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

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Background—Advanced glycation end products (AGEs), modification products of glycation or glycoxidation of proteins and lipids, have been linked to premature atherosclerosis in patients with diabetes as well as in nondiabetic subjects.

Methods and Results—Serum levels of AGEs were measured with an immunoassay in samples obtained at baseline examination of a random sample of 1141 nondiabetic individuals (535 men and 606 women), aged 45 to 64 years, living in Kuopio, East Finland, or Turku, West Finland in 1982 to 1984. After 18 years of follow-up, all-cause mortality, cardiovascular disease (CVD), and coronary heart disease (CHD) mortality were registered on the basis of copies of death certificates. Multivariate Cox regression model showed a significant association of serum AGEs with all-cause (P=0.012), CVD (P=0.018), and CHD (P=0.008) mortality in women but not in men. Fasting serum AGEs in the highest quartile were an independent risk factor for all-cause (hazards ratio [HR], 1.90; 95% CI, 1.16 to 3.11; P=0.011) and CHD (HR, 6.51; 95% CI, 1.78 to 23.79; P=0.005) mortality in women, even after the adjustment for confounding factors, including highly sensitive C-reactive protein.

Conclusions—The present study is the first to show that serum levels of AGEs can predict total, CVD, and CHD mortality in nondiabetic women.

Key Words: advanced glycation end products ▪ coronary heart disease ▪ cardiovascular disease ▪ mortality

Advanced glycation end products (AGEs), the short- and long-term modification products of glycation or glycoxidation of proteins and lipids,1,2 have been linked to premature atherosclerosis in diabetic patients3 as well as in nondiabetic subjects.4 AGEs are a heterogeneous group of compounds that have multiple biological effects, some of which are mediated by interacting with receptors, including the receptor for AGE (RAGE), on endothelial cells, smooth muscle cells, and macrophages.5–7 Furthermore, AGEs have been found in atherosclerotic plaques.8 AGEs may contribute to development of atherosclerosis by activating the transcription factor nuclear factor κB (NF-κB) through RAGE binding, resulting in induction of cellular adhesion molecule expression and cytokine activation,9,10 or through glycoxidation of lipoproteins and increased foam cell formation.11,12 AGEs might also quench NO and mediate impaired endothelial function.13 Increased AGE modification of long-lived proteins such as collagen increases cross-linking and stiffening of arteries.14 Experimental studies in animals and in humans have shown that a cross-link breaker treatment results in greater vascular compliance.15,16 Recently, elevated levels of circulating AGEs were reported in nondiabetic patients with coronary heart disease (CHD) that correlated significantly with the number of vessels with stenosis.17

Because no study has been undertaken to examine whether there is any association of serum levels of AGEs with the subsequent development of cardiovascular disease (CVD) in a large nondiabetic population, the present study was aimed to investigate whether increased serum levels of AGEs predict total mortality, CVD mortality, or CHD mortality in Finnish nondiabetic subjects.

Materials and Methods

Baseline Study
A random sample of nondiabetic subjects born and currently living in Kuopio, East Finland, or in Turku, West Finland, was taken from the population register including all individuals aged 45 to 64 years in 1982 through 1984.18 Of the 827 individuals in eastern Finland and 863 in western Finland originally eligible for the study, a total of 638 men and 735 women were examined: 313 men and 336 women from East Finland (participation rate 79%) and 325 men and 399 women from West Finland (participation rate 81%).

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women from West Finland (participation rate 85%). All subjects with a previous history of diabetes or newly diagnosed diabetes according to the World Health Organization (WHO) criteria²⁰ at the baseline study were excluded from the present analyses. Clinical characteristics and background variables did not differ between participants and nonparticipants.

The study program for subjects was performed during an outpatient visit at the clinical research unit of the University of Kuopio or the rehabilitation research center of the Social Insurance Institution in Turku. These methods have been described previously.³⁸ The visit included an interview on the history of chest pain suggestive of CHD, smoking, alcohol intake, physical activity, and drug use. All medical records of subjects who reported on the interview that they had been admitted to hospital for chest pain symptoms were reviewed. Review of the medical records was performed by M.L. in Kuopio and T.R. in Turku after careful standardization of the methods between the reviewers. The WHO criteria for verified definite or possible myocardial infarction (MI), based on chest pain symptoms, ECG changes, and enzyme determinations, were used to define previous MI.²⁰

Smoking status was based on an interview. In all statistical analyses, subjects were classified as nonsmokers or current smokers. Blood pressure was measured in the sitting position after a 5-minute rest with a mercury sphygmomanometer and read to the nearest 2 mm Hg. Subjects were classified as having hypertension if they were receiving drug treatment for hypertension or if their systolic blood pressure was ≥160 mm Hg or diastolic blood pressure ≥95 mm Hg. All laboratory specimens were drawn after a 12-hour fast at 8:00 AM and subsequently stored frozen at −20°C until analysis. Serum levels of AGEs were measured with a competitive immunoassay developed in our laboratory. The method has been described previously.²¹ Briefly, we used polyclonal anti-AGE antibodies from rabbit developed in our laboratory. The method has been described previously.²¹ The final serum concentrations of AGE were adjusted for total protein concentration.²² Interassay coefficient of variation was 15% for the control in the median range of the assay curve and 24% in the lower range. Two batches of antibody obtained from the same rabbits with dextran sulfate–MgCl₂.²³ LDL cholesterol was calculated using the Friedewald formula.²⁴ Total protein concentration (interassay variation was 3.36% at the mean level of 1.5 mg/L and 2.65% at the mean level of 2.5 mg/L).

Follow-Up Study

The follow-up period was until January 1, 2001. Information on the vital status of the participants and copies of death certificates of all deceased subjects were obtained from the Cause-of-Death Register (Statistics Finland). In the final classification of causes of death, hospital records and autopsy records also were used, if available. The causes of death were reviewed by A.J. and S.I. The end points used in this study were all-cause death, non-CVD death, CVD death, and CHD death. CVD death was defined by codes 390 to 459 and CHD death by codes 410 to 414, based on the International Classification of Diseases, 9th Revision.

Statistical Methods

Data analyses were conducted with the SPSSX, SPCC/PC+, and SPSS 11.0.1 programs (SPSS). Results for continuous variables are given as means±SD or percentages. The bivariate correlation of continuous variables with AGEs was assessed by Spearman’s correlation coefficient. The differences among the groups were assessed by the χ² test or Student’s 2-tailed t test for independent samples, when appropriate. Univariate and multivariate Cox regression models and Kaplan–Meier survival curves were used to investigate the association of cardiovascular risk factors with total, non-CVD, CVD, and CHD mortality. In multivariate Cox models, the adjustment was done for area of residence, gender, body mass index (BMI), current smoking, hypertension (blood pressure ≥160/95 mm Hg or drug treatment for elevated blood pressure), total cholesterol, triglycerides, HDL cholesterol, and menopausal status (in women).

Approval of Ethics Committee

This study was approved by the ethics committee of Kuopio University Hospital and the Turku University Central Hospital. All study subjects gave informed consent.

Results

The median follow-up was 17.8 years. During this period, altogether 253 subjects died (170 men and 83 women). A total of 114 subjects died of cardiovascular causes (92 men and 22 women), and 84 subjects died of CHD (69 men and 15 women).

Baseline characteristics by gender are given in Table 1. Compared with men, women had higher BMI and total and HDL cholesterol, but lower total triglycerides and fasting plasma glucose. Fewer women than men were smokers or had a history of previous MI, but more women had hypertension. Serum levels of AGEs were significantly higher in men than in women. Subjects in the highest AGE quartile were more often current smokers (26% versus 19%; P=0.009) and had
higher total cholesterol (6.92 ± 1.27 versus 6.74 ± 1.32 mmol/L; \( P = 0.048 \)), total triglycerides (1.48 ± 0.74 versus 1.40 ± 0.74 mmol/L; \( P = 0.032 \)), and lower HDL cholesterol (1.44 ± 0.39 versus 1.50 ± 0.39 mmol/L; \( P = 0.023 \)) than subjects in other quartiles; whereas no difference in age, BMI, or the prevalence of hypertension was observed. There were no statistically significant correlations between serum AGE level and age, fasting plasma glucose, total cholesterol, HDL cholesterol, total triglycerides, and BMI (range of correlations from 0.015 to 0.081 in men and from 0.022 to 0.079 in women). The lack of significant correlations is not surprising given the fact that absolute levels of cardiovascular risk factors were not substantially different between the highest quartile of AGEs versus the other quartiles. Exclusion of 2 patients from statistical analyses who had serum creatinine \( >200 \) \( \mu \)mol/L did not change the results.

Table 2 gives unadjusted and multiple-adjusted hazard ratios (HRs) for serum levels of AGEs as a continuous variable to predict all-cause, non-CVD, CVD, and CHD mortality (Cox regression model). Because AGE levels and gender had a statistically significant interaction in their effects on total mortality (\( P = 0.023 \)) and CHD mortality (\( P = 0.012 \)), even after the adjustment for confounding variables, results are presented separately for men and women. Unadjusted serum AGEs were significantly related to total (\( P = 0.007 \)), CVD (\( P < 0.001 \)), and CHD (\( P < 0.001 \)) mortality in men but not in women. Adjusted HRs remained essentially similar.

Total and CHD mortality in men and women according to the gender-specific AGE quartiles (6.0 U/mL, 7.6 U/mL, and 9.9 U/mL in men; 5.6 U/mL, 7.1 U/mL, and 9.0 U/mL in women) is shown in Figure 1. The highest quartile of serum AGEs versus the 3 other quartiles predicted total (\( P = 0.032 \)) and CHD mortality (\( P = 0.001 \)) in women but not in men (Figure 2). In women, high AGE levels increased mortality from all causes by 1.6-fold (\( P = 0.042 \)) and CHD mortality by 4-fold (\( P = 0.014 \)), independently of confounding factors. AGE levels were not associated with increased mortality from noncardiovascular causes (Table 3). Additional adjustment for hs-CRP increased further the HRs for all-cause mortality.
mortality up to 1.9-fold \( (P=0.011) \) and CHD mortality up to 6.5-fold \( (P=0.005) \).

In Kaplan–Meier survival analysis, total \( (P=0.032) \) and CHD mortality \( (P<0.001) \) were significantly higher in subjects in the highest AGE quartile compared with the 3 other quartiles in women (Figure 2). In men, no differences in mortality according to the AGE quartiles were observed.

### Discussion

The present study is the first to show that increased AGE serum levels, measured with a polyclonal anti-AGE antibody, can predict total, CVD, and CHD mortality, and that serum AGE level in the top quartile \( (\geq 9.0 \text{ U/mL}) \) is an independent risk factor for total and CHD mortality in nondiabetic women.

AGE-modified proteins are a heterogeneous group of compounds formed through glycation, glycoxidation, or, like \( \text{N-(carboxymethyl)lysine (CML)} \), through glycoxidation/lipoxidation from lipids.\(^2,25\) The dominant AGE epitope for binding to the RAGE receptor is CML.\(^26\) Through binding to the RAGE receptor, CML may induce NF-\( \kappa B \) activation\(^26\) and vascular cell adhesion molecule-1 expression,\(^27\) which might contribute to accelerated atherosclerosis. Circulating AGES may arise from intracellularly formed reactive AGE precursors,\(^28\) and AGES can be formed in the circulation, for example, on lipids.\(^29\) We measured serum AGES with a polyclonal anti-AGE antibody to detect most of the circulating AGES. The polyclonal anti-AGE antibody has been shown previously to recognize CML,\(^30\) as well as a number of as yet unidentified AGE epitopes. Thus, variations in AGE levels could mirror changes in CML levels that might be related to inflammation through NF-\( \kappa B \) activation, reflecting some of the burden of inflammation as well as that of glucose-modification of proteins.

Our finding of a significant association of AGE serum levels with CHD and CVD mortality in women might indicate an etiologic role of AGES in atherosclerosis in patients without diabetes. So far, only 1 animal study has demonstrated a causal involvement of AGES in atherosclerosis. In that study, diabetic apolipoprotein E (apoE–null mice were given normal chow, which increased atherosclerosis, but by adding soluble RAGE, development of atherosclerosis was almost completely inhibited.\(^31\) There was also a nonsignificant trend toward a reduction in atherosclerosis in nondiabetic apoE–null mice given soluble RAGE in addition to normal chow compared with those continuing on normal chow. Recently, treatment with soluble RAGE significantly reduced atherosclerotic lesion area in apoE–null diabetic mice having established atherosclerosis.\(^32\)

Fasting serum AGES in the highest quartile \( (\geq 9.0 \text{ U/mL}) \) in this population-based study were an independent risk factor for total and CHD mortality in women. High AGE levels might reflect some of the increase in inflammation or

### Table 3. High Levels of Serum AGES as a Risk Factor for Total, CHD, and Non-CVD Mortality in 1141 Nondiabetic Subjects During 18-Year Follow-Up

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>Total Mortality</th>
<th>CHD Mortality</th>
<th>Non-CVD Mortality</th>
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<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>( P ) Value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age, sex, and area</td>
<td>All 1.25 (0.96–1.64) 0.102 1.61 (1.03–2.53) 0.038 1.14 (0.78–1.66) 0.507</td>
<td>Men 0.98 (0.68–1.41) 0.922 1.10 (0.63–1.92) 0.745 0.99 (0.59–1.68) 0.973</td>
<td>Women 1.63 (1.03–2.56) 0.036 4.86 (1.73–13.66) 0.003 1.33 (0.76–2.30) 0.315</td>
</tr>
<tr>
<td>Age, sex, area, total cholesterol, smoking, hypertension, BMI, HDL cholesterol, triglycerides, and postmenopausal status (in women)</td>
<td>All 1.23 (0.94–1.61) 0.138 1.57 (1.00–2.49) 0.052 1.17 (0.80–1.71) 0.421</td>
<td>Men 0.99 (0.69–1.42) 0.961 1.10 (0.62–1.93) 0.746 1.02 (0.60–1.72) 0.905</td>
<td>Women 1.62 (1.02–2.59) 0.042 4.03 (1.32–12.26) 0.014 1.38 (0.79–2.43) 0.257</td>
</tr>
<tr>
<td>Age, sex, area, total cholesterol, smoking, hypertension, BMI, HDL cholesterol, triglycerides, and postmenopausal status (in women)</td>
<td>All 1.29 (0.97–1.72) 0.076 1.72 (1.06–2.79) 0.028 1.19 (0.79–1.77) 0.403</td>
<td>Men 0.96 (0.65–1.41) 0.828 1.12 (0.61–2.05) 0.724 0.89 (0.51–1.57) 0.695</td>
<td>Women 1.90 (1.16–3.11) 0.011 6.51 (1.78–23.79) 0.005 1.56 (0.86–2.81) 0.143</td>
</tr>
</tbody>
</table>

The cutoff point is the sex-specific highest quartile of AGES \( (\geq 9.0 \text{ U/mL}) \) in men; \( \geq 9.0 \text{ U/mL} \) in women. \( HR \) is from the Cox proportional hazards model (the highest quartile vs the lowest 3 quartiles).
oxidative stress in atherosclerosis because anti-AGE antibody recognizes oxidatively modified proteins as well as glucose-modified compounds. However, high AGE levels predicted mortality even after adjustment for hs-CRP, indicating that AGEs probably increase mortality, at least in part, independently of inflammation in the vascular wall.

Circulating AGEs predicted mortality only in women. Therefore, it is possible that AGEs represent an additional risk for CVD only in low-risk individuals (compared with men, women in this study had a lower frequency of smoking, higher levels of HDL cholesterol, a lower ratio of total cholesterol/HDL cholesterol, and low alcohol intake). Further studies are needed to substantiate this notion, including therapeutic studies with AGE-lowering agents. Lowering of dietary AGEs in short-term studies has been shown to reduce inflammatory mediators such as peripheral blood mononuclear cell tumor necrosis factor-α and hs-CRP, but the data on cardiovascular complications are missing.

Serum levels of AGEs did not correlate with fasting plasma glucose in our study, perhaps reflecting that the anti-AGE antibody recognizes the more heavily modified glucose compounds as well as oxidatively modified proteins. Furthermore, because CML can be formed from glucose and lipid modification of proteins, this might also contribute to the lack of correlation with fasting plasma glucose because the antibody we used in the assay recognizes CML.

This study has limitations. In spite of a long follow-up period, the number of end points was relatively low, particularly in women. Furthermore, the relatively high interassay coefficient of variation might possibly underestimate the full etiologic contribution of increased serum levels of AGEs in atherosclerosis development. Long-term storage of serum samples at –20°C might influence the measured AGE serum levels. However, all samples were stored under identical conditions, and consequently, differences in AGE levels should represent differences initially present in the samples when they were drawn. It is not likely that the storage had a different effect on AGE levels among those who died compared with those who did not. Furthermore, the staff of the laboratory in Oslo, where all AGE measurements were done, was unaware of the mortality status of participants. Increased AGE levels did not predict atherosclerotic events in men, which may be because of a high frequency of smoking and other risk factors in men. Other studies have shown increased serum AGE levels in smokers and tobacco smoke does contain AGEs. In summary, we demonstrate for the first time that high AGE serum levels predict total, CVD, and CHD mortality in nondiabetic women. Therefore, the measurement of serum AGEs might identify high-risk individuals in a low-risk population.

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