Involvement of Adiponectin in Early Phase of Acute Myocardial Infarction with ST-Segment Elevation (STEMI)

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Adiponectin is secreted by fatty tissue; it has a peptide biochemical structure and among other roles, it seems to inhibit endothelial inflammation. C-reactive protein has an essential role in host defense. Troponin is a protein that is part of cardiac and skeletal muscle contraction. Myocardial infarction is an acute event defined as myocardial cell necrosis, caused by sudden and sustained ischemia. The purpose of our search work was to analyze the connection between various biomarkers over the early phase of acute STEMI: adiponectin, high sensitive-C reactive protein (hs-CRP), high sensitive-Troponin T (hs-Troponin T), triglycerides, total -, LDL- and HDL-cholesterol. Our study included two groups of patients - one group of 30 patients diagnosed with STEMI in the first 12 h after onset, and the second group of 30 patients with unstable angina pectoris, normal hs-Troponin T, and normal findings at coronary angiography computed tomography (CT). Subjects in the STEMI-arm had a higher serum level of hs-CRP, hs-Troponin T, total cholesterol, LDL cholesterol, triglycerides, and decreased levels of HDL-cholesterol and adiponectin than those in the angina-arm. We identified diminished adiponectin plasma concentrations during the first hours of STEMI evolution. We also found out a directly proportional ratio among adiponectin and HDL-cholesterol and an inverse report between this hormone and all other studied biomarkers. Our results may support the anti-inflammatory and anti-atherogenic features of adiponectin.

Keywords: adiponectin, hs-CRP, hs-Troponin T, STEMI

According to its universal definition, an acute myocardial infarction is diagnosed when acute myocardial injury is detected by high and evolutive serum levels of hs-Troponin T (above the 99 percentiles) of the reference scale for a healthy population), together with at least one proof for myocardial ischemia (clinical/ electrocardiography/ imagistic/ coronarography/ autopsy) [1].

Troponin is a protein found in myocardium and skeletal muscle; it is made out of three substructures: Troponin C, T and I; a significant difference is that Troponin C in the skeletal muscle cell contains four calcium-binding sites, while myocardial Troponin C contains only three.

Troponin T and I are released in the blood flow after the loss of cell membrane integrity. Troponin T begins to increase one hour after myocardial infarction outset, gains significant serum levels after 3-4 hours and persists above normal ranges 6-14 days. This prolonged serum persistence is due to the slow release of troponin complex from myocytes and is used for myocardial infarction diagnostics in acute setting and two weeks after [2].

Adiponectin is a plasma cytokine with mainly anti-inflammatory properties. It takes part in glucose metabolism and oxidation of fatty acids [3]. Besides a small amount which is coming from the placenta during pregnancy, it is secreted only by the adult adipose tissue; its plasma level is inversely proportional with adult body adipose mass [4]. Adiponectin secretion has a circadian rhythm, with an early-morning peak and a nocturnal decrease in plasma levels [5].

C reactive protein is the prototype of the acute phase reactants in humans, playing an essential role in host defense. CRP bloodstream levels are low in normal conditions but dramatically increases (about ten thousand fold) in the first hours of all-cause inflammation. CRP is a polypeptide secreted by liver cells as a non-glycosylated monomer, consecutively forming the pentameric structure specific for the pentraxin family of proteins [6].

Cholesterol is found in high amounts in all animal cell membranes, where it takes part in maintaining its structure and also in intercellular signaling [7]. Cholesterol is carried through the bloodstream by the lipoproteins, like LDL and HDL. LDL particles have variable dimensions and density. Clinical trials have shown that small and dense LDL (B model) is a higher risk factor than large and less dense LDL (A model) since smaller particles are more capable of penetrating endothelium. HDL can extract free cholesterol from the cell membrane and attaches it to fatty acids, resulting in cholesterol esters; these are yielded to LDL molecules, in exchange to triglycerides and lipid-soluble vitamins (e.g. vitamin E). In healthy subjects, 30% of plasma cholesterol is transported by HDL [8].

Triglycerides are esters of glycerin with fatty acids, in which all the three hydroxyl groups are esterified. Triglycerides are synthesized by liver cells from carbohydrates. It enters in the structure of VLDL (59%), HDL (3%) and chylomicrons (81-88%), playing a central role in lipid metabolism [9].

The purpose of our search work was to analyze the connection between various biomarkers over the early phase of acute STEMI: adiponectin, high sensitive-C reactive protein (hs-CRP), high sensitive-Troponin T (hs-Troponin T), triglycerides, total -, LDL- and HDL-cholesterol.

Experimental part

Material and method

The active arm of our study enrolled 30 consecutive patients with acute STEMI, admitted in the Cardiology Clinic of Constanta County Hospital in the first 12 h from symptoms onset - during a period of six months (June 2018 - November 2018). The control arm enrolled 30 patients

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	Control group	STEMI group	*P* value
Age (mean, years) ± SD	55.4±16.6	58.6±19.3	0.15
Gender (M/F)	18/12	19/11	0.25
Urban/Rural	22/8	21/9	0.34
BMI (kg/m^2)	28.3±6.4	28.7±6.9	0.09
eGFR (ml/min/m2)	62.4±30.8	54.6±24.9	0.04
Systolic blood pressure (mmHg)	154.2±35.4	148.5±34.6	0.03
Diastolic blood pressure (mmHg)	78.6±21.9	76.8±20.8	0.004

Table 1
CHARACTERISTICS OF PATIENTS FROM BOTH STUDY GROUPS

with unstable angina pectoris, who display normal hs-Troponin T and normal findings at coronary angiography CT. Patients in both arms had comparable demographic characteristics (table 1).

Excluding criteria were: chronic inflammatory diseases, active neoplasia, recent (<3 months) trauma or surgery, sepsis or extensive burnings -conditions in which acute phase reactants may be modified; patients who did not agree to participate in our study were also excluded.

The diagnostic of acute STEMI was made on: prolonged acute chest pain, dynamic ST-segment elevation in at least two concordant derivations on ECG, positive markers for myocardial injury (hs-Troponin T) and localized ventricular wall motion anomalies at transthoracic ultrasound examination.

All patients included in our study accomplished anamnesis, physical control and paraclinical tests. Subjects in the STEMI arm underwent all these within the first 12 hours from STEMI onset.

Anamnesis included: age, gender, urban/rural environment, level of education, smoking status, family and personal pathological history, reasons for hospital admission, date and time of symptoms onset and actual disease conditions together with treatment followed by patients until hospitalization.

Physical examination consisted of general status appreciation, BMI calculation and study of respiratory, cardiovascular, digestive, renal apparatus, together with cutaneous/subcutaneous, osteo-articular and central nervous systems.

Paraclinical investigations consisted of:

-ECG: using a 12-derivations Nyhon-Kohden device, in standard conditions (recumbent resting patients); for evidencing of posterior and right ventricle infarction we used extreme left, respectively correct derivations;

-transthoracic echocardiography: using a General Electric Vivid S6 device and making all standard measurements (valves, chambers, systolic and diastolic LV function, segmental wall kinetics, Doppler flows evaluations and great vessels appreciation);

-biochemical blood determinations: using venous blood obtained at the moment of hospital admission; hs-Troponin T was determined using Eclecsys test, adiponectin was determined using the ELISA method and hs-CRP using latex-immunoturbidimetric assay. Triglycerides, total- and HDL-cholesterol plasma concentrations were obtained by the spectrophotometric method, and LDL-cholesterol was obtained by the fourth homogeneous assay. This assay removes non-LDL cholesterol via a selective reaction with cholesterol esterase and cholesterol oxidase, and the resulting peroxide byproduct is eliminated by reaction with catalase.

Normal lipid profile was defined as follows: HDL-cholesterol >40 mg/dL, LDL= cholesterol <100 mg /dL, total cholesterol <200 mg/dL and triglycerides <150 mg/dL.

Statistical analysis was performed with t-test and Pearson correlation coefficient. Continuous variables were summarized as mean ± SD and compared using Student's *t*-test.

Results and discussions

The patients' baseline features are given in table 1, along with the P-values for the univariate analysis.

Of the 30 patients included in the active arm of the study, 20 patients (66%) were diagnosed with dyslipidemia. High-

	Control group *Mean±SD*	STEMI group *Mean±SD*	*P* value
Adiponectin (mg/L	9.05±1.27	6.42±1.41	<0.00001
Total Cholesterol (mg/dL)	200.8±17.69	234.2±38.03	0.005
HDL-Chol (mg/dL)	51±8.32	44±5.43	0.001
LDL-Chol (mg/dL)	104.6±10.17	129.9±32.07	0.009
Triglycerides (mg/dL)	135.8±16.75	181.13±58.56	0.010
hs CRP (mg/L)	0.92±0.29	3.37±2.28	0.0009
hs-Troponine T (pg/mL)	9.29±2.88	1211.15	0.029

Table 2
COMPARISON BETWEEN PATIENTS IN ACTIVE ARM AND CONTROL ARM

	Female Control *Mean±SD*	Female STEMI *Mean±SD*	*P* value
Adiponectin (mg/L)	9.96±1.00	7.24±1.30	0.0005
Total Cholesterol (mg/dL)	192.2±18.61	229.81±39.17	0.031
HDL-Chol (mg/dL)	56±5.47	45.72±5.04	0.001
LDL-Chol (mg/dL)	98.8±4.54	126.81±27.81	0.022
Triglycerides (mg/dL)	129±18.50	161.81±50.56	0.093
hs CRP (mg/L)	0.85±0.26	3.11±1.18	0.0004
hs-Troponine T (pg/mL)	8.94±3.01	725.45	0.026

Table 3
COMPARISON BETWEEN FEMALES-STEMI GROUP AND THE CONTROL GROUP

sensitive troponin T, hs C-reactive protein, adiponectin, triglycerides, total- , HDL- and LDL- cholesterol were analyzed and compared with the group of controls (table 2, 3, 4).

Patients with acute myocardial infarction had increased serum level of triglycerides, total- and LDL- cholesterol, high-sensitive Troponin T and high sensitive C-reactive protein ($P < 0.05$), but decreased plasma levels of adiponectin and HDL- cholesterol ($p < 0.05$) than controls. Concerning equality, these correlations were stronger in females (except triglycerides); in males, these correlations were available only for hs-Troponin T, hs-CRP and adiponectin.

We found a strong inverse ratio between adiponectin and total cholesterol among angina arm females (r=-0.55), and among all patients in agina arm (r=- 0.6) (Table 3), and also a moderate same inverse ratio among male STEMI patients (r=-0.28) (table 4). Adiponectin and HDL-cholesterol exhibited a powerful positive relation among females in the angina arm (r=0.56), and a moderate positive one for the male STEMI arm (r=0.42), total angina arm (r=0.42), and all subjects (r=0.39). Important inverse ratio was found between adiponectin and LDL cholesterol in the male angina arm (r=-0.77), total angina arm (r=-0.64), and also a slight inverse ratio in the male STEMI arm (r=- 0.49), and all subjects (r=-0.36). We found a solid inverse ratio between adiponectin and triglycerides in female angina arm (r=-0.53), in female STEMI arm (r=-0.58), and a moderate inverse one for total angina arm (r=-0.41), and in all subjects (r=-0.38). Strong negative correlation with high sensitive C-reactive protein was found in the male control group (r=-0.66), in male STEMI patients (r=-0.89), and all patients (r=-0.64). Strong negative correlation with high sensitive troponin T was recorded for female STEMI subjects (r=-0.57), male STEMI subjects (r=-0.86), and all STEMI subjects (r=-0.74) (table 5).

There have been numerous in vitro studies, as well as studies in animal models and clinical studies, which highlighted the link between circulating adiponectin levels and cardiovascular diseases.

In vitro studies:

We must mention the most popular in vitro studies, as follows:

-Ouchi et al. showed that adiponectin inhibits the shift of macrophages into foam cells, may be acting as a mediator for this conversion, and thus making a connection between atherosclerosis and endothelial inflammation [10];

-another study showed that adiponectin might play an important role in adjusting the effects of insulin; subjects with type 2 diabetes mellitus recorded low adiponectin concentrations, which may concur to tissue insulin resistance [11];

-Matsuda et al. conclude that adiponectin exerts an inhibitory outcome on the HB-EGF (heparin-binding epidermal growth factor-like) expression in endothelial cells treated with TNFα and down-regulates the migration of activated smooth muscle cells [12];

-Yokota underlined the inhibitory effect of adiponectin on the proliferation of myelomonocytic cell line and mature macrophage functions such as TNFα production and phagocytosis [13];

-according to Chen, adiponectin inhibits the synthesis of nitric oxide in endothelial cells [14], and Arita et al concluded that adiponectin has a negative effect on the multiplication and migration of smooth muscle cells set by platelet-derived growth factor [15].

All these experimental studies have concluded that adiponectin has direct anti-atherogenic and anti-inflammatory effects on the endothelium, interfering all stages of plaque formation.

	Male Control *Mean±SD*	**Male STEMI** *Mean±SD*	***P* value**
Adiponectine (mg/L)	8.13±0.74	5.95±1.27	0.0007
Total Cholesterol (mg/dL)	209.4±13.16	236.73±38.20	0.067
HDL-Chol (mg/dL)	46±7.96	43±5.53	0.167
LDL-Chol (mg/dL)	110.4±11.32	131.68±34.90	0.099
Triglycerides (mg/dL)	142.6±13.16	178.89±71.78	0.139
hs-CRP (mg/L)	1.002±0.33	3.54±2.74	0.027
hs-Troponine T (pg/dL)	9.64±3.04	1471.29	0.040

Table 4
COMPARISON BETWEEN MALES- STEMI GROUP AND THE CONTROL GROUP

Table 5
ADIPONECTIN AND LIPID PROFILE/ HS-CRP/ HS-TROPONIN T' RELATIONSHIP AMONG THE STUDIED GROUPS

	Female Control	**Male Control**	**Female STEMI**	**Male STEMI**	**Total Control**	**Total STEMI**
Total Cholesterol (mg/dL)	-0.55	-0.02	0.02	-0.28	-0.6	-0.19
HDL-Chol (mg/dL)	0.56	-0.75	0.14	0.42	0.42	0.39
LDL-Chol (mg/dL)	0.22	-0.77	-0.12	-0.49	-0.64	-0.36
Triglycerides (mg/dL)	-0.53	0.57	-0.58	-0.3	-0.41	-0.38
hs-CRP (mg/L)	- 0.05	-0.66	0.04	-0.89	-0.24	-0.64
hs-Troponine T (pg/dL)	-0.23	-0.11	-0.57	-0.86	-0.08	-0.74

Studies in animal models

-Kubota et al. used adiponectin knockout animals and showed that neointimal proliferation in response to injury is accelerated. [16]

-Okamoto et al. observed, by the use of immuno-histochemical techniques in mice, the accumulation of the adiponectin in the mechanically destroyed carotid artery and afterward demonstrated that the administration of adiponectin to apolipoprotein E knockout mice significantly reduce the progression of atherosclerotic lesions [17, 18].

Clinical studies

-Jansson et al have reported the inverse correlation between adiponectin plasma concentration and endothelial dysfunction [19];

-Hotta et al emphasized that in subjects associating coronary artery disease and type 2 diabetes mellitus plasma adiponectin was significantly decreased [20];

-Zoccali et al followed a group of patients with end-stage renal disease and observed that low adiponectin was an independent predictor of cardiovascular events [21];

-Adamczak et al noticed decreased circulating adiponectin in subjects with essential hypertension compared with normotensive subjects [22];

-Okamoto et al have shown that adiponectin is bound to collagen type I , III and V found in the vessels' frame, particularly in the event of an injury at this level, taking part in the repair process [17];

-Margaritis et al emphasized that adiponectin raises nitric oxide (NO) production by PI3 kinase/Akt-mediated phosphorylation and activation of eNOS [23];

-Cheng et al pointed out that disturbances in adiponectin and insulin signaling paths increase the risk of metabolic and vascular disorders [24].

Our study revealed an opposite relation between adiponectin and triglycerides, total- and LDL-cholesterol, and a linear relation between adiponectin and HDL-cholesterol, disclosing adiponectin as a protective factor against atherosclerosis. The opposite relationship between adiponectin and high-sensitive troponin T may denote an increased accumulation of adiponectin in the injured myocardial region, with a possible tisular regenerating role, as other studies suggest [25, 26].

Concordant to literature data [27, 28], our study pointed out that an important acute reaction of CRP accompanies myocardial infarction. Other studies concluded that CRP and cardiovascular diseases are complement-related: CRP promotes endothelial expression of adhesion molecules, in a genotype-dependent manner [29, 30]. These facts sustain the theory that CRP has a main role in the atherosclerosis inflammatory pathway [31].

A recent update [32] emphasized that hypoadiponectinemia correlates with an increased cardiovascular risk given the fact that low adiponectinemia promotes insulin resistance [33] and that serum adiponectin levels grew concordant with insulin resistance's improvement after renin-angiotensin system blockade [34].

A large trial [35] has shown that high adiponectinemia was independently correlated with augmented risk of death by all-cause (including cardio-vascular) in subjects associating type 2 diabetes mellitus and recent acute coronary syndrome; the same correlation was found in an elderly cohort [36] and in non-diabetic patients discharged after an acute myocardial infarction [37,38].

However, the conflicting results were recently analyzed [39], but sustainable explanations – concerning adiponectin tissue resistance, genetic variants, sex-related effects or simply altered results by unexpected confounders - were not yet offered for the *adiponectin paradox* on cardiovascular and all-cause mortality.[40]

Limitations

Nowadays, an important biochemical issue is the fact that all adiponectin isoforms are recognized by commercial assay kits measuring the total adiponectin concentrations [41]. The ELISA technique used in our study detects the globular and full-length isoforms. It is well known right now that each adiponectin isoform is playing a different biological role.[42-44] The proportion of isoform expression is altered in various physiological and pathological conditions. For example, to predict risk mortality in heart failure populations, total adiponectin is usually recommended [42,43].

Newly developed adiponectin assays can measure each isoform separately, so further research is needed to establish the role of isoforms in different pathologies [45].

Another shortcoming is the reduced number of patients included in this study. Larger studies are needed to further refine the relationship among adiponectin and other biomarkers in the acute phase of STEMI.

Conclusions

In our study we revealed an inverse ratio between hs-CRP and adiponectin during the first hours of STEMI evolution, which may support the anti-inflammatory and anti-atherogenic role of adiponectin. We identified diminished adiponectin plasma concentrations during the first hours of STEMI evolution.On the other hand, the study revealed a directly proportional ratio among adiponectin and HDL-cholesterol and an inverse report between this hormone and all other studied biomarkers.

Low circulating adiponectin concentrations in the setting of acute STEMI, its inverse correlation with LDL-cholesterol, hs-troponin T, hs-CRP together with its positive correlation with HDL- cholesterol, gave us arguments to assume that this adipokine may be a protective cardiovascular factor, but the result is difficult to be interpreted.

Abreviation

BMI=body mass index
CRP= C-reactive protein
CT= computed tomography
ECG= electrocardiography
eGFR= estimated glomerular filtration rate
eNOS= endothelial nitric oxide synthase 3
HB-EGF= heparin binding epidermal growth factor-like
HDL-cholesterol, HDL-chol= high density lipoproteins cholesterol
hs Troponin T= high sensitive Troponin T
hs-CRP= high sensitive C reactive-protein
LDL-cholesterol, LDL-chol= low density lipoprotein cholesterol
NO= nitric oxide
STEMI= myocardial infarction with ST-segment elevation
TNFα=tumor necrosis factor α
VLDL= very low density lipoproteins

References

1.THYGESEN, K., ALPERT, J.S., JAFFE, A.S., CHAITMAN, B.R., BAX, J.J., MORROW, D.A., WHITE, H.D., Eur. Heart J., **40**,nr.3, 2019,p. 237.
2.GAZE, D.C., COLLINSON, P.O., Ann. Clin. Biochem., **45**,nr.4,2008,p.349.
3.DÍEZ, J.J., IGLESIAS, P., Eur.J. Endocrinol.,**148**,nr.3,2003,p.293.
4.UKKOLA, O., SANTANIEMI, M., J.,Mol.Med.,**80**,nr.11,2002,p.696.
5.GAVRILA, A., PENG, C.K., CHAN, J.L., et al., J.Clin.Endocrinol.Metab., **88**, 2003,p.2838.

6.MANTOVANI,A.,GARLANDA,C.,DONI,A.,BOTTAZZI,B.,J.Clin. Immunol., **28**,nr.1,2008, p.1.
7.YEAGLE, P.L,. Biochimie,**73**,nr.10, 1991,p.1303.
8.OLSON,R.E., J. Nutr.**128**,Suppl 2,1998,p. 439S.
9.NELSON, D.L., COX, M.M., Lehninger Principles of Biochemistry, 3rd, Ed. Worth Publishing, New York, 2000.
10.OUCHI, N., KIHARA, S., ARITA, Y., NISHIDA, M., MATSUYAMA, A., OKAMOTO, Y., et al., Circulation **103**,2001,p. 1057.
11.CHANDRAN, M., CIARALDI, T., PHILIPS, S., HENRY, R., Diabetes Care, **26**,nr.8, 2003,p.2442.
12.MATSUDA, M., SHIMOMURA, I., SATA, M., et al., J. Biol. Chem., **277**, 2002,p.37487.
13.YOKOTA, T., ORITANI, K., TAKAHASHI, I., et al., Blood, **96**,2000,p. 1723.
14.CHEN, H., MONTAGNANI, M., FUNASHASHI, T., SHIMOMURA, I., QUON, M.J., J. Biol. Chem., **278**,2003,p. 45021.
15.ARITA, Y., KIHARA, S., OUCHI, N., et al., Circulation,**105**, 2002,p. 2893.
16.KUBOTA, N., TERAUCHI, Y., YAMAUCHI, T., et al., J. Biol. Chem., **277**,2002,p.25863.
17.OKAMOTO, Y., ARITA, Y., NISHIDA, M., et al., Horm. Metab. Res., **32**,2000,p.47.
18.OKAMOTO, Y., KIHARA, S., OUCHI, N., et al., Circulation,**106**,2002,p.2767.
19.JANSSON, P.A., PELLME, F., HAMMARSTEDT, A., et al., FASEB J.,**17**,2003,p. 1434.
20.HOTTA, K., FUNAHASHI, T., ARITA, Y., et al., Arterioscler. Thromb. Vasc. Biol.**20**,2000,p.595.
21.ZOCCALI, C., MALLAMACI, F., TRIPEPI, G., BENEDETTO, F.A., CUTRUPI, S., PARLONGO, S., et al., J. Am. Soc. Nephrol.**,13**,2002,p. 134.
22.ADAMCZAK, M., WIECEK, A., FUNAHASHI, T., et al., Am. J. Hypertens.**16**,2003,p.72.
23.MARGARITIS, M., ANTONOPOULOS, A.S., DIGBY, J., et al., Circulation, **127**,2013,p. 2209.
24.CHENG, K.K., LAM, K.S., WANG, B., XU, A., Best Pract. Res. Clin. Endocrinol. Metab., **28**, nr.1,2014,p. 3.
25.KRASNODEBSKI, P., KARNAFEL, W., OPOLSKI, G., Kardiologia Polska,**69**, nr.9, 2011,p.924.
26.SHIBATA, R., et al. Cardiovascular Research,**74** ,nr.32007,p. 471.
27.GRISELLI, M., HERBERT, J., HUTCHINSON, W.L., et al., J. Exp. Med.,**190**, 1999, p.1733.
28.INOUE, T., KATO, T., UCHIDA, T., et al., J. Am. Coll. Cardiol., **46**, 2005,p.239.
29.JIALAL, I., DEVARAJ, S., SINGH, U., JACC,**47**, nr.7,2006,p.1379.
30.RAAZ-SCHRAUDER, D. et al., Thromb. Res., **133**,nr.3,2014,p.426.
31.PAFFEN, E., DEMAAT, P.M.M., Cardiovascular Research,**71**,nr.1, 2006,p. 30.
32.GEAGEA, A.G., et al., Open Medicine Journal,**5**, 2018,p. 20.
33.GOTO, A., NODA, M., GOTO, M., et al., Diabetes Res. Clin. Pract.,**127**,2017,p.254.
34.FURUHASHI, M., URA, N., HIGASHIURA, K., et al., Hypertension, **42**, nr.1,2003,p.76.
35.BERGMARK, B.A., CANNON, C.P., WHITE, W.B., JAROLIM, P. , LIU, Y., BONACA, M.P., ZANNAD, F.,MORROW, D.A., Diabetes Obes. Metab.**19**,2017,p.962.
36.CHOI, S.H., KU, E.J., HONG, E.S., et al., Int. J. Cardiol.,**183**,2015,p.91.
37.RITSINGER, V., BRISMAR, K., MALMBERG, K., MELLBIN, L., NASMAN, P., RYDEN, L., SODERBERG, S., TENERZ, A., NORHAMMAR, A., Diab. Vasc. Dis.Res.,**14**,nr.2,2017,p.77.
38.CAPATINA, C., GHINEA, A.,DUMITRASCU, A.,et al., International Journal Of Diabetes In Developing Countries,**36**,nr.4,2016,p. 393.
39.MENZAGHI, C., TRISCHITTA, V., Diabetes, **67**,nr.1,2018,p.12.
40.PADURARU, D.N., BOUARIU,A.,DANIEL, I. et al., Romanian Biotechnological Letters,**23**,nr.5,2018,p.14056.
41.EBINUMA, H., MIYAZAKI, O., YAGO, H., HARA, K., YAMAUCHI, T., KADOWAKI, T., Clin. Chim. Acta., **372**,2006,p. 47.
42.TSUTAMOTO, T., TANAKA, T., SAKAI, H., ISHIKAWA, C., FUJII, M., YAMAMOTO, T., HORIE, M., EUR. HEART J.,**28**,2007,P.1723.0.
43.TOTU, E.E., MANUC, D., Rev.Chim.(Bucharest), **59**, no. 9, 2008, p. 947.
44.TANTU, M.M., MAN, G.M., PAUNESCU, A., et al., Rev.Chim. (Bucharest), **69**, no.11, 2018,p.30015.
45.TAKEMURA, Y., OSUGA, Y., KOGA, K., TAJIMA, T., HIROTA, Y., HIRATA, T., MORIMOTO, C., HARADA, M., YANO, T., TAKETANI, Y.,J. Reprod. Immunol.,**73**,2007,p.60.

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