RELATION BETWEEN INFLAMMATION AND OXIDATIVE STRESS MARKERS IN DIABETIC FOOT PATIENTS

BOGDANA VÎRGOLICI[*], *MARIA MOHORA**, *LAURA GĂMAN**, *DANIELA LIXANDRU**, *B. MANOLESCU**, *ANCA COMAN*****, *IRINA STOIAN**

*Department of Biochemistry, "Carol Davila" University of Medicine and Pharmaceutics, Bucharest, Romania

**"N. Paulescu" Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

Abstract. Oxidative stress and carbonylic stress are associated with diabetes mellitus. It is not known if they are cause or effect in this illness. Also, chronic low-grade inflammation is involved in the pathogenesis of type 2 diabetes. The aim of this study is to assess the relationship between inflammation markers levels and blood redox status in diabetic foot patients.

Forty newly hospitalized diabetic foot patients, aged between 45–75, were enrolled. Considering C-reactive protein (CRP) values, patients were divided into two groups: group 1 with CRP 0,1–1 mg/dL and group 2 with CRP values above 1 mg/dL. Blood samples were also collected from twenty healthy controls, age and sex-matched. Spectrophotometric methods were applied for oxidative stress markers.

Plasma concentrations for dROM (determinable reactive oxygen metabolites), CRP, ceruloplasmin, dicarbonyls, uric acid and blood glutathione were higher in diabetic patients *vs*. controls. Plasma total antioxidant capacity was not significantly modified. Comparing the diabetic patients, plasma concentrations of dROM, ceruloplasmin, uric acid and glycated hemoglobin were significantly increased in group 2 *vs*. group 1. Blood glutathione, plasma dicarbonyls and total antioxidant capacity values were similar in both groups. Correlations between plasma dROM and ceruloplasmin and also between uric acid and glycated hemoglobin were calculated for both groups.

This study suggests that the link between obesity and diabetes mellitus may be represented by inflammation and oxidative stress. Modulating these processes using therapeutic methods and, of course, weight control may reduce the incidence of diabetic complications.

Key words: inflammation, oxidative stress, obesity, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by hyperglycemia, metabolic dysfunction involving carbohydrates, lipids, and proteins and by long-term complications characterized by microvascular disease with capillary basement membrane thickening, macrovascular disease with accelerated arteriosclerosis, neuropathy involving both the somatic and autonomic nervous systems,

Received September 2008.

ROMANIAN J. BIOPHYS., Vol. 18, No. 4, P. 273–282, BUCHAREST, 2008

neuromuscular dysfunction with muscle wasting, and decreased resistance to infections [1]. Diabetic complications in target organs arise from chronic elevations of glucose. The pathogenic effect of high glucose, possibly in concert with fatty acids, is mediated to a significant extent *via* oxidative stress, carbonylic stress and inflammation [6, 13].

Oxidative stress causes insulin resistance, β-cell dysfunction and late diabetic complications [8]. The carbonylic stress is reflected by the high values of glyoxal and methylglyoxal (the main dicarbonyls). These compounds are the major precursor of advanced glycation end-products (AGE) implicated in the development of diabetic complications [24].

There has been a recent explosion of interest in the notion that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of type 2 diabetes [19, 27]. It was demonstrated that markers of inflammation predict or/and are associated with type 2 diabetes and that inflammation is involved in the pathogenesis of atherosclerosis, a common feature of type 2 diabetes [19].

Present evidence supports the notion that atherosclerosis develops in parallel with type 2 diabetes, with both conditions sharing the common antecedent of activated innate immunity, but like hyperglycemia and possibly some other manifestations of type 2 diabetes such as obesity, macroangiopathy, once present, would presumably further enhance inflammation [19].

It is known that obesity usually precedes diabetes mellitus type 2. Subcutaneous and intra-abdominal adipose tissue is a major source of TNF-α (tumor necrosis factor) and IL-6 (interleukin-6) production. These cytokines stimulate the liver synthesis of the acute-phase proteins. This raises the question of whether the acute-phase reaction of type 2 diabetes is mainly secondary to obesity. For many researchers the answer is an affirmative one even if some of them consider the high values of these inflammatory markers as surrogate markers of hypoadiponectinemia. Obesity was strongly related to elevated circulating levels of inflammatory markers, mainly CRP (C-reactive protein), in several cross-sectional studies in the general population and type 2 diabetes [11, 19].

The exact effect of inflammatory cytokines on glucose metabolism in humans is still unclear.

Interestingly, insulin is itself an inhibitor of acute-phase protein synthesis and in animal models of diabetes, the acute-phase response is increased by insulin deficiency. This indicates that there could be a positive feedback in type 2 diabetes whereby cytokine-induced insulin resistance further augments the acute-phase response. The relatively normal levels of acute-phase reactants in type 1 diabetes suggest that insulin replacement and the much lesser degree of hepatic insulin resistance in this type of diabetes is sufficient to restrain acute-phase protein production [19].

Because the acute-phase response and cytokinemia are so closely related to insulin resistance, the relationship with hyperglycemia is not unexpected. Lowering of blood glucose levels in type 2 diabetic patients is accompanied by reduced levels of inflammation markers [19].

During inflammation, the blood antioxidant defence systems try to counter the increase in oxygen metabolites. Diabetic patients with high values of CRP have an increased vascular risk. Antioxidant therapy was proposed to prevent cardiovascular events and improve diabetes mellitus prognosis but conflicting results were obtained [3].

The aim of this study was to assess the relationship between CRP plasma levels and blood redox status in diabetic foot patients.

MATERIALS AND METHOD

STUDY SUBJECTS

Forty newly hospitalized diabetic patients, aged between 45–75, with stage III or IV foot ulceration according to the Wagner classification, were enrolled. They were recruited from Bucharest "Paulescu" Hospital. Severity of neuropathy and vascular disease were assessed.

Blood samples were taken into 10 ml vacutaine tubes, containing heparine, from a peripheral vein after 12 hours of fasting and drugs break. The study protocol was approved by the Ethical Commission of "Carol Davila" University of Medicine and Pharmaceutics, Bucharest, and a written informed consent was obtained from each study participant.

Routine blood tests including glycemia, plasma cholesterol, plasma triglycerides were analysed in the laboratory of Bucharest "Paulescu" Hospital. Also, the CRP concentrations and HbA1c values were obtained from the hospital laboratory. In the "Carol Davila" University, at the biochemistry department, blood samples were analyzed for oxidative and carbonylic stress markers, using spectrophotometric methods.

The oxidative stress status was evaluated by measuring blood glutathione, total plasma antioxidant capacity, plasma uric acid and products of lipid peroxidation. The last one is estimated as dROM (determinable reactive oxygen metabolites).

The carbonylic stress status was evaluated by measuring plasma dicarbonyls.

The inflammation status was evaluated by using CRP and ceruloplasmin plasma values.

Considering CRP values, patients were divided into two groups. Group 1 (n = 17) had CRP concentration between 0.1–1 mg/dL and group 2 (n = 23) had CRP value above 1 mg/dL.

Data were analyzed using Statistic and Excel software. Differences between groups were assessed by Student's t-test. The relationship between the various parameters was assessed by correlation, stepwise multiple regression. Two-tailed *p*-values were considered statistically significant.

ANALYTIC METHODS

The plasma ceruloplasmin level determination is based on the oxidizing activity of the protein towards p-phenylenediamine [22].

Plasma antioxidant determination is based on the ability of the antioxidants contained in the sample to reduce the preformed radical $ABTS^{\cdot+}$. The etalonation curve is done with Trolox and the AC is expressed in mmol/L Trolox [20].

The dROM's measuring principle is based on the Verde *et al.* method. Peroxides present in the plasma, in the presence of Fe^{2+} ions, can produce radicals by a Fenton-like reaction. These are chemically trapped by a fenolic derivative (n,n-diethyl-p-phenyldiamine) which is then itself transformed into a red-colored radical which absorbs at 512 nm wavelength [26].

Reduced glutathione (GSH) in whole blood was measured by a colorimetric method using Ellman's reagent [2].

The dicarbonyls react with Girard's reagent T [(carboxymethyl)trimethyl-ammonium chloride hydrazide] at 294 nm. Glioxal was used for the etalonation curve [16]. Plasma uric acid was determined using kit (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden Germany). Total plasma proteins concentration was determined by Biuret method.

RESULTS

The characteristics of the healthy subjects and diabetic patients are shown in Table 1 and the measured markers of oxidative and carbonylic stress are illustrated in Table 2.

Table 1

The clinical characteristics of the healthy subjects and diabetic patients

Parameters	**Healthy subjects** ***n*** **= 20**	**Diabetic patients** ***n*** **= 40**	***p*** **value Healthy subjects** ***versus*** **diabetic patients**
Age (years)	64 (44–82)	62 (45–75)	ns
Duration of diabetes (years)	–	17 (1–41)	–
BMI (kg/m^2)	24.9 (19–35.8)	27.27 (19.5–35.6)	$p < 0.05$
Sex (female/male)	12/8	28/12	ns
Smokers/Nonsmokers	4/16	8/32	ns

Table 1 (continued)

Plasma HDL cholesterol (mg/dL)	59 (36–119)	35 (18–55)	$P = 0.005$
Plasma LDL cholesterol (mg/dL)	136 (82–194)	112 (55–257)	$p < 0.05$
Plasma triglycerides (mg/dL)	100 (47–239)	119 (24–851)	ns
Plasma total cholesterol (mg/dL)	218 (164–277)	174 (126–304)	$p = 0.005$
Atherogenic index	3.6 (2.1–6.3)	5.2 (2.9–12)	$p = 0.005$

Diabetic patients compared to controls had higher plasma dROM (3.92 ± 1.09 vs 2.37 ± 1.06 mM TBHP, $p = 0.001$), CRP (2.14 vs 0.09 mg/dL, $p < 0.001$), higher plasma ceruloplasmin (38.78 ± 12.54 vs 25.94 ± 7.54 mg/dL, $p = 0.001$), higher plasma dicarbonyls (0.49 ± 0.14 vs 0.31 ± 0.83 micromol/g protein, $p = 0.02$), higher plasma uric acid (0.37 ± 0.1 vs 0.24 ± 0.02 mmol/L, $p = 0.01$) higher blood glutathione (6.55 ± 1.74 vs 5.63 ± 0.77μmol/g Hb, $p = 0.04$) and almost the same values for the plasma total antioxidant capacity (1.78 ± 0.2 vs 1.78 ± 0.79 mM Trolox).

Group 2 of diabetic patients compared to group 1 of diabetic patients had higher plasma dROM (4.42 ± 1.03 vs 3.25 ± 1.83 mM TBHP, $p = 0.001$), higher plasma ceruloplasmin (43.12 ± 14.36 vs 32.91 ± 7.01 mg/dL, $p = 0.01$), higher plasma uric acid (0.45 ± 0.14 vs 0.32 ± 0.07 mmol/L, $p = 0.01$) and higher glycated hemoglobin (9.94% ± 0.65 vs 8.62% ± 0.59, $p = 0.048$).

Comparing group 2 *versus* group 1, there were not any significant differences for blood glutathione (6.47 ± 1.7 vs 6.53 ± 1.7 μmol/g Hb), plasma dicarbonyls (0.50 ± 0.13 vs 0.48 ± 0.16 micromol/g protein) and total antioxidant capacity (1.79 ± 0.23 vs 1.74 ± 0.15 mM Trolox).

There were not any significant differences for the BMI (body mass index) between the two diabetic groups. We could not find significant correlations between the BMI value and markers of inflammation for any group of subjects.

Table 2

The markers of oxidative and carbonylic stress in the healthy subjects and in the two groups of diabetic patients

Parameter	**Healthy subjects $n = 20$**	**Diabetic patients $n = 40$**	**Group 1 CRP = 0.1–1 mg/dL $n = 17$**	**Group 2 CRP > 1 mg/dL $n = 23$**	***p* value control vs. diabetic patients**	***p* value group 2 vs. group 1**
Plasma dROM (mM TBHP)	2.37±1.06	3.92±1.09	3.25±1.83	4.42±1.03	$p = 0.001$	$p = 0.001$
Plasma Ceruloplasmin (mg/dL)	25.94±7.54	38.78±12.54	32.91±7.01	43.12±14.36	$p = 0.001$	$p = 0.01$

Table 2 (continued)

Plasma total antioxidant capacity (mM Trolox)	1.78±0.79	1.78±0.2	1.74±0.15	1.79±0.23	$p > 0.05$	$p > 0.05$
Blood GSH (μmol/g Hb)	5.63 ± 0.77	6.55 ± 1.74	6.53 ± 1.7	6.47 ± 1.7	$p = 0.04$	$p > 0.05$
Plasma Oxoaldehydes (dicarbonyls) (μmol/g protein)	0.31 ± 0.83	0.49 ± 0.14	0.48 ± 0.16	0.50 ± 0.13	$p = 0.02$	$p > 0.05$
HbA_{1C} (%)	5.2 ± 0.2	9.84 ± 0.62	8.62 ± 0.59	9.94 ± 0.65	$p = 0.01$	$p = 0.048$
Plasma uric acid (mmol/L)	0.24 ± 0.02	0.37 ± 0.1	0.32 ± 0.07	0.45 ± 0.14	$p = 0.01$	$p = 0.01$

A positive correlation between plasma dROM and ceruloplasmin ($r = 0.83$, $p < 0.05$) was calculated for all forty diabetic patients. In both groups of diabetic patients, the above correlation was present and had the same value ($r = 0.80$, $p < 0.05$). Also, a positive correlation between uric acid and glycated hemoglobin concentrations ($r = 0.50$, $p < 0.05$) were calculated for diabetic patients.

DISCUSSION

It seems that in type 2 diabetes, there is an ongoing cytokine-mediated acute-phase response (part of a wide-ranging activation of innate immunity), and this is closely involved in the pathogenesis of the disease [19, 27].

Ceruloplasmin and CRP are acute–phase proteins. The changes in the plasma concentration are due largely to changes in their production by hepatocytes. The magnitude of the increase may be about 50 percent for ceruloplasmin to as much as1000 fold for CRP. In general, the acute-phase proteins limit injury or aid healing [9].

On the one hand, cytokines are the chief stimulators of the production of acute-phase proteins [9]. On the other hand, cytokines can lead to: insulin resistance, impaired insulin secretion, dyslipidemia, and accelerated atherosclerosis. In the study by Leinonen *et al.* [14], all markers of inflammation, including CRP, serum amyloid A, secretory phospholipase A_2 and IL-6, and endothelial dysfunction (soluble cell adhesion molecules) are correlated with the homeostasis model-measured insulin resistance. Also, anti-inflammatory agents like Aspirin and Statins decrease the acute-phase response, may reduce the risk of developing type 2 diabetes and improve control in established diabetes [19].

Mechanisms by which cytokines such as TNF-α can cause insulin resistance have been further clarified recently and include activation of the prototype stress-

induced kinase, c-Jun NH_2-terminal kinase, which serine phosphorylates many signaling proteins including insulin receptor substrate (IRS)-1 and IRS-2, thereby inhibiting insulin signaling and stimulation of expression of SOCS (suppressor of cytokine signaling) proteins, which bind IRS-1 and -2 and mediate their degradation [17]. Inflammatory cytokines such as: TNF-α, IL-1β, and IL-6, also downregulate PPAR-γ [peroxisome proliferator-activated receptor-γ] expression [23].

In this study, in diabetic foot patients, the increased values for the acute phase proteins, CRP and ceruloplasmin reflect the presence of inflammation. In group 2 of diabetic patients (with CRP > 1 mg/dL), ceruloplasmin had higher plasma levels. The effects of these high values deserve some comments. It is known that ceruloplasmin has antioxidant properties because of its ferroxidase activity. Alternatively, ceruloplasmin is thought to be a scavenger of reactive oxygen species [5] and plays an important role in nitrosothiol formation, which may contribute to its potent antioxidant activities [12]. Recently, unexpected, prooxidant effects of plasma ceruloplasmin were demonstrated by some authors. An increase in serum ceruloplasmin in type 2 diabetes could generate excess oxidized LDL, which causes atherosclerosis [7]. The prooxidant site was localized to a region containing His^{426}. These observations support the hypothesis that copper bound at specific sites on protein surfaces can cause oxidative damage to macromolecules in their environment [18]. Ceruloplasmin could also cause vascular injury by generating free radicals, such as hydrogen peroxide, in the course of oxidization of serum homocysteine [21].

The positive correlation between plasma ceruloplasmin and dROM ($r = 0.83$, $p < 0.05$) in diabetic patients is based on the fact that inflammation increases both lipid peroxides and acute phase protein levels.

In this study, increased oxidative stress is reflected by the high values for the lipid peroxides (dROM) and the increased carbonylic stress is demonstrated by the high values for the plasma dicarbonyls. Comparing the two groups of diabetic patients, group 2 has an increased plasma oxidative stress, reflected by the higher values of dROM.

The high blood values for glutathione (known as a powerful antioxidant) are unexpected in diabetic patients, but increased concentration of this tripeptide is mentioned as an acute-phase phenomenon [9]. Another explanation for the high blood values for glutathione may be inferred from the next lines.

Glutathione is a key molecule in aerobic cells. The essential functions it fulfills include the detoxification of toxic oxygen and electrophilic metabolites, the preservation of essential thiol groups and the transport of cystein. It is an important antioxidant and modulates cell proliferation. The availability of GSH to various tissues is determined by the liver and kidney, which synthesize and release GSH and GSH precursors into the plasma. The main source of plasma GSH is hepatic GSH transported across the sinusoidal membrane and this process appears to respond to the needs of peripheral tissues. In the liver major factors that determine

the availability of cysteine are diet, membrane transport activities of the three sulfur aminoacids (cysteine, cystine and methionine) and the conversion of methionine to cysteine *via* the trans/sulfuration pathway. Many conditions alter GSH level *via* changes in GCS (γ-glutamylcysteine synthetase) activity and GCS gene expression. This includes oxidative stress, activators of Phase II detoxifying enzymes, antioxidants, drug resistant tumor cell lines, hormones, cell proliferation and diabetes mellitus [15]. Oxidative stress increases the transcription of GCS. Urata *et al.* showed that GCS m RNA level and transcriptional rates in mouse endothelial cells were increased by treatment with cytokines such as TNF and IL-1β. The basal GCS mRNA level was decreased and the effect of cytokines disappeared when cells were grown in media containing high glucose concentration [25].

It is important to underlie that in the diabetic group, the increased carbonylic stress is reflected by the high values of glyoxal and methylglyoxal (the main dicarbonyls). Glyoxal is formed in the lipid peroxidation process and by the slow oxidative degradation of glucose and glycated proteins. Methylglyoxal is formed by the non-enzymatic and enzymatic fragmentation of glyceraldehyde-3-phosphate and dihydroxyacetonephosphate. It is also formed in ketone body metabolism from acetone catalysed by cytochrome P450, and in the metabolism of threonine. The concentrations of α-oxoaldehydes in human tissues and body fluids are usually low. Such low concentrations of α-oxoaldehydes are important, however, because of the high reactivity of α-oxoaldehydes with DNA, RNA and proteins [4]. The accumulation of α-oxoaldehydes modified nucleotides is associated with crosslinking of proteins, protein degradation and activation of a proinflammatory response of monocytes and macrophages.

Even if we could not find any significant correlation between the dicarbonyls concentration and inflammation markers, there must be a relation between α-oxoaldehydes and lipid peroxidation, glucose concentration and inflammation.

On the one hand, a link between inflammation and uric acid is provided by the fact that uric acid stimulates human mononuclear cells to produce interleukin-1β, IL-6 and TNF-α [10]. On the other hand, these cytokines are the chief stimulators of the acute phase proteins (including ceruloplasmin) [9]. The significant higher values of uric acid and ceruloplasmin in group 2 *versus* group 1 must be carefully interpreted. These antioxidants may become prooxidant in certain situations. As an example, uric acid "works" as a prooxidant in the atherosclerotic vascular milieu of diabetic patients [10]. So, in group 2 of diabetic patients, plasma uric acid, at the high calculated concentrations, may have harmful effects.

The positive correlation between uric acid and glycated hemoglobin concentrations ($r = 0.50$, $p < 0.05$) in diabetic patients has importance for treatment approach. It is known that a good glycemic control prevents diabetic complications. Maybe a low purine diet, increased exercise to combat overweight as well as controlling the consumption of alcohol should be recommended to diabetic foot patients with benefic effects.

Even though the diabetic patients have high plasma levels of uric acid, the plasma antioxidant capacity is not significantly increased in comparison with healthy subjects. Probably, an increased production of free radicals, directly caused by hyperglycemia, results in an increased consumption of antioxidants *in vivo* and thus, decreases antioxidants stores.

In conclusion, in this study, we demonstrate that diabetic patients with high values for inflammatory markers have an increased oxidative stress. Even if we could not find any correlation between the BMI values and inflammation markers, we suggest that the link between obesity and diabetes mellitus may be represented by inflammation and oxidative stress. Modulating these processes using therapeutic methods and, of course, weight control may reduce the incidence of diabetic complications.

REFERENCES

1. BROWNLEE, M., The pathobiology of diabetic complications. A unifying mechanism, *Diabetes,* 2005, **54,** 1615–1625.
2. BEUTLER, E., Reduced glutathion (GSH), In: *Red Cell Metabolism; A Manual of Biochemical Methods*, E. Beutler, F.L. Orland (eds.): Grune&Stratton Inc., 1975, 131–132.
3. CERIELLO, A., Pathophysiology of diabetic vascular complications: the role of oxidative stress, *Medicographia*, 1999, **21**, 309–312.
4. CHETA, D., *New insights into experimental diabetes*, Editura Academiei Române, Bucureşti, 2002.
5. COLLIER, A., R. WILSON, H. BRADLEY, J.A. THOMSON, M. SMALL, Free radical activity in type 2 diabetes, *Diabet. Med.,* 1990, **7**, 27–30.
6. DAVI, G., A. FALCO, C. PATRONO, Lipid peroxidation in diabetes mellitus, *Antioxid. Redox Signal,* 2005, **7**, 256–268.
7. EHRENWALD, E., G.M. CHISOLOM, P.L. FOZ, Intact human ceruloplasmin oxidatively modifies low density lipoprotein, *J. Clin. Invest*., 1994, **93**, 1493–1501.
8. EVANS, J.L., I.D. GOLDFINE, B.A. MADDUX, G.M. GRODSKY, Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction?, *Diabetes*, 2003, **52**, 1–8.
9. GABAY, C., I. KUSHNER, Acute-phase proteins and other systemic response to inflammation, *The New England Journal of Medicine*, 1999, **340**, 448–454.
10. HAYDEN, M.R., S.C. TYAGI, Uric acid: A new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: The urate redox shuttle*, Nutr. Metab. (Lond)*, 2004, **10**, 1–15.
11. HIGDON, J.V., B. FREI, Obesity and oxidative stress. A direct link to CVD?, *Arterioascler. Throm. Vasc. Biol.,* 2003, **23**, 365–367.
12. INOUE, K., T. AKAIKE, Y. MIYAMOTO, T. OKAMOTO, T. SAWA, M. OTAGIRI, S. SUZUKI, T. YOSHIMURA, H. MAEDA, Nitrosothiol formation catalyzed by ceruloplasmin. Implication for cytoprotective mechanism *in vivo*, *J. Biol. Chem.,* 1999, **274**, 27069–27075.
13. KALOUSOVÁ, M., J. ŠKRHA, T. ZIMA, Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus, *Physiol. Res.,* 2002, **51**, 597–604.
14. LEINONEN, E., E. HURT-CAMEJO, O. WIKLUND, L.M. HULTÉN, A. HIUKKA, M.R. TASKINEN, Insulin resistance and adiposity correlate with acute-phase reaction and soluble cell adhesion molecules in type 2 diabetes, *Atherosclerosis,* 2003,**166**, 387–394.

15. LU, S.C., Regulation of hepatic glutathione synthesis: current concepts and controversies, *FASEB J*., 1999, **13**, 1169–1183.
16. MITCHEL, R.E.J., H.C. BIMBOIM, The use of Girard T reagent in a rapid and sensitive method for measuring glyoxal and certain other alfa dicarbonyl compounds, *Anal. Biochem.*, 1977, **81**, 47–56.
17. MORRIS, M.F., Insulin receptor signalling and regulation, In: *Textbook of Diabetes*. 3rd ed., J.C. Pickup, G. Williams, (eds.), Oxford, U.K., Blackwell, 2003, pp. 141–147.
18. MUKHOPADHYAY, C.K., B. MAZUMDER, P.F. LINDLEY, P.L. FOX, Identification of the prooxidant site of human ceruloplasmin: a model for oxidative damage by copper bound to protein surfaces, *Proc. Natl. Acad. Sci. U.S.A*., 1997, **9**4, 11546–11551.
19. PICKUP, J.C., Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes, *Diabetes Care*, 2004, **27**, 813–823.
20. RICE EVANS, C., N.J. MILLER, Total antioxidant status in plasma and body fluids, *Methods Enzymol.*, 1994, **234**, 279–293.
21. STARKEBAUM, G., J.M. HARLAN, Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine, *J. Clin. Invest.,* 1986, **71,** 1370–1376.
22. SUNDERMAN, F.W., Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity, *Clin. Chem.*, 1970, **16**, 903–910.
23. TANAKA, T., H. ITOH, K. DOI, K. FUKUNAGA, M. SHINTANI, J. YAMASHITA, T.H. CHUN, M. INOUE, K. MASATSUGU, N. SAWADA, T. SAITO, H. NISHIMURA, Y. YOSHIMASA, K. NAKAO, Down regulation of peroxisome proliferator-activated receptor γ-expression by inflammatory cytokines and its reversal by thiazolidinediones, *Diabetologia*, 1999, **42**, 702–710.
24. THORNALLEY, P., Glutathione-dependent detoxification of α-oxoaldehydes by glyoxalase system: involvement in disease mechanism and antiproliferative activity of glyoxalase I inhibitors, *Chemico-Biological Interactions*, 1998, **111**, 137–151.
25. URATA, Y., H. YAMAMOTO, S. GOTO, H. TSUSHIMA, S. AKAZAWA, S. YAMASHITA, S. NAGATAKI, T. KONDO, Long exposure to high glucose concentration impairs the responsive expression of gamma-glutamylcysteine synthetase by interleukin-1β and tumor necrosis factor-α in mouse endothelial cells, *J. Biol. Chem*., 1996, **271**,15146–15152.
26. VERDE, V., V. FOGLIANO, A. RITIENI, G. MAIANI, F. MORISCO, N. CAPORASO, Use of N,N dimethyl-p-phenylendiamine to evaluate the oxidative status of human plasma, *Free Rad. Res.*, 2002, **368**, 869–873.
27. WELLEN, K.E., G.S. HOTAMISLIGIL, Inflammation, stress and diabetes, *The Journal of Clinical Investigation*, 2005, **5**, 1111–1119.