Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 55 (2006) 912–917

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Positive association of serum levels of advanced glycation end products with thrombogenic markers in humans

Mika Enomoto[a,b], Hisashi Adachi[a,b,*], Sho-ichi Yamagishi[a,b], Masayoshi Takeuchi[c], Kumiko Furuki[a,b], Asuka Hino[a,b], Akiko Hiratsuka[a,b], Yoshinori Takajo[a,b], Tsutomu Imaizumi[a,b]

[a]*The Third Department of Internal Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan*
[b]*Cardiovascular Research Institute, Kurume University School of Medicine, Kurume 830-0011, Japan*
[c]*Department of Biochemistry, Faculty of Pharmaceutical Science, Hokuriku University, Kanazawa 920-1181, Japan*

Received 29 October 2005; accepted 27 February 2006

Abstract

Advanced glycation end products (AGEs) are elevated in diabetes. We have demonstrated that AGEs trigger thrombogenic responses in cultured cells. We investigated here whether serum AGE levels were positively correlated with thrombogenic markers in humans. Data for fasting serum AGE levels of 186 nondiabetic subjects were obtained from a general population in Japan. We measured body mass index, blood pressure, total cholesterol, low-density lipoprotein and high-density lipoprotein cholesterol, triglycerides, fasting plasma glucose, glycosylated hemoglobin A_{1c}, insulin, creatinine, uric acid, high-sensitive C-reactive protein, plasminogen activator inhibitor 1 (PAI-1), and fibrinogen. Uni- and multivariate analyses were applied for the determinants of serum AGE levels. The average AGE levels were 4.11 ± 0.74 U/mL in males and 4.10 ± 0.93 U/mL in females. In the univariate analysis, PAI-1 ($P < .05$) and fibrinogen ($P < .05$) were significantly associated with AGE levels. After performing multivariate analyses, PAI-1 ($P < .05$) and fibrinogen ($P < .05$) still remained significant independently. In conclusion, the present study is the first demonstration that PAI-1 and fibrinogen levels were positively associated with serum AGE levels. Advanced glycation end products may be associated with thrombogenesis in humans.

1. Introduction

Several types of thrombogenic abnormalities such as attenuated fibrinolytic activity due to the elevation of plasminogen activator inhibitor 1 (PAI-1), hypercoagulability, and platelet hyperaggregation are demonstrated in patients with diabetes [1], thus contributing to the development and progression of diabetic vascular complications [2]. Although various hyperglycemia-induced metabolic and hemodynamic derangements are considered to participate in the thrombogenic tendency, it is plausible that advanced glycation end products (AGEs), senescent macroproteins, could play a central role in the thrombogenic abnormalities in patients with diabetes. Glucose can react nonenzymatically with the amino groups of proteins to form reversible Schiff bases and, then, Amadori products. These early glycation products undergo further complex reactions and rearrangements to become irreversibly cross-linked fluorescent protein derivatives termed AGEs. Advanced glycation end products were originally characterized by a yellow-brown fluorescent color and an ability to form cross-links with and between amino groups, but the terminology of AGEs is now used for a broad range of advanced products of the glycation process (the so-called Maillard reaction), including *N*-carboxymethyllysine and pyrraline, which show neither color nor fluorescence and do not cross-link with proteins [3-7]. The formation and accumulation of AGEs in various tissues as well as in serum have been known to progress at an extremely accelerated rate in diabetes [3-7]. AGEs are notorious to cause tissue damages via oxidative stress; there is a growing body of evidence that AGEs are implicated in the pathogenesis of diabetic retinopathy, nephropathy, and accelerated atherosclerosis [8,9]. We have previously demonstrated in cultured endothelial cells that AGEs not only inhibit prostacyclin

* Corresponding author. The Third Department of Internal Medicine, Kurume University School of Medicine, Kurume 830-0011, Japan. Tel.: +81 942 35 3311x3746; fax: +81 942 33 6509.
E-mail address: hadac@med.kurume-u.ac.jp (H. Adachi).

0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.02.019

production, but also induce functional PAI-synthesis through a decrease in intracellular cyclic AMP levels [10,11]. However, it remains to be elucidated whether AGEs play a role in thrombogenicity in humans.

Evolving evidence of the central role for fibrinogen and PAI-1 in mediating thrombosis supports the theory that these markers are one of the important risk factors for coronary artery disease, not only in patients with diabetes [12-15], but also in those without diabetes [16,17]. Therefore, in this study, we investigated whether serum AGE levels were associated with PAI-1 and fibrinogen in humans.

2. Methods

2.1. Subjects

In 2004, in a fishing community in southwestern Japan (Uku town), a total of 204 people received a health examination. This town is an isolated island near Fukue city, located in Nagasaki prefecture, and the total population is about 3700. This community is a typical island of fishermen. We evaluated their diet composition by use of a self-administered food frequency questionnaire [18]. They are consuming the diet that approximately consists of 292.9 g/d of carbohydrate, 58.1 g/d of fat, and 77.6 g/d of protein with salt intake of 13.0 g/d, and total energy intake of 8631.6 kJ/d (2063 kcal/d). The fish and shellfish they consume are the main sources of protein. We have been performing an epidemiologic study every year for 4 years. The subjects' demographic backgrounds this year were almost the same as those from the previous years. Of these, 8 subjects rejected a blood test. We excluded 10 subjects receiving medications for diabetes mellitus and/or with glycosylated hemoglobin A_{1c} (HbA_{1c}) levels of more than 6.5%. Finally, complete data set for 186 nondiabetic subjects (64 males and 122 females) was available in this study.

2.2. Data collection

The medical history, smoking, and alcohol intake were ascertained by a questionnaire. We carefully interviewed them. They did not have apparent autoimmune or inflammatory disorders. Smoking and alcohol intake were classified as either current habitual use or not. Height and weight were measured, and body mass index (weight in kilograms divided by the square of height in meters) was calculated as an index of obesity. Blood pressure (BP) was measured twice with the subjects in the sitting and supine position. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP measurement. The supine BP with the fifth-phase diastolic pressure was used for analysis.

Blood was drawn from the antecubital vein in the morning after 12-hour fast for determinations of lipids (total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], and triglycerides), fasting plasma glucose (FPG), HbA_{1c}, insulin, serum urea nitrogen, creatinine, uric acid, high-sensitive C-reactive

Table 1
Clinical characteristics of subjects

Characteristics	Male (n = 64)	Female (n = 122)	Total (N = 186)
Age (y)	66.7 ± 9.1	65.0 ± 9.5	65.6 ± 9.4
AGEs (U/mL)	4.13 ± 0.75	4.11 ± 0.91	4.12 ± 0.85
Body mass index (kg/m^2)	23.5 ± 3.7	23.5 ± 3.3	23.4 ± 3.4
Systolic BP (mm Hg)	131.9 ± 15.4	139.4 ± 20.0	136.8 ± 18.8
Diastolic BP (mm Hg)	82.6 ± 11.5	81.4 ± 12.6	81.8 ± 12.2
Total cholesterol (mg/dL)	191.4 ± 34.3	207.6 ± 34.1	202.0 ± 34.9
HDL-C (mg/dL)	58.6 ± 15.9	60.4 ± 13.9	59.8 ± 14.6
LDL-C (mg/dL)	106.7 ± 27.7	123.7 ± 31.4	117.9 ± 31.2
Triglycerides (mg/dL)[a]	92.6 ± 5.6	86.3 ± 5.2	88.4 ± 5.3
FPG (mg/dL)	97.5 ± 15.6	91.5 ± 8.4	93.6 ± 11.7
HbA_{1c} (%)	5.2 ± 0.4	5.2 ± 0.3	5.2 ± 0.3
Insulin (μU/mL)[a]	4.3 ± 0.3	4.1 ± 0.2	4.1 ± 1.1
Serum urea nitrogen (mg/dL)	18.9 ± 5.0	17.2 ± 4.2	17.8 ± 4.5
Creatinine (mg/dL)	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Uric acid (mg/dL)	5.7 ± 1.4	4.6 ± 1.1	5.0 ± 1.3
hs-CRP (mg/dL)[a]	0.047 ± 0.003	0.033 ± 0.002	0.037 ± 0.002
Fibrinogen (mg/dL)	291.6 ± 61.8	300.8 ± 58.1	297.2 ± 59.4
PAI-1 (ng/mL)[a]	23.6 ± 1.4	18.1 ± 1.1	19.8 ± 1.2
Smoking (% yes)	23.4	0.8	8.6
Alcohol intake (% yes)	73.0	14.8	34.6
Medication			
Hypertension (% yes)	28.1	30.3	29.6
Hyperlipidemia (% yes)	7.8	9.0	8.6

Data are expressed as mean ± SD or %, unless otherwise indicated.

[a] Mean values, and upper and lower 95% confidence limits were exponentiated and presented as geometric means (SD), where the SD was approximated as the difference of the exponentiated confidence interval divided by 3.92.

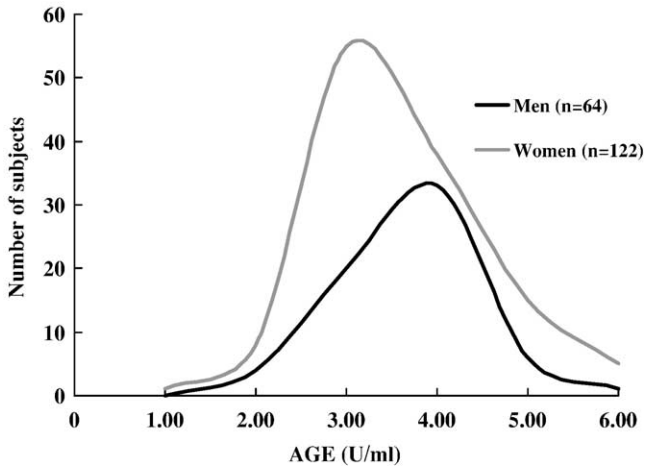

Fig. 1. Distributions of serum AGE levels in men and women.

protein (hs-CRP), and fibrinogen. PAI-1 was measured in citrated plasma, using enzyme-linked immunosorbent assay (ELISA) [17] that is sensitive to free PAI-1, but not to PAI-1 complexed with tissue plasminogen activator (t-PA). The citrated sample was centrifuged for a minimum of 10 minutes to make sure that there was no contamination from platelet PAI-1. AGE levels were measured with an ELISA as described previously [19]. In this study, 1 U corresponds to 2 μg of glyceraldehyde-derived AGE–bovine serum albumin standard as described previously [19]. Other chemistries, such as serum total cholesterol, HDL-C (enzymatic assay method), and creatinine (enzymatic assay method) were measured at a commercially available laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan).

The mayor and the welfare section of Uku town approved this study. The ethical committee of Kurume University also approved this study. All participants gave informed consent.

2.3. Statistical methods

Because of skewed distributions, the natural logarithmic transformations were performed for triglycerides, insulin, hs-CRP, and PAI-1. Mean values, and upper and lower 95% confidence limits were exponentiated and presented as geometric mean ± SD, where the SD was approximated as the difference of the exponentiated confidence interval (CI) divided by 3.92, which is the number of SD in a 95% CI where data are normally distributed. Results are presented as mean ± SD. The medications for hypertension and hyperlipidemia were coded as dummy variables. Univariate analysis was performed for determinants of serum AGE levels. Multiple linear regression analysis was performed to determine independent determinants of AGE levels. Mean plasma PAI-1 and fibrinogen levels by tertiles of the increasing AGE levels were compared using analysis of covariance, adjusted for age and sex as covariates. Statistical significance was defined as $P < .05$. All statistical analyses were performed with the use of the SAS system (SAS Institute, Cary, NC).

3. Results

Backgrounds of the subjects are presented in Table 1. AGE levels did not differ between men and women. Fig. 1 shows distributions of AGE levels in men and women, which show normal distributions in both sexes. Table 2 shows results of a univariate analysis for determinants of serum AGE levels. Parameters statistically and significantly related to AGE levels were fibrinogen ($P < .05$) and PAI-1 ($P < .05$). Because these significant parameters were closely correlated with each other, multiple linear regression analysis was performed. Finally, fibrinogen ($P < .05$) and PAI-1 levels ($P < .05$) remained significant (Table 3) and were independently related to serum AGE levels ($R^2 = 0.052$). The use of alcohol and smoking status did not affect the relationships between AGEs and PAI-1 or fibrinogen in either sex by multivariate analysis. Even after including 10 subjects with diabetes, fibrinogen ($P < .05$) and PAI-1 levels ($P < .05$) still remained significant. A statistical significance and a dose-response relationship were demon-

Table 2
Univariate analyses for determinants of serum AGE levels

Characteristics	β	SE	P
Age	.095	0.007	.221
Sex	−.007	0.135	.878
Body mass index	.029	0.019	.700
Systolic BP	.086	0.003	.219
Diastolic BP	.120	0.005	.116
Total cholesterol	.097	0.002	.191
HDL-C	−.086	0.004	.236
LDL-C	.138	0.002	.076
Triglycerides[a]	.037	0.143	.618
FPG	.129	0.005	.097
HbA_{1c}	.086	0.197	.247
Insulin[a]	.127	0.082	.092
Serum urea nitrogen	.140	0.014	.068
Creatinine	.056	0.432	.516
Uric acid	.093	0.050	.230
hs-CRP	.063	0.057	.488
Fibrinogen	.142	0.001	.042
PAI-1[a]	.154	0.104	.039
Smoking	−.037	0.234	.835
Alcohol intake	.074	0.135	.283
Medication			
Hypertension	.058	0.140	.373
Hyperlipidemia	.200	0.223	.065

[a] The natural logarithmic transformation was performed for triglycerides, insulin, hs-CRP, and PAI-1 concentrations.

Table 3
Multiple regression analysis for determinants of serum AGE levels

Characteristics	β	SE	P
PAI-1[a]	.180	0.104	.014
Fibrinogen	.169	0.001	.015
$R^2 = 0.052$			

[a] The natural logarithmic transformation was performed for PAI-1 concentration.

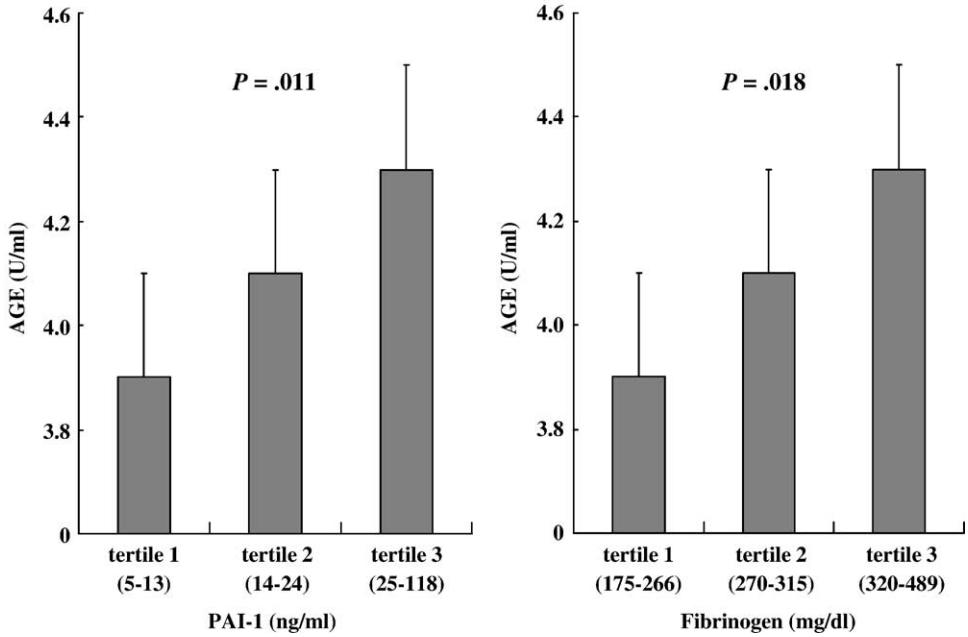

Fig. 2. Age- and sex-adjusted mean serum AGE levels stratified by plasma PAI-1 and fibrinogen tertiles.

strated for PAI-1 (P = .011) and fibrinogen (P = .018) levels in Fig. 2, respectively.

4. Discussion

In the present study, we demonstrated for the first time the link between thrombogenic abnormalities and serum AGE levels in humans; serum AGE levels in nondiabetic subjects were positively associated with PAI-1 and fibrinogen, important risk factors for acute coronary events [20-25]. These observations suggest that subclinical thrombogenic abnormalities might be mediated, at least in part, by serum AGEs, even in a nondiabetic general population without apparent cardiovascular disease.

4.1. Subject selection

We limited our analysis to nondiabetic subjects for the following reasons. First, several hyperglycemia-related metabolic derangements were reported to elicit various thrombogenic abnormalities in diabetes, which could confound the relationship between the thrombogenic markers and serum AGE levels. Second, among 204 subjects enrolled in this study, only 10 patients had diabetes. Third, it is well known that diabetic subjects have elevations of both AGEs and thrombogenic markers compared with healthy subjects. Thus, there will be a possibility of a positive association between AGEs and thrombogenic markers if they are analyzed all together. Finally, serum AGE levels are reported to be somewhat elevated and associated with coronary artery disease in nondiabetic subjects [26]. These rationales let us to study the association between serum levels of AGEs and thrombogenic markers in nondiabetic subjects.

4.2. Serum levels of AGEs

There have been several studies reporting serum AGE levels in the nondiabetic general population [15,26-28]. Kilhovd et al [15] reported in whites that serum levels of AGEs were significantly increased in patient with diabetes compared with nondiabetic subjects (7.4 [4.4-10.9] vs 4.2 [1.6-6.4] U/mL, $P < .0001$). They also reported in their recent article that serum levels of AGEs predicted coronary heart disease mortality in nondiabetic women [27]. Tan et al [28] reported that serum AGE levels were increased in diabetic patients compared with healthy control subjects (4.6 ± 0.7 vs 3.1 ± 0.8 U/mL, $P < .01$) and its high levels were associated with endothelial dysfunction. In Japanese population, Kanauchi et al [26] reported that serum levels of AGEs were higher in nondiabetic subjects with coronary artery disease than in control subjects (2.42 ± 0.65 vs 1.96 ± 0.40 mU/mL, $P < .001$). In our study, the mean levels of AGEs were 4.13 U/mL in men and 4.11U/mL in women. Therefore, the levels of AGEs in our subjects were almost same as the levels in control subjects reported by Kilhovd [15] or Tan et al [28]. It is surprising that the AGEs were so low in the order of microunits per milliliter in the study reported by Kanauchi et al [26]. We have one possible explanation for these low levels. In their article, 4 μg of AGE–bovine serum albumin standard was arbitrarily defined as 1 mU of AGEs, whereas in our article, 1 U corresponded to 2 μg of AGE–bovine serum albumin standard. Thus, the AGE levels of control subjects in their report corresponded to 3.92 U/mL in our study and were not necessarily low compared with our study and those of others. Several researchers prepare AGE-modified proteins under different conditions and use them as a standard in their ELISA assay. This is one of the reasons why the value

of serum AGE levels varies among the reports. It is proposed to establish the gold standard method for measuring serum AGE levels in humans.

As reported by the above-mentioned investigators [15,26], we did not find any association between AGE levels and blood glucose control (FPG, HbA_{1c}, and insulin levels) or age as shown in Table 1. It is well known that renal function affects serum AGEs [29]. Our study subjects all had normal renal function. Taken together, our study was the first one reporting the association of serum AGE levels with subclinical thrombogenic abnormalities in a large number of the population without apparent cardiovascular disease and without diabetes.

We found a positive relationship between AGEs and PAI-1 or fibrinogen. However, from the multiple correlation coefficients, these 2 factors explained only 5.2% of the variation of AGEs. In addition to PAI-1 and fibrinogen, a number of factors may potentially confound our results, although from our study, we cannot determine factors contributing to the variability in serum AGE levels. The following 2 possibilities are considered. One may be an environmental factor and the other a genetic one. Diet is a major environmental source of AGEs. It has been reported that two thirds of orally absorbed dietary AGEs (about 10% of the amount ingested) are retained in tissues in bioreactive forms [30,31]. Genetic and diabetes-independent factors are recently recognized to influence HbA_{1c} and AGE levels [32-34]. Therefore, it is plausible that genetic factors could influence serum AGE levels as well. Further studies are needed to elucidate the factors other than PAI-1 and fibrinogen determining variability in serum AGE levels in nondiabetic or even in diabetic subjects.

4.3. Relationship between AGEs and thrombogenetic markers

Our present study has extended the in vitro findings, showing that AGEs may be one of the causal factors for PAI-1 expression [10]. Although we cannot clarify the molecular mechanism underlying the link between AGEs and PAI-1, endothelial release of PAI-1 upon stimulation of RAGE with AGEs [10] could account for the elevation of this antifibrinolytic factor in humans.

In this study, we measured PAI-1 levels in an ELISA that is sensitive to free PAI-1, but not to PAI-1 complexed with t-PA. Therefore, although PAI-1 activity was not measured here, our present observations may suggest that serum AGE levels could increase free PAI-1 levels, thus suggesting the association between impaired fibrinolytic activity and subclinical thrombogenic abnormalities in nondiabetic subjects.

In the present study, we also demonstrated for the first time that fibrinogen levels were positively correlated with serum AGEs in nondiabetic subjects. Fibrinogen is an acute-phase reactant to inflammation, produced by the liver. Therefore, elevated AGEs may imply subclinical inflammation. Supporting this notion, Tan et al [35] recently reported that serum concentration of AGEs was an independent determinant of plasma CRP, another acute-phase protein, in type 2 diabetic patients [35]. However, in this study, we did not find any association between serum AGE levels and hs-CRP in nondiabetic patients.

5. Limitation

First, although the number was small, we found the same relationship between AGEs and thrombogenic markers when diabetic subjects (n = 10) with elevated AGEs and PAI-1 were included. However, our study was a cross-sectional one and, therefore, still does not elucidate the causal relationship between AGEs and elevations of thrombogenic markers. Second, our study was limited to nondiabetic subjects without apparent cardiovascular disease. It is interesting to study a large number of subjects including nondiabetic as well as diabetic subjects with or without cardiovascular disease.

Acknowledgment

This study was supported in part by the Kimura Memorial Heart Foundation, Fukuoka; by Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology; and by Grants of Collaboration with Venture Companies Project from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

We express our gratitude to the elected officials and residents of the Uku town, and the team of physicians in the Third Department of Internal Medicine, Kurume University School of Medicine, for their help in carrying out the health examinations.

References

[1] Osende JI, Badimon JJ, Fuster V, Herson P, Rabito P, Vidhun R, et al. Blood thrombogenicity in type 2 diabetes mellitus patients is associated with glycemic control. J Am Coll Cardiol 2001;38:1307-12.

[2] Rauch U, Crandall JI, Osende JI, Fallon JT, Chesebro JH, Fuster V, et al. Increased thrombus formation relates to ambient blood glucose and leukocyte count in diabetes mellitus type 2. Am J Cardiol 2000; 86:246-9.

[3] Vlassara H, Bucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Lab Invest 1994;70:138-50.

[4] Brownlee M. Advanced protein glycosylation in diabetes and aging. Ann Rev Med 1995;46:223-34.

[5] Dyer DG, Blackledge JA, Thorpe SR, Baynes JW. Formation of pentosidine during nonenzymatic browning of proteins by glucose. J Biol Chem 1991;18:11654-60.

[6] Grandhee S, Monnier VM. Mechanism of formation of the Maillard protein cross-link pentosidine. J Biol Chem 1991;18:11649-53.

[7] Takeuchi M, Kikuchi S, Sasaki T, Suzuki N, Watai T, Iwaki M, et al. Involvement of advanced glycation end-products (AGEs) in Alzheimer's disease. Curr Alzheimer Res 2004;1:39-46.

[8] Schmidt AM, Stern D. Atherosclerosis and diabetes: the RAGE connection. Curr Atheroscler Res 2000;5:430-6.

[9] Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM. 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 1997;272:17810-4.
[10] Yamagishi S, Fujimori H, Yonekura H, Yamamoto Y, Yamamoto H. Advanced glycation end products inhibit prostacyclin production and induce plasminogen activator inhibitor-1 in human microvascular endothelial cells. Diabetologia 1998;41:1435-41.
[11] Yamagishi S, Yamamoto Y, Harada S, Hsu CC, Yamamoto H. Advanced glycosylation end products stimulate the growth but inhibit the prostacyclin-producing ability of endothelial cells through interactions with their receptors. FEBS Lett 1996;384:103-6.
[12] Kislinger T, Tanji N, Wendt T, Qu W, Lu Y, Ferran LJ, et al. Receptor for advanced glycation, end products mediates inflammation and enhanced expression of tissue factor in vasculature of diabetic apolipoprotein E–null mice. Arterioscler Thromb Vasc Biol 2001; 21:905-10.
[13] Shen GX. Impact and mechanism for oxidized and glycated lipoproteins on generation of fibrinolytic regulators from vascular endothelial cells. Mol Cell Biochem 2003;246:69-74.
[14] Yamagishi S, Takeuchi M, Inagaki Y, Nakamura K, Imaizumi T. Role of advanced glycation end products (AGEs) and their receptor (RAGE) in the pathogenesis of diabetic microangiopathy. Int J Clin Pharm Res 2003;23:129-34.
[15] Kilhovd BK, Berg TJ, Birkeland KI, Thorsby P, Hanssen KF. Serum levels of advanced glycation end products are increased in patients with type 2 diabetes and coronary heart disease. Diabetes Care 1999;22: 1543-8.
[16] Montalescot G, Collet JP, Choussat R, Thomas D. Fibrinogen as a risk factor for coronary heart disease. Eur Heart J 1998;19(Suppl H):H11-7.
[17] Declerck PJ, Alessi MC, Verstreken M, Kruithof EKO, Juhan-Vague I, Collen D. Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. Blood 1988;71:220-5.
[18] Sasaki S, Shimoda T, Katagiri A, Tsuji T, Amano K. Eating frequency of rice vs. bread at breakfast and nutrient and food-group intake among Japanese female college students. J Community Nutr 2002;4:83-9.
[19] Takeuchi M, Makita Z, Bucala R, Suzuki T, Koike T, Kameda Y. Immunological evidence that non-carboxymethyllysine advanced glycation end-products are produced from short chain sugars and dicarbonyl compounds in vivo. Mol Med 2000;6:114-25.
[20] Saigo M, Hsue PY, Waters DD. Role of thrombotic and fibrinolytic factors in acute coronary syndromes. Prog Cardiovasc Dis 2004;46:524-38.
[21] Ottani F, Galvani M. Prognotic role of hemostatic markers in acute coronary syndromes patients. Clin Chim Acta 2001;311:33-9.
[22] Sato S, Nakamura M, Iida M, Naito Y, Kitamura A, Okamura T, et al. Plasma fibrinogen and coronary heart disease in urban Japanese. Am J Epidemiol 2000;152:420-3.
[23] Smith FB, Lee AJ, Fowkes FGR, Prince JF, Rumley A, Lowe GDO. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. Arterioscler Thromb Vasc Biol 1997; 17:3321-5.
[24] Sakata T, Mannami T, Baba S, Kokubo Y, Kario K, Okamoto A, et al. Potential of free-form TFPI and PAI-1 to be useful markers of early atherosclerosis in a Japanese general population (the Suita Study): association with the intimal-medial thickness of carotid arteries. Atherosclerosis 2004;176:355-60.
[25] Sowers JR, Lester MA. Diabetes and cardiovascular disease. Diabetes Care 1999;22:C14-C20.
[26] Kanauchi M, Hashimoto T, Tsujimoto N. Advanced glycation end products in nondiabetic patients with coronary artery disease. Diabetes Care 2001;24:1620-3.
[27] Kilhovd BK, Juutilainen A, Lehto S, Ronnemaa T, Torjesen PA, Birkeland KI, et al. High serum levels of advanced glycation end products predict increased coronary heart disease mortality in nondiabetic women but not in nondiabetic men. A population-based 18-year follow-up study. Arterioscler Thromb Vasc Biol 2005;25: 815-20.
[28] Tan KCB, Chow WS, Ai VHG, Metz C, Bucala R, Lam KSL. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care 2002;25:1055-9.
[29] Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, et al. Advanced glycation end products in patients with diabetic nephropathy. N Engl J Med 1991;325:836-42.
[30] Koschinsky T, He CJ, Mitsuhashi T, Bucala R, Liu C, Buenting C, et al. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. Proc Natl Acad Sci U S A 1997;94:6474-9.
[31] He C, Sabol J, Mitsuhashi T, Vlassara H. Dietary glycotoxins: inhibition of reactive products by aminoguanidine facilitates renal clearance and reduces tissue sequestration. Diabetes 1999; 48:1308-15.
[32] Hempe JM, Gomez R, McCarter RJ, Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. J Diabetes Complications 2002; 16:313-20.
[33] Snieder H, Sawtell PA, Ross L, Walker J, Spector TD, Leslie RDG. HbA1c levels are genetically determined even in type 1 diabetes. Diabetes 2001;50:2858-63.
[34] Leslie RD, Beyan H, Sawtell P, Boehm BO, Spector TD, Snieder H. Level of an advanced glycation end product is genetically determined: a study of normal twins. Diabetes 2003;52:2441-4.
[35] Tan KCB, Chow WS, Tam S, Bucala R, Betteridge J. Association between acute-phase reactants and advanced glycation end products in type 2 diabetes. Diabetes Care 2004;27:223-8.