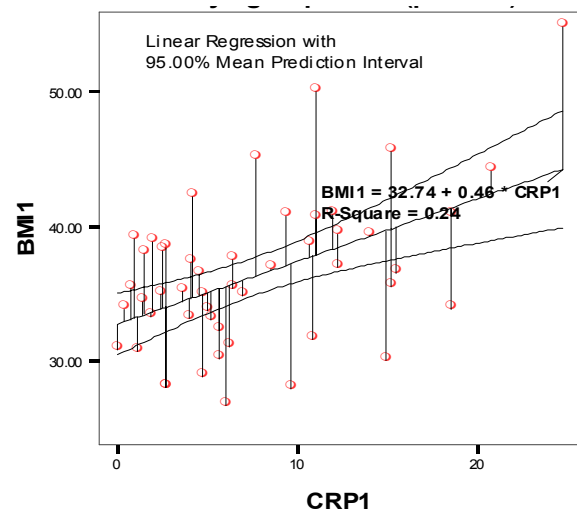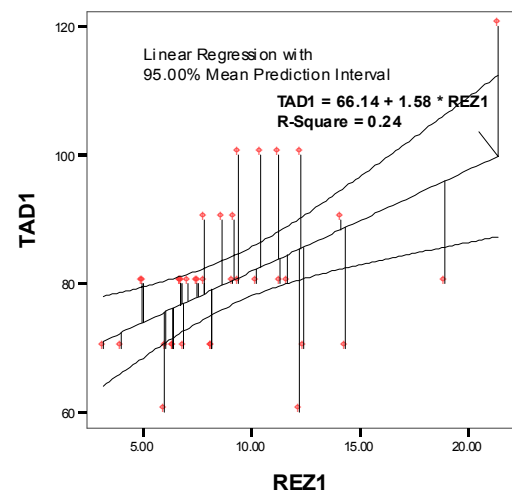Fig. 5. Correlation BMI-CRP MetSyn group visit 1 (p=0.002).



Fig. 6. Correlation TAD-resistin MetSyn group visit 1 (p=0.002).

Hip circumference correlated positive with glucose values (p=0.035) and CRP (p=0.014).

Triglycerides correlated negatively with HDL-cholesterol (p< 0.001) and positively with the number of criteria used to diagnose the MetSyn (p=0.006).

HDL-cholesterol correlated negatively with triglycerides and with criteria number (p=0.032). There were positive correlations between the diastolic and systolic tension (p< 0.001), resistin (p=0.002) (Fig. 6) and numbers of criteria used to diagnose the metabolic syndrome (p=0.032).

Adiponectin correlated positively with resistin (p< 0.001) (Fig. 7), diastolic tension (p< 0.001), glycaemia (p=0.003) and although not statistically significant we observed a tendency towards negative correlations for adiponectin with age, triglycerides, total cholesterol, insulinemia, leptin and positive ones with HDL-cholesterol.
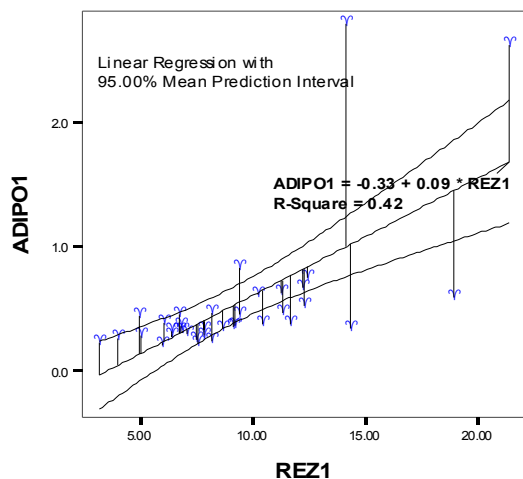


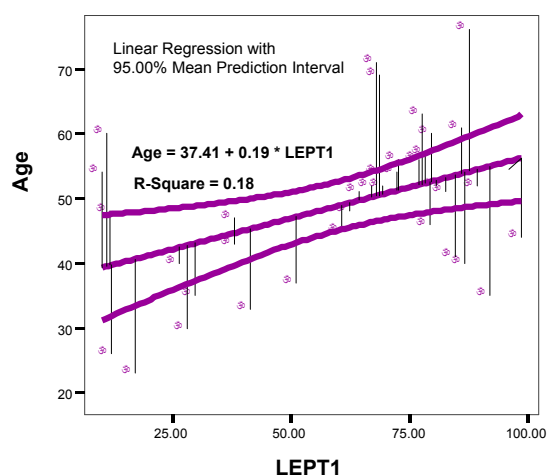Fig. 7. Adiponectin-resistin correlation MetSyn group visit 1 (p<0.001).



Fig. 8. Age-leptin correlation in MetSyn group visit 1 (p=0.01).

Leptin correlated positive with age (p=0.01) (Fig. 8) and CRP (p=0.049). We also observed a tendency towards positive correlations for leptin with BMI, waist circumference, triglycerides, total cholesterol, glycaemia, insulinemia, HOMA index, systolic blood pressure and negative correlations with adiponectin, resistin and diastolic blood pressure.

Resistin correlated positively with diastolic blood pressure (p< 0.001) and adiponectin (p< 0.001) and negative with insulin, HOMA, leptin, HDL-cholesterol, triglycerides and cholesterol although statistically insignificant.

Glycaemia correlated also positively with hip circumference (p=0.04) and the number of criteria (p=0.022).

TNFα did not reach any statistically significant value in correlation with the studied parameters, but we observed a positive tendency for TNFα's correlations with BMI, waist circumference, hip circumference, systolic tension, leptin, insulin, HOMA, CRP and negative with resistin.

Correlations in the control group, during the first visit were positive for age with BMI (p=0.03), waist circumference (p=0.041), triglycerides (p=0.024), systolic blood pressure (p=0.01) and insulinemia (p=0.044).

BMI correlated positive with waist circumference (p< 0.001), hip circumference (p< 0.001) and waist hip ratio (p=0.03). Waist circumference correlated positive with hip circumference (p< 0.001), CRP (p=0.014) and TNFα (p=0.016).

Hip circumference correlated positive with diastolic blood pressure (p=0.033). WHR correlated positive with CRP (p=0.025), BMI and waist circumference.

Triglycerides correlated positive with age and cholesterol (p=0.043).

Systolic tension correlated positively with age, cholesterol (p=0.016), diastolic tension (p< 0.001), CRP (p=0.021), TNFα (p=0.008). Diastolic tension correlated positively with hip circumference (p=0.003), systolic tension (p< 0.001), CRP (p=0.031) and the numbers of criteria (p=0.029).

Adiponectin correlated positively with leptin (p=0.043) and resistin (p=0.008) and negatively with the numbers of criteria (p=0.045).

Leptin correlated positively with adiponectin (p=0.043) and the numbers of criteria (p=0.007).

Insulin correlated positive with TNFα (p=0.016), HOMA (p< 0.001), TNFα correlated also positive with age (p=0.011) and negative with resistin (p=0.045).

A reduction in the numbers of criteria could be demonstrated after 3 month diet in the MetSyn group. Age still correlated with waist

circumference (p=0.05), BMI kept positive correlations with leptin (p=0.004), resistin (p=0.02), insulinemia (p=0.03) and HOMA (p=0.008) although weight was lost. Waist circumference correlated positive supplementary with BMI, hip circumference (p<0.001), triglycerides (p=0.043), leptin (p=0.034), glycaemia (p=0.012), insulinemia (p=0.04) and HOMA (p=0.005). Hip circumference correlated supplementary with triglycerides (p=0.025), leptin (p=0.02) and kept a positive correlation with glycaemia (p=0.022). WHR correlated supplementary with HOMA (p=0.028).

Triglycerides correlations were positive with waist circumference and hip circumference as mentioned above. HDL-cholesterol correlated negatively with resistin (p=0.042) (Fig. 9).
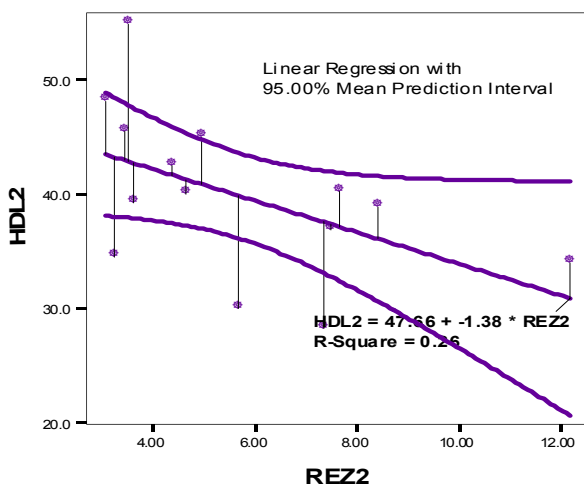


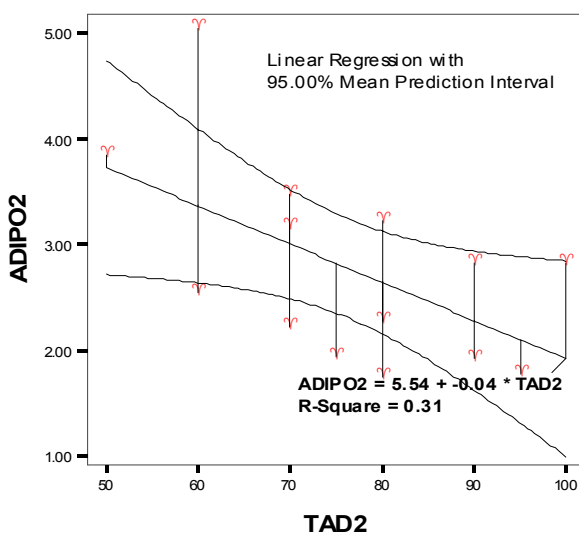Fig. 9. HDL-Cholesterol-resistin correlation in MetSyn group visit 2 (p=0.042).



Fig. 10. Tad-adiponectin correlation in MetSyn group visit 2 (p=0.021).

Diastolic blood pressure correlated negatively with adiponectin (p=0.021) (Fig. 10), and kept the positive correlation with systolic blood pressure (p=0.002). After weight reduction, heart rate correlated with insulin levels (p=0.05).

Adiponectin correlated negatively with diastolic blood pressure (p=0.021), positive with resistin (p=0.047) and leptin (p=0.01).

Leptin correlated positively with BMI (p<0.001), waist circumference (p=0.034), hip circumference (p=0.02), adiponectin (p=0.01), resistin (p<0.001) and CRP (p=0.005).

Resistin correlated positively with BMI (p=0.02), adiponectin, leptin and CRP (p<0.001) and negatively with HDL-cholesterol as mentioned above. Resistin's correlation were similar with adiponectin's correlation excepting systolic and diastolic tension.

Glycaemia correlated positively with waist circumference (p=0.012), hip circumference (p=0.02), cholesterol (p=0.047) and did not correlate any longer with adiponectin and criteria number.

Insulin values were positively associated with BMI (p=0.03), hip circumference (p=0.035), heart rate (p=0.049), correlations that weren't present during visit 1. HOMA-IR was positively associated with BMI (p=0.01), waist circumference (p=0.005) and WHR (p=0.028).

CRP's correlations were positive with leptin and resistin.

Unfortunately TNFα didn't correlate significantly with any studied parameter, not even after diet.

In the control group, at visit 2, age correlated positively with adiponectin (p=0.028) and negatively with cholesterol (p=0.03). BMI correlated positively with glycaemia (p=0.014).

Waist circumference and glycaemia associated positively (p=0.031). Hip circumference correlated also positively with glycaemia (p=0.017). WHR correlated positively with systolic blood pressure (p=0.036), diastolic blood pressure (p=0.023), insulinemia (p=0.009) and HOMA-IR (p=0.016). Systolic blood pressure correlated positively just with WHR (p=0.04), diastolic blood pressure correlated supplementary with heart rate (p=0.013), insulinemia (p=0.007) and HOMA (p=0.016).

Adiponectin's correlations were different after weight loss, positive with age (p=0.028), negative with cholesterol (p=0.01) and statistically unsignificant with leptin, glycaemia and resistin.

Leptin and resistin didn't reach any statistically significant correlations.

Glycaemia correlated postively with BMI (p=0.014), waist circumference (p=0.031) and hip circumference (p=0.017); insulinemia correlated positively with diastolic blood pressure (p=0.007) and HOMA (p<0.001).

CRP and TNFα didn't show significant correlations in the control group.

## DISCUSSIONS

The simultaneously evaluation of adiponectin, leptin, resistin, CRP and TNFα in two groups-of obese subjects with MetSyn and controls revealed extremely low adiponectin and high leptin levels in both groups, with lower adiponectin and higher leptin levels in the MetSyn group, normal resistin values, with a tendency towards higher values in the control group, high CRP values in both groups, although higher in the MetSyn group and high TNFα levels in the MetSyn group.

Low levels of adiponectin are due to excessive fat masses especially with visceral disposition[17–20], insulin resistance, proved by high HOMA index values in the MetSyn group compared to control group (p=0.033).

Adiponectin's correlations with resistin and leptin in the control group, although insignificant, point out to adipokines' variations in obesity associating insulin sensibility when even low levels of adiponectin can be benefic. In the control group, visceral fat associates high levels of CRP correlating with elevated blood pressure proving the existence of pro-inflammatory state with significant cardiovascular risk[21].

Adiponectin correlated surprisingly with resistin and diastolic blood pressure, justified by low adiponectin mean values and lack of its protective effect. Mean values for leptin are high in both groups, with higher values for the MetSyn group due to insulin resistance and probably leptin resistance.

In the control group, although obese, triglycerides, HDL-cholesterol and blood pressure values are normal, adiponectin concentrations low and leptin values high, probably due to subcutaneous fat mass excess. It is known that leptin synthesis happens mainly in the subcutaneous fat stores, reflecting subcutaneous more than omental adipocytes hypertrophy.

Leptin receptor mutations, SOCS 3 blocking leptin uptake and the presence of brain barrier transfer defect may lead to high leptin levels[22–24], as a leptin resistance consequence. This can be the way high levels of leptin can be explained in both groups.

Leptin correlated positively with age in the MetSyn group, suggesting that aging associates a tendency towards weight gain (especially abdominal fat storages), due to IGF1, GH, DHEA and DHEA-S deficiencies, sedentary life style and deregulations of the lipid and glucose metabolism, justifying the positive correlations of leptin. In preexisting glucose metabolism deregulations, leptin cannot act normally. High levels of leptin can predict worsening of glucose metabolism[25]. Leptin can stimulate insulin secretion; receptors for leptin have been described in β pancreatic cells[26]. Leptin can induce the alteration of insulin action in adipocytes and liver[27], proven by the positive correlations between leptin and insulin, glycaemia, triglycerides, cholesterol.

The negative correlations of leptin with adiponectin and resistin suggest that low values of adiponectin and normal high resistin values associated with high leptin concentrations can contribute to the adverse lipid profile.

Leptin's positive correlations in the control group with adiponectin and resistin (although insignificant) may be in the context of high normal leptin value and low adiponectin levels due to the fat mass excess that does not associate insulin resistance.

Resistin has been considered to be involved in insulin resistance by counteracting insulin action, high levels being present in obese rats[7,28]. In overweight adults[7,29], resistin levels were described as low by several studies, while others proved high resistin levels in insulin resistant states, while others demonstrated high resistin levels in insulin sensitive states[30].

In this study resistin values were high normal in both groups, but with slightly lower levels in the MetSyn group. It correlated positively with adiponectin and negatively with leptin in the study group, suggesting resistin's possible involvement in insulin sensitivity[31].

High resistin levels present simultaneously with high insulin levels may suggest resistin resistance[31]. The positive correlation between diastolic blood pressure and resistin can be explained through NOS inhibition and stimulation and stimulation of endothelin 1 secretion[9].

CRP mean values in the MetSyn group were very high suggesting important cardiovascular risk, the inverse correlation between CRP and endothelial vasodilatation being already known, especially in PCOS with insulin resistance[32]. CRP is involved in endothelial dysfunction through

raising soluble adhesion molecules and monocytes chemoattractant proteins expression, favoring LDL-cholesterol uptake in macrophages[33, 34].

CRP's positive correlations with BMI, waist circumference and leptin are suggestive for the presence of the inflammatory syndrome, which contribute to cardiovascular risk.

TNFα levels seamed not to correlate with insulin resistance. Local TNFα production is a homeostatic mechanism that prevents the variation of adipocytes volume. It also suppresses adiponectin gene expression and so reduces adiponectin concentrations. The reduction of adiponectin levels may stimulate LPS and NF kB induced TNFα raises, and so the anti-inflammatory efect of adiponectin (inhibition of monocytes and endothelial cells adhesion molecule expression) is not exerted.

Several studies proved that adiponectin levels raise[16, 35], leptin levels are reduced[27], resistin levels are lowered or do not change[36] after weight loss. This study demonstrates that leptin levels normalise, adiponectin, resistin and CRP levels reduce after weight loss due to a low caloric intake, in both groups disregarding the associated endocrinopathy. TNFα showed lower levels just in the MetSyn group.

Weight loss was associated with reductions of waist circumference, hip circumference, WHR, triglycerides, total cholesterol, diastolic tension, insulinemia, glycaemia, HOMA, CRP and TNFα and a mild raise of HDL-cholesterol.

Comparing mean values showed persisting higher values for triglycerides, cholesterol, blood pressure, insulinemia, glycaemia, HOMA-IR in the MetSyn group, with higher adiponectin and resistin values and lower leptin, HDL-cholesterol, CRP and TNFα. Although adiponectin mean values were lower after 3 month, they were slightly higher in the MetSyn group and showed different correlations.

Adiponectin correlated positively with resistin and leptin and negatively with the numbers of criteria due to insulin resistance reduction (demonstrated by lower insulinemia and HOMA index values). Although adiponectin mean values are still low, adiponectin can exert its positive effects on blood pressure and glucose metabolism, pointed out by its negative correlations with diastolic blood pressure (p=0.021) and glycaemia. HMW (high molecular weight) adiponectin is more frequent in women, especially during weight loss and lowers glucose values, raises NO levels and inhibits endothelial cells' apoptosis[18, 37]. It can also lower free fatty acids levels and stop triglycerides

storage in the liver and muscles[4], indicated by the negative associations between adiponectin and triglycerides in the MetSyn group.

Adiponectin correlating positively with insulin, after weight reduction and the improvement of insulin resistance and glycaemia, can be explained by the adiponectin involvement in insulin secretion (reduction of pancreatic islets apoptosis)[38]. The negative correlation between adiponectin and the numbers of criteria demonstrates adiponectin effects in counteracting insulin resistance and its consequences.

Leptin correlated positively with BMI, waist circumference and hip circumference due to leptin levels being dependent on BMI and subcutaneous adipocytes' volume.

High leptin levels in obesity can be caused by a leptin resistance; leptin being otherwise known to improve insulin resistance when infused intraventricullary[39].

Resistin correlated postively with adiponectin and leptin in the MetSyn group and negatively with HDL-cholesterol. Resistin is an inflammatory protein[8] and can stimulate the adhesion molecule expression, an action that cannot be counteract by low HDL-cholesterol levels.

Leptin and resistin, correlated both positively with CRP, explaining their involvement in inflammation.

Even though TNFα' correlations have been insignificant, weight reduction, especially waist circumference reduction lowered TNFα levels. TNFα's positive correlations with lipids, blood pressure, glycaemia and adipokines can be suggestive for the lack of insulin resistance influence on TNFα and so cardiovascular risk.

Waist circumference correlated positively with BMI, hip circumference, WHR, triglycerides, glycaemia, insulinemia, HOMA index and leptin proved already by multiple studies[40]. Triglycerides correlated positively with waist circumference and hip circumference; HDL-cholesterol correlated negatively with resistin, being known that a big waist circumference associates low HDL-cholesterol levels and inflammation[40], high leptin and resistin values[6] and low adiponectin concentrations.

Leptin determines hypertension through sympathetic nervous system activation and so vasoconstriction[3], while resistin inhibits NOS activation in endothelial cells; adiponectin inhibits neo-intimal proliferation and determines vasodilatation by NO release, explaining so the positive correlations between leptin and systolic blood pressure, the negative correlations of adiponectin with diastolic tension and the positive ones with leptin.

Glycaemia correlated positively with waist circumference, negatively with adiponectin pointing out to muscles glucose utilization[4]. Resistin correlated also, negatively with insulin suggesting its possible involvement in insulin sensibility.

The negative correlations of glycaemia with HDL-cholesterol, the positive ones with triglycerides, systolic and diastolic blood pressure, insulinemia and HOMA prove that hyperglycaemia stimulates insulin synthesis, raises the hypertension risk and favors synthesis and deposition of triglycerides due to FFA and HDL-cholesterol catabolism[41].

Correlations changed in the control group, once weight was lost. Although insignificant adiponectin correlated negatively with blood pressure, insulin, leptin and positive with resistin and HDL-cholesterol due to adiponectin role in insulin sensibility, reduction in triglycerides and antihypertensive effect through NO vasodilatation [4,18], sustained by HDL-cholesterol anti-atherogenic effects.

Resistin-adiponectin positive correlation points to a resistin resistance, or resistin involvement in insulin sensibility rather than in insulin resistance. Resistin involvement in hypertension in the control group is stating resistin direct influence on hypertension[42].

The activation of renin-angiotensin-aldosteron and sympathetic nervous system by insulin and stimulation of endothelin 1 secretion states by correlations between blood pressure, insulinemia and HOMA index.

## CONCLUSIONS

1. The metabolic syndrome alters profoundly hormones and biochemical parameters.

2. Insulin levels and insulin resistance grade are directly correlated with BMI, waist circumference without any regard towards age. Waist circumference is an excellent way to quantify obesity.

3. Adiponectin levels are low, leptin, CRP, TNFα concentrations are high and resistin values are high normal in obesity.

4. Obesity associating insulin resistance is characterized by lower adiponectin and resistin levels and higher leptin, CRP and TNFα levels compared with obesity without insulin resistance

5. High normal values for resistin (higher than the literature given normal range), may be due to resistin resistance.

6. We found a positive correlation between adiponectin and resistin suggesting a possible involvement of resistin more in insulin sensibility rather than insulin resistance.

7. MetSyn is characterized by high CRP levels proving the presence of a pro-inflammatory and pro-atherosclerotic state.

8. Resistin effects on blood pressure and inflammatory profile may involve insulin resistance.

9. Adipokines and cytokines levels were lower after 3 month low caloric intake, with a tendency towards higher adiponectin and resistin and lower leptin, CRP and TNFα levels in the MetSyn group

10. Although adiponectin levels were even lower after weight reduction, adiponectin had positive metabolic effects due to improved insulin sensibility.

11. Adipokines and CRP variations seamed to be the consequence and not the cause of insulin resistance. They can be considered as cofactors for insulin resistance.

## REFERENCES

1. Goulis DG, Tarlatzis BC Metabolic syndrome and reproduction: I. testicular function. *Gynecol Endocrinol.* **2008**;24(1):33–9.
2. Hu E., Liang P.,Spiegelman B.M.-AdipoQ is a novel adipose-specific gene dysregulated in obesity *.J.Biol.Chem.*, **1996**;271:10697–10703.
3. Gainsford T, Willson TA, Metcalf D, Handman E Leptin can induce proliferation, differentiation and functional activation of hemopoietic cells. *Proc Natl Acad Sci USA* **1996**;93:14564–14568.
4. Weyer C, Funahashi T, Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J.Clin Endocrinol Metab* **2001**;86;1930–1935.
5. Gasperikova D., Stencl J., Skopkova M., Vlcek M., Pechan J, Radikova Z., *et al.*, Different expression of the adipocytokines in subcutaneous and visceral adipose tissue of extremely obese subjects- *Diabetologia* **2007**; 50:(suppl1):S308.
6. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM-The hormone resistin links obesity to diabetes. *Nature,* **2001**; 409:307–312.
7. Lee J.H, Chan JL, Yiannakouris N., Kontogianni M.,Estrada E, Seip R.,Orlova C.,Mantzoros C.S., Circulating Resistin Levels Are Not Associated with Obesity or Insulin Resistance in Humans and Are Not Regulated by Fasting or Leptin Administration: Cross-Sectional and Interventional Studies in Normal, Insulin-Resistant, and Diabetic Subjects *J Clin Endocrinol Metab,* **2003**; 88(10): 4848–4856.
8. Steppan C, Brown E, Wright C, A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci USA* **2004**; 98:502–506.
9. Kougias P, Chai H, Lin PH, Yao Q, Effects of adipocyte-derived cytokines on endothelial functions: implication of vascular disease. *J Surg Res* **2005**;126(1):121–9.

10. Pitteloud N.,Hardin M., Dwyer A.,Valassi E.,Yialamas M.,Elahi D.,Hayes F Increasing Insulin Resistance Is Associated with a Decrease in Leydig Cell Testosterone Secretion in Men *J. Clin. Endocrinol. Metab*., **2005**; 90: 2636–2641.

11. Kern P.A.,Saghizadeh M.,Ong J.M.,Bosch R.J.,Deem R., Simsolo R.B.-The expression of tumor necrosis factor in human adipose tissue:regulation by obesity, weight loss and relationship to lipoprotein lipase. *J.Clin.Invest*., **1995**;95:2111–2119.

12. Nogueiras R., Gualillo O., Caminos E., Casanueva F.F., Dieguez C.- Regulation of Resisitin by Gonadal, Thyroid Hormone, and Nutritional Status *Obesity Research* **2003**;11(3):408–14.

13. Krassas G.E., Pontikides N., Loustis K., Koliakos G., Constantinidis T, Panidis D. Resistin levels in hyperthyroid patients before and after restoration of thyroid function: relationship with body weight and body composition *Eur. J. Endocrinol*., **2005**;153: 217–221.

14. Fernández-Real H.M, López-Bermejo A., Casamitjana R.,Ricart W.- Novel Interactions of Adiponectin with the Endocrine System and Inflammatory Parameters, *J Clin Endocrinol Metab* **2003**; 88(6): 2714–2718.

15. Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Panagiotakos DB, Koukkou E., Tzanela M, Thalassinos N., Raptis SA Insulin Action in Adipose Tissue and Muscle in Hypothyroidism *J. Clin. Endocrinol. Metab.*,**2006**; 91:4930–4937.

16. Xu A., Chan K., Hoo R L C, Tan K C.B., Zhang J,Chen B Testosteron Selectively Reduces the High Molecular Wieght Form of Adiponectin by Inhibiting its Secretion from Adipocytes *JBC papers in Press* **2005**; 1–23.

17. Perrini S., Laviola L., Cignarelli A., Melchiorre M., Stefano F.D, Caccioppoli C *et al.*, Fat depot related differences in gene expression, adiponectin secretion, and insulin action and signalling in human adipocytes differentiated in vitro from precursor stromal cells-*Diabetologia* **2008**;51:155–164.

18. Yamauchi T, Kamon J, Waki H, Imai Y, Shimozawa N-Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. *J.Biol Chem* **2003**;278:2461–2468.

19. Vlădoiu S, Oros S, Popa O, Dinu-Drăgănescu D,Ianăş O Serum levels of C-reactive protein in relation to hormonal parameters in metabolic syndrome. In press **2008**.

20. Carmina E,Orio F. Evidence for Altered Adipocyte Function in Polycystic Ovary Syndrome. *European Journal of Endocrinology* **2005**;152:389–394.

21. Mantzoros CS.The Role of Leptin in Human Obesity and Disease.A review of Current Evidence. *Annals of Internal Medicine* **1999**;130:671–680.

22. Lundgren M., Svensson M., Lindmark S., Fat cell enlargement is an independent marker of insulin resistance and 'hyperleptinaemia'. *Diabetologia* 2007; 50:625–633.

23. Schmidt M.I., Duncan B.B., Vigo A., Leptin and incident type 2 diabetes: risk or protection?. *Diabetologia* 2006; 49:2086–2096.

24. Kiefer TJ, Haller RS, Habner JF.-Leptin receptors expression on pancreatic β-cells. *Biochem Biophys Res Commun* 1996; 224: 522–27.

25. Näslund E., Andersson I, Degerblad M.Kogner P., KralJ.C., Rössner S.,Hellström P.M., Associations of leptin, insulin resistance and thyroid function with long-term weight loss in dieting obese men *Journal of Internal Medicine* **2000**; 248:299–308.

26. Roşca R, Ianăş O, Oros S, Manda D,Vlădoiu S,Dumitrache C. Adiponectina şi rezistina în obezitate şi comorbidităţi *Craiova Medicala* **2007**; 9(1):95–105.

27. Juan C, Au L, Fang V, Kang S, Ko Y, Kuo SF-Supressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. *Biochem Biophys Res Commun* 289:1328–1333(2001).

28. Perseghin G, Burska A, Lattuada G, Increased serum resistin in elite endurance athletes with high insulin sensitivity. *Diabetologia* **2006**, 49:1893–1900.

29. Pittas AG, Nandini JA, Greenberg AS-Adipocytokines and insulin resistance. *J.Clin, Endocrinol Meta.,* **2004**; 89(2):447–452.

30. Ilhan T, Berrin C. Endothelial Dysfunction in Young Women with Polycystic Ovary Syndrome: Relationship with Insulin Resistance and Low-Grade Chronic Inflammation. *J Clin Endocrinol Metab.***2004**;89:5592–5596.

31. Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy R, Haffner S Chronic subclinical inflammation as part of insulin resistance syndrome: the insulin resistance atherosclerosis study (IRAS). *Circulation* **2000**;102:42–47.

32. Barinas-Mitchell E, Cushman M, Meilahn EN, Tracy RP, Kuller LH Serum levels of C-reactive protein are associated with obesity, weight gain, and hormone replacement therapy in healthy postmenopausal women. *Am J Epidemiol* **2001**;153:1094–1101.

33. Swarbrick M, Austrheim-Smith I, Stanhope K, Circulating concentrations of high-molecular-weight adiponectin are increased following Roux-en-Y gastric bypass surgery. *Diabetologia,* 2006; 49:2552–2558.

34. Iqbal N, Seshadri P, Stern L, Serum resistin is not associated with obesity or insulin resistance in humans. *Eur Rev Med Pharmacol Sci.* **2005**;9(3):161–5.

35. Beulens J.W.J., Van Loon L.J.C, Kok F.J., Pelsers M., Bobbert T., Spranger J., Helander A., Hendriks H.F.J-The effect of moderate alcohol consumption on adiponectin oligomers and muscle oxidative capacity: a human intervention study.*Diabetologia,* **2007**;50:1388–1392.

36. Okamato M., Ohara-Imaizumi M., Kubota N., Hashimoto S et al-Adiponectin induces insulin secretion in vitro an in vivo at low glucose concentration-*Diabetologia* **2008**;5:827–835.

37. Kusminski C, McTernan P, Schraw T, Adiponectin complexes in human cerebrospinal fluid: distinct complex distribution from serum. *Diabetologia,* **2007**; 50:634–642.

38. Alexander CM, Landsman PB, Teutsch SM, Haffner SM (Third National Health and Nutrition Examination Survey [NHANES III], National Cholesterol Education Program [NCEP]). NCEP-defined metabolic syndrome,diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes*. **2003**;52:1210–1214.

39. Azen SP, Peters RK, Berkowitz K, Kjos S, Xiang A,Buchanan TA. TRIPOD (Troglitazone in the Prevention of Diabetes): a randomized, placebo-controlled trial of troglitazone in women with prior gestational diabetes mellitus. *Contrl Clin Trials*. **1998**;19:217–231.

40. Oros S, Ianaş O, Lerescu L, Sălăgeanu A,Giurcăneanu M, Dumitrache C Nivelul circulant al adipochinelor la pacienţii endocrini cu sindrom metabolic**.** Al III-lea Congres AECR, Cluj Napoca, septembrie **2008**.