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CORRELATIONS OF VISFATIN WITH THE LIPIDIC METABOLISM IN DIABETIC AND OBESE PATIENTS

RAMONA GLIGOR[1], DANA ZDREMȚAN[1], LUMINIȚA PILAT[1], IOAN MATEI[2], CONSTANTIN IONESCU-TÎRGOVIŞTE[3] and IOAN CRÎSNIC[1]

[1]Department of Biochemistry,"Vasile Goldiş" Western University in Arad, Romania
[2]University of Medicine and Pharmacy, Carol Davila, Bucharest, Romania
[3]National Institute of Diabetes, Nutrition and Metabolic Diseases N.C.Paulescu, Bucharest

Corresponding author: Ramona GLIGOR, E-mail: ramona_gligor@yahoo.com

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Obesity and type 2 diabetes mellitus are the most frequent nutritional disorders found in the developed countries and are associated with a high cardiovascular mortality and morbidity. They are multifactorial associations and nutritional disorders, resulting from a genetic component and a multitude of environmental factors. The clinical observation determined the association of the obesity and diabetes mellitus with high blood pressure, dislipidemia and atherosclerosis. These conditions represent a public health problem, due to the high prevalence and its association with high risk cardiovascular diseases; both conditions have a high incidence in developed countries. Visfatin is an adipocytokine identified in 2005, its name suggesting the idea that it is predominantly produced in the visceral fat. It has a molecular weight of 52 kDa and it has in its structure, 491 aminoacid residues. It is identical to the PBEF, that was described in 1994 as a lymphocytes produced cytokine.

Key words: visfatin, diabetes mellitus, cholesterol, obesity, LDL, HDL.

INTRODUCTION

Diabetes mellitus, hypertension, dyslipidemia and atherosclerosis, were defined as conditions specific for a certain lifestyle. A common feature for these diseases is overfeeding and its consequence, obesity. The researches in the biology of the adipose tissue have shown that the adipose tissue is not only an energy storing organ but also an endocrine secretion organ[1,2].

Visfatin is an adipocytokine identified in 2005[3,4], its name suggesting the idea that it is produced predominantly by the visceral fat. It has a molecular weight of 52 kDa and it has in its structure 491 aminoacid residues. It is identical to PBEF[5], described in 1994 as a lymphocyte produced cytokine[6]. Visfatin has an expression in hepatocytes and muscles[7,8] as well as in the adypocytes, kidneys and heart, in animals[9]. Numerous publications reported various effects and correlations of visfatin with different medical conditions[10,11,12,13,14]. In this way, increasing of the visfatin level can be observed in atherosclerosis[11], endothelial dysfunctions[15,16,13,17], metabolic syndrome[18,14], renal insufficiency[13], obesity[10,19]. The relationship with diabetes mellitus[20,21,22,13] and visceral fat[10,21,3,13] is controversed.

In some studies it was reported that visfatin is strongly expressed in foam cells in atherosclerotic lesions with the increase of expressivity, especially in atheroma plaques in symptomatic plaques[11,23,24,17]. Chang *et al.* have not find in their study, on a 53 group of Taiwanese adults, differences in the visfatin expression between the visceral and subcutaneous adipose tissue[11,23,24,17]. Revollo et al., have shown that visfatin is an essential enzyme in the NAD production[9].

This protein is also, a cytokine that promote the B cell maturation and inhibits the neutrophiles apoptosis. The enzyme belongs to the family of glycosyltransferases, being a pentosyltransferase. This enzyme plays an important role in the metabolism of the nicotinic derivatives and nicotinamides[5].

Dogru *et al.*[21] studied the visfatin values in 40 patients with recently discovered diabetes or with altered tolerance to glucose and discovered high levels of visfatin in diabetic partients as compared to the control group.

Takebayashi *et al.* didn't find any correlation between visfatin and diabetes[13], and other study proved that there is a positive correlation between the decrease of visfatin and type 1 diabetes and negative correlation between HbA1c and visfatin levels[25].

It was proven that oxidized LDL increases the visfatin expression in the monocytes cell cultures. More than that, visfatin determines the expression increase of some extracellular degrading molecules, causing the instability of the atheroma plaque[11,26].

The high interest for the adipose tissue, as a secretory active organ and the observation of similarities between adipocytes and inflammatory immune cells, generated a high amount of information that revolutionized the understanding of the adipose tissue role in the physiologic and pathologic processes. The researches performed are too recent to allow therapeutic indications due to the fact that we must understand all the details regarding the adipokines and cytokines produced by the adipose tissue, as well as the differences between the visceral and subcutaneous adipose tissue and its exact role[27].

MATERIAL AND METHODS

Study group characteristic

We studied a total number of 60 subjects. These were divided into three groups according to the original condition, as it follows: group I – 25 patients with type 2 diabetes, group 2 – 15 obese patients and control group – 20 normal weight, apparently healthy volunteers (Table 1).

Group I included type 2 diabetic patients with duration of diabetes higher then 5 years, registered in *Arad Antidiabetes Center database*. The inclusion criteria for type II diabetes were according to the IDF recommendations[27].

Group II was formed from 15 obese patients, without diabetes and cardiovascular diseases. The inclusion criteria was the BMI>30kg/m^2, according to the WHO recommendations[29].

The control group was formed from subjects that were clinically healthy having a normal weight (BMI<25 kg/m^2).[30]

Laboratory methods

Biochemical determinations were perfomered in the biochemistry lab department of the Faculty of Medicine, Pharmacy and Dentistry at the "Vasile Goldis" Western University and in a private medical laboratory, SC Laborator Analize SRL, accredited by the Romanian Accreditation Association, RENAR.

Plasma lipids determinations were performed from a blood sample collected in the morning, after an overnight fasting of minimum 10 hours. The total cholesterol and HDL-cholesterol and triglycerides were determined through an enzymatic, photometrical method. LDL-cholesterol was calculated by the Friederwald formula:

LDLc = Total cholesterol – HDLc – (triglycerides/2,2).

There were determined the ratios between total cholesterol/ HDLc and LDLc/HDLc, the values over 5 being considered having a high atherogenic risk.

The plasma levels of lipids was appreciated according to the recommendations of the National Cholesterol Education Program, National Heart, Lung, and Blood Institute[20]. Dyslipidemia was considered at total cholesterol values > 4.5 mmol/l, LDLc > 2.5 mmol/l, triglycerides > 1.7 mmol/l and HDLc < 1.00 mmol/l in males and < 1.3 mmol/l in females.

The changes that appeared in the carbohydrates metabolism were interpreted according to the criteria from the glycometabolic classification of WHO[29].

The basal plasma glucose was determined through enzymatic method with glucose-6 phosphate. The normal values of plasma glucose were considered at a plasma glucose value below 6.1 mmol/l.

The glycosilated hemoglobin (HbA1c) was appreciated according to the IDF criteria. It was analyzed through the latex turbidimetry. The glycosylated hemoglobin level, according to the European guides, for non-diabetics, is < 6.1 % and for diabetics, < 6.5 %.

The determination of visfatin was done from blood collected in the morning, in a fasting state, through ELISA (R&D System), according to the reagents working protocol attached to the analyzing kit.

To determine the correlation, we used the Pearson coefficient.

Statistically significant were considered the differences in the cases where the bilateral value, $p < 0.05$.

In all the groups, including the control, we determined the visfatin levels.

Table 1

Clinical characteristics and risk factors in the population studied.

	Group I Diabetic	**Group II Obese**	**Control group**
Subjects	25	15	20
Age (years)	60.7±11.55	42±3.64	41±11.17
BMI (kg/m^2)	28.79±4.55	36.85±5.28	23.02±1.65

RESULTS AND DISCUSSION

Analyzing the lipid spectra to determine the dyslipidemia frequency, we obtained total cholesterol values >4.5 mmol/l in 21 cases in group I and 15 in group II.

Values of LDLc>2.5 mmol/l were found in 23 patients in group I and 15 patients in group II. (Table 2).

In Table 3 it can be observed the visfatin values in the volunteers taken into our study according to their mean BMI.

We obtained a statistically significant value higher in diabetic group as compared to the control one $p < 0.0001$. Also in the case of the obese group there was a statistical significance $p = 0.0002$. Comparing visfatin of the diabetic group divided according to their BMI, with the control group, we obtained statistical significance with each one of the groups studied (with the obese diabetics $p = 0.001$, with overweight diabetics $p = 0.0002$, with normal weight diabetics $p < 0.0001$).

Regarding the visfatin distribution, compared to the lipid metabolism parameters in the case of diabetic patients, we obtained positive correlations with LDLc ($r = 0.64$, $p = 0.0006$) (Figure 1) and with the ratio LDL/HDL ($r = 0.50$, $p = 0.01$) (Figure 2) and negative correlations with the ratio TC/HDL ($r = 0.43$, $p = 0.03$) (Figure 3) and HDL-cholesterol ($r = -0.30$, $p = 0.15$) (Figure 4). With the other lipid metabolism parameters total cholesterol ($r = 0.10$, $p = 0.63$), triglyceride ($r = -0.24$, $p = 0.17$), we didn't obtain linear correlations.

Table 2

The characteristics of the lipid metabolism in the group studied

Parameters	Group I (n=25)	Group II (n=15)	Control group (n=20)
Cholesterol (mmol/l)	5.44±0.95	5.54±0.46	4.13±0.35
LDLcholesterol (mmol/l)	3.27±0.65	3.56±0.56	2.18±0.42
HDLcholesetrol (mmol/l)	1.26±0.56	1.09±0.30	1.51±0.22
Triglycerides (mmol/l)	1.93±1.09	1.82±0.56	0.96 ± 0.24
Cholesterol/HDL	4.98±2.02	5.45±1.17	2.72±0.49
LDL/HDL	3.15±1.70	3.59±1.35	1.48±0.43

Table 3

Visfatin values compared to IMC.

	BMI (Kg/m^2)	Visfatin (ng/ml)
Group I	< 25	77 ± 6.93
	25 – 29.9	58.85 ± 31.0
	≥ 30	59.56± 39.13
Group II	≥ 30	41.13,2±8.43
Control group	< 25	26.10±12.13

In the case of visfatin distribution compared to the lipid metabolism parameters in group II, obese patients, there was a negative linear correlation in case of visfatin *vs.* HDLc ($r = -0.45$, $p = 0.09$) (Figure 5) and a positive linear correlation in the case of visfatin *vs.* TC/HDL ($r = 0.30$, $p = 0.27$) (Figure 6) and in the case of visfatin *vs* LDL/HDL ($r = 0.33$, $p = 0.22$) (Figure 7); in all other cases there wasn't any linear correlation: visfatin *vs* triglycerides ($r = 0.05$, $p = 0.85$); visfatin vs LDLc ($r = 0.16$, $p = 0.56$); visfatin *vs* total cholesterol ($r = 0.06$, $p = 0.83$).

Regarding the visfatin values in the control group, there is a linear correlation with: HDLc ($r = 0.45$, $p = 0.04$) (Figure 8), LDLc ($r = -0.45$, $p = 0.04$) (Figure 9) and with the ratio CT/HDL ($r = -0.43$, $p = 0.05$) (Figure 10), LDL/HDL ($r = -0.44$, $p = 0.05$) (Figure 11); correlation is missing in the case of triglycerides ($r = -0.11$, $p = 0.64$) and total cholesterol ($r = -0.28$, $p = 0.23$).

The changes in the lipid metabolism in most patients with type 2 diabetes mellitus and obese, determined a boom in this research area and to enlighten in other areas of the lipid metabolism. In this way it was introduced the term "lipid triad", characterized by the decrease of HDL, increase of triglycerides and the appearance of some small and dense LDL particles[31].

High plasmatic values of visfatin are found in obesity associated with high LDL and low HDL-colesterol. However, the effects of visfatin remain controversial and the relationship with carbohydrates metabolism are uncertain. [32,33]

When discovered visfatin, Fukuhara *et al.*[3] have shown that this molecule was characterized by effects similar to insulin. In this study, the authors have shown that this adipocytokine is a strong adipogenic agent through the in vitro glucose transport stimulation and lipogenesis. In reality it seems to induce an increase of the in vitro glucose transport in 3T3 pre-adypocytes or L6 myocytes. The administration of visfatin in diabetic mice

seemed to ameliorate the insulin sensitivity. This hypothesis is confirmed through the presence of an original hyperglycemia in heterozygous animals to invalidate visfatin. This thing is explained by the fact that visfatin can bind to the insulin receptors with an affinity similar to insulin, but at a different site[3].

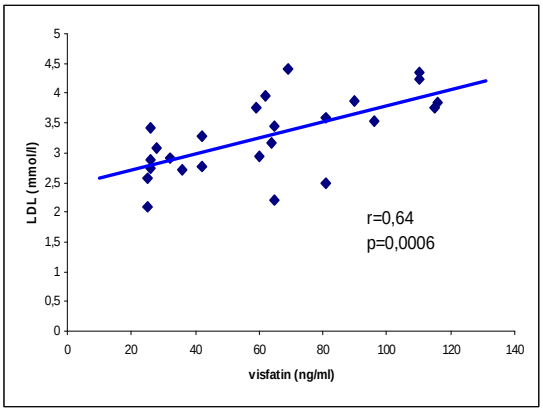

Fig. 1. Visfatin distribution as compared to the LDLc in the diabetic patients group.

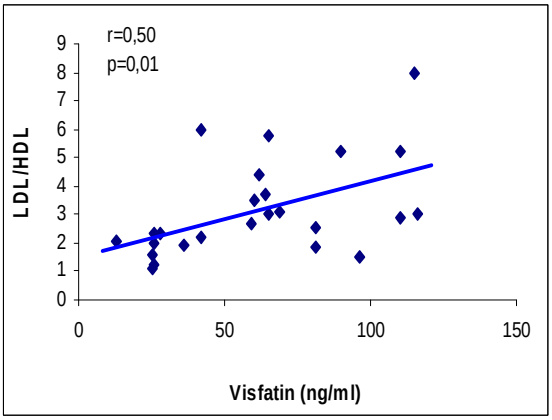

Fig. 2. Visfatin distribution as compared to the LDL/HDL in the diabetic patients group.

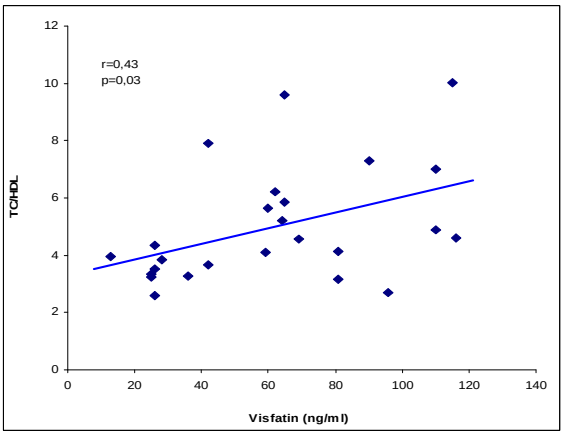

Fig. 3. Visfatin distribution as compared to the TC/HDL in the diabetic patients group.

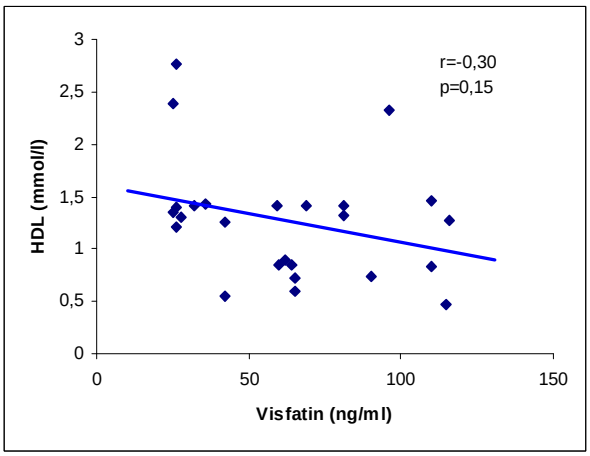

Fig. 4. Visfatin distribution as compared to the HDLc in the diabetic patients group.

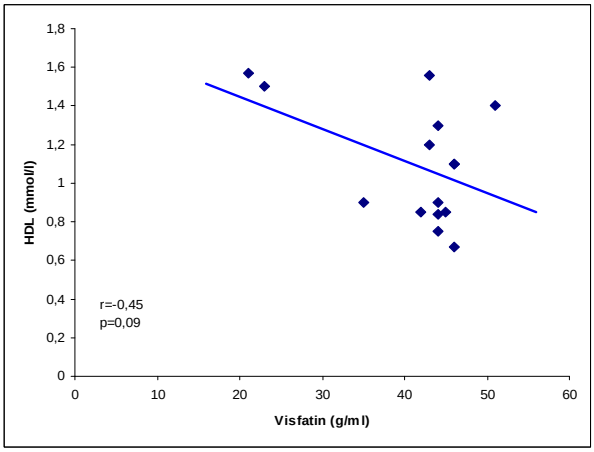

Fig. 5. Visfatin distribution as compared to the HDLc in the obese patients group.

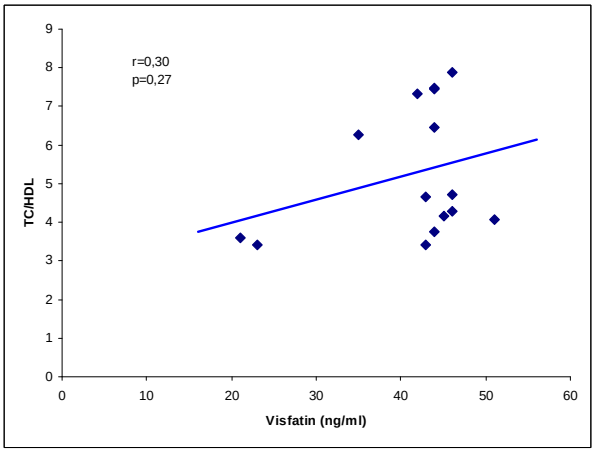

Fig. 6. Visfatin distribution as compared to the TC/HDL in the obese patients group.

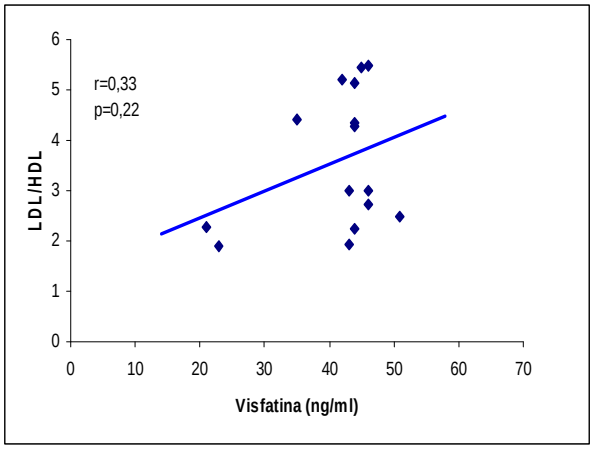

Fig. 7. Visfatin distribution as compared to the LDL/HDL in the obese patients group.

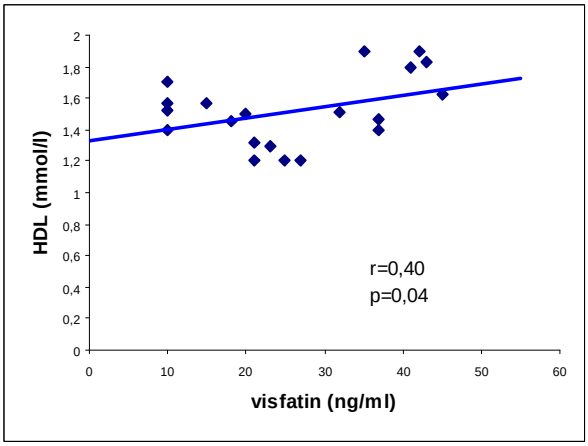

Fig. 8. Visfatin distribution as compared to the HDLc in the control group.

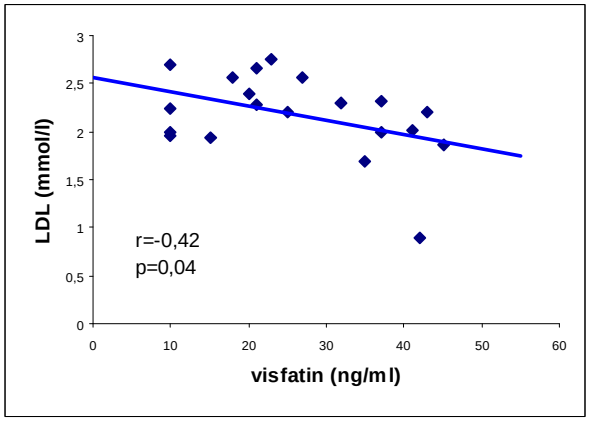

Fig. 9. Visfatin distribution as compared to the LDLc in the control group.

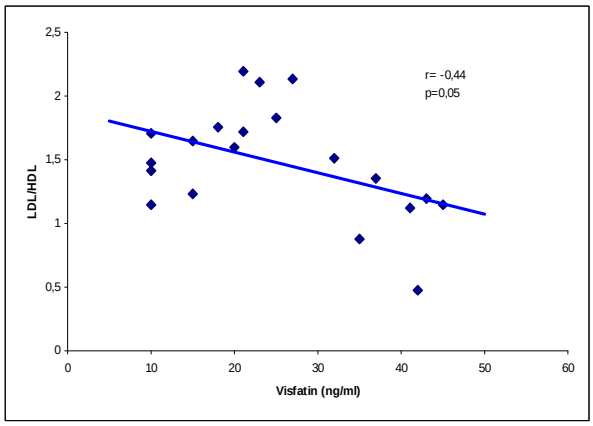

Fig. 10. Visfatin distribution as compared to the LDL/HDL in the control group.

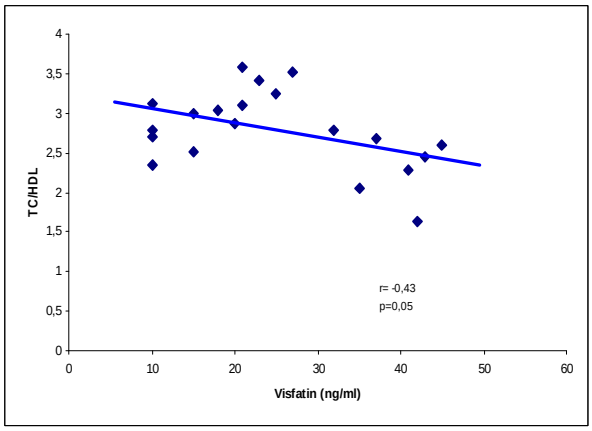

Fig. 11. Visfatin distribution as compared to the TC/HDL in the control group.

In humans, the preliminary studies have shown that the plasma levels of visfatin increased during obesity, without any correlation with insulin resistance. There are some studies showing that the plasma levels of visfatin are not correlated with the amount of visceral adipose tissue and there was not observed any differences in expression of visfatin in subcutaneous vs visceral adipose tissue. Even though more studies have shown a relationship between visfatin and obesity, its real metabolic role must be clarified[34].

In our study there were not observed any significant changes of visfatin in diabetic patients as compared to the control group. Neither, did we obtain values that were statistically significant with the obese group; the values in group II, obese, were higher than in the control group but statistically non-significant.

Positive correlations we obtained in the case of visfatin with LDLc, TC/HDLc ratio and LDL/HDL ratio. Knowing the fact that LDL is an atherogenic

molecule, we can state that visfatin could be involved or used as marker in the atherosclerosis pathology.

CONCLUSIONS

The mean values of visfatin were higher in diabetic group as compared to the obese and control group.

We obtained negative correlations of visfatin with triglycerides, in diabetic patients.

In the case of visfatin against HDLc, there were obtained negative correlations in diabetic and obese patients and positive correlations in the control group. Could visfatin also have anti-inflammatory properties?

Even though the role of visfatin in the diabetes mellitus pathogeny is far from being revealed, there are studies that indicate the fact that the visfatin values are independent and significantly associated with type 2 diabetes, plasma visfatin being increased in type 2 diabetes patients, while adiponectin is decreased and resistin doesn't show differences in comparison to the control group[35].

REFERENCES

1. Beaser RS, and the Staff of Joslin Diabetes Centes, Joslin's Diabetes Deskbook, a guide for primary care providers, second edition, Lippincott Wiliams & Wilkins, 2007.
2. Fortuňo A, Rodriguez A, Gómez-Ambrosi J, *et al.*, Adipose tissue as an endocrine organ: role of leptin and adiponectin in the pathogenesis of cardiovascular diseases. J. Physiol. Biochem.; 59:51-60, 2003.
3. Fukuhara A, Matsuda M, Nishizawa M *et al.*, Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 307,426, 2005.
4. Saddi-Rosa P, Oliveira CSV, Giuffrida FMA, Reis AF. Visfatin, glucose metabolism and vascular disease: a review of evidence. Diabetology & Metabolic Syndrome; 2:21-29, 2010.
5. Samal B, Sun Y, Stearns G, *et al.*, Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony enhancing factor. Mol Cell Biol 14:1431-1437, 1994.
6. Sattar N., Wannamethee SG, Forouhi NG, Novel biochimical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? Diabetologia, 51: 926-940, 2008.
7. Cosford SR, Bajpeyi S, Pasarica M, *et al.*, Scheletal muscle NAMPT is indiced bz exercise in humans Am J Physiol Endocrinol Metab, 98:E117-E126, 2010.
8. Garten A, Petzold S, Barnikol-Oettler A, Körner A *et al.*, Nicotinamide phosphoribosyltransferase (NAMPT/ PBEF-visfatin) is contitutively realsed from human hepatocytes. Biochem Biophys Res Commun 391(1): 376-81, 2010.
9. Revollo JR, Korner A, Mills KF *et al.*, Nampt/PBEF/ Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab. 6(5):363/375, 2007.
10. Berndt J, Kloting N, Kralisch S *et al.*, Plasma visfatin concentrations and fat depot-specific mRNA expressions in humans. Diabetes, 54(10): 2911-2916, 2005.
11. Dahl TB, Yndestad A, Skjelland M *et al.*, Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization. Circulation, 115: 972-980, 2007.
12. Ingelsson E, Larson MG, Fox Cs *et al.*, Clinical correlated of circulating visfatin levels in a community-based sample. Diabetes Care, May, vol 30 No 5: 1278-1280, 2007.
13. Takebayashi K, Suetsugu M, Wakabayashi S, *et al.*, Association between plasma visfatin and vascular endothelial function in patients with type 2 diabetes mellitus. Metabolism, 56(4):451-458, 2007.
14. Zhong M, Tan HW, Gong HP, Wang SF, Zhang Y, Zhang W. Increased serum visfatin in patients with metabolic syndrome and carotid atherosclerosis. Clin Endocrinol (Oxf); 69(6):878-84, 2008.
15. Romacho T, Azcutia V, Vazquez-Bella M *et al.*, Extracellular PBEF/NAMPT/visfatin activates pro-inflammatory signaling in human vascular smooth muscle cells through nicotinamide phosphoribosyl-transferase activity. Diabetologia; 52:2455-1463, 2009.
16. Stofkova A. Resistin and visfatin: regulators of insulin sensitivity inflammation immunity. Endocrine Regulations vol 44, 25-36, 2010.
17. Wang LS, Yan J-J, Tang N-P and colab., A polymorphism in the visfatin gene promoter in related to decreased plasma levels on inflammatory markers in patients with coronary artery disease. Mol Biol Rep; 38:819-825, 2011.
18. Moreno-Aliaga MJ, Lorente-Cebrián S, Pérez-Echarri N, Hernándes JAM. Visfatina, apelina ų nuevas moléculas del síndrome metabólico. Revista Espaňola de Obesidas vol. 6 Num 4 Julio-agosto (205-214), 2008.
19. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ, Owverwight, obrsity and mortality from cancer in a prospectively studies cohort of US adults, N Engl. J.Med, 348; 171625-1638, 2003.
20. Arner Peter. Visfatin - a true or false trail to type 2 diabetes mellitus. J Clin Endocrinol Metab, January, 91(1):28-30, 2006.
21. Dogru T, Sonmez A, Tasci I *et al.*, Plasma visfatin levels in patients with newly diagnosed and untreated type 2 diabetes mellitus and impaired glucose tolerance. Diabetes res Clin Pract, 76 (1):24-29, 2007.
22. Lopez-Bermejo A, Chico-Julia B, Fernandez-Balselles M *et al.*, Serum visfatin increases with progressive β/cell deterioration. Diabetes, 55(10): 2871-2875, 2006.
23. Görsoy G, Akçayöz ŞS, Acar Y, Demirbaş B. Visfatin in hyperlipidemic female patients. Journal of Medicine and Medical Science vol 1(4) pp. 120-125, 2010.
24. Moschen AR, Kaser A, Enrich B *et al.*, Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol;178:1748-1758, 2007.
25. Lim SY, Davidson SM, Paramanathan AJ *et al.*, The novel adipocytokine visfatin exerts direct cardioprotective effects J Cell Mol Med; 12(4):1395-1403, 2008.
26. Wang P, van Greevenbroek MMJ, Bouwman FG *et al.*, The circulating PBEF/NAMPT/visfatin level is associated with a beneficial blood lipid profile. Eur J Physiol;454: 971-976, 2007.

27. Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin: adiponectin ratio as an atherosclerotin index in patients with type 2 diabetes: realtionship of the index to carotid intima-media thickness. Diabetologia; 48:2684-2686, 2005.
28. Bountin P Dina C, Vasseur F *et al*., GAD2 on Chromosone 10p12 is a candidate gene for human obesity Plos Biol 3:361-371, 2003.
29. World Health Organization. Definition, Diagnosis, and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Part I: Diagnosis and Classification of Diabetes Mellitus (PDF), 1999. Accessed January 26, 2011.
30. *** National Heart, Lung and Blood Institute in cooperation with The national Institute of diabetes and Digestive and Kidney Diseases, Clinical Guidelines on the Identification, Evaluation and Treatment of overweight and obesity in Adults, the evidence report, NIH publication, sepember No. 98-4083, 1998.
31. Tilgh H and Moschen A. Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. Clinical science; 114:275-288, 2008.
32. Dray C, Valet P, Castan-Laurell I. Adipokines: quelles nouvelles. Obes; 3:33-41, 2008.
33. Sun G, Bishop J, Khalili S et colab., Serum visfatin concentration are positively correlated with serul triacylglycero and down-regulated by overfeering in healthy young men. Am J Clin Nutr; 85:399-404, 2007.
34. Trayhurn P and Wood IS., Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochemica Society Transactions, vol 33, part 5:1078-1081, 2005.
35. Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF *et al.*, Elevated plasma level of visfatin/pre-B cell colony enhancing factor in patients with type 2 diabetes mellitus, J Clin Endocrinol Metab, 91(1) 295-9, 2006.