Advanced Glycation End Products in Nondiabetic Patients With Coronary Artery Disease

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OBJECTIVE — To investigate whether advanced glycation end products (AGEs) participate in the development of coronary artery disease (CAD) in nondiabetic and diabetic subjects.

RESEARCH DESIGN AND METHODS — Serum concentrations of AGEs were measured using a newly established enzyme-linked immunosorbent assay in 48 nondiabetic patients (normal glucose tolerance, n = 20; impaired glucose tolerance, n = 28) who received coronary angiography for the study of chest pain or suspected CAD. Insulin sensitivity was examined by the euglycemic-hyperinsulenic glucose clamp technique and was estimated as the mean glucose infusion rate during the last 30 min of clamp time (M value).

RESULTS — Patients were classified into four groups based on the number of significantly stenosed vessels, defined as 0-, 1-, 2-, or 3-vessel disease. Serum concentrations of AGEs were significantly higher in nondiabetic subjects with CAD than in control subjects (2.42 ± 0.65 vs. 1.96 ± 0.40 μM/ml, P < 0.01) and significantly correlated with the number of significantly stenosed vessels (r = 0.678, P < 0.001). M values significantly inversely correlated with serum concentrations of AGEs (r = -0.490, P < 0.05). In multiple regression analysis, with the number of significantly stenosed vessels as the dependent variable, serum concentrations of AGEs, 2-h plasma glucose, and areas under the plasma glucose response curve were independently associated.

CONCLUSIONS — This pilot study indicates the relation between AGEs and the severity of CAD in nondiabetic patients. The measurement of serum AGE concentrations may be predictive of vascular damage.

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here is a large body of evidence linking both glucose intolerance and insulin resistance to an increased risk of coronary artery disease (CAD) (1). One of the potential mechanisms by which hyperglycemia may contribute to CAD is through the formation of advanced glycation end products (AGEs) (2). AGE formation may lead to oxidative stress (3). Several pieces of data seem to indicate that oxidative stress might be responsible for a decline in insulin-mediated glucose uptake, leading to insulin resistance (4). Moreover, AGE deposits have been demonstrated in the atherosclerotic plaques of patients with atherosclerosis and diabetes (5–7). It has recently been reported that serum concentrations of AGEs are increased in patients with type 2 diabetes and CAD (8). However, no study has demonstrated a clear relation between AGEs and insulin resistance in nondiabetic patients with CAD. This is the first study to measure serum concentrations of AGEs using a newly established enzyme-linked immunosorbent assay (9) and the first to show that AGEs may participate in the development of CAD, even in nondiabetic subjects.


Serum AGE concentrations were measured using an enzyme-linked immunosorbent assay (Special Reference Laboratories, Tokyo) (9). Intra- and interassay coefficients of variation were 4.8 and 3.5–6.2%, respectively (9).

Analysis of the coronary angiograms was performed by an independent experienced observer but not by quantitative coronary angiography. The presence of CAD was defined as >50% diameter narrowing in one major coronary artery or its major branches. Coronary arteries were grouped as left anterior descending artery or diagonal and septal branch, left circumflex artery or obtuse marginal branch, and right coronary artery or posterior descending and posterolateral branch for defining 1-, 2-, or 3-vessel disease. The study protocol was in accordance with the Helsinki Declaration and was approved by the local ethics committee.

**Statistical analysis**

Values are presented as means ± SD or median (range). Statistical analysis was performed using the unpaired Student's t test for parametric data or the Mann-Whitney U test for nonparametric data. The significance of differences between percentages was evaluated with the χ² test. Correlations were assessed using Spearman’s rank correlation test. Differences were considered significant at P < 0.05. Multiple regression models using serum concentrations of AGEs, fasting plasma glucose, 2-h plasma glucose, fasting plasma insulin, and 2-h plasma insulin as independent variables were analyzed for associations with the number of significantly stenosed vessels.

**RESULTS**—Subjects were classified into four groups based on the number of significantly stenosed vessels, defined as 0-, 1-, 2-, or 3-vessel disease (Table 1). Subjects were also divided into two subgroups: normal glucose tolerance (NGT) (n = 20) and IGT (n = 28). The number of NGT/IGT subjects in four subgroups are shown in Table 1. There were no significant differences among these groups with respect to mean age, BMI, systolic and diastolic blood pressure, serum level of total cholesterol, fasting plasma glucose, fasting plasma insulin concentration, or incidence of smoking. Serum level of triglycerides in the groups with 2- and 3-vessel disease tended to be higher than in the other groups (not significant), but they significantly correlated with the number of significantly stenosed vessels (r = 0.298, P = 0.039). Serum creatinine concentration showed a slight increase with an increase in the number of significantly stenosed vessels (r = 0.303, P = 0.043). AUCglu and 120-min plasma glucose were significantly higher in the group with 3-vessel disease than in the 2-vessel disease group.

### Table 1—Clinical characteristics of the study groups

<table>
<thead>
<tr>
<th>Number of significantly stenosed vessels</th>
<th>r</th>
<th>P</th>
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<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td></td>
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<tr>
<td>3</td>
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</tbody>
</table>

Data are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure. *P < 0.01 vs. 0-vessel disease; †P < 0.05 vs. 1-vessel disease; ‡P < 0.05 vs. 0-vessel disease; §P < 0.01 vs. 1-vessel disease; ||P < 0.05 vs. 2-vessel disease.
groups with 0- and 1-vessel disease and significantly correlated with the number of significantly stenosed vessels (r = 0.333, P = 0.027 and r = 0.427, P = 0.004, respectively).

Serum concentrations of AGEs were significantly higher in all subjects with CAD than in control subjects (2.42 ± 0.65 vs. 1.96 ± 0.40 mg/ml, P < 0.01) and significantly correlated with the number of significantly stenosed vessels (r = 0.678, P < 0.001) (Fig. 1). In subjects with NGT, serum concentrations of AGEs were significantly higher in the group with 3-vessel disease than in the groups with 0- and 1-vessel disease and significantly correlated with the number of significantly stenosed vessels (r = 0.701, P < 0.001). In subjects with IGT, serum concentrations of AGEs were significantly higher in the group with 3-vessel disease than in the groups with 0- and 1-vessel disease and significantly correlated with the number of significantly stenosed vessels (r = 0.661, P < 0.001) (Table 1).

There was no correlation between serum concentrations of AGEs and fasting plasma glucose (r = 0.060, P = 0.695), 2-h plasma glucose (r = 0.151, P = 0.321), AUCglu (r = 0.281, P = 0.064), or serum creatinine concentration (r = 0.273, P = 0.070). A similar pattern prevailed between the subgroups (NGT and IGT).

M values significantly inversely correlated with serum concentrations of AGEs (r = −0.490, P < 0.05) (Fig. 2). Multiple regression analysis suggested that serum concentrations of AGEs and 2-h plasma glucose were independently associated with the number of significantly stenosed vessels (Table 2).

Sufficient data are not available to accurately assess the impact of serum AGE levels on the development of macrovascular complications in diabetic patients. However, AGEs may be involved in macrovascular disease. These include activation of monocytes, production of cytokines and growth factors, impairment of endothelial function, modification of LDL, and depletion of nitric oxide. Binding of AGEs to RAGE (receptor of AGE) on cell surfaces, including endothelial and smooth muscle cells, induces an intracellular oxidative stress response characterized by increased activation of transcription factors, e.g., nuclear factor-κB (3). AGE deposits have been demonstrated in the atherosclerotic plaques and myocardium of patients with atherosclerosis (5–7). Circulating AGEs (tissue-derived degradation products) may be produced either by extracellular proteolytic systems or by catabolism by macrophages. Little is known concerning serum levels of AGE in patients with CAD, especially nondiabetic subjects, including those with IGT. We investigated whether serum concentrations of AGEs were elevated in nondiabetic patients with CAD and whether AGE concentration correlated with severity of CAD.

AGEs are very heterogeneous and include pyrraline, pentosidine, crossline, and carboxymethyllysine (CML). CML is a major chemical component of AGEs accumulated in tissue proteins (13) and is found in atherosclerotic plaques (6). Recently, a reliable enzyme-linked immunosorbent assay for AGEs was established. This assay was used in this study and detected primarily CML (and pentosidine to a much lesser degree) (9). Kilhovd et al. (8) reported that serum concentrations of AGEs were significantly increased in patients with type 2 diabetes and CAD than in patients without CAD. However, the diagnostic procedures that were used for CAD, e.g., clinical examination and medical history of angina pectoris or myocardial infarction and resting electrocardiogram (ECG) measurement, with/without exercise ECG (8), are not sufficiently sensitive. In our study, even in nondiabetic subjects, including those with IGT, serum concentrations of AGEs were significantly increased with increasing severity of angiographically documented CAD.

Chronic slight hyperglycemia are pro-oxidant factors. However, Ono et al. (9) reported that serum levels of AGE did not correlate with HbA1c in patients whose creatinine levels were <4.0 mg/dl. Kilhovd et al. (8) also found a lack of significant correlation between serum AGEs and HbA1c. Although we did not determine HbA1c values in all subjects, we consider that the lack of correlation between AGEs and HbA1c (or AUCglu) is probably caused by a different turnover and/or a time lag between AGE production and removal from tissue AGE accumulation. This issue has yet to be investigated.

Renal function also affects serum levels of AGE. Makita et al. (14) reported that serum AGE levels elevated in end-stage renal disease but not in normal renal function. In our study, all subjects had normal renal function (serum creatinine <133 μmol/l). Multiple regression analysis also demonstrated that serum creatinine is not independently associated with the number of significantly stenosed vessels.

Moreover, M values were significantly decreased with increasing serum concentrations of AGEs in our study. Several lines of evidence indicate a relation between hyperinsulinemia/insulin resistance and free-radical production (15). AGE formation may also lead to oxidative stress, but whether oxidative stress precedes or follows insulin resistance is still a question. It has been demonstrated that increased oxidative stress, even in nondiabetic subjects, has a role in insulin resistance because different antioxidative agents, e.g., vitamin C or vitamin E, improve whole-body glucose disposal (16). In vitro studies using an adipocyte cell line model exposed to H2O2 reduced insulin-stimulated glucose transport activity (17). These studies provided evidence to suggest an association between oxidative stress and insulin resistance.
Interestingly, it has been demonstrated that oxidative stress may be associated with a reduced GLUT4 exposition and an impairment of insulin signaling (4). These data are consistent with a pathophysiologic role for glycoxidation of tissue proteins in CAD and suggest that measurement of serum AGE concentrations may be predictive of vascular damage.

There were some limitations in this study. In simple regression analyses, there was no correlation between serum concentrations of AGE and fasting plasma glucose, 2-h plasma glucose, or AUClu because the number of subjects was relatively small. But, we confirmed by multiple regression analysis that 2-h plasma glucose is independently associated with the number of significantly stenosed vessels.

In conclusion, this pilot study indicates the relation between AGEs and the severity of CAD in nondiabetic patients.

References

Table 2—Multiple regression analysis for the number of significantly stenosed vessels as an dependent variable

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>β</th>
<th>SE</th>
<th>P</th>
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<td>AGEs</td>
<td>0.668</td>
<td>0.163</td>
<td>&lt;0.0001</td>
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<tr>
<td>2-h plasma glucose</td>
<td>0.544</td>
<td>0.004</td>
<td>0.0002</td>
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<tr>
<td>AUClu</td>
<td>-0.384</td>
<td>0.058</td>
<td>0.0365</td>
</tr>
<tr>
<td>Serum creatinine</td>
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<td>0.006</td>
<td>0.0609</td>
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<tr>
<td>Fasting plasma glucose</td>
<td>0.258</td>
<td>0.013</td>
<td>0.0751</td>
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β, standard regression coefficient.