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ORIGINAL CONTRIBUTION

Advanced glycation end products strongly activate platelets

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Abstract
Background Diabetes mellitus is characterized by hyperglycemia that plays an important role in the pathogenesis of diabetic complications including cardiovascular diseases. Moreover, hyperglycemia induces increased generation of advanced glycation end products (AGEs). The activation of platelets is associated with the development of cardiovascular diseases.
Aim of the study The question whether AGEs acutely induce platelet activation as a response to exogenous stimulus is addressed.
Materials and methods The effect of AGEs derived from food and human serum being purified by lysozyme affinity chromatography was examined by incubating in vitro freshly isolated blood platelets from fasted subjects at various concentrations and different time points. Platelet activation, determined as expression of surface markers CD62 and CD63, and the presence of the receptor for AGEs (RAGE) in platelet membranes was measured by flow cytometric analysis using specific antibodies.
Results Incubation with food-derived as well as serum-derived AGEs stimulated significantly the expression of CD62 up to 7.1-fold and CD63 up to 2.2-fold at the platelet surface membrane as a function of concentration and time. Incubation with thrombin or AGEs significantly increased RAGE expression twofold at the platelet surface membrane.
Conclusions The increase in surface activation marker and RAGE expression in platelets, resulting from concentrations of AGEs that occur in vivo after a meal or a drink as a source of exogenous AGEs, points to signaling mechanisms for food AGEs that could favor the precipitation of acute postprandial ischemic events.

Keywords Advanced glycation end products · Atherothrombosis · Diabetes mellitus · Flow cytometry · Platelet activation · RAGE

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Introduction

Platelets represent an important linkage between inflammation, thrombosis and atherogenesis. Platelet-induced chronic inflammatory processes at the vascular wall result in development of atherosclerotic lesions and atherothrombosis [7]. Activated platelets expressing P-selectin (CD62) rapidly adhere to human endothelial cells as well as to blood leukocytes. Adherence of activated platelets to endothelial cells is a key event in the sequence of thrombus formation, and platelet recruitment by activated leukocytes plays an important role in modulating an inflammatory reaction [13]. The activation of platelets is induced by binding of different agonists (ADP, collagen and thrombin)

to specific receptors on the platelet surface. Thrombin, as the strongest agonist, in high concentrations can mobilize sufficient signal molecules to cause complete activation of all platelet responses [34]. Thrombin activates platelets via the protease-activated receptors and by interaction with GPIbα, which is described to be the high-affinity receptor for thrombin [9, 10, 23]. Postprandial hyperglycemia is an early finding in type 2 diabetes mellitus caused by the loss of the early phase insulin response [24]. Yngen et al. were able to show that the postprandial state is associated with enhanced platelet reactivity in patients with type 2 diabetes mellitus [36]. Chronic hyperglycemia is associated with accumulation of advanced glycation end products (AGEs) [33]. AGEs are formed endogenously by non-enzymatic reactions between a carbonyl group of reducing sugars and free amino groups from macromolecules, such as proteins, phospholipids and nucleic acids. In vivo AGEs, however, also derive from exogenous sources like cigarette smoking and high-temperature processed food and may be major contributors to the AGE pool [17, 21, 22, 29, 32]. AGE contents differ depending on the origin and the processing of the food. A typical Western diet may vary regarding AGE content, up to five- to eightfold, if the components are roasted instead of being steam boiled [8]. Beverages also contain AGEs in different amounts with cacao being a prominent source of AGEs [1, 8]. For instance, with a 250-ml drink of cola 16.3 kU of AGEs are delivered, the same amount of diet cola contains only 3 kU AGEs [8]. Recent studies show that a single AGE-rich meal rapidly increases serum AGEs and biomarkers of inflammation, as well as endothelial dysfunction, in patients with type 2 diabetes mellitus [20, 27, 30]. Significant increases in serum AGEs after exposure to AGE-containing beverages can occur together with altered clinical measures of endothelial function in diabetic and nondiabetic subjects [30]. Besides receptor-independent effects, AGEs induce receptor-dependent effects via the interaction with several receptors, in particular with the receptor for AGEs (RAGE) [2, 5, 31]. Binding of AGEs to RAGE enhances oxidative stress and induces a state of cell activation and dysfunction [35]. RAGE is expressed by different cells such as endothelial cells, monocytes, macrophages, neurons and smooth muscle cells [3, 26].

The aim of the present study was to investigate the impact of food AGEs as well as serum-derived AGEs on platelet activation in vitro by using activation-specific surface markers CD62 and CD63. For this purpose, the well-established method for the preparation of AGEs from different sources using affinity chromatography with lysozyme is applied. Lysozyme is an important antibacterial defense protein found in saliva, nasal secretions, mucus, serum and in the lysosomes of neutrophils and macrophages. Lysozyme binds AGEs noncovalently, with high affinity ($Kd = 50$ nM). A conserved AGE-binding cysteine-bounded domain was identified. Among the soluble AGE-binding proteins, lysozyme has the highest affinity for AGEs [14]. This specific interaction is the basis to use an AGE affinity lysozyme matrix, prepared from lysozyme conjugated to Sepharose 4B, to bind and enrich AGEs from the sera of patients or other sources. The binding of both in vitro- and in vivo-derived AGEs to the lysozyme matrix was demonstrated by Mitsuhashi et al. [18, 19].

Materials and methods

Patient population

A total of 50 subjects [43 patients with diabetes mellitus (22 T1DM and 21 T2DM)], aged 18–79 years, with or without diabetic complications, as well as 7 healthy subjects, aged 25–58 years, participated in this study after obtaining informed consent. For each assay, the given number (n) of blood samples of this study population was analyzed.

Blood sampling

Fasting blood was obtained by venipuncture from an antecubital vein of healthy subjects and diabetes mellitus patients using a 0.9-mm caliber needle, without tourniquet, into a 10-ml Monovette® Z (Sarstedt, Nümbrecht, Germany) depleted of granulate, but containing 10% of an anticoagulant solution (EDTA 134 mM, hydroxychloroquine sulfate 0.7%, heparin 20 U/ml). Platelet-rich plasma was prepared by differential centrifugation ($200 \times g$, 10 min) and enriched platelets ($700 \times g$, 5 min) were resuspended in 1 ml phosphate buffered saline (PBS), counted and diluted to 100,000 platelets/µl. For baseline measurements, one part was immediately fixed by addition of an equal volume of formaldehyde solution in PBS, pH 7.4 and 0.5% final concentration. Further parts were incubated with various AGEs or thrombin and fixed with formaldehyde in PBS for 15 min. As a control, buffer only incubated platelets were analyzed. Platelets were counted again and adjusted to 50,000 platelets/µl. As much as 200 µl of the platelet suspension was incubated for 1 h with 50 µl monoclonal antibodies against activation-dependent epitopes and in a second step for 30 min with 100 µl anti-mouse IgG-FITC (SIGMA, Saint Louis, USA) for fluorescence labeling. Finally, the stained platelets were diluted with 1 ml PBS, centrifuged ($700 \times g$, 5 min) and resuspended in FACSFlow™ (Becton Dickinson, Heidelberg, Germany). As much as 10,000 platelets per sample were analyzed with a FACScan flow cytometer (Becton

Dickinson) with an analysis rate of 1,000/s and at 488 nm excitation wavelength. All data were processed using LYSYS II software (Becton Dickinson). The proportion of specific fluorescence-positive platelets was obtained after electronic subtraction of non-specific mouse IgG binding. Fluorescence signal discrimination and intensity were calibrated daily using FluoroSpheres (DAKO, Denmark) at logarithmic photomultiplier settings. Serum was obtained from blood as described above using 9 ml Serum-Monovette® Z (Sarstedt, Nümbrecht, Germany) and centrifuged for 10 min at 2,000 × *g*.

Isolation of AGEs

Advanced glycation end product-rich extracts were isolated from Coca-Cola®, the chocolate powder Cocoa Sarotti® and from serum of patients with diabetes mellitus using a lysozyme (SIGMA ALDRICH, Taufkirchen, Germany)-linked Sepharose 4B column [18]. For the binding of serum-derived AGEs, serum was than diluted 1:5 with PBS just before loading on the lysozyme column. Coca-Cola was diluted 1:4 with PBS. Cocoa Sarotti (175 mg/ml) was diluted 1:4 with PBS after chloroform/methanol extraction and digestion with proteinase K. The lysozyme-bound AGE fractions were eluted with 0.1 M NaOH and neutralized with HCl for the following experiments. A competitive AGE-specific ELISA using anti-AGE-RNase rabbit antiserum was employed to measure AGEs in fractions eluted from the lysozyme column and original samples. The procedure has been described previously [16, 19] and AGE units were standardized by the normal human serum method [19]. The protein concentration of eluted lysozyme-bound fractions was determined by Lowry protein assay. The endotoxin content of each preparation was assessed by Limulus amoebocyte lysate assay (Pharmatox, Germany) and was below the detection limit of 1 ng/mg (0.03 U = 0.83 pg). The AGE-rich extracts were stored at −80 °C until performing the experiments. Various concentrations of AGEs (0.006–7.5 AGE U/ml) were used for the incubation of freshly isolated platelets from human subjects for 5–120 min at 37 °C in vitro.

Antibodies

Activation markers CD62 (clone 2.17, directed against P-selectin) and CD63 (clone 2.28, against a lysosomal platelet glycoprotein 53) were generously provided by H.K. Nieuwenhuis, Utrecht, Netherlands. Isotype control mouse IgG from Beckman-Coulter, Krefeld, Germany was used for non-specific control staining. Fluorescence labeling was performed in a second step with anti-mouse IgG-FITC conjugate (Sigma). The presence of RAGE in platelets' membranes was examined by flow cytometric (FACS) analysis of freshly isolated platelets. A specific antibody directed against the N-terminus of RAGE N16 (Santa Cruz Biotechnology, Heidelberg, Germany) was used.

Statistical analysis

Results of the experimental studies are reported as mean ± SEM. Differences were analyzed by Student's *t* test or one-way ANOVA, followed by Bonferroni or Dunnett's multiple comparison post-test. A P value of < 0.05 was regarded as statistically significant.

For the presentation of results, absolute values were related to those of the controls. Therefore, in all figures, control appears as 100%. Statistical analyses were performed on original values.

Results

Effects of food and serum AGEs on expression of CD62 and CD63

The stimulatory effect of Cola-derived AGEs (7.5 AGE U/ml) on the expression of P-selectin (CD62) increased over time. After an incubation period of 10 min, the expression of CD62 on platelets from ten subjects was enhanced 7.1-fold ($^{**}P < 0.01$ vs control) compared to baseline and remained rather constant until 60 min of incubation (Fig. 1a).

The proportion of CD62 and LIMP (CD63)-positive platelets was determined after incubation of human platelets with Cola-derived AGEs (15 min at 37 °C) as compared to controls. In human platelets from seven subjects, Cola-derived AGEs stimulated dose dependently (1.5–7.5 AGE U/ml) the expression of CD62 and CD63. The effect was significant at the concentration of 7.5 AGE U/ml resulting in a 6-fold (CD62) and 2.2-fold (CD63) ($^{**}P < 0.01$ vs control) stimulation compared to baseline (Fig. 1b).

Treatment of platelets from six subjects with serum AGEs resulted in a dose-dependent (6–30 mU/ml AGE) expression of CD62 and CD63 after 120 min of incubation at 37 °C. Compared to control, the expression of CD62 and CD63 was significantly increased 3.7- and 2.2-fold ($^{**}P < 0.01$ vs control), respectively, by incubation with 30 AGE mU/ml (Fig.1c). Figure 2 shows the induction of CD62 and CD63 by Cola AGE in comparison to the physiological platelet activator thrombin (2 nM) (CD62: $n = 31$; $^{***}P < 0.001$ vs control) (Fig. 2a); (CD63: $n = 19$; $^{***}P < 0.001$ vs control) (Fig. 2b). Regarding the expression of CD62, the stimulation of platelets with AGEs derived from Cocoa Sarotti showed similar results to Coca-Cola AGEs (Fig. 2c). Furthermore, regarding the stimulation with AGEs, no differences between platelets

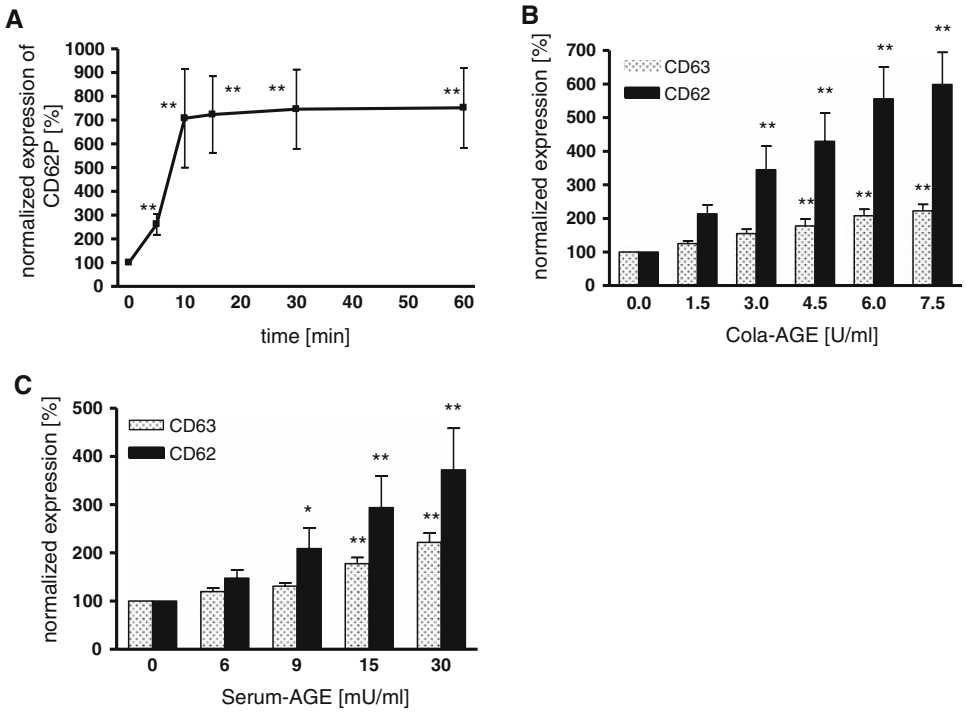

Fig. 1 Effects of AGEs on platelet P-selectin (CD62) and LIMP (CD63) expression. **a** Time-dependent impact of Cola-derived AGE (0–60 min, 7.5 AGE U/ml) on platelet expression of CD62. $^{**}P < 0.01$ vs control; mean ± SEM; $n = 10$. **b** Dose-dependent effect of Cola-derived AGE (1.5–7.5 AGE U/ml) on expression of CD62 and CD63 on fresh isolated human platelets after 15 min incubation at 37 °C. $^{**}P < 0.01$ vs control; mean ± SEM; $n = 7$. **c** Dose-dependent impact of serum AGE (6–30 AGE mU/ml) on expression of CD62 and CD63 on fresh isolated human platelets after 15 min incubation at 37 °C. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs control; mean ± SEM; $n = 6$

from patients with diabetes mellitus and healthy people were detected (data not shown).

Impact of food-derived AGEs on the expression of RAGE

Platelets were incubated with Cola-derived AGEs or thrombin and afterwards the expression of RAGE on the platelet surface was examined by flow cytometry. Cola-derived AGEs (7.5 AGE U/ml) significantly stimulated RAGE expression twofold ($^{**}P < 0.01$), similar to thrombin (1 nM), which led to a 1.5-fold increase in the expression of RAGE (Fig. 3b). The stimulatory effect of Cola-derived AGEs (7.5 AGE U/ml) on RAGE expression increased over time, with a maximum increase after 15 min of incubation ($^{**}P < 0.01$ vs control) (Fig. 3c).

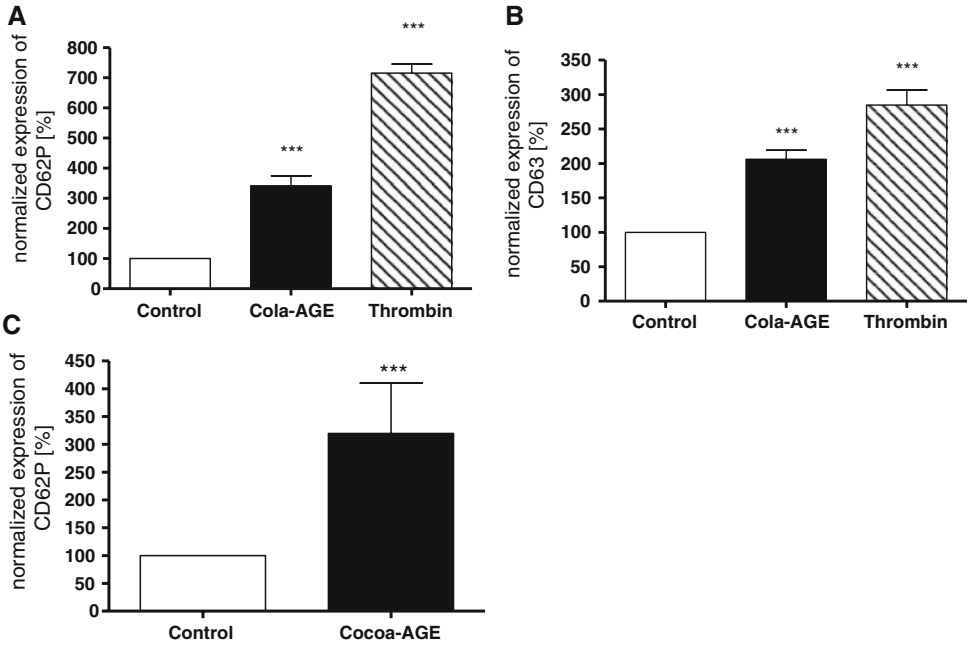

Fig. 2 Effects of AGEs and thrombin on platelet activation. **a** Expression of CD62 on platelet surface after stimulation with Cola-derived AGEs (7.5 U/ml; $n = 31$) and thrombin (2 nM; $n = 9$). $^{***}P < 0.001$ vs control; mean ± SEM. **b** Impact of Cola-derived AGE (7.5 U/ml; $n = 19$) on expression of CD63 on human platelets compared to thrombin (2 nM; $n = 9$) and control. $^{***}P < 0.001$ vs control; mean ± SEM. **c** Expression of CD62 on platelet surface after stimulation with Cocoa-derived AGEs (4 U/ml; $n = 6$). $^{***}P < 0.001$ vs control ($n = 6$); mean ± SEM

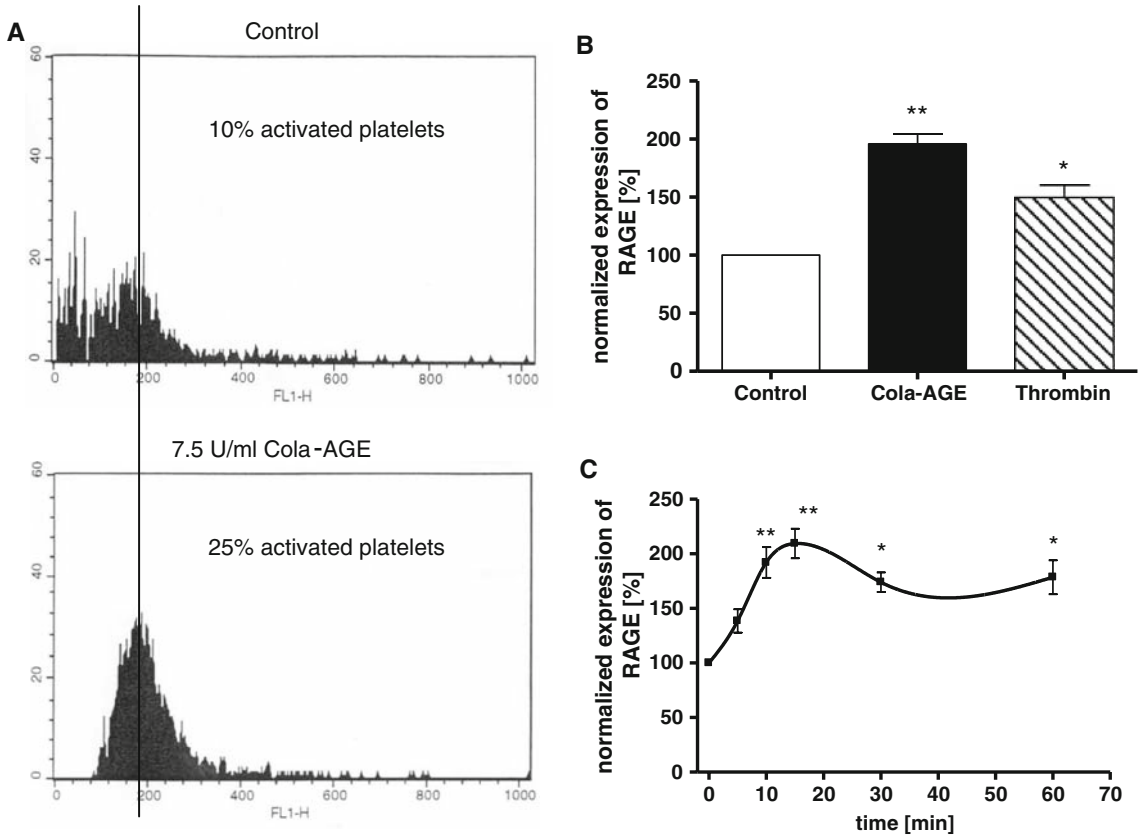

Fig. 3 Expression of RAGE in human platelets. **a** Representative flow cytometric histograms of the expression of RAGE by stimulated (7.5 U/ml Cola AGE) and non-stimulated platelets. **b** Effect of Cola-derived AGEs (15 min; 7.5 AGE U/ml) on expression of RAGE on human platelets compared to thrombin (15 min; 1 nM) and control. $*P < 0.05$ and $**P < 0.01$ vs control; mean ± SEM; $n = 8$. **c** Time-dependent impact of Cola-derived AGEs (0–60 min, 7.5 AGE U/ml) on platelet expression of RAGE. $*P < 0.05$ and $**P < 0.01$ vs control; mean ± SEM; $n = 4$

Discussion

The present observations demonstrate that both exogenous (food-derived) and endogenous (serum-derived) AGEs are strong in vitro activators of platelets. Diabetes mellitus is associated with enhanced reactivity of platelets and leukocytes [15, 28]. In addition, postprandial hyperglycemia induces enhanced platelet reactivity in patients with type 2 diabetes mellitus [36]. Hyperglycemia leads to increased formation of AGEs such as carboxymethyllysine [25]. Moreover, cigarette smoking and food, especially the standard Western-style diet, are additional sources for in vivo AGEs [8, 21]. Even a single oral AGE challenge results in a rapid increase of serum AGEs in both diabetic and healthy subjects [11]. From previous experiments, it is known that RAGE is expressed in human platelets [4].

In the present study, we have shown that AGEs derived from different food sources as well as from serum of patients with diabetes mellitus significantly increased platelet activation, determined as increased expression of P-selectin (CD62) and LIMP (CD63). AGEs stimulated platelets activation as a function of concentration and time, suggesting that increased levels of AGEs should also activate platelets in vivo. Taken together, these data support the hypothesis that elevated circulating concentrations of AGEs could be responsible for the hyperactivity of platelets in diabetes mellitus, particularly in the postprandial state.

The interaction of AGEs with specific cell receptors activates cellular signaling pathways including the p21RAS and mitogen-activated protein kinase (MAPK) pathways [6, 12]. The best-characterized AGE receptor is RAGE. Therefore, AGE-induced platelet activation may be a result of the interaction between AGEs and RAGE. The present study showed for the first time that in vitro stimulation with AGEs or thrombin induced increased expression of RAGE on the surface of platelets from healthy subjects and patients with diabetes mellitus. RAGE was detected in all preparations and there were no significant differences between the investigated samples. It was already shown that the AGE-induced tissue factor expression by cultured endothelial cells and monocytes is

mediated via the interaction of AGEs with RAGE [2]. Platelets include specific storage granules, which fuse with the plasma membrane, with the diffusion of internal granular membrane proteins during activation. This mechanism can possibly lead to the translocation of RAGE to the platelet surface. Additional studies are required to investigate the interaction of AGEs with RAGE, as well as other receptors for AGEs on the platelet surface.

In conclusion, the results of the present study indicate that AGEs of different sources induce a significant activation of platelets that may be mediated through increased RAGE expression. This platelet activation is a likely link between hyperglycemia and diet, and the subsequent development of atheroembolic complications in patients with diabetes mellitus.

Reference

1. Ahmed N, Mirshekar-Syahkal B, Kennish L, Karachalias N, Babaei-Jadidi R, Thornalley PJ (2005) Assay of advanced glycation end products in selected beverages and food by liquid chromatography with tandem mass spectrometric detection. Mol Nutr Food Res 49:691–699
2. Bierhaus A, Illmer T, Kasper M, Luther T, Quehenberger P, Tritschler H, Wahl P, Ziegler R, Muller M, Nawroth PP (1997) Advanced glycation end product (AGE)-mediated induction of tissue factor in cultured endothelial cells is dependent on RAGE. Circulation 96:2262–2271
3. Brett J, Schmidt AM, Yan SD, Zou YS, Weidman E, Pinsky D, Nowygrod R, Neeper M, Przysiecki C, Shaw A et al (1993) Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. Am J Pathol 143:1699–1712
4. Buenting CE, Koschinsky T, Schwippert B, Ruetter R, Weiss J, Roesen P, Tschoepe D (2001) Food advanced glycation end products induce activation of platelets by increasing expression of receptors for AGE. Diabetologia 44:A33
5. Cai W, He JC, Zhu L, Lu C, Vlassara H (2006) Advanced glycation end product (AGE) receptor 1 suppresses cell oxidant stress and activation signaling via EGF receptor. Proc Natl Acad Sci USA 103:13801–13806
6. Chang PC, Chen TH, Chang CJ, Hou CC, Chan P, Lee HM (2004) Advanced glycosylation end products induce inducible nitric oxide synthase (iNOS) expression via a p38 MAPK-dependent pathway. Kidney Int 65:1664–1675
7. Gawaz M, Langer H, May AE (2005) Platelets in inflammation and atherogenesis. J Clin Invest 115:3378–3384
8. Goldberg T, Cai W, Peppa M, Dardaine V, Baliga BS, Uribarri J, Vlassara H (2004) Advanced glycoxidation end products in commonly consumed foods. J Am Diet Assoc 104:1287–1291
9. Harmon JT, Jamieson GA (1986) The glycocalicin portion of platelet glycoprotein Ib expresses both high and moderate affinity receptor sites for thrombin. A soluble radioreceptor assay for the interaction of thrombin with platelets. J Biol Chem 261:13224–13229
10. Kahn ML, Nakanishi-Matsui M, Shapiro MJ, Ishihara H, Coughlin SR (1999) Protease-activated receptors 1 and 4 mediate activation of human platelets by thrombin. J Clin Invest 103:879–887
11. Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C, Heitmann K, Vlassara H (1997) Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. Proc Natl Acad Sci USA 94:6474–6479
12. Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM (1997) Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. J Biol Chem 272:17810–17814
13. Li N, Hu H, Lindqvist M, Wikstrom-Jonsson E, Goodall AH, Hjemdahl P (2000) Platelet-leukocyte crosstalk in whole blood. Arterioscler Thromb Vasc Biol 20:2702–2708
14. Li YM, Tan AX, Vlassara H (1995) Antibacterial activity of lysozyme and lactoferrin is inhibited by binding of advanced glycation-modified proteins to a conserved motif. Nat Med 1:1057–1061
15. Lupu C, Calb M, Ionescu M, Lupu F (1993) Enhanced prothrombin and intrinsic factor X activation on blood platelets from diabetic patients. Thromb Haemost 70:579–583
16. Makita Z, Vlassara H, Cerami A, Bucala R (1992) Immunochemical detection of advanced glycosylation end products in vivo. J Biol Chem 267:5133–5138
17. Mauron J (1981) The Maillard reaction in food; a critical review from the nutritional standpoint. Prog Food Nutr Sci 5:5–35
18. Mitsuhashi T, Li YM, Fishbane S, Vlassara H (1997) Depletion of reactive advanced glycation end products from diabetic uremic sera using a lysozyme-linked matrix. J Clin Invest 100:847–854
19. Mitsuhashi T, Vlassara H, Founds HW, Li YM (1997) Standardizing the immunological measurement of advanced glycation end products using normal human serum. J Immunol Methods 207:79–88
20. Negrean M, Stirban A, Stratmann B, Gawlowski T, Horstmann T, Gotting C, Kleesiek K, Mueller-Roesel M, Koschinsky T, Uribarri J, Vlassara H, Tschoepe D (2007) Effects of low- and high-advanced glycation end product meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus. Am J Clin Nutr 85:1236–1243
21. Nicholl ID, Bucala R (1998) Advanced glycation end products and cigarette smoking. Cell Mol Biol (Noisy-le-grand) 44:1025–1033
22. O'Brien J, Morrissey PA (1989) Nutritional and toxicological aspects of the Maillard browning reaction in foods. Crit Rev Food Sci Nutr 28:211–248
23. Okumura T, Hasitz M, Jamieson GA (1978) Platelet glycocalicin. Interaction with thrombin and role as thrombin receptor of the platelet surface. J Biol Chem 253:3435–3443
24. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E (1988) Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. N Engl J Med 318:1231–1239
25. Schiekofer S, Andrassy M, Chen J, Rudofsky G, Schneider J, Wendt T, Stefan N, Humpert P, Fritsche A, Stumvoll M, Schleicher E, Haring HU, Nawroth PP, Bierhaus A (2003) Acute hyperglycemia causes intracellular formation of CML and activation of ras, p42/44 MAPK, and nuclear factor kappaB in PBMCs. Diabetes 52:621–633
26. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D (1994) Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb 14:1521–1528
27. Stirban A, Negrean M, Stratmann B, Gawlowski T, Horstmann T, Gotting C, Kleesiek K, Mueller-Roesel M, Koschinsky T, Uribarri J, Vlassara H, Tschoepe D (2006) Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative

stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. Diabetes Care 29:2064–2071
28. Stratmann B, Tschoepe D (2005) Pathobiology and cell interactions of platelets in diabetes. Diab Vasc Dis Res 2:16–23
29. Uribarri J, Cai W, Sandu O, Peppa M, Goldberg T, Vlassara H (2005) Diet-derived advanced glycation end products are major contributors to the body's AGE pool and induce inflammation in healthy subjects. Ann NY Acad Sci 1043:461–466
30. Uribarri J, Stirban A, Sander D, Cai W, Negrean M, Buenting CE, Koschinsky T, Vlassara H (2007) Single oral challenge by advanced glycation end products acutely impairs endothelial function in diabetic and nondiabetic subjects. Diabetes Care 30:2579–2582
31. Vlassara H, Li YM, Imani F, Wojciechowicz D, Yang Z, Liu FT, Cerami A (1995) Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. Mol Med 1:634–646
32. Vlassara H, Palace MR (2003) Glycoxidation: the menace of diabetes and aging. Mt Sinai J Med 70:232–241
33. Wendt T, Tanji N, Guo J, Hudson BI, Bierhaus A, Ramasamy R, Arnold B, Nawroth PP, Yan SF, D'Agati V, Schmidt AM (2003) Glucose, glycation, and RAGE: implications for amplification of cellular dysfunction in diabetic nephropathy. J Am Soc Nephrol 14:1383–1395
34. Willoughby S, Holmes A, Loscalzo J (2002) Platelets and cardiovascular disease. Eur J Cardiovasc Nurs 1:273–288
35. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D (1994) Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 269:9889–9897
36. Yngen M, Ostenson CG, Hjemdahl P, Wallen NH (2006) Meal-induced platelet activation in type 2 diabetes mellitus: effects of treatment with repaglinide and glibenclamide. Diabet Med 23: 134–140