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Original Article

Lipocalin-2 plasmatic levels are reduced in patients with long-term type 2 diabetes mellitus

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Abstract: Background: De-regulation of adipocytokines synthesis and secretion appears to be involved in the pathogenesis of different metabolic diseases. Aims: We assessed a possible association between plasmatic levels of lipocalin-2 (LCN2) and type 2 diabetes mellitus (T2DM), as well as among levels of LCN2 and those of adiponectin, ghrelin, leptin and resistin, in Mexican diabetic patients. Subjects and methods: Fifty-three healthy individuals and fifty-three with long-term T2DM were included. Measurements from all patients for BMI, fasting glucose, insulin, lipids and adipocytokine profiles were obtained. Results: Comparison of data between the corresponding for diabetic subjects and those of healthy individuals showed significant differences in every anthropometric and metabolic parameter analyzed ($P < 0.001$). In diabetic subjects, lipocalin-2 and ghrelin plasmatic levels were statistically diminished ($P < 0.001$) in comparison with the levels registered in healthy subjects. In conclusion, in this study we found that LCN2 plasmatic levels are reduced in Mexican subjects with long-term diabetes and this reduction in circulating concentrations is similar to the one reported for anti-inflammatory adipocytokines, which suggests that lipocalin-2 is somehow involved in insulin resistance and cardiometabolic alterations through an uncharacterized mechanism generated by the inflammation process.

Keywords: Lipocalin-2, diabetes, adipocytokines, inflammation

Introduction

At present, the prevalence of type 2 diabetes mellitus (T2DM) has reached worldwide epidemic proportions, representing the associated morbidity and mortality rates, a great economic burden especially for developing countries [1]. Therefore, the early identification of the risk factors related to this pathology in apparently healthy subjects results imperative. It is well established that in metabolic alterations such as insulin resistance, dyslipidemia, hypertension and T2DM, a deregulation of adipocytokines synthesis and secretion, characterized mainly by adipocyte hypertrophy and macrophage infiltration occurs, leading to a chronic low-grade inflammatory state that interferes in the morbidity of these diseases [2-4]. In this context, in patients with metabolic diseases, serum concentrations of different adipocytokines may be either increased or reduced. In fact, high concentrations of leptin, interleukin 6 (IL-6), Tumor Necrosis Factor alpha (TNF-alpha) or resistin, as well as reduced levels of adiponectin or ghrelin, are considered hallmarks of inflammation derived from an impaired metabolism [5]. Leptin, the protein product of the *obese (ob* or *Lep)* gene, is a hormone synthesized by adipocytes that signals available energy reserves to the brain, thereby influencing development, growth, metabolism and reproduction. In mammals, leptin acts as an adiposity signal: circulating leptin fluctuates in proportion to fat mass, exerting its action on the hypothalamus to suppress food intake. Hyperleptinemia positively correlates with production of inflammatory cytokines and metabolic syndrome [6, 7]. IL-6 and TNF-alpha are inflammatory cytokines, directly involved in the development of insulin resistance. IL-6 is highly expressed in adipose tissue and its circulating concentrations are increased in obese humans. Interleukin-6 role in the development of metabolic alterations remains controversial; when

administered peripherally, this adipokine disrupts insulin signaling by enhancing expression of the suppressor of cytokine signaling (SOCS), an insulin inhibitory molecule, on the contrary, when IL-6 is administered centrally an enhancement in energy expenditure, as well as a decrement in body mass index are observed [8]. TNF-alpha alters insulin sensitivity through changes in the phosphorylation pattern of the insulin receptor substrate-1 [9]. Resistin is another pro-inflammatory molecule, which is also involved in the development of metabolic alterations. It is predominantly expressed in macrophages. Thus the release of resistin is stimulated by IL-6, hyperglycemia as well as by growth and sex hormones [10]. On the other hand, anti-inflammatory adipocytokines like adiponectin or ghrelin promote glucose uptake and improve insulin sensitivity. Adiponectin exerts its anti-inflammatory properties by inhibiting the activation of NF-κB and consequently the production of both lipopolysaccharide and TNF-alpha in macrophages. Adiponectin also stimulates the production of IL-10, also considered an anti-inflammatory cytokine [8]. Ghrelin is a 28 amino acid peptide hormone synthesized mainly by the stomach. Its orexigenic function was accidentally identified, while studying the effect of ghrelin on growth hormone release in healthy humans [11]. Studies conducted in ob/ob mice, where ghrelin's synthesis was ablated, demonstrated an improvement on glucose tolerance, consequence of an increment of serum insulin levels and insulin sensitivity, whereas the body weight was not reduced [12, 13]. In fact, ablation of either ghrelin or its receptor in adult mice under the administration of a high fat diet, did not prevent obesity, but increased insulin sensitivity, which suggests that ghrelin's primary role is not appetite control but the regulation of glucose homeostasis [13].

Recently, lipocalin-2, also known as Neutrophil Gelatinase-Associated Lipocalin-2 (LCN2 or NGAL) has drawn the attention of many researchers, due to its implication in metabolic alterations [14]. Lipocalin-2 is a member of the lipocalin superfamily comprised by small secreted proteins, characterized by the presence of three conserved motifs, conforming a single eight-stranded anti-parallel beta-barrel similar to a calyx that presents the ability to bind organic ligands, specific cell surface receptors or to form complexes with soluble macromolecules. These three specific features confer a vast functional diversity. Thus, lipocalins are involved in different roles such as: retinol transport, cryptic coloration, olfaction, pheromone transport and enzymatic synthesis of prostaglandins. They are also implicated in the regulation of the immune response and cell homeostasis [15].

Lipocalin-2 is reported to be associated with obesity and insulin resistance [16], but recent investigations have revealed conflicting results. Despite the high prevalence of type 2 diabetes among Mexican people, data on lipocalin-2 plasmatic concentrations as well as its association with T2DM are not available; therefore, the aims of this study were to quantify lipocalin-2 circulating levels both in healthy individuals and T2DM Mexican patients and to assess the correlation between plasma levels of this adipocytokine and metabolic components of diabetes, as well as between the levels of LCN2 and those of six other adipocytokines (adiponectin, ghrelin, IL-6, leptin, resistin and TNF-alpha).

Material and methods

Study design & patients

A non-randomized cross-sectional assay was conducted on a total of 53 healthy individuals and 53 patients with type 2 diabetes mellitus, who were receiving medical attention at a secondary or primary care level hospital at Mexico City. Written informed consent was obtained from all patients before screening was undertaken. Upon medical consultation, each subject was interviewed for familiar history of diabetes, metabolic syndrome and/or cardiopathies. Exclusion criteria included: acute or chronic infectious diseases, liver or renal dysfunction, cardiopathies, as well as any disease generating inflammation. The present study was authorized by the Bioethics Committee of the Instituto Mexicano Del Seguro Social in accordance with the ethical guidelines comprised in the 1975 Declaration of Helsinki (1964).

Measurements of body weight and composition

All anthropometrical and biochemical measurements were taken as reported previously [17]. Briefly, in the standing position, weight and height were measured with the subjects in light

Table 1. Baseline characteristics of control subjects and patients with type 2 diabetes mellitus

Variables	Control (n = 53)	T2DM (n = 53)
Sex (Male/Female)	(24/29)	(33/20)
Age (years)	42.5 ± 3	48 ± 3
Body mass index (BMI, Kg/m^2)	23.4 ± 1	31.5 ± 2***
Total cholesterol (mg/dL)	165 ± 2.6	263 ± 4***
LDL (mg/dL)	95.4 ± 2.7	130 ± 18***
HDL cholesterol (mg/dL)	50 ± 1.2	45.3 ± 1.2***
Triglycerides (TG, mg/dL)	107.7 ± 5.4	244.4 ± 15**
Fasting glucose (mg/dL)	88.9 ± 1.5	191.8 ± 7.2***
Fasting insulin (μU/mL)	10.6 ± 0.5	25 ± 2***
HOMA-IR	2.3 ± 0.1	10 ± 1***
HbA1c (%)	5.4 ± 0.6	8.5 ± 0.2**
SBP (mmHg)	111 ± 9.3	136 ± 2.5***
DBP (mmHg)	72 ± 7	85 ± 2***
Creatinine (mg/dL)	0.6 ± 0.05	0.6 ± 0.06
Obesity (%)		55
Hypertension (%)		80
Dyslipidemia (%)		75
Hypercholesterolemia (%)		57

Data are expressed as mean ± standard error. Values were compared between groups employing a Mann-Whitney test for independent samples; **P < 0.001, ***P < 0.0001. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin glycated; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment index-insulin resistance; HC, hypercholesterolemia; T2DM, type 2 diabetes mellitus.

clothing and without shoes using a fixed scale with stadimeter (Tanita TBF-215. Tokyo, Japan). The increments of weight and height measurements were 0.1 kilograms and 0.01 meters, respectively. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Obesity was defined by a BMI of 30 kg/m^2 or more, and overweight by a BMI of 25 kg/m^2 or more, but less than 30 kg/m^2. Blood pressure measurements were taken in a supine position after 5 min of rest, following guidelines recommended in the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (The sixth report of the joint national committee, 1997).

Assays

In order to obtain fasting plasma glucose (FPG) levels as well as lipid profiles, glycated hemoglobin (HbA1c), and adipocytokine levels, venous blood samples were drawn after an overnight fasting of 8 to 10 hours and immediately stored at -70°C for subsequent assays. The population of study was categorized according to the criteria of the American Diabetes Association (The expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1999), considering control subjects, those with FPG of less than 100 mg/dL and patients with type 2 diabetes mellitus those presenting an impaired fasting glucose (IFG) of 100 mg/dL or more, but less than 126 mg/dL and Hb_{A1c} < 5.7%.

The glucose-oxidase method was employed to measure plasma glucose. Triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and HbA1c were assayed using a Technicon RA1000 analyzer (Bayer Diagnostics, Puteaux, France). Insulin was centrally assayed on serum by a specific radioimmunoassay (Linco Research Inc., St Charles, MO, USA). Intra e inter-assay coefficients of variation for all measurements were < 7%, respectively. Insulin resistance (IR) was estimated using the homeostasis model assessment index-insulin resistance (HOMA-IR). HOMA-IR is defined as fasting glucose (mg/dL) multiplied by fasting insulin (μU/mL), divided by 405. Dyslipidemia was defined as anyone of the following: total cholesterol above 200 mg/dL, HDL cholesterol below 40 mg/dL, LDL cholesterol above 130 mg/dL, or triglycerides (TG) above 150 mg/dL.

Measurements of adipokines

Plasma adiponectin and ghrelin levels were measured by a specific radioimmunoassay (Linco Research Inc., St Charles, MO, USA). Plasmatic levels of resistin, leptin, IL-6 and Tumor TNF-alpha were measured by enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Minneapolis, MN, USA); lipocalin-2 circulating levels were quantitated with a NGAL ELISA kit (BioPorto Diagnostics, Denmark). All ELISA experiments were determined in duplicate as recommended by the manufacturer. Intra e inter-assay coefficients of variation for all measurements were < 7%, respectively. Also a standard curve was included within each assay.

Table 2. Baseline plasmatic concentrations of LCN2 and six other adipocytokines in both, control and diabetic subjects

Variables	Control (n = 53)	T2DM (n = 53)
Lipocalin-2 (ng/mL)	98.2 ± 5.1	65.44 ± 8**
Ghrelin (pg/mL)	1592 ± 205	806 ± 37**
Adiponectin (μg/mL)	16.3 ± 2	8.9 ± 1**
Leptin (ng/mL)	3.4 ± 1	17.7 ± 2***
Resistin (ng/mL)	3.7 ± 2	10.2 ± 0.2***
TNF-alpha (pg/mL)	4.2 ± 1.2	22.5 ± 7.5**
IL-6 (pg/mL)	24.8 ± 7	108 ± 20**

Data are expressed as mean ± standard error. Values were compared between groups employing a Mann-Whitney test for independent samples; **P < 0.001, ***P < 0.0001.

Table 3. Spearman rank correlation test for three metabolic parameters

	Body Mass Index	Glucose	Insulin
Body Mass Index	1.000	-0.30**	0.32**
Glucose	-0.30**	1.000	-0.4**
Insulin	0.32**	-0.4**	1.000

Spearman rank correlation test between body mass index (BMI) and glucose; BMI and insulin and between glucose and insulin within patients with type 2 diabetes mellitus; **P < 0.01.

Statistical analysis

Data are presented as mean value per group and standard error (SE); for categorical variables, number and percentage were determined. In order to analyze both anthropometric and biochemical differences between the control group and the diabetic group, we employed a Mann-Whitney test for independent samples. Also, to assess within patients with T2DM a possible correlation between lipocalin-2 concentrations (considered to be the independent variable) and all the anthropometric and biochemical variables analyzed (considered dependent variables), a Spearman rank correlation test was performed. A P value ≤ 0.05 was considered as statistically significant. All statistical analyses were performed with the Graph Pad Prism version 5.1.

Results

A total of 106 subjects, 53 healthy individuals (24 men and 29 women) and 53 subjects with T2DM (33 men and 20 women) were included; 16 men and 14 women with hypercholesterolemia (HC). Data of anthropometrical and biochemical parameters from both groups are shown in **Table 1**. As expected, all biochemical parameters were considerably higher in the diabetic subjects, in comparison with the ones registered in the control group, excepting the one reported for creatinine, which was almost identical between groups; all biochemical differences, between the control group and the diabetic individuals were statistically significant (P < 0.0001).

Interestingly, the results from the quantification of lipocalin-2 plasmatic levels showed that in control subjects these levels were higher than in patients with diabetes (98 ± 5.1, vs 65 ± 8 ng/mL), such difference was statistically significant (P < 0.01) (**Table 2**). In the same manner, the results derived from the plasmatic quantification of the other six adipocytokines studied (ghrelin, adiponectin, leptin, resistin, TNF-alpha and IL-6), showed statistical difference between control and study groups for all adipocytokines, being the reduction of ghrelin's plasmatic levels the most significant (1 592 ± 205 vs 806 ± 37) (P < 0.0001) (**Table 2**). We also analyzed the correlation among levels of all the anthropometric and biochemical parameters within the diabetic group. The Spearman rank correlation test indicated that only the correlations between BMI and glucose, BMI and insulin, as well as between glucose and insulin, were statistically significant (P < 0.003, P < 0.009 and P < 0.003, respectively) (**Table 3**). Correlations among the plasmatic levels of the six adipocytokines studied were no statistically significant within the diabetic group.

Discussion

In an attempt to contribute in the understanding of the role that lipocalin-2 may exert in metabolic diseases, in this study we quantitated LCN2 plasmatic concentrations in both healthy subjects and diabetic individuals of Mexican origin and assessed a possible correlation between circulating levels of LCN2 and metabolic components of T2DM; Firstly, our results showed a significant decrease of lipocalin-2 circulating levels in patients with diabetes in comparison with the levels observed in the plasma of healthy subjects (**Table 2**). Moreover and in disagreement with what has been reported by

different research groups [18-21], regarding an increase of LCN2 levels in subjects with obesity and obesity-related metabolic disorders, in our study this decrement of lipocalin-2 circulating levels of diabetic patients, was observed despite a BMI > 30 in the majority of them. When performing a correlation test for all the variables of the diabetic group, a statistically significant correlation between BMI and both glucose and insulin levels was observed. The latter was to be expected, since a relationship between a greater BMI and impaired levels of glucose and/or insulin is well documented [22, 23], but is also suggests that the decrease in lipocalin-2 levels may not be a direct consequence of either increased BMI or impaired glucose metabolism.

Unfortunately, the cross-sectional design of our study limits the possibility to determine if a reduction of LCN2 plasmatic levels may indeed constitute a risk factor for type 2 diabetes mellitus. On the other hand, it is well established that adipocytokine levels, may reflect the inflammatory state in metabolic disorders. Some of them pose protective anti-inflammatory effects, such as adiponectin and ghrelin, and others like resistin or interleukin-6 have the opposite effect [24]; in our study, we assessed a possible correlation between LCN2 levels and those of several adipocytokines in patients with T2DM. Even though there was not a statistically significant correlation between LCN2 and any of the six adipocytokines, our results showed a difference between the adipocytokines levels of the control group versus the diabetic group, which is in accordance with what has been reported regarding pro-inflammatory cytokines; that is, leptin, resistin and IL-6 concentrations were increased in the diabetic groups as opposed to the respective concentrations observed in healthy individuals. Conversely, levels of the anti-inflammatory adipocytokines, adiponectin and ghrelin were reduced in subjects with diabetes in comparison with the levels reported in control subjects (**Table 2**) [7]. In this context, it has been demonstrated that both adiponectin and ghrelin exert cardioprotective actions through different mechanisms [25, 26] and their low plasmatic levels are also associated with insulin resistance, and prevalence of T2DM [27]; based on the latter, we could speculate that like adiponectin and ghrelin, LCN2 acts as a protective cytokine and the lower concentrations observed in diabetic patients are due to specific mechanisms activated by the inflammatory condition present in these subjects which is also related to a higher risk for developing cardiometabolic diseases. A study performed by Alkharfy *et al*. [28], demonstrated a reduction of LCN2 levels in obese and non-obese diabetic individuals, whereas in the same study, LCN2 levels were increased in patients with only cardiovascular anomalies. Likewise, a recent study reported low serum levels of LCN2 in women with polycystic ovarian syndrome (PCOS) as opposed to the levels observed in controls, while both the carotid intima media thickness and the aortic stiffness were significantly increased in PCOS patients [29]. Although the authors of both studies stated no significant correlation between serum LCN2 levels and such cardiovascular anomalies, these results may suggest that the reduction or increment on lipocalin-2 levels within cardiometabolic alterations depend on the level of inflammation, the disease stage and whether or not any of the inflammatory associated pathologies to diabetes mellitus, like acute kidney injury or cardiovascular diseases (which in our case neither of both were present in any of the diabetic patients), coexist.

In the same manner, studies performed on plasma of patients with essential hypertension, demonstrated an independent association between increased levels of lipocalin-2 and mean arterial pressure [30]. Recent investigations also demonstrated an elevated concentration of LCN2 in both urinary and plasmatic samples of patients with cardiac surgery [19, 31]. Huang *et al*., analyze the circulating levels of LCN2 in a large Chinese population, indicating a significant increment of lipocalin-2 levels in patients with glucose alterations, but the majority of patients were pre-diabetic and the ones reported as diabetic were diagnosed while conducting such study [28]. Taking this into account, it is worth mentioning that in our study, a non-significant increment of LCN2 levels in pre-diabetic patients was observed (data not shown). The latter supports the idea of lipocalin's ambivalent behavior depending on both the grade of tissular damage caused by diabetes, and the amount of time the patient has been suffering this disease, which in the case of the patients analyzed herein, the average was 8.5 years. Additionally, the interrelationship among altered levels of the other cyto-

kines in patients with T2DM may be eliciting the reduction in lipocalin-2 plasmatic concentrations.

In conclusion, in this study we found that LCN2 plasmatic levels are reduced in Mexican subjects with long-term diabetes and this reduction in circulating concentrations is similar to the one reported for ghrelin, which suggests that lipocalin-2 is somehow involved in cardiometabolic alterations through an uncharacterized mechanism generated by the inflammation process.

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Disclosure of conflict of interest

None.

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References

[1] Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Blood Glucose). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 2011; 378: 31-40.

[2] Basurto L, Galvan R, Cordova N, Saucedo R, Vargas C, Campos S. Adiponectin is associated with low bone mineral density in elderly men. Eur J Endocrinol 2009; 160: 289-93.

[3] Esteve E, Ricart W, Fernandez-Real JM. Adipocytokines and Insulin Resistance The possible role of lipocalin-2, retinol binding protein-4, and adiponectin. Diabetes Care 2009; 32 suppl 2: S362-S7.

[4] Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89: 2548-56.

[5] Saucedo R, Zarate A, Basurto L, Hernandez M, Puello E, Galvan R. Relationship between circulating adipokines and insulin resistance during pregnancy and postpartum in women with gestational diabetes. Arch Med Res 2011; 42: 318-23.

[6] Manuel-Apolinar L, Lopez-Romero R, Zarate A, Damasio L, Ruiz M, Castillo-Hernandez C. Leptin mediated ObRb receptor increases expression of adhesion intercellular molecules and cyclooxygenase 2 on murine aorta tissue inducing endothelial dysfunction. Int J Clin Exp Med 2013; 6: 192.

[7] Cruz-Domínguez MP, Corte DHM, Zarate A, Tapia-González MA, Álvarez-Acosta S, Damasio L, Manuel AL. Relationship of ghrelin, acid uric and proinflammatory adipocytokines in different degrees of obesity or diabetes. Int J Clin Exp Med 2014; 7: 1435.

[8] Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Front Endocrinol 2013; 4: 71.

[9] Costagliola C, Romano V, De Tollis M, Aceto F, Romano MR, Pedicino C, Semeraro F. TNF-alpha levels in tears: a novel biomarker to assess the degree of diabetic retinopathy. Mediators Inflamm 2013; 2013: 629529.

[10] Al-Suhaimi EA, Shehzad A. Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. Eur J Med Res 2013; 18: 12.

[11] Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F, Ghigo E. Preliminary evidence that Ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. J Endocrinol Invest 2000; 23: 493-5.

[12] Sun Y, Asnicar M, Saha PK, Chan L, Smith RG. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. Cell Metab 2006; 3: 379-86.

[13] Sun Y, Butte NF, Garcia JM, Smith RG. Characterization of adult ghrelin and ghrelin receptor knockout mice under positive and negative energy balance. Endocrinology 2008; 149: 843-50.

[14] van Dam RM, Hu FB. Lipocalins and insulin resistance: etiological role of retinol-binding protein 4 and lipocalin-2? Clin Chem 2007; 53: 5-7.

[15] Flower D. The lipocalin protein family: structure and function. Biochem J 1996; 318: 1-14.

[16] Wang Y, Lam KS, Kraegen EW, Sweeney G, Zhang J, Tso AW, Chow WS, Wat NM, Xu JY, Hoo RL, Xu A. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. Clin Chem 2007; 53: 34-41.

[17] Guerrero-Romero F, Rodríguez-Morán M, Pérez-Fuentes R, Sánchez-Guillén MC, González-Ortiz M, Martínez-Abundis E, Brito-Zurita O, Madero A, Figueroa B, Revilla-Monsalve C, Flores-Martínez SE, Islas-Andrade S, Rascón-Pacheco RA, Cruz M, Sánchez-Corona J. Prediabetes and its relationship with obesity in Mexican adults: the Mexican Diabetes Prevention (MexDiab) Study. Metab Syndr Relat Disord 2008; 6: 15-23.

[18] Auguet T, Quintero Y, Terra X, Martínez S, Lucas A, Pellitero S, Aguilar C, Hernández M, del Castillo D, Richart C. Upregulation of lipocalin 2 in adipose tissues of severely obese women: positive relationship with proinflammatory cytokines. Obesity 2011; 19: 2295-300.

[19] Huang Y, Yang Z, Ye Z, Li Q, Wen J, Tao X, Chen L, He M, Wang X, Lu B, Zhang Z, Zhang W, Qu S, Hu R. Lipocalin-2, glucose metabolism and chronic low-grade systemic inflammation in Chinese people. Cardiovasc Diabetol 2012; 11: 11.

[20] Yan QW, Yang Q, Mody N, Graham TE, Hsu CH, Xu Z, Houstis NE, Kahn BB, Rosen ED. The adipokine lipocalin 2 is regulated by obesity and promotes insulin resistance. Diabetes 2007; 56: 2533-40.

[21] Choi KM, Lee JS, Kim EJ, Baik SH, Seo HS, Choi DS, Oh DJ, Park CG. Implication of lipocalin-2 and visfatin levels in patients with coronary heart disease. Eur J Endocrinol 2008; 158: 203-7.

[22] El Assar M, de Adana JCR, Angulo J, Martinez MaLP, Matias AHn, Rodriguez-Mañas L. Preserved endothelial function in human obesity in the absence of insulin resistance. J Transl Med 2013; 11: 263.

[23] DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia 2010; 53: 1270-87.

[24] Kotnik P, Fischer-Posovszky P, Wabitsch M. RBP4: a controversial adipokine. Eur J Endocrinol 2011; 165: 703-11.

[25] Kishimoto I, Tokudome T, Hosoda H, Miyazato M, Kangawa K. Ghrelin and cardiovascular diseases. J Cardiol 2012; 59: 8-13.

[26] Rojas E, Rodríguez-Molina D, Bolli P, Israili ZH, Faria J, Fidilio E, Bermúdez V, Velasco M. The role of adiponectin in endothelial dysfunction and hypertension. Curr Hypertens Rep 2014; 16: 1-8.

[27] Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 2003; 52: 2546-53.

[28] Alkharfy KM, Al-Daghri NM, Vanhoutte PM, Krishnaswamy S, Xu A. Serum retinol-binding protein 4 as a marker for cardiovascular disease in women. PLoS One 2012; 7: e48612.

[29] Gencer M, Gazi E, Hacıvelioğlu S, Binnetoğlu E, Barutçu A, Türkön H, Temiz A, Altun B, Vural A, Cevizci S, Kumcular T, Coşar E. The relationship between subclinical cardiovascular disease and lipocalin-2 levels in women with PCOS. Eur J Obstet Gynecol Reprod Biol 2014; 181: 99-103.

[30] Park CG, Choi KM. Lipocalin-2, A-FABP and inflammatory markers in relation to flow-mediated vasodilatation in patients with essential hypertension. Clin Exp Hypertens 2014; 36: 478-83.

[31] Liebetrau C, Dorr O, Baumgarten H, Gaede L, Szardien S, Blumenstein J, Rolf A, Möllmann H, Hamm C, Walther T, Nef H, Weber M. Neutrophil gelatinase-associated lipocalin (NGAL) for the early detection of cardiac surgery associated acute kidney injury. Scand J Clin Lab Invest 2013; 73: 392-9.