

THE DYNAMICS OF ADIPONECTIN AND LEPTIN ON METABOLIC SYNDROME PATIENTS AND AGE MATCHED HEALTHY SUBJECTS

MOHAMAD ABDULWAHABE MOHAMAD¹, NICULINA MITREA¹, ALINA-CRENGUȚA NICOLAE^{1*}, MARIA-ZINAIDA CONSTANTINESCU¹, CRISTINA MANUELA DRĂGOI¹, ANDREEA-LETIȚIA ARSENE¹, CARMEN GABRIELA BARBU^{2,3}

¹*Biochemistry Department, „Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Traian Vuia St. 6, Bucharest, Romania*

²*Endocrinology Department, „Carol Davila” University of Medicine and Pharmacy*

³*Elias Emergency University Hospital, Mărăști St. 17, Bucharest*

* *corresponding author: alinanicolae29@gmail.com*

Abstract

The metabolic syndrome is a complex combination of interrelated risk factors of metabolic origin that, when occurring together, increase the risk of developing cardiovascular disease and diabetes.

Adipocytokines are bioactive mediators released from the adipose tissue including adipocytes and other cells present at the level of fat tissues. These include several novel and highly active molecules released abundantly by adipocytes like leptin, resistin, adiponectin, as well as some more classical cytokines released possibly by inflammatory cells infiltrating fat, like tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6). The most abundantly expressed cytokines within the adipose tissue are leptin and adiponectin [1, 3].

Adiponectin, is highly expressed in adipose cells, and circulates in high concentrations in the blood. Decreased serum levels of adiponectin are associated with insulin resistance. The role of adiponectin in systemic inflammation and critical illness is not well defined. The variability in the serum adiponectin levels is partly attributed to various options for modulation of this hormone.

Leptin, correlates directly with the mass of adipose tissue and its main function is to control the appetite and exerts its various actions on glucose metabolism and energy expenditure *via* binding to the leptin-receptor in the brain and peripheral tissues as liver, pancreas, adipose tissue and in the immune system [15].

In this study, we assessed the levels of adiponectin and leptin in lean and obese subjects with associated dislipidemy and diabetes.

Rezumat

Sindromul metabolic reprezintă o "constelație" de factori de risc, o combinație de afecțiuni medicale care, atunci când sunt asociate, cresc riscul de a dezvolta boli cardiovasculare și diabet.

Adipocitokinele sunt mediatori bioactivi eliberați de țesutul adipos, în deosebi de către adipocite. Acestea includ mai multe molecule noi și foarte active eliberate din abundență de adipocite precum leptina, rezistina, adiponectina, dar și a unor citokine clasice

proinflamatoare, cum ar fi factorul alfa de necroză tumorală (TNF- α) și interleukina 6 (IL-6). Cele mai pregnant exprimate în țesutul adipos sunt leptina și adiponectina.

Adiponectina, este exprimată în celulele adipoase, și circulă în concentrații foarte mari în sânge. Nivelele serice scăzute ale adiponectinei sunt asociate cu rezistența la insulină. Variabilitatea nivelurilor serice ale adiponectinei este atribuită diferitelor efecte de modulare ale acestui hormon. Nivelele serice de leptină sunt corelate direct cu masa de țesut adipos, iar funcția sa principală este de a controla apetitul. Leptina exercită diversele sale acțiuni asupra metabolismului glucozei și a consumului de energie prin legarea de receptorul leptinei din creier și din țesuturile periferice cum ar fi ficatul, pancreasul, țesutul adipos.

În acest studiu, s-a evaluat nivelul seric al adiponectinei și leptinei la subiecți sănătoși și pacienți obezi cu dislipidemie asociată și diabet.

Keywords: adiponectin, leptin, inflammation, metabolic syndrome, obesity.

Introduction

The prevalence of obesity is increasing worldwide, accompanied by a high incidence of type 2 diabetes mellitus and cardiovascular diseases. There is convincing evidence that obesity is accompanied by unfavorable metabolic profiles, such as impaired glucose tolerance, dyslipidemia, elevated blood pressure, or low-grade systemic inflammation.

Metabolic syndrome is considered a distinct pathology that implies the presence of a series of imbalances: an increased central distribution of body fat, insulin resistance, dyslipidemia, elevated blood pressure and an increased hypercoagulable and proinflammatory state. The presence of three or more risk factors qualifies for the diagnosis of metabolic syndrome, depending on the different criteria.

Criteria for the diagnosis of metabolic syndrome are the following [4,8]:

- ✓ **Adult Treatment Panel III (ATP-3) criteria** (any 3 of the following):
 - Waist circumference > 88 cm;
 - High density lipoprotein cholesterol (HDL-C) < 50 mg/dL;
 - Triglycerides (TG) > 150 mg/dL;
 - Blood pressure (BP) > 130/ > 85mmHg;
 - Fasting plasma glucose (FPG) \geq 110 mg/dL.
- ✓ **World Health Organization criteria**
 - (One major criterion):
 - Diabetes; insulin resistance; abnormal glucose tolerance; and
 - (Two minor criteria):
 - Body mass index (BMI) > 30; elevated BP; abnormal triglycerides with/without abnormalities in HDL-C or microalbuminuria.
- ✓ **International Diabetes Federation (IDF) criteria**
 - central obesity defined as waist circumference specific values (>80 cm in female and 94 cm in male population) plus any two of the following factors:

raised TG concentration: >150mg/dL,
reduced HDL cholesterol: <40mg/dL in males and <50mg/dL in females,
raised blood pressure: systolic BP >130 or diastolic BP >85 mmHg,
raised fasting plasma glucose (FPG):>100mg/dL.

It has recently been appreciated that adipose tissue, in addition to its role as an energy reservoir, modulates energy metabolism via secretion of circulating adipocytokines. The most abundant depots are visceral and subcutaneous adipose tissues, which produce unique profiles of biologically active adipokines with diverse functions. Adipokines are pharmacologically active, low molecular weight proteins that exert pleiotropic functions through several metabolic pathways. Adipokines are important due to their crucial mediator role and active participation in metabolic functions. These hormones also easily cross the blood-brain barrier, reach the main site of action located in the hypothalamic region, exert their actions and mediate the balance of satiety and hunger. The adipokines have a central role in the control of energy metabolism, communicating the nutrient status of the organism including energy intake and expenditure as well as insulin sensitivity.

Adiponectin is an intriguing adipokine with its serum level inversely correlated with fatness. It is related to the enhancement of insulin sensitivity, anti-inflammatory effects, anti-atherogenic actions, and regulation of metabolic homeostasis. It is particularly important in regulating insulin sensitivity. Although obesity, particularly visceral obesity, is associated with insulin resistance, the mechanism whereby adipose tissue modulates insulin sensitivity is controversial [7].

Leptin, a 16-kD protein, correlates directly with the mass of adipose tissue and its main function is to control the appetite. Leptin exerts its various actions on glucose metabolism and energy expenditure *via* binding to the leptin-receptor in the brain and peripheral tissues as liver, pancreas, adipose tissue and in the immune system. Leptin, an anti-obesity adipocytokine, regulates body weight by modifying energy levels and increasing metabolic rate while decreasing food intake. Most overweight and obese patients show resistance to leptin at the receptor level, and therefore have higher leptin levels than lean individuals [6].

In order to investigate the relationship among these adipocytokines, insulin resistance and obesity, we assessed the levels of adiponectin and leptin in lean and obese subjects with associated dislipidemy and diabetes.

Materials and Methods

The study was conducted in a private clinic in Bucharest from December 2011 until June 2012. Subjects were evaluated in terms of

anthropometric and biochemical markers, after these evaluations, there were selected 60 patients diagnosed with metabolic syndrome.

The study was performed in comparison with a control group consisting of 40 healthy volunteers. The two groups studied were divided according to age and gender, as follows: males and females between 30 and 50 years old, between 50 and 60 years old and over 60 years old, as depicted in table I and II.

The study protocol complies with the Declaration of Helsinki as well as with local institutional guidelines and was approved by the local ethics committees. Written informed consent was obtained from all participants.

Table I

The distribution of male subjects included in the study, patients and healthy subjects (control), according to their age

Male Healthy subjects	30-50 years old	25%
	50-60 years old	35%
	> 60 years old	40%
Male Patients	30-50 years old	20%
	50-60 years old	40%
	> 60 years old	40%

Table II

The distribution of female subjects included in the study, patients and healthy subjects (control), according to their age

Female Healthy subjects	30-50 years old	45%
	50-60 years old	35%
	> 60 years old	45%
Female Patients	30-50 years old	36%
	50-60 years old	44%
	> 60 years old	20%

In this study, a medical history to retrieve information about health status, current medications, alcohol consumption and a physical examination including height, weight, body mass index, a fasting blood sampling for the determination of adiponectin, leptin and usual biochemical markers was performed for all participants.

Patients were excluded from the study if they had taken any of the following: thyroid hormone therapy, medications that might affect glucose metabolism and insulin sensitivity such as glucocorticoids, oral contraceptives, and patients taking oral glucose-lowering medication or lipid lowering drugs. All subjects were studied in the morning between 08:00 am and 09:30 am after an overnight fast.

Waist circumference was measured on bare skin as the narrowest circumference between the 10th rib and the iliac crest with tailor meter. Weight was measured by a physician using a balanced-beam scale with light

clothing without shoes and expressed in kilograms (kg). Height was measured using a wall-mounted stadiometer and expressed in centimetres (cm). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Fasting venous blood samples were collected for the determination of biochemistry panel, lipid profile status, leptin and adiponectin. Cholesterol and triglycerides in serum were measured by an enzymatic colorimetric method. Blood glucose was measured using an automated glucose oxidase method. Adiponectin and insulin were measured by RIA using reagents from Linco Research Inc. (St Charles, MO, USA) and Pharmacia-Upjohn Diagnostics (Uppsala, Sweden) respectively. Serum leptin was determined using an ELISA kit (Human Endocrine LINCOplex Kit, LINCO Research, Inc., St. Charles, MO, USA) (MDC=1 ng/mL in 100 μL sample). Insulin resistance was assessed using the homeostasis model assessment ratio (HOMA-IR) formula (Fasting insulin x Fasting glucose / 405) [2,11].

Samples were measured in duplicate in the same assay run. The average of the two measures was used in the analyses.

Statistical analysis

Values are reported as mean \pm SD. Statistical analyses were performed using SPSS, Inc-PC (Chicago, IL). Statistical differences between groups were determined by analysis of variance (ANOVA). Correlations between parameters of adiponectin with anthropometric and metabolic variables were determined using Pearson's correlation coefficient. Level of statistical significance was chosen to be 0.05.

Results and Discussion

The first group of patients and the matched control group was represented by men aged 30 to 50 years old. The group of study was composed of 14 patients and 7 healthy subjects.

The average age was approximately the same (44-42 years old) for the patients and controls, and considering the median BMI, which was $35.21 \text{ kg}/\text{m}^2$ for patients compared with $22.71 \text{ kg}/\text{m}^2$ calculated for the healthy subjects, the waist circumference and the levels of serum glucose and insulin, we can surely point out the well defined characteristics of the metabolic syndrome group of patients and the age matched healthy controls group.

In addition, the lipidic profile, given by the triglycerides, total cholesterol, HDL and LDL cholesterol serum levels, confirmed the existence of a metabolic unbalanced general state in the case of patients group.

Considering the two well contured groups of study, we assessed the adiponectin serum concentration as a biochemical marker useful for depicting the evolution of this cluster of multiple metabolic disorders. In the case on healthy volunteers, the level of circulating adiponectin was 10.42 ± 0.53 $\mu\text{g/mL}$. The results registered for the patients group were much lower, 6.78 ± 1.62 $\mu\text{g/mL}$.

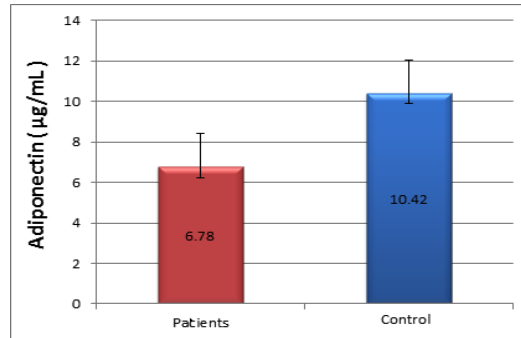


Figure 1

Serum adiponectin levels in 30-50 years old men patients and control

We also assessed the leptin serum concentration for the 30-50 years old men groups. In the case of healthy volunteers, the level of circulating leptin was 20.14 ± 2.11 ng/mL . The results registered for the patients group were much higher, 30.85 ± 2.95 ng/mL . (figure 2)

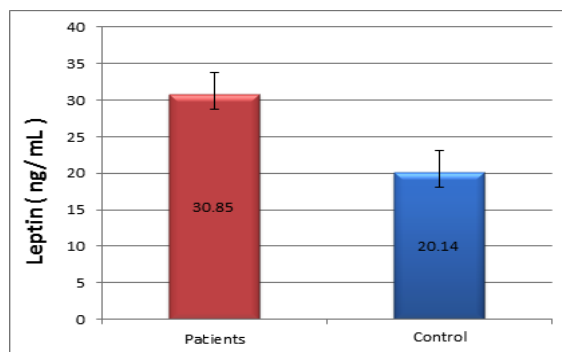


Figure 2

Serum leptin levels in 30-50 years old men patients and control

The second group of study was represented by women aged 30 to 50 years old. The groups of study were composed of 9 patients and 9 healthy subjects.

Serum adiponectin levels were higher in the healthy group 14.01 ± 4.86 $\mu\text{g/mL}$ of people compared to the studied patients, 11.55 ± 4.13 $\mu\text{g/mL}$, as it can be seen in figure 3.

Considering the age matched studied groups of patients, men *versus* women, we observed significantly higher levels of adiponectin in women, both in control and patients groups. In men population, the adiponectin level is lower.

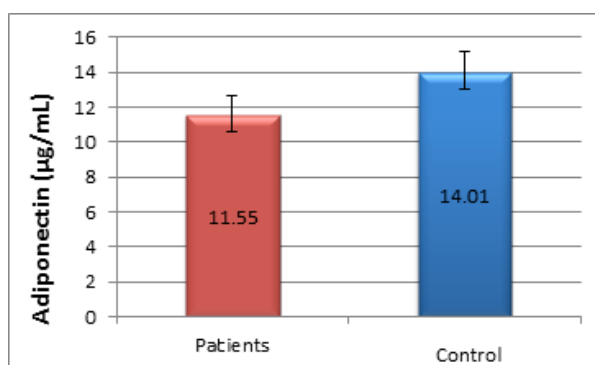


Figure 3

Serum adiponectin levels in 30-50 years old women patients and control

Considering leptin concentration, the most obvious difference was seen in women patients that registered 32.88 ± 2.61 ng/mL of circulating leptin, compared with control (17.22 ± 0.83 ng/mL), as also depicted in Figure 4. These values are highly correlated with HOMA-IR levels, denoting the fact that leptin resistance is a synergic process with insulin resistance.

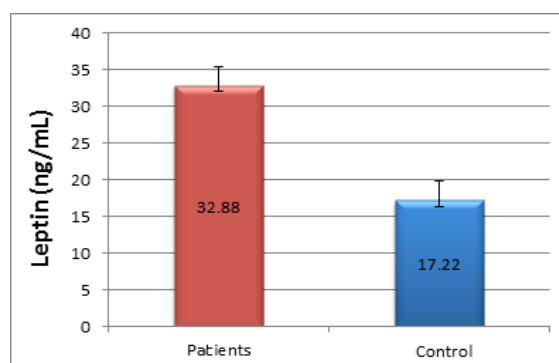


Figure 4

Serum leptin levels in 30-50 years old women patients and control

For the next group of study, 50-60 years old men, the adiponectin levels in patients presented a dramatical decrease 5.42 ± 1.22 µg/mL, compared to 50-60 years old healthy men, 8.23 ± 1.3 µg/mL.

We can also state that ageing, both in patients and controls, caused an important decrease of the adiponectin levels, by comparing the 30-50 years old groups with the 50-60 years old study population. For the patients groups, the

registered levels of adiponectin were 6.78 ± 1.62 $\mu\text{g/mL}$ for the 30-50 years old, compared to 5.42 ± 1.22 $\mu\text{g/mL}$ for the 50-60 years old patients. A similar dynamics of the adiponectin level was observed in the healthy men population: 10.42 ± 0.53 $\mu\text{g/mL}$, for 30-50 years old controls, *versus* 8.23 ± 1.3 $\mu\text{g/mL}$, for 50-60 years old controls.

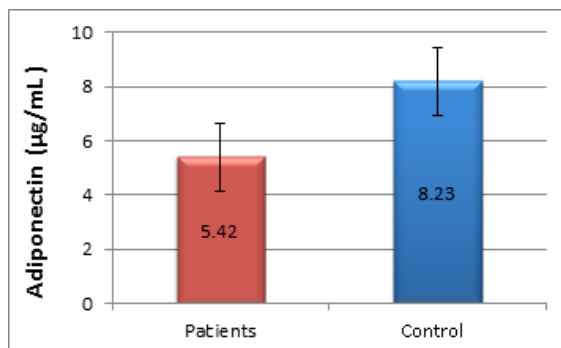


Figure 5

Serum adiponectin levels in 50-60 years old men patients and control

Serum leptin levels were higher in the patients group 31.5 ± 3.05 ng/mL compared to the healthy volunteers group, 16.87 ± 1.64 ng/mL , as it can be seen also in Figure 6.

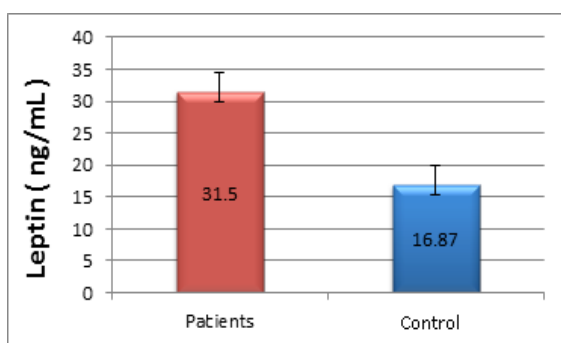


Figure 6

Serum leptin levels in 50-60 years old men patients and control

Considering the two age groups of men (30-50 years old *versus* 50-60 years old), we observed no significant differences in the leptin levels of young men, both in control and patients groups, compared to older men.

The next group of study was represented by women aged 50 to 60 years old. The groups of study were composed of 11 patients and 7 healthy subjects. For the patients groups, the registered levels of adiponectin were 7.45 ± 1.5 $\mu\text{g/mL}$, compared to 9.85 ± 3.21 $\mu\text{g/mL}$ for the 50-60 years old healthy controls (Figure 7).

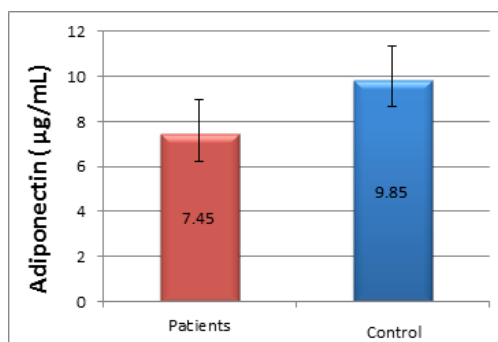


Figure 7

Serum adiponectin levels in 50-60 years old women patients and control

Among the parameters related to the metabolic syndrome, plasma adiponectin concentration varied inversely proportional with total cholesterol, LDL, triglycerides, fasting plasma glucose and serum insulin.

Serum leptin is closely associated with obesity and diabetes, has a functional role in the pathogenesis of severe illness and clearly correlates with markers of glucose and lipids metabolism, registering high levels in metabolic syndrome patients 33.45 ± 4 ng/mL (Figure 8).

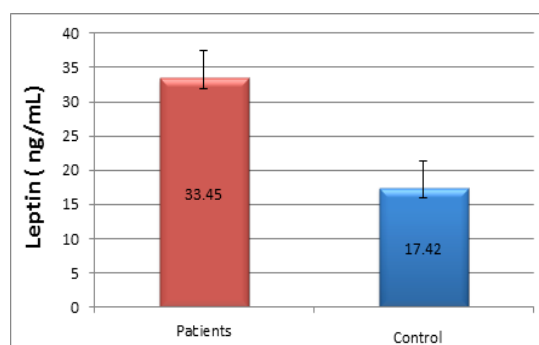


Figure 8

Serum leptin levels in 50-60 years old women patients and control

The last group of patients and control studied, was represented by men and women aged over 60. This was of particular interest considering the advanced pro-inflammatory general state of individuals, at this age, and moreover, for the women sub-population, the dramatic changes occurred after menopause, and the crucial role of hormones on various components of the metabolic syndrome.

The most obvious change in adiponectin synthesis is seen in men patients that registered $3.71 \pm 1.11 \mu\text{g/mL}$ of circulating adiponectin, compared with controls ($7.11 \pm 1.58 \mu\text{g/mL}$), as depicted in Figure 9.

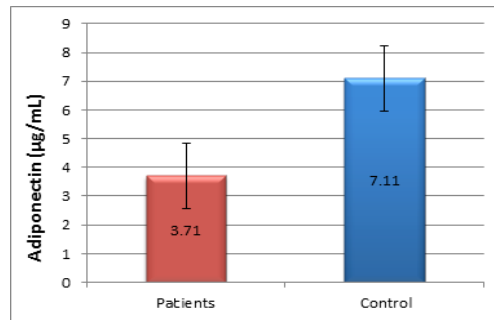


Figure 9

Serum adiponectin levels in over 60 years old men patients and control

In this age category, men registered a similar dynamics of the serum leptin levels, as the two previously studied age groups. Serum leptin levels in patients with metabolic syndrome are higher than those in healthy controls.

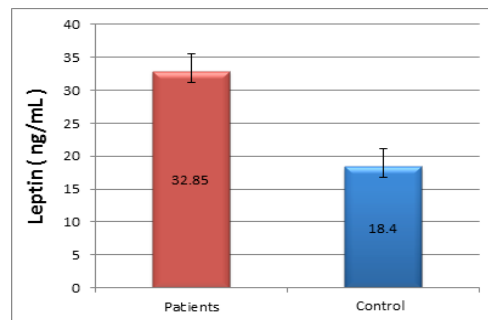


Figure 10

Serum leptin levels in over 60 years old men patients and control

The last group of study was represented by over 60 years old women, being of high interest considering the general state of individuals, at this age, and the profound changes occurred after menopause, considering hormonal and metabolic imbalances.

Women aged patients registered $5.02 \pm 1.2 \mu\text{g/mL}$ of circulating adiponectin, compared with controls levels of $8.25 \pm 0.95 \mu\text{g/mL}$.

Considering the previously studied women groups of patients and controls, we observed significantly higher levels of adiponectin in young

women, both in control and patients groups, levels that decreased considerably once the ageing process has evolved.

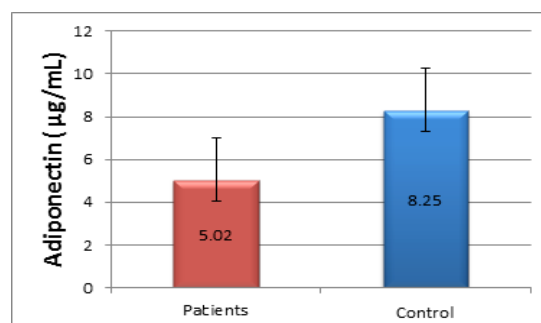


Figure 11

Serum adiponectin levels in over 60 years old women patients and control

We investigated the relationship between plasma adiponectin concentration and each component of the metabolic syndrome and the other adipocytokines assessed during this study. With regard to the anthropometric parameters, plasma adiponectin concentration correlated for some age groups of patients and controls, with a series of important parameters.

As it can be observed, leptin levels in patients presented a dramatical increase 32.2 ± 2.28 ng/mL, compared to over 60 years old healthy women, 18 ± 2.58 ng/mL. The correlation with other studied parameters reveals once more the close relationship among leptin levels and dislipidemia, diabetes and insulin resistance.

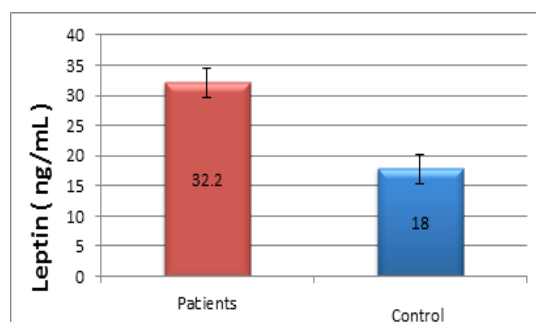


Figure 12

Serum leptin levels in over 60 years old women patients and control

For the male patients 50-60 years old it was obtained a correlation of the adiponectin level with the circulating leptin. For this statistical

correlation, the Pearson correlation coefficient was $r=0.6586$, as can be observed in Figure 13.

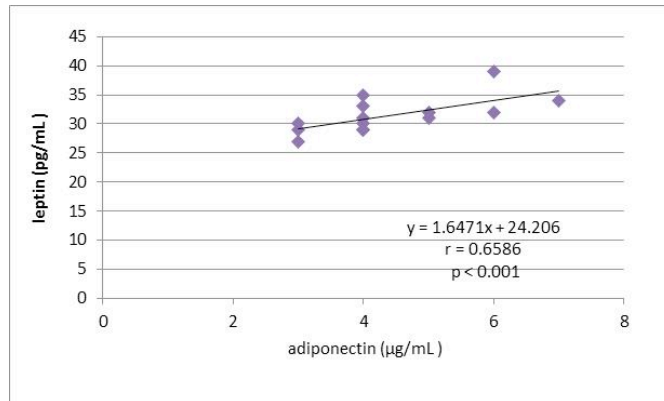


Figure 13

Correlation between adiponectin and leptin in male patients 50-60 years old

For the female patients over 60 years old we also obtained a negative correlation of the adiponectin level with the serum leptin. For this statistical correlation, the Pearson correlation coefficient was -0.9866 , as can be observed in Figure 14.

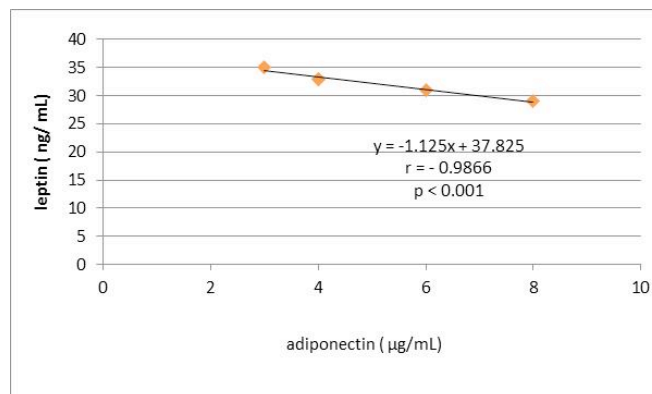


Figure 14

Correlation between adiponectin and leptin in female patients over 60 years old

Conclusions

It is now well known that the adipose tissue is not simply a reservoir for fat storage, but rather, adipose tissues constitute an active endocrine organ with multiple roles. One of these important roles is the contribution of adipose tissue to the inflammatory process in both vascular and nonvascular

tissues. Activated macrophages, together with adipocytes, secrete a number of proteins, the adipokines, that have both pro- and anti-inflammatory activities.

Adipocyte dysfunction occurs as a result of excess fat accumulation in the body, which causes dysregulated production of adipokines, thereby contributing to the pathogenesis of obesity-associated metabolic and cardiovascular complications [14].

The study of adiponectin and leptin profiles, one of the most important adipokines, therefore, is of particular interest for the understanding of the biochemical mechanisms implied in developing obesity-associated complications.

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