Serum Levels of Advanced Glycation End Products Are Increased in Patients With Type 2 Diabetes and Coronary Heart Disease

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OBJECTIVE — To investigate whether serum levels of advanced glycation end products (AGEs) and the glycoxidation product N^{ε}-(carboxymethyl)lysine (CML) are increased in patients with type 2 diabetes compared with nondiabetic control subjects and whether levels of AGEs and/or CML differ in patients with type 2 diabetes with or without coronary heart disease (CHD).

RESEARCH DESIGN AND METHODS — Serum levels of AGEs and CML were measured with an immunoassay in 32 men and 21 women aged 59.3 ± 6.2 years (means ± SD) with type 2 diabetes for 7.3 + 3.1 years and in 17 men and 17 women aged 56.2 ± 4.2 years without diabetes. Of the patients with diabetes, 18 had CHD.

RESULTS — The serum levels of AGEs and CML were significantly increased in patients with type 2 diabetes compared with nondiabetic control subjects (median [5th–95th percentile]: AGEs 7.4 [4.4–10.9] vs. 4.2 [1.6–6.4] U/ml, $P < 0.0001$; CML 15.6 [5.6–29.9] vs. 8.6 [4.4–25.9] U/ml, $P < 0.0001$). The median level of AGEs but not CML was significantly increased in patients with type 2 diabetes and CHD compared with patients without CHD (8.1 [6.4–10.9] vs. 7.1 [3.5–9.8] U/ml, $P = 0.03$). There were significant positive correlations between serum levels of AGEs and CML in both patients and control subjects.

CONCLUSIONS — Levels of AGEs and CML were significantly increased in patients with type 2 diabetes compared with nondiabetic control subjects, and levels of AGEs but not CML were significantly higher in patients with type 2 diabetes and CHD than in patients without diabetes. These results may indicate a role for non-CML AGEs in the development of macrovascular disease in patients with type 2 diabetes.

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Patients with type 2 diabetes have a two- to fourfold increased risk of cardiovascular morbidity and mortality compared with age-matched nondiabetic subjects (1–3). Known risk factors, such as smoking, dyslipidemia, and hypertension, can explain only part of this increase, and recent epidemiological studies have suggested that hyperglycemia contributes to increased cardiovascular risk (4–6).

One of the potential mechanisms by which hyperglycemia may induce cardiovascular disease is through the formation of advanced glycation end products (AGEs). AGEs have been suggested to participate in the development of both micro- and macrovascular complications in diabetes.

AGE deposits have been demonstrated in atherosclerotic plaques and myocardium by immunohistochemistry in patients with diabetes and atherosclerosis (7), and the cross-linking abilities of AGEs may contribute to the increased stiffening of collagen and possibly to vascular hypertrophy (8,9). Because calcification of the media of arteries and reduced arterial compliance have been shown to be significant predictors of future coronary heart disease (CHD) in patients with type 2 diabetes (10), formation of AGEs may be one important mechanism.

AGEs are also, through interaction with receptors, able to increase the level of NF-κB, a transcription factor suggested to be involved in the development of atherosclerosis and in apoptosis (11). In addition, AGEs can quench nitric oxide and may impair endothelial function (12,13). Modification of LDL as a result of glycation may contribute to foam cell formation and increased atherosclerosis (14,15).

Given these potential contributions of AGEs to the development of atherosclerosis, and the fact that increased serum levels of AGEs have been demonstrated to be increased and to predict development of microvascular complications in patients with type 1 diabetes (16,17), we investigated whether serum levels of AGEs were elevated in patients with type 2 diabetes and whether the levels were different in patients with type 2 diabetes with and without CHD. In addition, we examined whether there were any differences in serum levels of the glycoxidation epitope, N^{ε}-(carboxymethyl)lysine (CML) by using a newly developed immunoassay (18).

RESEARCH DESIGN AND METHODS

Subjects

The study involved 53 patients with type 2 diabetes (32 men and 21 women) with a

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Abbreviations: AGE, advanced glycation end product; BSA, bovine serum albumin; CHD, coronary heart disease; CML, N^{ε}-(carboxymethyl)lysine; CV, coefficient of variation; ECG, electrocardiogram; GDR, glucose disposal rate; GDRI, glucose disposal rate index; ICD-9, *International Classification of Diseases, Ninth Revision*; OCTOPUS, Oslo Comparative Trial of Peroral Versus Insulin Treatment in Type 2 Diabetes.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Serum levels of AGEs and CML in patients with type 2 diabetes and control subjects*

	Patients	Control subjects
n	53	34
Serum AGEs (U/ml)	7.4 (4.4–10.9)	4.2 (1.6–6.4)*
Serum CML (U/ml)	15.6 (5.9–29.9)	8.6 (4.4–25.9)*

Data are medians (5th–95th percentiles). *$P < 0.0001$.

mean age of 59.3 ± 6.2 years (means ± SD) and a known duration of diabetes of 7.3 ± 3.1 years. Patients with established type 2 diabetes of >2 years duration with an initial HbA_{1c} of 7–10% who had stable glycemic control during a run-in period of 3 months consisting of diet and glibenclamide were randomized to either continue glibenclamide or start insulin treatment in the Oslo Comparative Trial of Peroral Versus Insulin Treatment in Type 2 Diabetes (OCTOPUS) Study (19). The patients were examined every 3 months for a median of 5 years. The OCTOPUS study was designed to investigate the relationship between long-term metabolic control and the development of micro- and macrovascular complications in type 2 diabetes.

Initially, half of the patients were randomized to start insulin treatment and the other half was to continue sulfonylurea treatment, but most of the patients (n = 51) took insulin 5 years from randomization to maintain acceptable blood glucose levels. At start of this study, mean BMI was 26.6 ± 3.7 kg/m^2, HbA_{1c} was 8.6 ± 1.2%, and fasting blood glucose was 11.0 ± 2.6 mmol/l. There were no significant differences between men and women regarding baseline values.

The control group for the AGE and CML measurements consisted of 34 randomly selected healthy blood donors (17 men and 17 women with a mean age of 56.2 + 4.2 years). The mean age in the control group was slightly but significantly lower than in the patient group.

CHD was diagnosed on the basis of a thorough clinical examination and a medical history of angina pectoris or myocardial infarction. Medical records were examined, and the diagnosis of myocardial infarction (*International Classification of Diseases, Ninth Revision* [ICD-9] codes 410 and 412) and angina pectoris (code 413) or the use of coronary intervention procedures (e.g., percutaneous transluminal coronary angioplasty or coronary artery bypass grafting) was verified. Angina pectoris was defined on the basis of a medical history of exercise-induced central chest pain relieved by stopping the activity or by sublingual nitroglycerin. Resting electrocardiogram (ECG) confirmed CHD when pathological Q-waves (i.e., Q-wave duration of >0.04 s or Q > 1/4 of the QRS complex in more than one lead) were present. Exercise ECG was defined as confirming CHD if horizontal ST-segment depressions of at least 1 mm combined with characteristic chest pain appeared during or immediately after exercise.

During follow-up, yearly clinical examinations with resting ECGs were performed, and ECGs were examined for newly developed Q-waves. Patients with new symptoms suggesting angina pectoris during follow-up were exercise tested and referred for coronary angiography if the exercise ECG indicated ischemia. Medical records from hospital admissions during the study were collected. Medical records with ICD-9 codes 410–414 confirmed CHD.

The number of patients with CHD was the sum of those who had CHD at the start of the study and those who developed it during follow-up.

Methods

Serum levels of AGEs were measured with a competitive immunoassay (16) by using

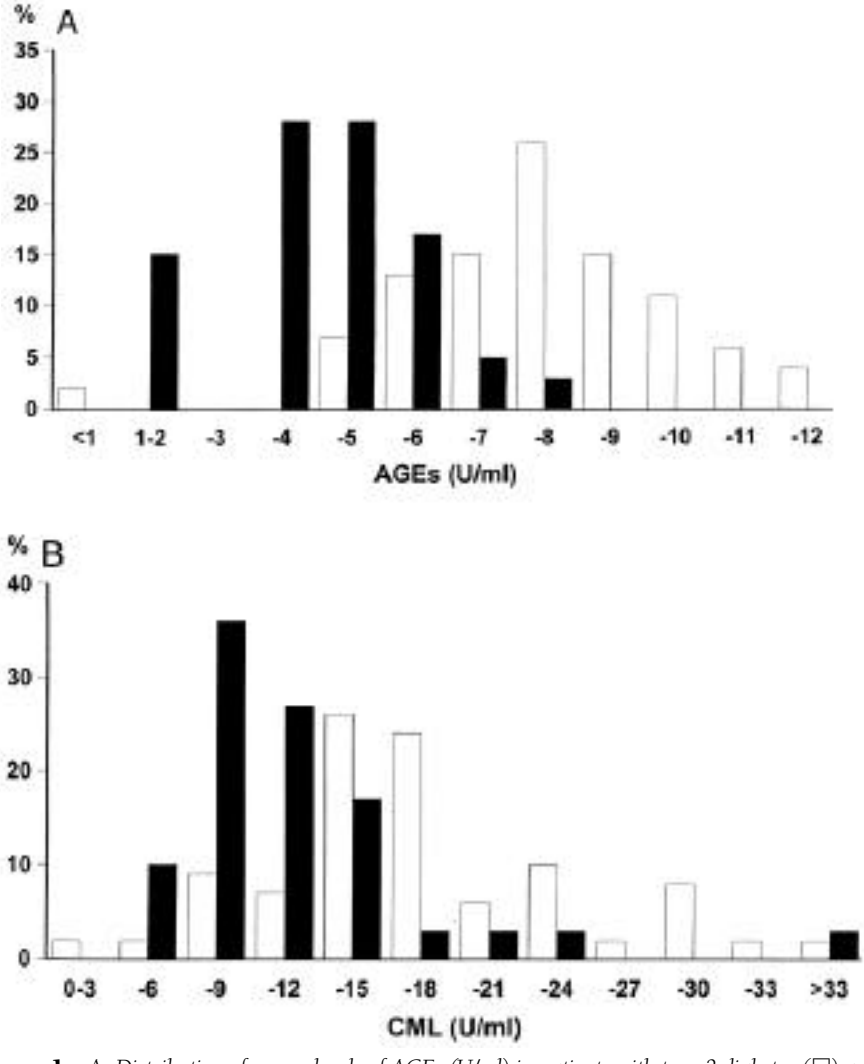

Figure 1—A: *Distribution of serum levels of AGEs (U/ml) in patients with type 2 diabetes (□) and nondiabetic control subjects (■). Percentages indicate patients with a given level of serum value of AGEs.* B: *Serum levels of CML in patients and control subjects.*

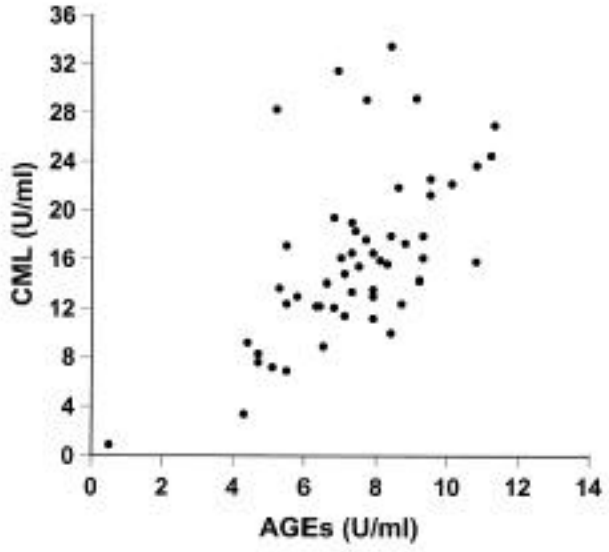

Figure 2—*Serum levels of AGEs correlate to serum levels of CML (r = 0.61, P < 0.0001) in patients with type 2 diabetes.*

polyclonal anti-AGE antibodies made from rabbit immunized with AGE-RNase (20) and europium-labeled anti-rabbit antibodies as detecting antibodies. AGE-bovine serum albumin (BSA) was used as a standard. Both AGE-BSA and polyclonal anti-AGE antibodies were provided by Prof. Richard Bucala of the Picower Institute for Medical Research (Manhasset, NY). Results are expressed as AGE units (1 U = 1 µg/ml AGE-BSA standard) and are adjusted for total serum protein concentration in each sample. All analyses were performed during the same run, and the intra-assay coefficient of variation (CV) was <12%.

Serum levels of CML were measured in a newly developed immunoassay (18) by using monoclonal anti-CML antibodies as detecting antibodies and CML-BSA as the standard. Both anti-CML antibodies and CML-BSA were supplied by Jes Clausen of Novo Nordisk AS (Bagsværd, Denmark). Europium-labeled anti-mouse antibodies were used as indicators. Results are expressed as CML units (1 U = 1 µg/ml CML-BSA standard) and are adjusted for total serum protein concentration in each sample. All analyses were performed during the same run, and the intra-assay CV was <12%.

HbA_{1c} was analyzed with a high-performance liquid chromatography method (Diamat analyzer; Bio-Rad, Richmond, CA) that has a normal range of 4.3–6.1% with an interassay CV of <3%.

Fructosamine was measured as a nitrobleu-tetrazolium reaction product in a photometric analyzer (Cobas BIO/Unimat Roche, Basel, Switzerland) with a normal range of 230–300 µmol/l for nondiabetic subjects and an interassay CV of <4%.

Serum total cholesterol, HDL cholesterol, and triglycerides were measured enzymatically (Boehringer Ingelheim/Boehringer Mannheim, Mannheim, Germany), and LDL cholesterol was calculated with the Friedewald formula (21). Total protein in serum samples was measured by using the Biuret method (Boehringer Mannheim, Mannheim, Germany) with a CV of <2%. Microalbuminuria was defined as an albumin excretion rate of >20 and <200 µg/min in an overnight timed urine sample. Blood pressure was measured with a standard sphygmomanometer with the patient sitting after a 5- to 10-min rest. Diastolic blood pressure was registered at the disappearance of Korotkoff's V sound.

The euglycemic glucose clamp technique was performed in the patients after an overnight fast. Insulin (7.2 pmol · kg^{-1} · min^{-1}) was infused at fixed rate until euglycemia was reached, and a variable glucose infusion (concentration 240 mg/l) was then started. The infusion rate of glucose was adjusted to maintain a stable blood glucose level at 5 mmol/l for 120 min, and the amount of glucose infused during the last 20 min was defined as the glucose disposal rate (GDR). The insulin concentration was measured in arterialized blood samples every 10–20 min. The GDR index (GDRI) was expressed as the GDR relative to the prevailing serum insulin levels.

Blood pressure and levels of HbA_{1c}, lipids, AGEs, and CML were measured after a 10-h fast before randomization into the OCTOPUS Study.

Statistical analyses were performed with the Number Cruncher Statistical System (NCSS, Kaysville, UT) and SAS statistical software (Cary, NC). Nonparametric tests

Table 2—*Characteristics of patients with type 2 diabetes with or without CHD*

	With CHD	Without CHD	*P*
n	18	35	—
Sex (M/F)	10/8	22/13	NS
Age (years)	61.5 (50–69)	60 (47.2–67.2)	NS
Diabetes duration (years)	6.5 (3–13)	7 (2.4–14.2)	NS
HbA_{1c} (%)	8.3 (6.4–10.9)	8.5 (6.9–11.1)	NS
Fasting blood glucose (mmol/l)	10.5 (6.8–15.3)	11.2 (7.5–16.8)	NS
Serum AGEs (U/ml)	8.1 (4.4–11.3)	7.1 (3.5–9.8)	0.03
Serum CML (U/ml)	16.2 (8.3–27)	13.7 (2.9–31.9)	NS
Total cholesterol (mmol/l)	6.8 (5.1–9.6)	6.0 (4.9–8.0)	NS
Triglycerides (mmol/l)	1.89 (0.60–6.65)	1.60 (0.47–3.61)	NS
HDL cholesterol (mmol/l)	1.07 (0.68–1.76)	1.12 (0.76–2.12)	NS
LDL cholesterol (mmol/l)	4.46 (3.37–7.18)	4.0 (2.90–6.19)	NS
Systolic blood pressure (mmHg)	160 (120–180)	140 (114–204)	0.03
Diastolic blood pressure (mmHg)	90 (80–120)	90 (74–106)	NS
BMI (kg/m^2)	26.0 (22.0–33.1)	26.1 (20.4–33.2)	NS
GDRI	4.31 (0.07–12.7)	4.43 (1.14–12.0)	NS
Smokers (current or former/never)	13/5	17/18	NS
Microalbuminuria (with/without)	4/14	6/29	NS

Data are medians (5th–95th percentiles). Microalbuminuria is defined as two out of three or one out of two consecutive measurements of albumin excretion rate >20 and <200 mg/min the last year before inclusion/never microalbuminuria.

Table 3—*Risk factors for CHD in a multivariate model using stepwise forward logistical regression analysis*

Variable	Odds ratio (95% CI)	*P*
Triglycerides (mmol/l)	2.4 (1.2–4.8)	0.018
Serum AGEs (U/l)	1.9 (1.2–3.1)	0.008
Age (years)	1.1 (1.0–1.3)	0.049

were used because of the nonequal distribution of data, and differences between groups were tested with a two-tailed Mann-Whitney *U* test. Univariate correlations were tested with the use of Spearman rank-order correlations, and a stepwise logistical regression analysis was performed with CHD as the dependent variable. The two-sided significance level was 5%.

RESULTS — Levels of serum AGEs were 7.4 (4.4–10.9) U/ml (median [5th–95th percentiles]) in the patients with type 2 diabetes and 4.2 (1.6–6.4) U/ml in the control group ($P < 0.0001$). Levels of serum CML were 15.6 (5.6–29.9) U/ml in the patients with type 2 diabetes, and 8.6 (4.4–25.9) U/ml in the control group ($P < 0.0001$) (Table 1). The frequency distribution of serum levels of AGEs and CML are shown in Fig. 1.

The serum levels of AGEs correlated significantly to levels of CML in both patients ($r = 0.61$, $P < 0.0001$) (Fig. 2) and control subjects ($r = 0.57$, $P < 0.001$). There were no significant correlations between age and serum levels of AGEs or CML in our material, neither in patients nor control subjects (Fig. 3).

The median serum levels of AGEs were 8.1 (6.4–10.9) U/ml in patients with type 2 diabetes and CHD compared with 7.1 (3.5–9.8) U/ml in patients without CHD ($P = 0.03$). Systolic blood pressure was significantly higher in patients with CHD than in those without CHD (Table 2).

Serum triglycerides showed a tendency toward higher values in patients with CHD compared with patients without CHD, but the difference did not reach significance ($P = 0.07$).

In a stepwise logistical regression analysis with CHD as the dependent variable (including blood pressure, diabetes duration, HbA_{1c}, GDRI, microalbuminuria, serum AGEs, serum CML, total cholesterol, triglycerides, HDL and LDL cholesterol, age, BMI, and smoking status as independent variables), serum triglyceride, serum AGEs, and patient age were significantly associated with CHD (Table 3).

There were no significant differences in serum levels of CML, HbA_{1c}, fasting blood glucose, or total, LDL, or HDL cholesterol between patients with and without CHD (Table 2), nor was there a difference in insulin resistance measured with the euglycemic hyperinsulinemic glucose clamp technique.

There were no significant correlations between serum levels of AGEs and CML and fasting blood glucose, HbA_{1c}, or cholesterol levels in patients with type 2 diabetes. There were no differences in serum levels of AGEs or CML between men and women.

A total of 10 patients had microalbuminuria, 4 in the group with CHD and 6 in the group without CHD.

CONCLUSIONS — This study showed highly significantly increased serum levels of both AGEs and CML in patients with type 2 diabetes. The differences are comparable with those in patients with type 1 diabetes (16,18).

We also found significantly increased serum levels of AGEs but not CML in patients with CHD ($n = 18$). This is the first study to show such a difference, and this raises the possibility that AGEs may participate in the development of CHD in patients with type 2 diabetes.

There may be several potential mechanisms for AGE involvement in macrovascular complications in diabetes. When examining the in vitro properties of AGEs such as activation of cytokines (22) and transcription factors (10), chemotaxis and activation of monocytes through the activation of adhesion molecules (23,24), and expression of platelet-derived growth factor (25), one finds possible mechanisms of action for AGEs in the development of atherosclerosis. There is also indirect evidence of the involvement of AGEs in the atherosclerotic process via the effect of aminoguanidine, an inhibitor of AGE formation, on the development of atherosclerosis in nondiabetic rats (26) and in cholesterol-fed rabbits (27). Aminoguanidine can prevent decreased myocardial compliance in diabetic rats (28) and age-related arterial stiffening and cardiac hypertrophy in nondiabetic rats (29). Recently, a soluble receptor for AGEs (RAGE) has been shown to inhibit atherosclerosis in mice (30).

The polyclonal anti-AGE antibody recognizes several epitopes, one of which is CML (18). However, anti-AGE antibodies recognize other epitopes as well, and some of them are not known. An increasing interest in non-CML immunoreactivity in diabetic complications is now evident (31–34).

The serum levels of CML were not significantly increased in patients with CHD versus the levels of AGEs. This may indicate that there are AGE fractions other than CML that are of importance in the development of atherosclerosis in patients with diabetes and CHD (7).

There were no correlations between age and serum levels of AGEs or CML in our study. So far, there has been a tendency to believe that serum levels of AGEs and CML increase with age because tissue lev-

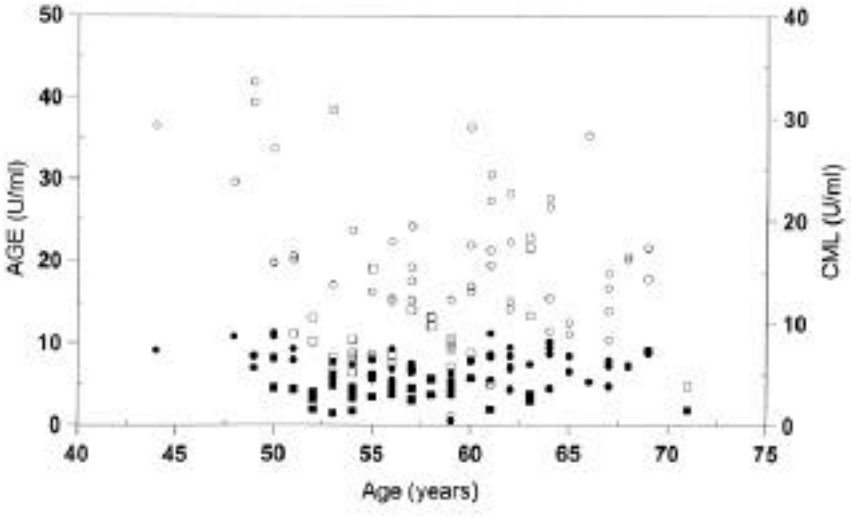

Figure 3—*Serum levels of AGEs in patients with diabetes (●) and in nondiabetic control subjects (■), and serum levels of CML in patients with diabetes (○) and in nondiabetic control subjects (□) according to age.*

els of AGEs and CML increase with age (20,35–38), possibly because a slower turnover in the tissues that allows AGEs and CML to accumulate with age. No studies so far have shown any increase in serum levels with age.

Some of the increase in serum AGEs may represent increased AGE-LDL, and glycation of LDL has been shown to increase its uptake by the macrophage scavenger receptors and contribute to foam cell formation (14,15). It is also possible that modification of serum proteins through glycation may contribute to increased atherosclerosis through activation of cytokines and growth factors as shown in vitro.

Our results alone cannot be considered evidence for the involvement of AGEs in atherosclerosis in humans, but they raise it as a hypothesis and may give rise to further investigations.

The lack of a significant correlation between serum AGEs and CML and HbA_{1c} in our study compared with the previously shown associations between skin collagen AGEs and CML and previous mean HbA_{1c} (39) may reflect the different turnover of the components in serum and skin (40) because these are different environments.

Only four patients in the study had both microalbuminuria and CHD, but there is no indication that patients with microalbuminuria and CHD in our study had any higher levels of serum AGEs or CML than patients with CHD alone (data not shown). This contrasts with previous publications that showed increased levels of AGEs (41,42) and CML (40,43) in patients with macroalbuminuria and on dialysis and may indicate that kidney damage in microalbuminuria is not severe enough to cause defects in handling AGEs in the kidneys.

We did not find any significant correlation between either previous or current smokers and levels of AGEs or CML, and thus our data do not support the hypothesis that persons with diabetes who are or have been smokers have increased ACE levels (44).

In summary, our results show significantly increased serum levels of both AGEs and CML in patients with type 2 diabetes compared with nondiabetic control subjects. We also found that patients with type 2 diabetes and CHD have a significantly higher serum level of AGEs (but not CML) than patients with diabetes but without CHD. These findings may suggest that AGEs contribute to the development of macrovascular complications in diabetes.

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References

1. Kannel WB, McGee DL: Diabetes and cardiovascular disease: the Framingham study. *JAMA* 241:2035–2038, 1979
2. Stamler J, Vaccaro O, Neeton JD, Wentworth D, for the Multiple Risk Factor Intervention Trial Research Group: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434–444, 1993
3. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
4. Uusitupa MIJ, Niskanen LK, Siitonen O, Voutilainen E, Pyörälä K: Ten-year cardiovascular mortality in relation to risk factors and abnormalities in lipoprotein composition in type 2 (non-insulin-dependent) diabetic and non-diabetic subjects. *Diabetologia* 36:1175–1184, 1993
5. Hanefeld M, Fischer S, Julius U, Schulze J, Schwanebeck U, Schmechel H, Ziegelsch HJ, Lindner J, the DIS Group: Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. *Diabetologia* 39:1577–1583, 1996
6. Turner RC, Millns H, Neil HAW, Stratton IM, Manley SE, Matthews DR, Holman RR for the United Kingdom Prospective Diabetes Study Group: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom prospective diabetes study (UKPDS: 23). *BMJ* 316:823–828, 1998
7. Nakamura Y, Horii Y, Nishino T, Shiiki H, Sakaguchi Y, Kagoshima T, Dhoi K, Makita Z, Vlassara H, Bucala R: Immunohistochemical localization of advanced glycosylation endproducts in coronary atheroma and cardiac tissue in diabetes mellitus. *Am J Pathol* 143:1649–1656, 1993
8. Sims TJ, Rasmussen LM, Oxlund H, Bailey AJ: The role of glycation cross-links in diabetic vascular stiffening. *Diabetologia* 39: 964–951, 1996
9. Rumble JR, Cooper ME, Soulis T, Cox A, Youssef S, Jasik M, Jerums G, Gilbert RE: Vascular hypertrophy in experimental diabetes: role of advanced glycation end products. *J Clin Invest* 99:1016–1027, 1997
10. Lehto S, Niskanen L, Suhonen M, Rönnemaa T, Laakso M: Medial artery calcification: a neglected harbinger of cardiovascular complications in non-insulin dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 16:978–983, 1996
11. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, Luther T, Berentshtein E, Tritschler H, Müller M, Wahl P, Ziegler R, Nawroth PP: Advanced glycation end product-induced activation of NF-κB is suppressed by α-lipoic acid in cultured endothelial cells. *Diabetes* 46: 1481–1490, 1997
12. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 87:432–438, 1991
13. Hogan M, Cerami A, Bucala R: Advanced glycosylation endproducts block the antiproliferative effect of nitric oxide. *J Clin Invest* 90:1110–1115, 1992
14. Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, Vlassara H: Modification of low density lipoprotein by advanced glycation endproducts contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci U S A* 91:9441–9445, 1994
15. Sobenin IA, Tertov VV, Koschinsky T, Bünting CE, Slavina ES, Dedov II, Orehkov AN: Modified low density lipoprotein from diabetic patients causes cholesterol accumulation in human intimal aortic cells. *Atherosclerosis* 100:41–54, 1993
16. Berg TJ, Torjesen PA, Bangstad H-J, Bucala R, Østerby R, Hanssen KF: Advanced glycation endproducts predict changes in kidney morphology in type 1 diabetic patients. *Metabolism* 46:661–665, 1997
17. Berg TJ, Torjesen PA, Dahl-Jørgensen K, Hanssen KF: Increased serum levels of advanced glycation end products (AGEs) in children and adolescents with IDDM. *DiabetesCare* 20:1006–1008, 1997
18. Berg TJ, Clausen JT, Torjesen PA, Dahl-Jørgensen K, Bangstad H-J, Hanssen KF: The advanced glycation end product N^{ε}-(carboxymethyl)lysine is increased in serum from children and adolescents with type 1 diabetes. *Diabetes Care* 21:1997–2002, 1998
19. Birkeland KI, Rishaug U, Hanssen KF, Vaaler S: NIDDM: a rapid progressive disease: results from a long-term, randomised, comparative study of insulin or sulphonylurea treatment. *Diabetologia* 39:1629–1633, 1996
20. Makita Z, Vlassara H, Cerami A, Bucala R: Immunochemical detection of advanced glycosylation end products in vivo. *J Biol*

Chem 267:5133–5138, 1992
21. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. *ClinChem* 18:499–502, 1972
22. Vlassara H, Brownlee M, Manogue KR, Dinarello CA, Pasagian A: Cachectin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodelling. *Science* 240:1546–1548, 1988
23. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D: Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human endothelial cells and in mice. *J Clin Invest* 96:1395–1403, 1995
24. Vlassara H, Fuh H, Donelly T, Cybulsky M: Advanced glycation endproducts promote adhesion molecule (VCAM-1, ICAM-1) expression and atheroma formation in normal rabbits. *Mol Med Today* 1:447–456, 1995
25. Kirstein M, Brett J, Radoff S, Ogawa S, Stern D, Vlassara H: Advanced protein glycosylation induces transendothelial human monocyte chemotaxis and secretion of platelet-derived growth factor: role in vascular disease and aging. *Proc Natl Acad Sci U S A* 87:9010–9014, 1990
26. Li YM, Steffes M, Donelly T, Liu C, Fuh H, Basgen J, Bucala R, Vlassara H: Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. *Proc Natl Acad Sci U S A* 93:3902–3907, 1996
27. Panagiotopoulos S, O'Brien RC, Bucala R, Cooper ME, Jerums G: Aminoguanidine has an anti-atherogenic effect in the cholesterol-fed rabbit. *Atherosclerosis* 136:125–131, 1998
28. Norton GR, Candy G, Woodiwiss AJ: Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation* 93:1905–1912, 1996
29. Corman B, Duriez M, Poitevin P, Heudes D, Bruneval P, Tedgui A, Levy BI: Aminoguanidine prevents age-related arterial stiffening and cardiac hypertrophy. *Proc Natl Acad Sci U S A* 95:1301–1306, 1998
30. Park L, Raman KG, Lee KJ, Lu Y, Ferran LJ Jr, Chow WS, Stern D, Schmidt AM: Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 4:1025–1031, 1998
31. Ikeda K, Higashi T, Sano H, Jinnouchi Y, Yoshida M, Araki T, Ueda S, Horiuchi S: N^{ε}-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. *Biochemistry* 35:8075–8083, 1996
32. Niwa T, Katsuzaki T, Ishizaki Y, Hayase F, Miyazaki T, Uematsu T, Tatemichi N, Takei Y: Imidazolone, a novel advanced glycation end product, is present at high levels in kidneys of rats with streptozotocin-induced diabetes. *FEBS Lett* 407:297–302, 1997
33. Shamsi FA, Partal A, Sady C, Glomb MA, Nagaraj RH: Immunological evidence for methylglyoxal-derived modifications in vivo: determination of antigenic epitopes. *J Biol Chem* 273:6928–6936, 1998
34. Yamaguchi M, Nakamura N, Nakano K, Kitagawa Y, Shigeta H, Hasegawa G, Ienaga K, Nakamura K, Nakazawa Y, Fukui J, Obayashi H, Kondo M: Immunochemical quantification of crossline as a fluorescent advanced glycation end product in erythrocyte membrane proteins from diabetic patients with or without retinopathy. *Diabet Med* 15:458–462, 1998
35. Brownlee M, Cerami A, Vlassara H: Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 318:1315–1321, 1988
36. Dunn JA, McCance DR, Thorpe SR, Lyons TJ, Baynes JW: Age-dependent accumulation of N^{ε}-(carboxymethyl)lysine and N^{ε}-(carboxymethyl)hydroxylysine in human skin collagen. *Biochemistry* 30:1205–1210, 1991
37. Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, Baynes JW: Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 91:2463–2469, 1993
38. Schleicher ED, Wagner E, Nerlich AG: Increased accumulation of the glycoxidation product N^{ε}-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* 99:457–468, 1997
39. Beisswenger PJ, Makita Z, Curphey TJ, Moore LL, Jean S, Brinck-Johnsen T, Bucala R, Vlassara H: Formation of immunochemical advanced glycosylation end products precedes and correlates with early manifestations of renal and retinal disease in diabetes. *Diabetes* 44:824–829, 1995
40. Papanastasiou P, Grass L, Rodela H, Patrikarea A, Oreoupoulos D, Diamandis EP: Immunological quantification of advanced glycosylation end-products in the serum of patients on hemodialysis or CAPD. *Kidney Int* 46:216–222, 1994
41. Makita Z, Bucala R, Rayfield EJ, Friedman EA, Kaufman AM, Korbet SM, Barth RH, Winston JA, Fuh H, Manogue KR, Cerami A, Vlassara H: Reactive glycosylation endproducts in diabetic uraemia and treatment of renal failure. *Lancet* 343:1519–1522, 1994
42. Wróbel K, Wróbel K, Garay-Sevilla ME, Nava LE, Malacara JM: Novel analytical approach to monitoring advanced glycosylation end products in human serum with on-line spectrophotometric and spectrofluorometric detection in a flow system. *Clin Chem* 43:1563–1569, 1997
43. Degenhardt TP, Grass L, Reddy S, Thorpe SR, Diamandis EP, Baynes JW: The serum concentrations of the advanced glycation end-product N^{ε}-(carboxymethyl)lysine is increased in uremia. *Kidney Int* 52:1064–1067, 1997
44. Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, Lee A, Al-Abed Y, Vlassara H, Bucala R, Cerami A: Tobacco smoke is a source of toxic reactive glycation products. *Proc Natl Acad Sci U S A* 94: 13915–13920, 1997