



Age-related changes in the glycation of human aortic elastin

Emiliana Konova^{a,*}, Stephan Baydanoff^b, Milena Atanasova^b, Angelika Velkova^c

^aCenter of Clinical Immunology, University School of Medicine, 1, Kl. Ohridski St., 5800 Pleven, Bulgaria

^bDepartment of Biology, University School of Medicine, Pleven, Bulgaria

^cDepartment of Social Medicine and Health Management, University School of Medicine, Pleven, Bulgaria

Received 1 September 2003; received in revised form 26 September 2003; accepted 9 October 2003

Abstract

Non-enzymatic glycation of proteins is a consequence of hyperglycemia in diabetes and correlates with aging. The aim of the study was to investigate age-related changes in the glycation of human aortic elastin in healthy subjects by two approaches: (1) assessment by fluorescence method of formed in vivo advanced glycation end products (AGEs) of elastins, purified from human aortas, obtained from different age groups; (2) in vitro glycation of elastins from different age groups and investigation of their capacity to form early (by colorimetric nitroblue tetrazolium method) and AGEs (fluorescence method). Human insoluble elastins were prepared from macro- and microscopic unaltered regions of thoracic aortas, obtained from 68 accident victims, distributed in 15 age-groups, using the method of Starcher and Galione. Soluble α -elastins were obtained by the method of Partridge et al. The direct assessment of Maillard reaction related fluorescence in the age groups showed increase of the fluorescence with age. The 'young' elastin had the highest capacity to form both fructosamine and AGEs under glycation in vitro. The glycation of 'old' elastin did not increase markedly during the incubation. These results are consistent with the interpretation that because of its long biological half-life, elastin is susceptible to the slow process of glycation and the following modifications would contribute to the age-related changes of connective tissue.

© 2003 Elsevier Inc. All rights reserved.

Keywords: Elastin; Non-enzymatic glycation; Fructosamine; Advanced glycation end products; Aging

1. Introduction

In 1912, Maillard established the non-enzymatic reaction of reducing sugars with amino groups of amino acids, leading to the formation of complex brown pigments (Maillard and Gautier, 1912). In the mid-1970s, the Maillard reaction has been assumed important in vivo and the first studies were focused on hemoglobin A1c (HbA1c)—an adduct of glucose with the β -chain of hemoglobin, elevated in diabetes. The significance of the late-stage Maillard processes as mediators of the complications of diabetes was recognized around 1980 (Bunn, 1979). It is now well known that the non-enzymatic glycation of long-lived proteins includes three types of reactions (Monnier et al., 1992): (a) early Maillard reactions—resulting in the formation of Schiff bases and their conversion to Amadori product (fructosamine); (b) intermediate Maillard reactions—formation of reactive free α -dicarbonyl glyoxal compounds (Thornalley et al.,

1999), able to cross-link proteins; (c) late Maillard reactions—formation of cross-links from glycated protein, known as advanced glycation end products, or AGEs (Bucala et al., 1992). AGE cross-links detected in vivo could be fluorescent (pentosidine (Sell et al., 1991), cross-links (Obayashi et al., 1996) and vespersylsines (Tessier et al., 1999)) and non-fluorescent (imidazolium dilysine cross-links (Odani et al., 1998), pyrrolyne cross-links, α -amino acid amide cross-links (Lederer et al., 1998), etc.). In vivo, AGEs are formed by oxidative and non-oxidative reactions, including not only glycation, but also glucoxidation with glucose auto-oxidation and the polyol pathway (Niwa, 1999).

The increased non-enzymatic glycation of proteins is a consequence of hyperglycemia in diabetes and correlates with the severity of diabetic complications (Vlassara et al., 1986; Vishwanath et al., 1986). The tissue accumulation of AGEs alters the structure and functions of long-lived proteins by two pathways: directly (cross-linking, polymerization, decreased sensitivity to enzyme degradation (Eble et al., 1983; Shnider and Kohn, 1981), trapping of plasma proteins (Brownlee et al., 1988), etc.) and

* Corresponding author. Tel.: +359-64-805-557; fax: +359-64-801-603.
E-mail address: eikonova@abv.bg (E. Konova).

indirectly via AGE-specific receptors and cell-mediated interactions (Yang et al., 1991; Horii et al., 1992; Peake et al., 1989; Kirstein et al., 1990; Vlassara et al., 1994; Skolnik et al., 1991).

In 1985, Cerami proposed a hypothesis for the biological role of glucose as a mediator of aging (Cerami, 1985). According to a later, combined theory, age-related changes are induced by the action of free radicals and Maillard reactions (Kristal and Bung, 1992). Proteins with long life spans serve as cumulators of exposure to chemical damage, resulting in formation of advanced glycation and lipoxidation end products. The two main structural proteins of the body — collagen and elastin, are primarily involved in the tissue aging by intermolecular cross-linking and side-chain modifications. Non-enzymatic glycation of human elastin is still not an object of extensive study. Studies carried out on animal models have demonstrated an age-dependent increase in AGEs of aortic elastin (Brüel and Oxlund, 1996). In humans, the possible role of increased elastin glycoxidation and lipid peroxidation for the atherogenesis has been studied in hemodialysis patients (Yamamoto et al., 2002). In our previous studies, we reported methods for *in vitro* glycation, biochemical and immunological characterization of glycated human aortic α -elastin (Baydanoff et al., 1994, 1996). To date, there have not been investigations on the glycation of human elastin with aging, in healthy subjects.

The aim of this study was to investigate age-related changes in the glycation of human aortic elastin in healthy subjects. In this connection, we used two approaches: (1) assessment of formed *in vivo* AGEs of elastins, purified from aortas, obtained from different age groups; (2) study of the capacity of different aged-elastins to form early (fructosamine) and AGEs (fluorescent AGE cross-links) under glycation *in vitro*.

2. Materials and methods

2.1. Preparation of human aortic elastins

Human insoluble elastins were prepared from macro- and microscopic unaltered regions of thoracic aortas, obtained from 68 accident victims, distributed in 15 age-groups (Table 1), using the method of Starcher and Galione, (1976). Amino acid analysis of the purified elastin (7th group) showed quantitative similarity to the proteins purified by others (Starcher and Galione, 1976) and the lack of methionine suggested a low level of contamination. Soluble α -elastin was obtained by the method of Partridge et al. (1955). Protein content of every sample was determined by measurement of the absorption of UV light at 280 nm wavelength and calculation according to a standard curve, constructed on the basis of different dilutions of lyophilized α -elastin from 7th aged-group.

Table 1
Aged-groups of the purified human aortic α -elastins

| Age group | Age (years) | No. of aortas |
|-----------|-------------|---------------|
| 1 | Under 5 | 3 |
| 2 | 6–10 | 3 |
| 3 | 11–15 | 4 |
| 4 | 16–20 | 5 |
| 5 | 21–25 | 5 |
| 6 | 26–30 | 5 |
| 7 | 31–35 | 6 |
| 8 | 36–40 | 5 |
| 9 | 41–45 | 5 |
| 10 | 46–50 | 6 |
| 11 | 51–55 | 4 |
| 12 | 56–60 | 5 |
| 13 | 61–65 | 5 |
| 14 | 66–70 | 4 |
| 15 | Above 70 | 3 |

2.2. Methods for assessment of glycation

1. Direct determination of AGEs formed *in vivo*: the fluorescence (FC) of the samples of α -elastins, purified from the 15 age-groups was measured as an index of advanced glycation (Baydanoff et al., 1994; Monnier et al., 1984). For FC estimation, 1.0 ml aliquot of the samples, diluted 1:3 with distilled water was placed in a 1 cm² quartz cuvette and Maillard reaction related fluorescence at 440 nm upon excitation of 365 nm was measured. Quinic sulfate (Fluka) 1 μ mol/l in 0.1N H₂SO₄ was used as a standard. AGE levels were expressed as arbitrary fluorescence units per mg of protein.

2. Investigation of the capacity of α -elastins from different aged-groups to form early and AGEs:

(A) *In vitro* glycation of α -elastins. α -Elastins (0.7 mg/ml) from 1st (less than 5 years), 7th (31–35 years) and 14th (66–70 years) age-groups were incubated with 100 mmol/l glucose in 0.2 M phosphate buffer, pH 7.8, containing 0.04% NaN₃, at 37 °C for 30 days (Baydanoff et al., 1994). Controls (α -elastins from the 1st, 7th and 14th age-groups), were set at the same conditions of incubations but without glucose.

(B) *Assessment of early glycation products (Amadori products, fructosamine)*. Colorimetric method with nitro-blue tetrazolium (NBT) for determination of fructosamine in glycated human α -elastin (Baydanoff et al., 1994; Johnson et al., 1982). The NBT colorimetric procedure is based upon the reducing ability of fructosamine in alkaline solution. Incubated samples (200 μ l) were added to 2 ml carbonate buffer, pH 10.8, containing 0.25 mmol/l NBT, at 37 °C and the absorbance of the mixtures measured after 5 and 10 min. The incubation times were selected after performing reaction kinetics on two samples. The absorbance differences (ΔE) of every sample were measured 3 times and the average established. A stabilized solution of glycated human serum — Precimat (Boehringer Mannheim,

Mannheim, Germany), containing 344 $\mu\text{mol/l}$ fructosamine, served as a standard. The glycation of the elastins was expressed as mmol fructosamine per g protein. The testing of the three incubated samples was performed every day from 1st to 15th after the beginning of incubation.

(C) Assessment of AGEs of the three incubated aged-samples and their controls (incubated without glucose) was performed by the described fluorescence method from day 1 to day 30 after the beginning of incubation. The fluorescence difference (sample FC – control FC) for each day was calculated.

2.3. Statistical analysis

The correlation between age and AGEs formed in vivo (fluorescence of the aged-groups) was evaluated by Pearson correlation coefficient. The results from the in vitro formation of fructosamine and AGEs in the three aged-groups were compared by the Kruskal–Wallis test because of the non-parametric distribution of the data.

3. Results

In Fig. 1, the results from the direct estimation of FC in the 15 aged groups of aortic α -elastins are shown. Samples from the first two groups (1st and 2nd) showed similar fluorescence — 7.24 and 7.53 arbitrary fluorescence units per mg of elastin. Samples from the last two aged groups (14th and 15th) showed the highest values — 23.97 and 22.82 arbitrary fluorescence units per mg of protein.

The fluorescence in the remaining groups tended to increase with age, demonstrated also by increase of fluorescence with aging calculated in percent of the 1st group. Pearson correlation coefficient was calculated to evaluate the significance of the correlation between the age and fluorescence of the probes. A highly significant correlation ($r = 0.944$, $p < 0.0001$) was established.

The capacity of three aged-groups α -elastins (1st, 7th and 14th) to form fructosamine under glycation in vitro is shown in Fig. 2. The fructosamine content has been established to be dependent on the length of incubation and reached equilibrium in a given time after the beginning of incubation. Even placed in the same conditions (elastin content and glucose concentration), the three probes showed different fructosamine formation in vitro. The highest value of fructosamine was found for group 1 — 0.48 mmol fructosamine/g elastin on day 2nd, intermediate — for group 7 (0.35, day 3rd) and lowest — for group 14 (0.22, day 3rd). After reaching the highest value, fructosamine levels in all groups tended to decrease with time of incubation, probably because of the formation of AGEs.

The statistical significance of differences between the means of fructosamine content of the three groups was calculated by Kruskal–Wallis test (Table 2) and showed significant differences in the fructosamine formation during the incubation period between the aged-groups.

The fructosamine content of the controls (samples of the same groups α -elastins incubated in the same conditions but without glucose) did not change during the incubation period and was 0.048 mmol/g (1st group), 0.09 mmol/g (7th group) and 0.023 mmol/g (14th group).

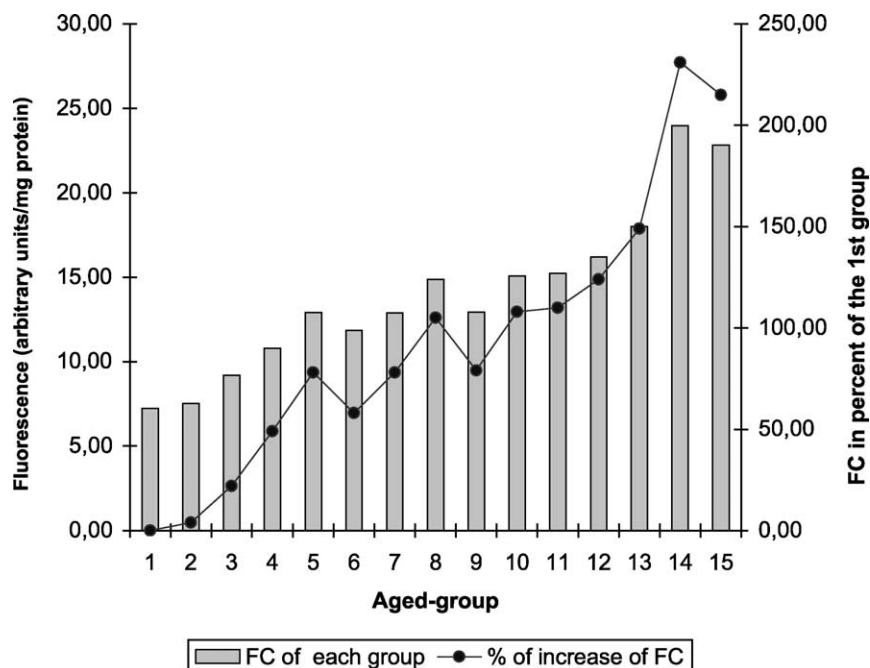


Fig. 1. Maillard reaction related fluorescence (arbitrary units/mg protein) detected in 15 aged groups of human aortic α -elastins and increase of fluorescence with aging calculated in percent of the 1st group.

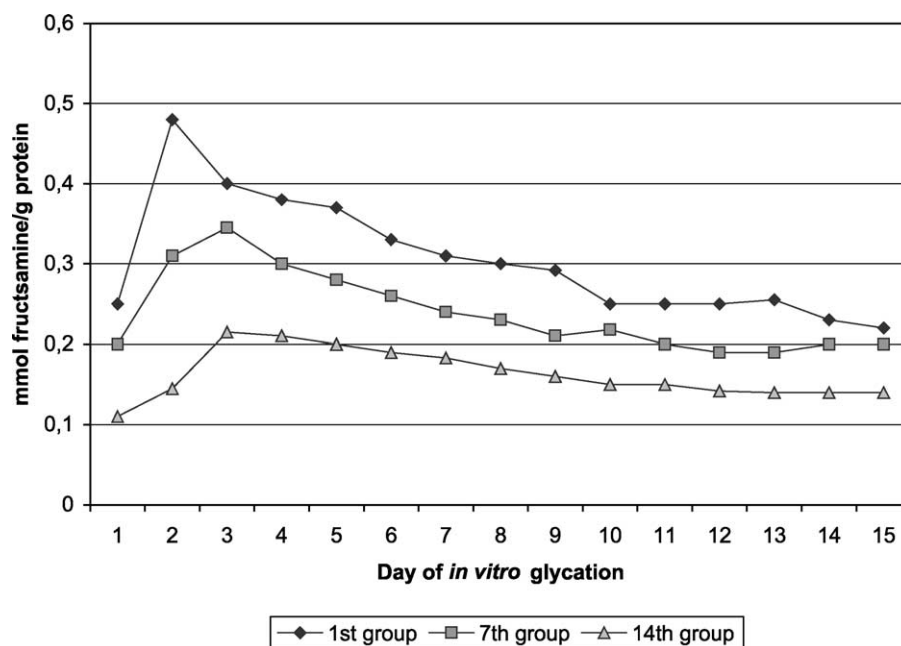


Fig. 2. In vitro glycation of α -elastins from the three aged-groups, measured by the NBT method, expressed as mmol fructosamine per mg protein.

The capacity for in vitro formation of fluorescent AGEs cross-links during a 30-day period of incubation was established by the estimated FC of the three α -elastin samples. Results are presented as the fluorescence differences (in arbitrary fluorescence units per mg of elastin) between the glycated probes and their controls for each day of the incubation (Fig. 3). The highest difference between a probe and control (16.3) was achieved for group 1 on day 22 after the beginning of incubation, followed by 8.9 for group 7 when the plateau was reached. For group 14, only 2.02 difference in arbitrary fluorescence units per mg of elastin was achieved during the period of incubation.

Kruskal–Wallis test applied for the calculated differences between the FC of probes and their controls for each day of incubation showed significant differences between the three aged-groups (Table 3).

4. Discussion

Elastin is an unusual biological substance. It is one of the most hydrophobic polypeptides, the least soluble protein in the body and extremely durable, lasting the lifetime of the organism. Elastin is an essential mechanical component of many tissues including arterial wall, skin and lung. It could be considered a polymer of linear polypeptide chains (tropoelastin) stabilized by lysine-derived cross-links, such as desmosine, isodesmosine, dehydrolysinonorleucine, or lysinonorleucine. The soluble precursor of elastin has a lysine content of about 40 residues/1000 amino acid residues. Quantitative studies of the known cross-links account for only about 30 of the residues probably including

the few lysines that remain intact in adult elastin. It has been suggested that the discrepancy indicates occurrence of another form of lysine-derived cross-links (Paz et al., 1976). The phenomenon of non-enzymatic glycation — by which glucose can directly condense with free amino groups on lysine residues or the N-terminal amino acids of proteins — is one of the main mechanisms of aging of long-lived proteins. Our previous study confirmed by three colorimetric and one fluorescence method that human aortic α -elastin is able to form early and AGEs under glycation in vitro (Baydanoff et al., 1996). Glycation in vitro has been reported also for rat aortic elastin (Tomizawa et al., 1993). We supposed that because of its very long biological half-life, elastin is susceptible to the slow process of glycation and the formation of stable, irreversible AGEs would be relevant for the aging process of connective tissue.

In this study, we investigated age-related changes in the glycation of human aortic elastins from healthy subjects. The direct assessment of Maillard reaction related fluorescence in the aged-groups showed increase with age, especially after the age of 66. Fluorescence of the last, 15th group was 215% (or 3.15 times) higher than those of the 1st group. These results suggest increase of non-enzymatic

Table 2
Kruskal–Wallis test of the results from fructosamine in vitro formation

| Aged-group | |
|------------|-------------------------------|
| 1st | $M = 0.3045$, $SEM = 0.0192$ |
| 7th | $M = 0.2382$, $SEM = 0.0012$ |
| 14th | $M = 0.1630$, $SEM = 0.0078$ |

Kruskal–Wallis test $\chi^2 = 29.18$, $df = 2$, $p < 0.001$.

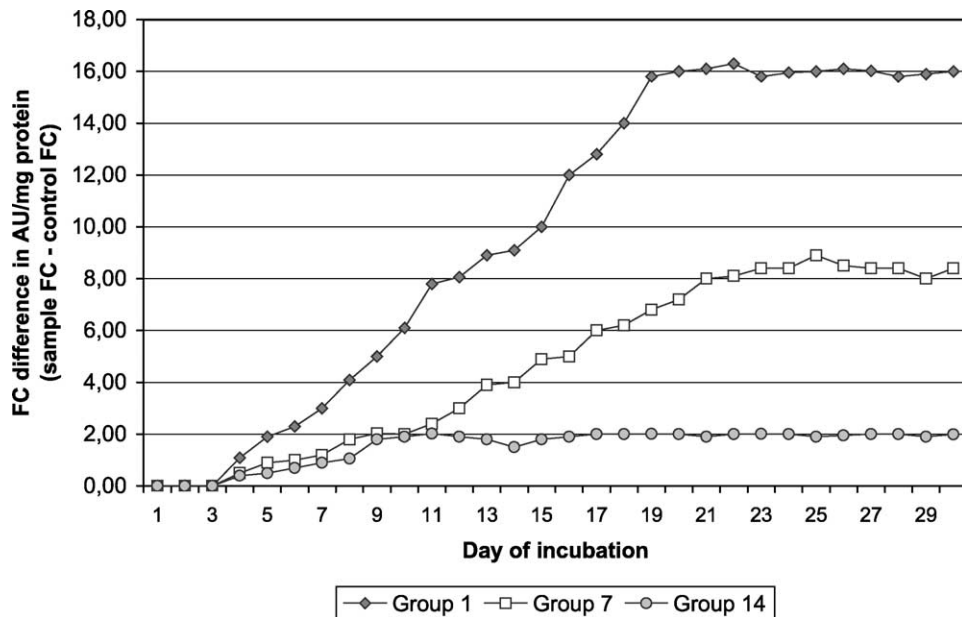


Fig. 3. Difference between fluorescence of α -elastins from the 1st, 7th, and 14th aged-groups, incubated with 100 mmol/l glucose and their controls (incubated without glucose) during the 30-day period of in vitro glycation.

glycation with aging, even in normoglycemia, and are in agreement with the demonstration of age-dependent glycation of rat aortic elastin (Brüel and Oxlund, 1996). Most probably in both — humans and animals, it is due to the life-long exposure to glucose.

To confirm age-related differences in the glycation of elastin, we investigated the capacity of three age-groups to form in vitro fructosamine and fluorescent AGEs. In both experiments, α -elastin from the 14th group showed the lowest capacity to form either fructosamine or AGEs. This is probably due to the fact that most of the free lysine residues have already been involved in AGEs cross-links. The fluorescence of the 'young' elastin (group 1), when incubated with glucose, increased with 16.3 fluorescence units, compared to the control. This value is comparable with the value we would obtain if we calculate the fluorescence difference between 14th group and 1st group — 15.58 fluorescent units (in the direct determination). Thus, the results of in vitro glycation experiments are in agreement with the results obtained from the direct AGEs determination. We can conclude then, that with aging, an increasing amount of free lysine residue becomes involved in AGEs cross-links and therefore, the 'old' elastin poorly forms new fructosamine and AGEs under in vitro glycation.

At this stage, we could not explain the fluorescence registered for the 1st and 2nd group. One possible reason could be the existence of another type of cross-link with similar excitation and fluorescence wavelength. Even if we assume the fluorescence registered in groups 1 and 2 is a 'fluorescent background', the increase of fluorescence with age is a fact. Another possible explanation could be the

hypothesis that glycation is a normal post-translational modification of proteins and occurs not only in diabetes and aging, but also in normoglycemia, at the beginning of individual life. The glycation products could be under homeostatic control and therefore, do not have any pathogenic effects. We could further speculate that AGE-specific receptors and anti-AGEs antibodies established in healthy subjects (Baydanoff et al., 1996), are perhaps homeostatic components which take part in the recognition, binding and removal of glycated structures. This way glycation could stimulate the renewal and remodeling of extra-cellular matrix. However, when it is increased (in diabetes and aging), the capacity of normal homeostasis seems to be inefficient. This would lead to accumulation of AGEs, which contributes to development of long-term diabetic complications and aging process.

In our opinion, these preliminary results confirm that non-enzymatic glycation of elastin in non-diabetic conditions is an age-dependent process. More studies are necessary to establish the role of glycation, both in the physiological turnover and aging of the elastin molecule.

Table 3

Kruskal–Wallis test analysis of the results from the formation of AGEs in vitro (applied for the differences between FC of probes and controls)

| Aged-group | |
|------------|---------------------------|
| 1st | $M = 9.93$, $SEM = 1.12$ |
| 7th | $M = 4.74$, $SEM = 0.59$ |
| 14th | $M = 1.52$, $SEM = 0.13$ |

Kruskal–Wallis test $\chi^2 = 30.62$, $df = 2$, $p < 0.001$.

References

- Baydanoff, S., Konova, E., Dosheva, I., Dorovski, P., 1994. Non-enzymatic glycation of elastin. *Glycosylation Dis.* 1, 53–58.
- Baydanoff, S., Konova, E., Ivanova, N., 1996. Determination of anti-AGE antibodies in human serum. *Glycoconj. J.* 13, 335–339.
- Brownlee, M., Cerami, A., Vlassara, H., 1988. Advanced glycosylation end-products in tissue and the biochemical basis of diabetic complications. *N. Engl. J. Med.* 318, 1315–1321.
- Brüel, A., Oxlund, H., 1996. Changes in biochemical properties, composition of collagen and elastin, and advanced glycation end products of the rat aorta in relation to age. *Atherosclerosis* 127, 155–165.
- Bucala, R., Vlassara, H., Cerami, A., 1992. In: Harding, J.J., Crabbe, M.J.C. (Eds.), *Post-Translation Modification of Proteins*, CRP Press, Inc., Boca Raton, FL, pp. 53–79.
- Bunn, H.F., 1979. Structural heterogeneity of human HbA due to nonenzymatic glycosylation. *Biol. Chem.* 254, 3892–3898.
- Cerami, A., 1985. Hypothesis—glucose as a mediator of aging. *J. Am. Geriatr. Soc.* 33, 624–634.
- Eble, A.S., Thorpe, S.R., Baynes, J.W., 1983. Nonenzymatic glycosylation and glucose-dependent cross-linking of proteins. *J. Biol. Chem.* 258, 9406–9412.
- Horii, Y., Skolnik, E., Suthanthiran, M., Vlassara, H., 1992. Novel T-cell receptors for advanced glycation end products (AGE) mediate production of IFN. *Diabetes* 41, 59A.
- Johnson, R.N., Metcalf, P.A., Baker, J.R., 1982. Fructosamine: a new approach to the estimation of serum glycosylprotein. An index of diabetic control. *Clin. Chim. Acta* 127, 87–95.
- Kristal, B., Bung, P., 1992. An emerging hypothesis: induction of aging by free radicals and Maillard reaction. *J. Gerontol.* 47, B107–B111.
- Kirstein, M., van Deventer, S., Vlassara, H., 1990. *J. Cell Biol.* 89, 14E.
- Lederer, M.O., Gerum, F., Severin, T., 1998. Cross-linking of proteins by Maillard processes—model reactions of D-glucose or methylglyoxal with butylamine and guanidine derivatives. *Bioorg. Med. Chem.* 6 (7), 993–1002.
- Maillard, L.C., Gautier, M.A., 1912. The reaction of amino acids with sugars: mechanisms of melanoid formation. *C R Seances Acad. Sci. III* (154), 66–68.
- Monnier, V.M., Kohn, R.R., Cerami, A., 1984. Accelerated age-related browning of human collagen in diabetes mellitus. *Proc. Natl Acad. Sci. USA* 81, 583–587.
- Monnier, V., Sell, D., Miyata, S., 1992. Advanced Maillard reaction products as markers for tissue damage in diabetes and uremia: relevance to diabetic nephropathy. *Acta Diabetol.* 29, 130–135.
- Niwa, T., 1999. 3-Deoxyglucosone: metabolism, analysis, biological activity, and clinical implication. *J. Chromatogr. B, Biomed. Sci. Appl.* 731 (1), 23–36.
- Obayashi, H., Nakano, K., Shigeta, H., Yamaguchi, M., Yoshimori, K., Fukui, M., Fujii, M., Kitagawa, Y., Nakamura, N., Nakamura, K., Nakazawa, Y., Ienaga, K., Ohta, M., Nishimura, M., Fukui, I., Kondo, M., 1996. Formation of crossline as a fluorescent advanced glycation end product in vitro and in vivo. *Biochem. Biophys. Res. Commun.* 226 (1), 37–41.
- Odani, H., Shinzato, T., Usami, J., Matsumoto, Y., Brinkmann Frye, E., Baynes, J.W., Maeda, K., 1998. Imidazolium crosslinks derived from reaction of lysine with glyoxal and methylglyoxal are increased in serum proteins of uremic patients: evidence for increased oxidative stress in uremia. *FEBS Lett.* 427 (3), 381–385.
- Partridge, S., Davis, H., Adair, G., 1955. The chemistry of connective tissues soluble proteins derived from partial hydrolysis of elastin. *Biochem. J.* 61, 11–15.
- Paz, M.A., Keith, D.A., Traverso, H.P., Gallop, P.M., 1976. Isolation, purification, and cross-linking profiles of elastin from lung and aorta. *Biochemistry* 15 (22), 4912–4918.
- Peake, P.W., Charlesworth, J.A., Timmermans, V., Deckert, T., 1989. Does non-enzymatic glycosylation affect complement function in diabetes? *Diabetes Res. Clin. Pract.* 11, 109–114.
- Sell, D.R., Nagaraj, R.H., Grandhee, S.K., Odetti, P., Lapolla, A., Fogarty, J., Monnier, V.M., 1991. Pentosidine: a molecular marker for the cumulative damage to proteins in diabetes, aging, and uremia. *Diabetes Metab. Rev.* 4, 239–251.
- Shnider, S.L., Kohn, R.R., 1981. Effects of age and diabetes mellitus on the solubility and nonenzymatic glycosylation of human skin collagen. *J. Clin. Invest.* 67, 1630–1635.
- Skolnik, E., Yang, Z., Makita, Z., Radoff, S., Kirstein, M., Vlassara, H., 1991. *J. Exp. Med.* 174, 931–939.
- Starcher, B., Galione, M., 1976. Purification of elastins from different animal species. *Anal. Biochem.* 74, 441–447.
- Tessier, F., Obrenovich, M., Monnier, V.M., 1999. Structure and mechanism of formation of human lens fluorophore LM-1. Relationship to vesperlysine A and the advanced Maillard reaction in aging, diabetes, and cataractogenesis. *J. Biol. Chem.* 274 (30), 20796–20804.
- Thornalley, P.J., Langborg, A., Minhas, H.S., 1999. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* 344 (Pt 1), 109–116.
- Tomizawa, H., Yamazaki, M., Kunika, K., Itakura, M., Yamashita, K., 1993. Association of elastin glycation and calcium deposit in diabetic rat aorta. *Diabetes Res. Clin. Pract.* 19 (1), 1–8.
- Vishwanath, V., Frank, K.E., Elmmets, C.A., Dauchot, P.J., Monnier, V.M., 1986. Glycation of skin collagen in type 1 diabetes mellitus. Correlation with long-term complications. *Diabetes* 35, 916–921.
- Vlassara, H., Brownlee, M., Cerami, A., 1986. Non-enzymatic glycosylation: role in the pathogenesis of diabetic complications. *Clin. Chem.* 32, B37–B41.
- Vlassara, H., Bucala, R., Liliane, S., 1994. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. *Lab. Invest.* 70, 138–151.
- Yamamoto, Y., Sakata, N., Meng, J., Sakamoto, M., Noma, A., Maeda, I., Okamoto, K., Takebayashi, S., 2002. Possible involvement of increased glycoxidation and lipid peroxidation of elastin in atherogenesis in hemodialysis patients. *Nephrol. Dial. Transplant.* 17, 630–636.
- Yang, Z., Makita, Z., Horii, Y., Brunelle, S., Cerami, A., Sehajpal, P., Suthanthiran, M., Vlassara, H., 1991. Two novel rat liver membrane proteins that bind advanced glycosylation end products: relationship to macrophage receptor for glucose-modified proteins. *J. Exp. Med.* 174, 515–524.