REVIEW

Parameters for Measurement of Oxidative Stress in Diabetes Mellitus: Applicability of Enzyme-Linked Immunosorbent Assay for Clinical Evaluation

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ABSTRACT

Investigations of the mechanisms involved in the onset and progression of diabetes have recently confronted the role of reactive oxygen species (ROS) and oxidative stress. Prolonged exposure to hyperglycemic conditions induces nonenzymatic glycation of protein via the so-called Maillard reaction, resulting in Schiff-base products and Amadori products that engender ROS production. These processes initiate and exacerbate micro- and macrovascular complications in diabetes. Increased oxidative stress is induced by excessive ROS production and inadequate antioxidant defenses. Recently, oxidative stress status markers have been associated directly with the severity and prognosis of diabetes. To examine oxidative stress, reliable and high-throughput methods are needed to examine large numbers of clinical samples. The emerging availability of enzyme-linked immunosorbent assay (ELISA) for oxidative stress status markers allows its application to assessment of various pathophysiologic conditions, including diabetes. This review outlines the recent achievements of ELISA application for clinical studies elucidating oxidative stress. It introduces the potential applicability of ELISA for investigating oxidative stress in diabetes.

Key Words: enzyme-linked immunosorbent assay, diabetes mellitus, advanced glycation end products, oxidative stress, lipid peroxide, nitric oxide, peroxynitrite, thioredoxin, fatty acid binding protein

A widely used indicator of diabetes mellitus, hemoglobin A_{1c} (HbA_{1c}) is a stable Amadori product converted from Schiff-base adducts on molecular rearrangement.[1] Schiff-base adduct was nonenzymatically generated from glucose and reducing sugars reacting with protein amino groups. Therefore, the involvement of reactive oxygen species (ROS) in the disease process of diabetes mellitus is conceivable. Amadori products are transformed gradually and over several weeks or months into advanced glycosylation end products (AGEs). This slow process mainly affects proteins with a slow turnover, such as matrix tissue proteins. Suzuki and Miyata and Horie and colleagues demonstrated the accumulation of AGE compounds in expanded mesangial matrix and nodular lesions in diabetic nephropathy.[2,3] The accumulation of AGEs was colocalized with lipid peroxidation end products (ALEs), such as malondialdehyde lysine (MDA), 4-hydroxynonenal protein adduct (HNE), and acrolein (ACR) protein adduct. From the perspective of clinical practice, measuring these indicators in tissue specimens is not useful to follow-up every case. Blood and urine analyses must be pursued.

Direct measurement of ROS in in vivo specimens is difficult. Many studies have measured so-called oxidative stress status markers using analytic chemistry techniques that combine high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC-MS). These markers are often measurable using stable protein adducts produced by oxidative processes in vivo or in vitro because it has become possible to produce specific antibodies against these epitopes with improved antibody production technologies. Therefore, use of the flexible and high-throughput enzyme-linked immunosorbent assay (ELISA) method has spread quickly to the analysis of clinical samples. Furthermore, analyses of oxidative stress in various disease processes are now performed predominantly using ELISA. Onerous pretreatments and expensive apparatus are virtually obviated by ELISA; it is a labor-saving, cost-saving method. Results are obtained quickly. Two or more markers can be measured simultaneously. Examination using a few specimen aliquots is possible. Its reproducibility is excellent. This technique is demonstrably suited for use in clinical medicine. For these reasons, this review is intended to assess the recent achievements of ELISA application for clinical studies that have elucidated oxidative stress. In so doing, we emphasize ELISA's potential application to investigation of oxidative stress in diabetes.

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Trapping ROS per se is difficult. Therefore, methods to measure them by ELISA are divisible into two systems. One is the ELISA system to detect proof of the existence of oxidative stress. The other is to detect antioxidative system changes in response to ROS.

Because the present review specifically addresses the clinical applicability of ELISA, AGEs are discussed in the first section as the method for detecting glycoxidation. The following section, on ALEs, examines the method for detecting lipid peroxidation. The subsequent section discusses 8-hydroxydeoxyguanosine (8-OHdG), which demonstrates the oxidative denaturation of deoxyribonucleic acid (DNA) as a marker of oxidative stress. The thioredoxin-thioredoxin reductase system, which has attracted attention in recent years, is discussed first to illustrate detection of defense system activity against oxidative stress. Subsequently, we describe bilirubin oxidative metabolites, representing the heme oxygenase–bilirubin antioxidant system, and, finally, the fatty acid binding protein system.

This review also briefly addresses the importance of the nitric oxide (NO)-generating system in diabetes and the nitroxidative stress produced in related reaction processes. To confirm the participation of oxidative stress in certain pathologic conditions, it is important to examine the combination of oxidative stress status markers constructively. Our recent achievements in this area are included.

APPROACHES TO TRACE DETECTION OF OXIDATIVE STRESS

Advanced Glycosylation End Products

Carbonyl groups do not exist in the amino acid constituent proteins in a steady-state condition. Carbonyl proteins exist at a negligible level in healthy individuals. For that reason, carbonyl groups are thought to form in response to the Amadori transition reaction in an oxidative environment. Therefore, carbonyl proteins and Amadori compounds are useful indicators for examining pathologic conditions. Under the hyperglycemic state of diabetes, the reducing sugar derived mainly from glucose reacts with the epsilon-amino group of a protein lysine residue, thereby forming a Schiff base. Amadori compounds possessing carbonyl groups are produced depending on the Schiff base, as shown in Figure 1 (upper panel). This process is known in many cases as the early step of the Maillard reaction. In the process of the Maillard reaction, highly reactive 3-deoxyglucosone is generated following several anhydration reactions, which react with the free amino group, especially with the imidazole group, promoting the bridging formation. This series of reactions is referred to as the late step of the Maillard reaction. AGEs bind with the specific receptor for advanced glycation end products, which initiate intracellular signal transduction. Distribution of the receptor for advanced glycation end products is rather broad and reportedly found in vascular endothelial cells, vascular smooth muscle cells, monocytes, and macrophages.[4] Apparently, the amount of Amadori compounds depends on both the level and duration of the hyperglycemic state.

ELISA for AGEs was developed for the first time by Araki and colleagues, who reported the age-dependent increase in AGEs in eye lens protein.[5] Later the same group reported that N -(carboxymethyl) lysine (CML) is the particular epitope recognized by this antibody.[6] Makita and colleagues developed a polyclonal antibody for AGEs. They demonstrated a significantly higher level of AGEs in the serum of patients with diabetes and end-stage renal disease. Furthermore, they suggested that AGEs were primarily metabolized and excreted by the kidney because the level of AGEs was reduced after hemodialysis or renal transplant.[7,8] Although CML is a component of AGEs that is capable of binding with the receptor for AGEs, [9] it is generated by the cleavage of fructose lysine and is also produced by auto-oxidation of ascorbic acid or glucose. For that reason, CML is inferred to reflect not only glycation but also the redox state of a living body. ELISA for CML was reported by Degenhardt and colleagues.[10] A polyclonal antibody used in their study, pDia1, recognized CML. Serum CML values of individuals from a healthy volunteer group, a peritoneal dialysis group, and a hemodialysis group showed significant correlation in measured CML levels by ELISA and GC-MS. Papanastasiou and colleagues applied fluorescence method to ELISA, thereby increasing its sensitivity. They found a positive correlation between serum CML and creatinine concentrations in patients with early-stage diabetes.[11]

If ELISA can measure Amadori compounds generated at the early stage of the Maillard reaction, it might detect early diabetic complications. Amadori compounds have been examined using the colorimetric reaction of dinitrophenylhydrazine, but that method requires time, effort, and a large sample volume. Buss and colleagues developed a polyclonal antibody to dinitrophenylhydrazine and used it to measure Amadori compounds in ELISA.[12] Subsequently, Himmelfarb and McMonagle used ELISA to

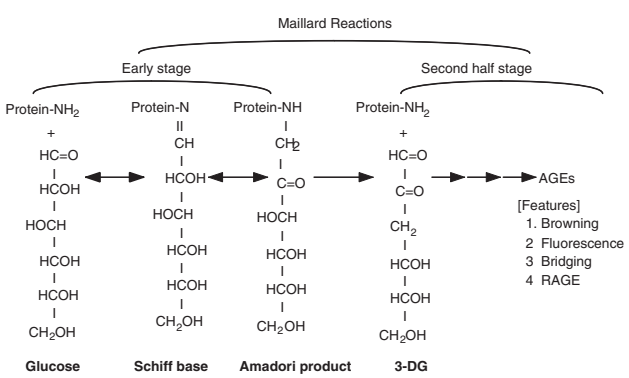

FIGURE 1 Advanced glycosylation end product (AGE) formation from glucose by the Maillard reaction. 3-DG = 3-deoxyglucosone; RAGE = receptor for advanced glycation end products.

measure Amadori compounds in the serum of hemodialysis patients. They found significantly higher quantities of compounds in the patients' serum than in that from healthy individuals; they reported these findings mainly on the basis of data from serum albumin.[13] Schalkwijk and colleagues used competitive ELISA to examine the serum Amadori albumin of 447 patients with type 1 diabetes. They found a significant increase in the beginning of the microalbuminuric stage; thereafter, it increased significantly according to the development of diabetic nephropathy.[14] These investigators claim an advantage of serum measurement of Amadori compound or albumin over AGEs because Amadori compounds constitute 2 to 10% of the serum protein of diabetic patients, in marked contrast to AGEs, which constitute 0.07% or less.

3-Deoxyglucosone (see Figure 1) is generated either nonenzymatically from Amadori compounds or enzymatically from fructose-3-phosphate via hydroxylation. It reacts with protein and generates pentosidine and pyrraline. Taneda and Monnier developed an antibody against pentosidine and used ELISA to demonstrate significantly high levels of pentosidine in the serum protein and skin collagen of diabetic patients.[15] Izuhara and colleagues applied the competitive ELISA method to pentosidine. They demonstrated higher sensitivity without using the protease used by Taneda and Monnier; they reported a significantly high correlation between values obtained using HPLC and ELISA.[16]

More than a decade is often necessary for overt development of diabetic nephropathy. In contrast, oxidative stress is inferred to accelerate this process according to the sustained hyperglycemic state. This difference is a crucial point for the further development of diabetic retinopathy and nephropathy. For an examination of type 2 diabetes, Shimoike and colleagues analyzed urine specimens using ELISA on AGEs and 8-OHdG.[17] The antibody against AGEs did not cross-react with Amadori compounds but cross-reacted with CML and components of pentosidine. The upper panel of Figure 2A shows that the urinary excretion of AGEs reflected the hyperfiltration state in diabetic nephropathy. This increase was normalized with the development of diabetic nephropathy. Because this increase in AGEs was already normalized at the period of microalbuminuria, it is quite possible that urinary AGEs are able to detect more rapidly a subtle change in diabetic kidney than urinary microalbuminuria. Moreover, regarding 8-OHdG (discussed later in detail) in urine, this oxidative stress status marker evidently increases with the development of diabetic nephropathy (lower panel of Figure 2A). Accumulation of serum AGEs in advanced type 2 diabetes nephropathy (correlation shown in Figure 2B) also increases the renal tissue level of AGEs and ALEs,[2,3] thereby inducing an increase in urinary 8-OHdG.

Type 1 diabetes often occurs during youth. Berg and colleagues examined AGEs in type 1 diabetes of patients with renal function with microalbuminuria. They found a

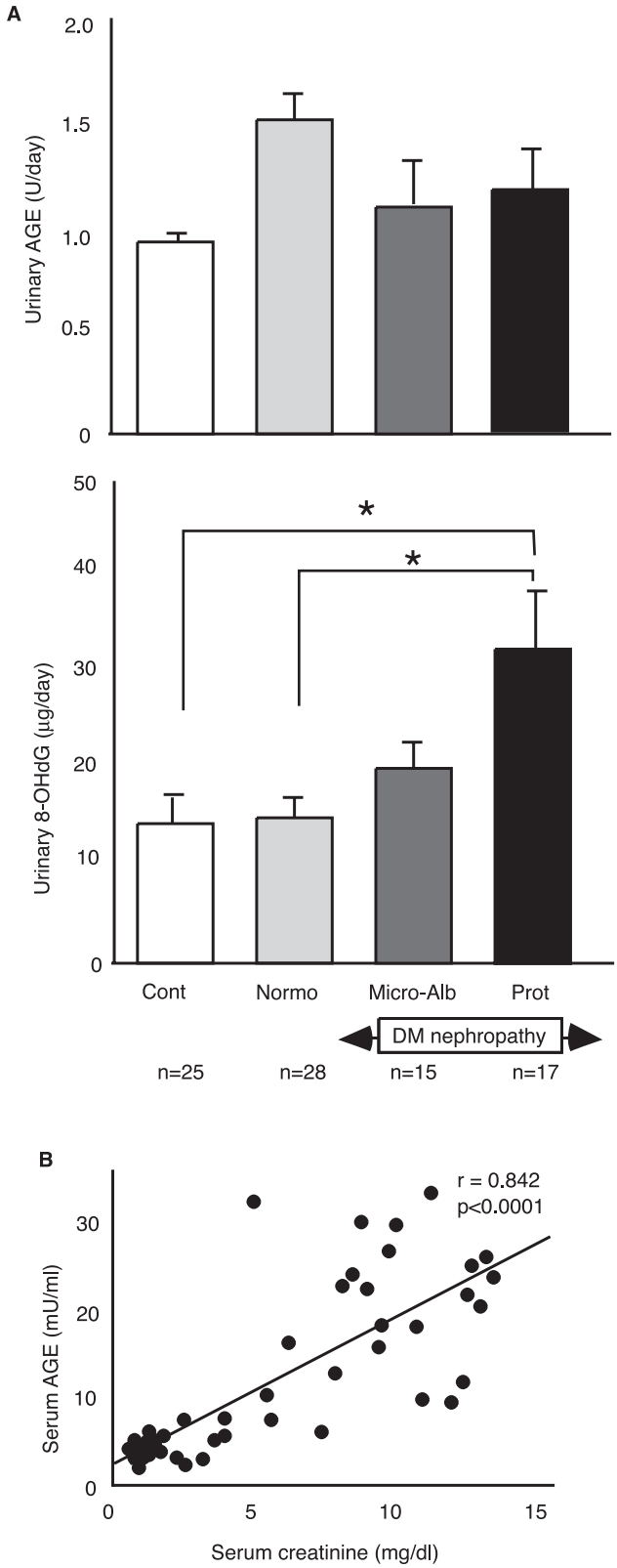

FIGURE 2 *A*, Comparison of the diabetic nephropathy exacerbation with urinary advanced glycosylation end product (AGE) (*upper panel*) or urinary 8-hydroxydeoxyguanosine (8-OHdG) level (*lower panel*). *B*, Correlation between the serum creatinine and the serum AGE level. Cont = control; DM = diabetes mellitus; Micro-Alb = microalbuminuria; Normo = normoalbuminuria; Prot = proteinuria. $*p < .05$. Reproduced with permission from Shimoike T et al.[17]

significant correlation between the level of serum AGEs and the subsequent severity of renal pathologic findings after 2 to 3 years.[18] These observations provide evidence against the conjecture that an accumulation of AGEs is a secondary phenomenon of reduced renal function.[17] More precisely, the sustained higher blood level of AGEs aggravates the regulatory milieu of microvasculature in a diabetic kidney.

Advanced Lipoxidation End Products

Various aldehydes of unsaturated fatty acid origin arise in the process of nonenzymatic oxidation of lipids. Because the 2-alkenal group in the volatile straight-chain aldehydes has the particular characteristic of inducing cellular response and signal transduction pathway in addition to inducing the common features of oxidative stress, such as cell injury and cell death, this group is often deemed important for the interpretation of cell physiology and pathophysiology. For example, MDA, HNE, 4-hydroxyhexenol, croton aldehyde, and ACR belong to this category. Among them, HNE and ACR are the best-studied aldehydes for human specimens by ELISA.[19] That work was made possible by the specific antibody to Lys and His residues that produce hemiacetal adducts in response to reactive ornamentation by these aldehydes. A recent study using sandwich ELISA in combination with the antibody to HNE and the antibody to albumin found that the serum level of HNE-modified albumin was significantly higher in members of the type 2 diabetic group than in those of a healthy volunteer group.[20] Recently, the existence of ACR-modified protein was also shown in the lumen of atherosclerotic aorta.[21]

Tsukahara and colleagues performed simultaneous measurements of carbonyl and oxidative stress status markers in the urine of young patients with type 1 diabetes.[22] They found a significant correlation between pentosidine (measured using HPLC) and ACR lysine (measured using ELISA), as shown in Figure 3. Noiri and colleagues found significantly higher levels of serum ACR lysine levels among a group of hemodialysis patients with end-stage renal disease than among healthy individuals.[23] That significance was more pronounced in end-stage renal disease in the type 2 diabetic group. The hemodialyzer was changed to a vitamin E–bonded hemodialyzer for 6 months in 41 patients who showed a higher serum ACR lysine value than its average in end-stage renal disease; a significant decrease in ACR was found 3 months after starting use of the vitamin E–bonded hemodialyzer. That significant drop in ACR lysine reached a level equivalent to that of healthy subjects after 6 months of treatment (Figure 4). When hemodialyzers were switched back to the original ones, the serum ACR lysine again rose to a statistically significant level.

Bucala and colleagues reported on the detectability of human low-density lipoprotein by the antibody to AGEs.[24] Therefore, CML was also expected to be involved in lipid peroxidation products: CML reportedly colocalizes to the atherosclerotic lesions of nondiabetic patients.[25] Thus, CML is generated not only by glucose oxidation but also by the lipid oxidation process. Moreover, CML was detected at senile spots of Alzheimer elastin under sunlight exposure.[26] Involvement of lipid peroxidation was elucidated in those pathophysiologic conditions. Therefore, CML has begun to be considered a comprehensive indicator of oxidative stress rather than a specific indicator of glycoxidation.

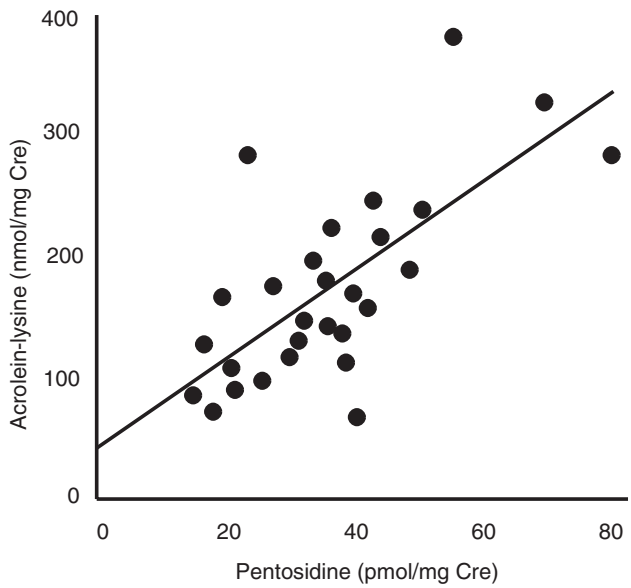

FIGURE 3 Correlation between urinary acrolein lysine and pentosidine in juvenile type 1 diabetes. Reproduced with permission from Tsukahara H et al.[22]

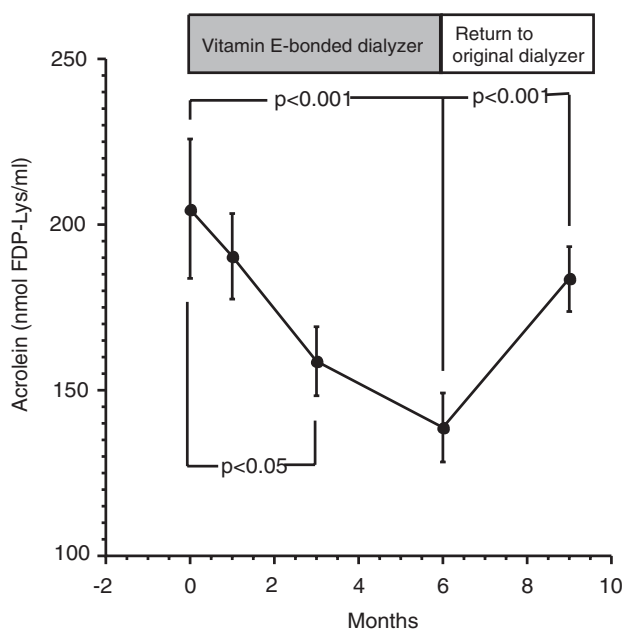

FIGURE 4 Efficacy of the vitamin E–bonded hemodialyzer in reducing the acrolein level in patients with end-stage renal disease (N = 41). Reproduced with permission from Noiri E et al.[23] FDP = fibrin/fibrinogen degradation products.

DNA Oxidative Damage Substance

DNA damage results from myriad diseases. It also occurs by physiologic processes, such as aging. 8-OHdG is derived from DNA or the nucleotide pool via an endonuclease or nucleotide excision repair; it has been used as a marker of oxidative stress.[27] Because 8-OHdG is not metabolized in vivo, it is emitted into the blood and is excreted in urine.

The participation of oxidative stress in DNA damage in diabetes has been demonstrated by Dandona and colleagues.[28] Serum 8-OHdG levels of patients with type 1 and type 2 diabetes were significantly higher than those of healthy individuals when measured using HPLC (upper panel in Figure 5). Further, patients' levels of serum ROS were significantly higher than those of healthy individuals when measured by chemiluminescence using luminol as the indicator (lower panel in Figure 5). In ELISA, the diabetic blood glucose was well correlated with the urinary 8-OHdG level when the blood glucose control was analyzed depending on the layer of the controlled level, as in $HbA_{1c} \leq 6.8\%$ (fair), $6.8\% < HbA_{1c} < 9.6\%$ (moderate), and $9.6\% \leq HbA_{1c}$ (poor).[29] Glucose toxicity occurring in the poor controlled group was loaded with oxidative stress that was 1.5 times higher than that in the fair controlled group. In diabetic nephropathy, Kanauchi and colleagues examined the relationship between renal histologic findings and the urinary 8-OHdG level. Their study found that the level of urinary 8-OHdG was correlated more with interstitial injury than with glomerular lesions.[30]

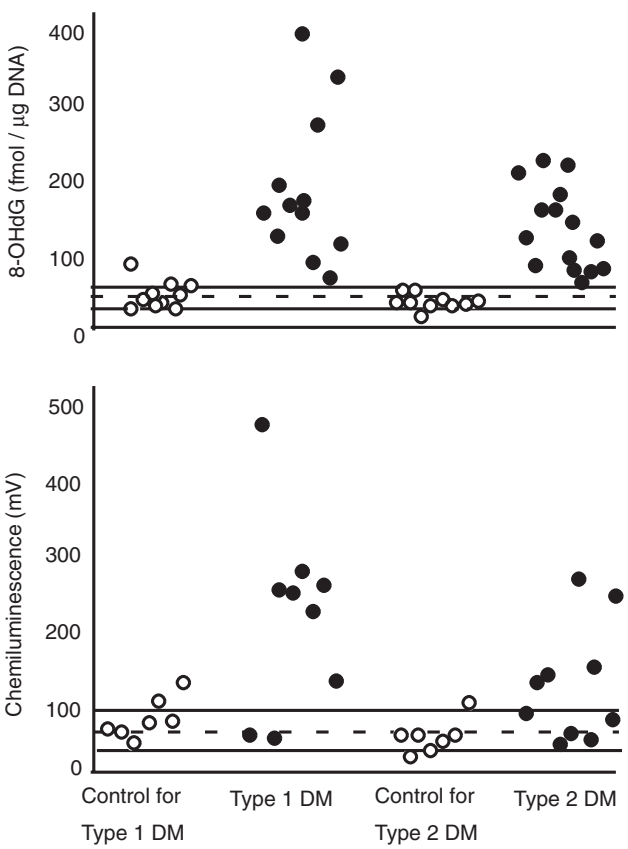

FIGURE 5 Serum 8-hydroxydeoxyguanosine (8-OHdG) in type 1 and type 2 diabetes. Reproduced with permission from Dondona P et al.[28] DM = diabetes mellitus; DNA = deoxyribonucleic acid.

APPROACHES TO TRACE DETECTION OF DEFENSE MECHANISMS AGAINST OXIDATIVE STRESS

Thioredoxin System

Thioredoxins are small disulfide-containing redox proteins that interact with a broad range of proteins by a mechanism based on reversible oxidation of two cysteine thiol groups to a disulfide, with accompanying transfer of two electrons and two protons. Thioredoxins control various cell functions and enzymatic activity by this reducing reaction to the sulfhydryl group on the cysteine of other proteins.[31,32]

In a diabetic laboratory animal model, when overexpression of thioredoxin was induced in nonobese diabetic mice with the autoimmunity background often showing symptoms of type 1 diabetes, the diabetes frequency was reduced significantly. Moreover, also in the streptozocin mice model, the diabetes frequency was reduced significantly in thioredoxin transgenic mice compared with wild-type mice.[33] Therefore, ROS presumably participates in the onset of type 1 diabetes; thioredoxin is able to control it and diminish the occurrence of diabetes. Kakisaka and colleagues reported that the serum thioredoxin levels in individuals of a type 2 diabetic group were significantly higher than those of healthy individuals, as determined by sandwich ELISA.[34] Although no correlation was found in the quality of blood glucose control and the level of thioredoxin, that study found significant correlations with insulin resistance, plasma free fatty acid, and the intrinsic insulin excretion level before breakfast.

Bilirubin Oxidative Metabolites

Heme oxygenase decomposes a heme into biliverdin, carbon monoxide, and iron ion. Bilirubin, the metabolite of biliverdin, shows a powerful antioxidative action under physiologic and pathophysiologic conditions.[35] Antibilirubin monoclonal antibody 24G7 in ELISA can be used to estimate the level of systemic oxidative stress in urine samples.[36] In other words, elevated levels of urinary bilirubin oxidative metabolites reflect heme oxygenase 1 induction under stressful conditions. No reports have examined bilirubin oxidative metabolites in diabetic specimens, but the clinical applicability to the human disease process addressed below will be useful for future analysis of diabetes.

Recently, urinary 8-OHdG, ACR lysine, and bilirubin oxidative metabolites in children with acute aggravation of atopic dermatitis were examined by Tsukahara and colleagues. The patient group exhibited significantly high levels of ACR lysine, 8-OHdG, and bilirubin oxidative

metabolites. Whereas ACR lysine and 8-OHdG were normalized with the improvement of clinical aspects in the patients, the high level of bilirubin oxidative metabolites was sustained after the improvement.[37]

These results suggest that an antioxidative system is activated by higher oxidative stress in serious illness and acute inflammation. Moreover, the antioxidative system is sustained for a certain period. That period is presumably related to the establishment of acquired resistance to subsequent exposure to oxidative stress, which has been reported in heme oxygenase 1.[38,39]

FATTY ACID BINDING PROTEIN

The metabolic pathway of highly reactive lipid peroxides and free fatty acids is a potential source of aldehydes. It is extremely important to maintain the redox condition in a living body. Fatty acid binding protein (FABP), a protein of 14 kDa capable of binding free fatty acid, promotes β-oxidation by transferring free fatty acids to mitochondria and peroxisome. Recently, FABP served as a target of HNE in an investigation of the E type of FABP (Figure 6). That investigation showed that the one hundred twentieth cysteine of E-type FABP participated especially in the capture of HNE.[40] Expression of the specific type of FABP has been discovered in many important organs. With this scope, it is suggested that FABP can metabolize highly reactive lipid peroxide. It plays an essential antioxidative role in each organ.

Indeed, the I type of FABP has been reported to increase in blood at the acute phase of intestinal artery thrombosis, which is difficult for diagnosis in light of contemporary practical skill.[41] It is expected to be the specific diagnostic marker of this disease. Moreover, in patients with acute myocardial infarction, it has been observed that the H type of FABP released from damaged or ischemic cardiac muscle appears in the blood at the acute phase.[42]

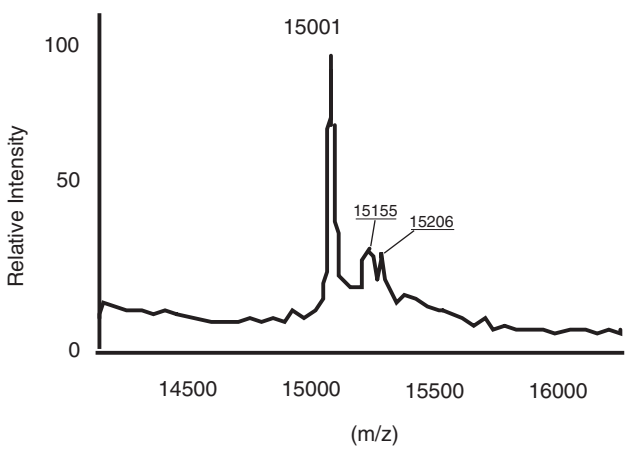

FIGURE 6 Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry linear mass spectrum of a mixture of native and hydroxynonenal (HNE)-modified fatty acid binding protein (FABP). The peak at m/z 15,001 corresponds to unmodified FABP, that at 15,155 to singly HNE-modified FABP, and that at 15,206 to the sinapinic acid adduct of FABP. Adapted from Bennaars-Eiden A et al.[40]

In the human kidney, the L type of FABP is expressed predominantly in the proximal tubules and is involved in the energy metabolism of β-oxidation; that metabolism is especially active in this site. Together with observations in other organs, L-type FABP is anticipated as an oxidative stress marker in proximal tubules. Recently, Kamijo and colleagues established a sandwich ELISA for L-type FABP and investigated its urinary level in progressive chronic renal disease. Their study concluded that the urinary level of this FABP was the most sensitive indicator able to substantiate the prognosis of renal disease: its use is preferable to that of the serum creatinine level, urinary protein level, or urinary α_1-macroglobulin level.[43] Given these encouraging results, future study in diabetic nephropathy appears to be promising.

NO AND RELATED SUBSTANCES

Macro- and microvascular complications accompanying diabetes are important prognostic factors. NO generated from the vascular endothelial cells serves an especially important role in vasodilatation. Its decrease disturbs the homeostasis of microvascular circulation and aggravates tissue injury. In diabetes, endothelial NO-dependent vasodilation is reduced because of the formation of AGEs at the vascular basement membrane under hyperglycemic conditions. Moreover, the following mechanisms are deemed to be key processes in the formation of intracellular oxidative stress in diabetes: (1) the decrease in the reduced nicotinamide adenine dinucleotide phosphate level, induced by activation of the polyol pathway and/or by the abnormality in the pentose pathway, perturbs superoxide dismutation[44,45]; (2) activation of protein kinase C induces phospholipase A_2, which activates prostaglandins and generates ROS generation[46,47]; (3) binding AGEs with the receptor for advanced glycation end products induces ROS[48]; (4) auto-oxidation processes of Amadori compounds generate ROS[49]; (5) AGEs induce inactivation of both low-molecular antioxidative factors, such as glutathione, vitamin C, and vitamin E, and the antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase; and (6) cytotoxic peroxynitrite is also generated by the reaction between NO and superoxide.[50]

Improvement in intracellular oxidative stress in a diabetic condition is expected by administration of antioxidants. An improved response to glyceryl trinitrate or methacholine has been observed in type 2 diabetes after either 1 week of vitamin E and glutathione medication or acute administration of vitamin C.[51,52] Nevertheless, peroxynitrite formation is difficult to trap because peroxynitrite is generated rapidly from superoxide and NO at the theoretic reaction velocity of $6.7 \times 10^9\ M^{-1} \cdot s^{-1}$, which is several times faster than the elimination of superoxide by superoxide dismutase. Peroxynitrite has a powerful oxida-

tive and nitroxidative action that engenders cell and tissue damage and promotes nitrosation of phenolic rings to produce protein nitration—the footprint of peroxynitrite. Beckmann and colleagues have reported on antinitrotyrosine antibody, which recognizes nitration of protein tyrosines.[53] That antibody is widely applied to analyses of various disease processes. ELISA has also been performed by Ceriello and colleagues on plasma from patients with type 2 diabetes. They reported significantly higher levels of nitrotyrosine in such patients compared with healthy individuals.[54] In contrast, Aydin and colleagues reported no significant difference in the plasma of patients with type 2 diabetes.[55] Rather, they found significantly elevated levels of nitrite/nitrate (NOx^-) in diabetic individuals with poor blood glucose control. They also found significantly lowered levels of plasma cyclic guanosine monophosphate (cGMP), ultimately concluding that cGMP may indicate endothelial dysfunction.

Such observations of type 2 diabetes suggest the following scenario. The relative increase in ROS is induced by endothelial injury of glucose toxicity, which promotes the relative decrease in physiologic NO, despite up-regulation of endothelial NO synthase. Therefore, although an increase is shown in the measurement of either NOx^- or peroxynitrite, cGMP will not increase sufficiently. Therefore, the physiologic signal transmission through NO becomes inadequate, thereby facilitating blood vessel malfunction.

DIABETES, OXIDATIVE STRESS, AND AGING

Increased oxidative stress in type 1 diabetes has been verified to occur through generation of MDA, the increase in Amadori compounds, the drop in thiol levels, and the decrease in erythrocyte glutathione concentration and glutathione peroxidase activity.[56–58] Tsukahara and colleagues measured pentosidine and pyrraline (by HPLC), as well as 8-OHdG and ACR lysine (by ELISA), in the urine samples of pediatric patients with type 1 diabetes (mean ± SD 12.8 ± 4.5 years of age). That study revealed significantly higher levels of pentosidine, 8-OHdG, and ACR lysine than in individuals of the control group.[22] Positive significant correlations were found between pentosidine and pyrraline, 8-OHdG, and ACR lysine, in addition to that between pyrraline and ACR lysine. These results demonstrate clearly that glycoxidation, lipoxidation, and nucleic acid oxidation are linked in young patients with type 1 diabetes. Because these parameters increase concomitantly with age, this study of young individuals without age-related vascular complication is important: it elucidates the heavy load of oxidative stress of type 1 diabetes. Although microalbuminuria is considered to be the index of glomerular endothelial dysfunction in diabetic nephropathy,[59,60] this report from Tsukahara and colleagues suggests the potentiality of oxidative stress for the onset and progression of diabetic nephropathy because the urinary levels of pentosidine, 8-OHdG, and ACR lysine

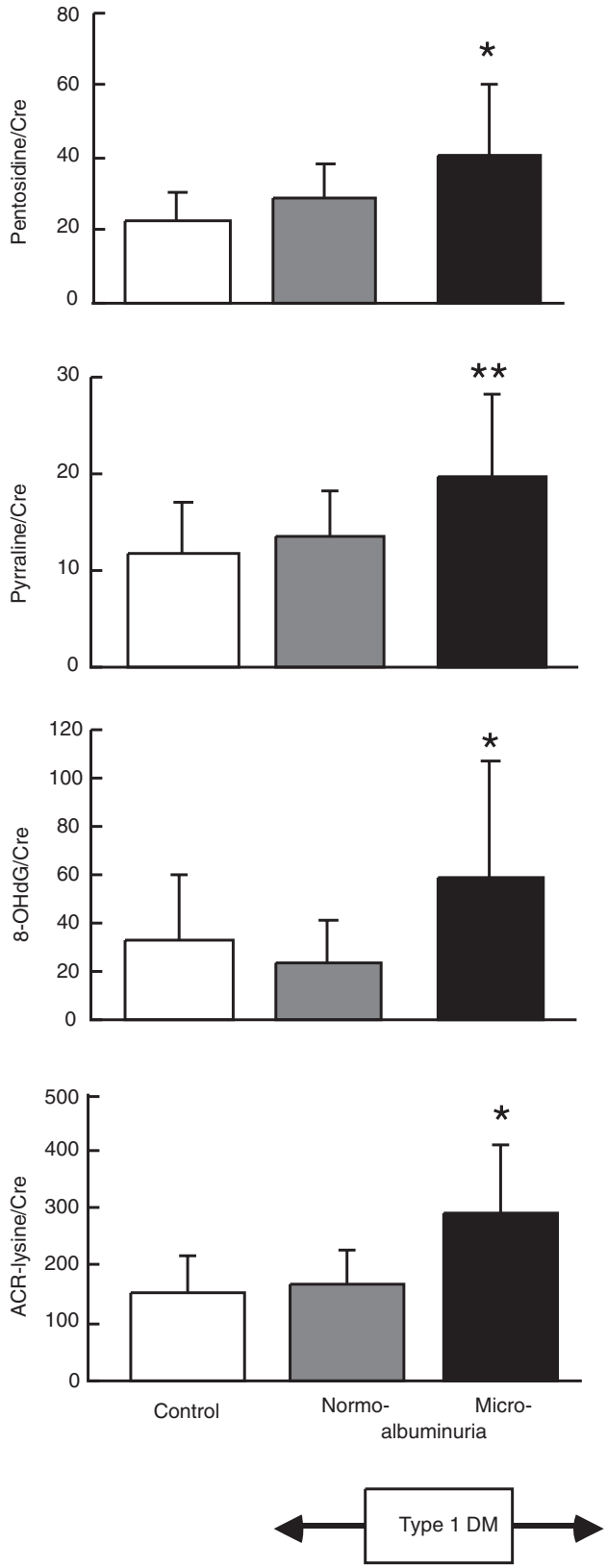

FIGURE 7 Urinary oxidative stress markers and the stage of nephropathy associated with type 1 diabetes. ACR = acrolein; Cre = urinary creatinine; DM = diabetes mellitus; 8-OHdG = 8-hydroxydeoxyguanosine. *p < .001;**p < .05. Error bar demonstrates mean ± SD.

are correlated significantly with microalbuminuria (Figure 7). Similarly, Suzuki and colleagues immunohistochemically examined renal biopsies from patients with type 2 diabetic nephropathy. They reported the deposition of oxidative markers, such as CML, pentosidine, MDA lysine, HNE lysine, and ACR lysine, in mesangial matrix and nodular lesion.[61]

FUTURE PERSPECTIVES

This review has specifically addressed ELISA using specific antibodies. In clinical medical practice, oxidative stress status markers are often taken from body fluids, such as blood, urine, spinal fluid, and synovial fluid. The discovery of a single marker for various disease processes remains an important challenge. Nonetheless, interpretation of certain pathologic conditions using ELISA with two or more markers is vastly more feasible. Even in large-scale clinical trials, a demand exists for markers that serve as decisive adjuncts with a more relevant clinical test in addition to conventional measurements. Oxidative stress status markers are promising as indicators that can contribute to the so-called enrichment of such clinical data. Recently, oxidative stress has become a focus in preventive medicine; interest in it is growing worldwide. Future development of ELISA for oxidative stress markers is expected.

Given that the parameters of oxidative stress are related directly to the severity and the prognosis of diabetes, recent achievement of ELISA applications in clinical studies to elucidate oxidative stress underscores its potential applicability for investigating oxidative stress in diabetes and, consequently, for advancing the management of this disease.

REFERENCES

1. Vlassara H, Bucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Lab Invest 1994;70:138–51.
2. Suzuki D, Miyata T. Carbonyl stress in the pathogenesis of diabetic nephropathy. Intern Med 1999;38:309–14.
3. Horie K, Miyata T, Maeda K, et al. Immunohistochemical colocalization of glycoxidation products and lipid peroxidation products in diabetic renal glomerular lesions: implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. J Clin Invest 1997;100:2995–3004.
4. Brett J, Schmidt AM, Yan SD, et al. Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissue. Am J Pathol 1993;143:1699–712.
5. Araki N, Ueno N, Chakrabarti B, et al. Immunohistochemical evidence for the presence of advanced glycation end products in human lens proteins and its positive correlation with age. J Biol Chem 1992;267:10211–4.
6. Ikeda K, Higashi T, Sano H, et al. N-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. Biochemistry 1996;35:8075–83.
7. Makita Z, Radoff S, Rayfield EJ, et al. Advanced glycation end products in patients with diabetic nephropathy. N Engl J Med 1991;325:836–42.
8. Makita Z, Vlassara H, Rayfield E, et al. Hemoglobin-AGE: a circulating marker of advanced glycosylation. Science 1992;258:651–3.
9. Kislinger T, Fu C, Huber B, et al. N-(Carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. J Biol Chem 1999;274:31740–9.
10. Degenhardt TP, Grass L, Reddy S, et al. The serum concentration of the advanced glycation end-product N-(carboxymethyl)lysine is increased in uremia. Kidney Int 1997;52:1064–7.
11. Papanastasiou P, Grass L, Rodela H, et al. Immunological quantification of advanced glycosylation end-products in the serum of patients on hemodialysis or CAPD. Kidney Int 1994;46:216–22.
12. Buss H, Chan TP, Sluis KB, et al. Protein carbonyl measurement by a sensitive ELISA method. Free Radic Biol Med 1997;23:361–6.
13. Himmelfarb J, McMonagle E. Albumin is the major plasma protein target of oxidant stress in uremia. Kidney Int 2001;60:358–63.
14. Schalkwijk CG, Chaturvedi N, Twaafhoven H, et al. Amadori-albumin correlates with microvascular complications and precedes nephropathy in type 1 diabetic patients. Eur J Clin Invest 2002;32:500–6.
15. Taneda S, Monnier VM. ELISA of pentosidine, an advanced glycation end product, in biological specimens. Clin Chem 1994;40:1766–73.
16. Izuhara Y, Miyata T, Ueda Y, et al. A sensitive and specific ELISA for plasma pentosidine. Nephrol Dial Transplant 1999;14:576–80.
17. Shimoike T, Inoguchi T, Umeda F, et al. The meaning of serum levels of advanced glycosylation end products in diabetic nephropathy. Metabolism 2000;49:1030–5.
18. Berg TJ, Bangstad HJ, Torjesen PA, et al. Advanced glycation end products in serum predict changes in the kidney morphology of patients with insulin-dependent diabetes mellitus. Metabolism 1997;46:661–5.
19. Satoh K, Yamada S, Koike Y, et al. A one-hour enzyme-linked immunosorbent assay for quantitation of acrolein- and hydroxynonenal-modified proteins by epitope-bound casein matrix method. Anal Biochem 1999;270:323–8.
20. Toyokuni S, Yamada S, Kashima M, et al. Serum 4-hydroxy-2-nonenal-modified albumin is elevated in patients with type 2 diabetes mellitus. Antiox Redox Signal 2000;2:681–5.
21. Uchida K, Kanematsu M, Sakai K, et al. Protein-bound acrolein: potential markers for oxidative stress. Proc Natl Acad Sci U S A 1998;95:4882–7.
22. Tsukahara H, Sekine K, Uchiyama M, et al. Formation of advanced glycosylation end products and oxidative stress in young patients with type 1 diabetes. Pediatr Res 2003;54:419–24.
23. Noiri E, Yamada S, Nakao A, et al. Serum protein acrolein adducts: utility in detecting oxidant stress in hemodialysis patients and reversal using a vitamin E-bonded hemodialyzer. Free Radic Biol Med 2002;33:1651–6.
24. Bucala R, Makita Z, Koschinsky T, et al. Lipid advanced glycosylation: pathway for lipid oxidation in vivo. Proc Natl Acad Sci U S A 1993;90:6434–8.
25. Kume S, Takeya M, Mori T, et al. Immunohistochemical and ultrastructural detection of advanced glycation end products

in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. Am J Pathol 1995;147:654–7.
26. Castellani RJ, Harris PL, Sayre LM, et al. Active glycation in neurofibrillary pathology of Alzheimer disease: N -(carboxymethyl)lysine and hexitol-lysine. Free Radic Biol Med 2001;31:175–80.
27. Toyokuni S. Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int 1999;49:91–102.
28. Dandona P, Thusu K, Cook S, et al. Oxidative damage to DNA in diabetes mellitus. Lancet 1996;347:444–5.
29. Leinonen J, Lehtimäki T, Toyokuni S, et al. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. FEBS Lett 1997;417:150–2.
30. Kanauchi M, Nishioka H, Hashimoto T. Oxidative DNA damage and tubulointerstitial injury in diabetic nephropathy. Nephron 2002;91:327–9.
31. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 2001;31:1287–312.
32. Masutani H, Yodoi J. Thioredoxin. Overview. Methods Enzymol 2002;347:279–86.
33. Hotta M, Tashiro F, Ikegami H, et al. Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. J Exp Med 1998;188:1445–51.
34. Kakisaka Y, Nakashima T, Sumida Y, et al. Elevation of serum thioredoxin levels in patients with type 2 diabetes. Horm Metab Res 2002;34:160–4.
35. Stocker R, Yamamoto Y, McDonagh AF, et al. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.
36. Yoshida R, Ogawa Y, Shioji I, et al. Urinary 8-oxo-7, 8-dihydro-2′-deoxyguanosine and biopyrrins levels among construction workers with asbestos exposure history. Ind Health 2001;39:186–8.
37. Tsukahara H, Shibata R, Ohshima Y, et al. Oxidative stress and altered antioxidant defenses in children with acute exacerbation of atopic dermatitis. Life Sci 2003;72:2509–16.
38. Vogt BA, Shanley TP, Croatt A, et al. Glomerular inflammation induces resistance to tubular injury in the rat. A novel form of acquired, heme-oxygenase-dependent resistance to renal injury. J Clin Invest 1996;98:2139–45.
39. Yang L, Quan S, Abraham NG. Retrovirus-mediated HO gene transfer into endothelial cells protects against oxidant-induced injury. Am J Physiol 1999;277:L127–33.
40. Bennaars-Eiden A, Higgins L, Hertzel AV, et al. Covalent modification of epithelial fatty acid-binding protein by 4-hydroxynonenal in vitro and in vivo. Evidence for a role in antioxidant biology. J Biol Chem 2002;277:50693–702.
41. Kanda T, Fujii H, Fujita M, et al. Intestinal fatty acid binding protein is available for diagnosis of interstitial ischaemia: immunochemical analysis of two patients with ischaemic intestinal diseases. Gut 1995;36:788–91.
42. van Nieuwenhoven FA, Kleine AH, Wodzig WH, et al. Discrimination between myocardial and skeletal muscle injury by assessment of the plasma ratio of myoglobin over fatty acid-binding protein. Circulation 1995;92:2848–54.
43. Kamijo A, Kimura K, Sugaya T, et al. Urinary fatty acid-binding protein as a new clinical marker of the progression of chronic renal disease. J Lab Clin Med 2004;143:23–30.
44. Kashiwagi A, Asahina T, Ikebuchi M, et al. Glycation, oxidative stress, and scavenger activity: glucose metabolism and radical scavenger dysfunction in endothelial cells. Diabetes 1996;45 Suppl 3:S84–6.
45. Lee AY, Chung SS. Contributions of polyol pathway to oxidative stress in diabetic cataract. FASEB J 1999;13:23–30.
46. Williams B, Schrier JW. Glucose-induced protein kinase C activity regulates arachidon acid release and eicosanoid production by cultured mesangial cells. J Clin Invest 1993;92:2889–96.
47. Haneda M, Araki S, Togawa M, et al. Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. Diabetes 1997;46:847–53.
48. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of advanced glycation end products in diabetic nephropathy. J Am Soc Nephrol 2003;14(8 Suppl 3):S254–8.
49. Glomb MA, Monnier VM. Mechanism of protein modification by glyoxal and glycoaldehyde, reactive intermediates of the Maillard reaction. J Biol Chem 1995;270:10017–26.
50. Brodsky SV, Gealekman O, Chen J, et al. Prevention and reversal of premature endothelial cell senescence and vasculopathy in obesity-induced diabetes by ebselen. Circ Res 2004;94:377–84.
51. Ting HH, Timimi FK, Boles KS, et al. Vitamin C improves endothelium-dependent vasodilatation in patients with non-insulin-dependent diabetes mellitus. J Clin Invest 1996;97:22–8.
52. Giugliano D, Marfella R, Verrazzo G, et al. Abnormal rheologic effects of glyceryl trinitrate in patients with non-insulin-dependent diabetes mellitus and reversal by antioxidants. Ann Intern Med 1995;123:338–43.
53. Beckmann JS, Ye YZ, Anderson PG, et al. Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. Biol Chem Hoppe Seyler 1994;375:81–8.
54. Ceriello A, Mercuri F, Quagliaro L, et al. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. Diabetologia 2001;44:834–8.
55. Aydin A, Orhan H, Sayal A, et al. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: effects of glycemic control. Clin Biochem 2001;34:65–70.
56. Dominguez C, Ruiz E, Gussinye M, et al. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care 1998;21:1736–42.
57. Elhadd TA, Kennedy G, Hill A, et al. Abnormal markers of endothelial cell activation and oxidative stress in children, adolescents and young adults with type 1 diabetes with no clinical vascular disease. Diabetes Metab Res Rev 1999;15:405–11.
58. Telci A, Cakatay U, Salman S, et al. Oxidative protein damage in early stage type 1 diabetic patients. Diabetes Res Clin Pract 2000;50:213–23.
59. Schiekofer S, Balletshofer B, Andrassy M, et al. Endothelial dysfunction in diabetes mellitus. Semin Thromb Hemost 2000;26:503–11.
60. Pomilio M, Mohn A, Verrotti A, Chiarelli F. Endothelial dysfunction in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2002;15:343–61.
61. Suzuki D, Miyata T, Saotome N, et al. Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. J Am Soc Nephrol 1999;10:822–32.