Plasma Levels of Soluble Receptor for Advanced Glycation End Products and Coronary Artery Disease in Nondiabetic Men

Colomba Falcone, Enzo Emanuele, Angela D’Angelo, Maria P. Buzzi, Chiara Belvito, Mariacarla Cuccia, Diego Geroldi

Objective—The receptor for advanced glycation end products (RAGE) is a cell surface receptor whose signaling pathway has been implicated in atherogenesis. RAGE has an endogenous secretory receptor form, called soluble RAGE (sRAGE), that could exert antiatherogenic effects by acting as a decoy. We sought to determine whether a decreased plasma level of sRAGE could be independently associated with the prevalence of coronary artery disease (CAD) in nondiabetic men.

Methods and Results—Plasma levels of sRAGE were determined in 328 nondiabetic male patients with angiographically proved CAD and in 328 age-matched healthy controls. The concentration of sRAGE in plasma was significantly lower (P<0.0001) in CAD cases [median (interquartile range): 966 (658–1372) pg/mL] than in control subjects [1335 (936–1954) pg/mL]. In logistic regression analysis, the multivariate-adjusted odds ratio for the presence of CAD was 6.719 (95% confidence interval, 3.773 to 11.964; P<0.0001) when the lowest quartile of the sRAGE level was compared with the highest quartile.

Conclusions—Our findings indicate that low levels of sRAGE in plasma are independently associated with the presence of CAD in nondiabetic men and suggest that sRAGE is one of the clinically important molecules associated with atherosclerosis. (Arterioscler Thromb Vasc Biol. 2005;25:1032-1037.)

Key Words: coronary artery disease ■ risk factor ■ soluble receptor for advanced glycation end products

The established cardiovascular risk factors, including age, male gender, hypercholesterolemia, smoking, hypertension, and diabetes, do not account for the overall risk of coronary artery disease (CAD).1,2 Therefore, there is a growing need for the assessment of novel biomarkers that may help to identify people more prone to CAD.3

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The receptor for advanced glycation end products (RAGE) is a transmembrane receptor of the immunoglobulin superfamily that engages diverse ligands relevant to the pathogenesis of atherosclerosis4 and that is highly hyperexpressed at sites of vascular pathology.5 Numerous lines of evidence indicate that ligand-triggered RAGE-dependent cellular activation results in several deleterious effects, including activation of nuclear factor-κB, increased expression of cytokines and adhesion molecules, and induction of oxidative stress.6–8 One class of RAGE ligands includes glycoxidation products, termed advanced glycation end products (AGEs), which accumulate at an extremely high rate in the diabetic state.9,10 Thus, the role of RAGE in diabetic vasculopathy has been extensively investigated in animal11–13 and human studies.14–16 In any case, it is important to note that studies in rodent models also support a role of RAGE not only in diabetes but also in euglycemic vascular disease.17,18 Despite this, only a few clinical studies have focused on the putative involvement of the AGEs–RAGE axis in human nondiabetic atherosclerosis.19,20

RAGE has a C-truncated secretory isoform of the receptor protein, termed soluble RAGE (sRAGE), that may neutralize the AGEs-mediated damage by acting as a decoy.21–23 Accordingly, administration of recombinant murine sRAGE immediately on the diagnosis of hyperglycemia resulted in a dose-dependent suppression of accelerated atherosclerosis in diabetic apolipoprotein E–null mice.11 In addition, treatment with sRAGE stabilized established atherosclerosis in both euglycemic and diabetic mice deficient in apolipoprotein E in the absence of alterations in glucose levels or lipid profile.17 Of interest, preliminary data have shown that diabetic subjects with
higher sRAGE levels may be more resistant to AGEs than those with lower concentrations.22

Based on these observations, we hypothesized that high levels of endogenous sRAGE may exert antiatherogenic effects by preventing ligand-triggered RAGE-dependent cellular activation. To study the relationship between sRAGE level and CAD in nondiabetic subjects, we measured plasma sRAGE concentrations in nondiabetic male patients with CAD and examined whether sRAGE level is significantly associated with CAD prevalence after adjustment for well-known CAD risk factors.

Methods

Study Population

The study comprised 328 nondiabetic male patients with angiographically documented CAD and 328 control subjects. The patients with CAD were recruited at the Cardiology Department of the University Hospital of Pavia and showed at least 1 coronary stenosis >75% at catheterization within the past 6 months before entry into the study.24 Because of the small number of females undergoing coronary angiography in our center,20 only male subjects were included in the present study. The indications for angiography were suspicion of CAD or preoperative screening for CAD in subjects with valvular disease. Subjects with acute ischemic syndromes, heart failure, or cardiology complications were excluded. Exclusion criteria for all study participants also comprised use of lipid-lowering drugs, acute infection, acute state of a chronic infectious or inflammatory disease, severe liver or renal disease, neoplasm, and hematologic disorders.25

The control subjects were selected from men who visited our affiliated hospitals or clinics for a physical check-up. Controls were characterized by no history of angina and other heart disease, a normal resting ECG, and normal exercise ECG stress testing. They were matched with CAD patients by age. Both patients and controls had no evidence of peripheral artery or cerebrovascular disease, all had normal echo duplex of cervical arteries, the aorta, and lower limbs, and/or resting and postexercise ankle/brachial pressure index >0.85.

All study participants underwent a standard clinical examination. Body mass index was calculated as weight divided by the square of height. Cigarette smoking was dichotomized into ever versus never, with ever smoking defined as having smoked daily for 1 year or more. Many patients had quit after onset of their CAD, hence the designation as ever smoking rather than current and former. Hypertension was defined as a blood pressure >140/90 mm Hg or the use of antihypertensive medications. None of the study participant had diabetes or impaired glucose tolerance according to the criteria of the American Diabetes Association.26 All study participants were white and Italian and gave written informed consent. The study protocol was approved by our local ethics committee.

Laboratory Methods

In patients and controls, blood samples were taken in EDTA-containing tubes after a 14-hour overnight fast for sRAGE quantification as well as determination of lipid parameters. Blood samples were centrifuged at 1000g for 30 minutes and immediately divided into aliquots. Plasma and serum specimens were then frozen and stored at −20°C until analysis. All laboratory determinations were performed in a blinded fashion. Serum triglycerides and total cholesterol were determined by using a standard enzymatic procedure. High-density lipoprotein (HDL) cholesterol was determined enzymatically after precipitation of other lipoproteins with dextran sulfate-magnesium. Plasma lipoprotein(a) [Lp(a)] levels were measured by using an enzyme-linked immunosorbent assay method insensitive to size variation in apolipoprotein(a), as described.27 The lower limit of detection was 0.5 mg/dL. In cases of Lp(a) levels <0.5 mg/dL, the value of 0.5 mg/dL was used for statistical analysis. Corrected low-density lipoprotein (LDL) cholesterol levels were calculated by adjusting for Lp(a) levels.28 Plasma glucose was determined by a glucose oxidase method. Plasma sRAGE levels were determined using a commercially available enzyme-linked immunosorbent assay kit (Quantikine; R&D systems) according to the manufacturer’s protocol. Briefly, a monoclonal antibody against sRAGE was used to capture sRAGE from plasma. Captured sRAGE was detected with a polyclonal antihuman sRAGE antibody. After washing, plates were incubated with streptavidin-HRP, developed with appropriate substrate, and OD₄₅₀ was determined using an enzyme-linked immunosorbent assay plate reader. Measurements were performed in duplicate and the results were averaged. The intra-assay and interassay coefficients of variation values were <6% and <8%, respectively.

Statistical Analyses

The study power was determined by using StatMate 2 for Windows (GraphPad Software). Our sample had a 95% power to detect, between CAD cases and controls, a difference of 172 pg/mL in sRAGE plasma levels with a significance level (alpha) of 0.05 (2-tailed). All other statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc). The Kolmogorov–Smirnov test of normality was used to verify whether the distribution of variables followed a gaussian pattern. Normally distributed data in groups were expressed as means±SDs. Logarithmic transformation was performed on all skewed variables, including triglycerides, Lp(a), fasting plasma glucose, and plasma sRAGE levels. These variables were expressed as median and interquartile ranges. For continuous variables, the differences between the 2 groups were evaluated with an unpaired t test or the Mann–Whitney U test, as appropriate. Categorical variables are presented by frequency counts, and intergroup comparisons were analyzed by a χ² test. The CAD patients and control subjects were categorized in quartiles based on the plasma sRAGE concentration in the entire study cohort. The interquartile cutoff points of plasma sRAGE level were 776, 1138, and 1647 pg/dL. Simple (univariate) and multiple (multivariate) logistic regression analyses were performed to determine the associations between CAD and all other parameters. All baseline variables related to CAD with P<0.1 in simple logistic regression analysis were included into the multivariate model. Crude and multivariate-adjusted odds ratios (ORs) are presented with 95% confidence intervals (CIs). Two-tailed P<0.05 was considered statistically significant.

Results

Subjects Characteristics

The basic characteristics of the study participants are shown in Table 1. The concentration of sRAGE in plasma was significantly lower (P<0.0001) in CAD cases [median (interquartile range): 966 (658–1372) pg/mL] than in control subjects [1335 (936–1954) pg/mL]. The number of subjects according to the quartiles of plasma sRAGE concentration are shown in the Figure. In the first quartile (plasma sRAGE level <776 pg/mL), the number of CAD patients was 2.64-fold higher that of the control subjects (P<0.0001). However, the number of CAD patients in the fourth quartile (plasma sRAGE level ≥1647 pg/mL) was nearly 3-times lower than that of the control subjects (P<0.0001). Univariately, there were not statistically significant differences in the prevalence of CAD patients and controls across the second and third quartiles.

CAD cases were more likely to be ever-smokers and hypertensive compared with controls. In addition, the CAD patients had significantly higher levels of total cholesterol, LDL cholesterol, triglycerides and Lp(a), and lower levels of...
HDL cholesterol. There were no significant differences in age, body mass index, and fasting plasma glucose between the 2 groups.

**sRAGE and CAD**

The results of logistic regression analysis are presented in Table 2. To evaluate each factor in Table 1, simple logistic regression analysis was performed. There were significant differences between the 2 groups in terms of plasma sRAGE level, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, Lp(a), hypertension, and smoking habits. Multiple logistic regression analysis after controlling for triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, Lp(a), hypertension, and smoking habits demonstrated sRAGE as independently correlated with CAD \((P<0.0001)\). The other independent predictors of CAD were hypertension, smoking, and HDL, which were the only fraction of lipids remaining independently associated with coronary atherosclerosis. To evaluate the risk associated with decreasing levels of sRAGE, we calculated crude and multivariate-adjusted ORs for each quartile (based on the entire study cohort) relative to the fourth. As shown in Table 3, the ORs for CAD in the first, second, and third quartiles of sRAGE levels were significantly higher as compared with the fourth.

**sRAGE Levels in the Subpopulation of Normolipidemic Subjects**

To address the clinical need for improved assessment of risk among persons with cholesterol levels currently considered safe, we performed a subgroup analysis of the study participants with LDL cholesterol levels of \(<3.4 \text{ mmol/L (130 mg/dL)}\), the target level for the primary prevention of coronary heart disease according to the National Cholesterol Education Program. By using this cutoff point, normal levels of LDL were found in 49.8% of the study participants \((n=134\) and \(n=193\) in the CAD and control group, respectively). Among this normolipidemic subgroup, CAD patients showed lower plasma levels of sRAGE in comparison with controls \([905 \text{ (658–1372)} \text{ versus } 1301 \text{ (936–1954)} \text{ pg/mL}, \text{ respectively; } P<0.0001]\). After allowance for confounding factors, multivariate logistic regression analysis demonstrated that sRAGE remained an independent determinant of CAD among the individuals with optimal/near optimal levels of LDL. The multivariate-adjusted ORs for CAD in the first, second, and third quartiles of sRAGE levels were 10.256 \((95\% \text{ CI, 3.923 to 26.813; } P<0.0001)\), 3.005 \((95\% \text{ CI, 1.316 to 6.860; } P=0.009)\), and 3.039 \((95\% \text{ CI, 1.181 to 7.821; } P=0.021)\) compared with the fourth quartile.

**Discussion**

To the best of our knowledge, our study is the first to demonstrate an association between plasma sRAGE levels and CAD in nondiabetic males. The multivariate-adjusted ORs for CAD revealed that male subjects with sRAGE levels below 776 pg/dL had a 6.719-fold increase in CAD preva-
ence, independent of established vascular risk factors and lipid parameters. Notably, the independent association between sRAGE levels and CAD was confirmed when analyzing a subgroup of patients with normal levels of LDL cholesterol. This finding appears of interest, because it is estimated that up to half of all CAD cases occur in subjects with moderate to low risk as determined by evaluation of LDL and total cholesterol.3

sRAGE is a soluble receptor produced by alternative splicing of RAGE mRNA21 and is abundantly present in the circulation.22 The mechanism by which plasma sRAGE levels were decreased in CAD patients, however, remains to be clarified. Because sRAGE has been shown to successfully bind to AGEs,30 it has been postulated that this soluble isoform could play an antagonistic role by competing with the cell surface receptor, thus preventing the adverse effects of RAGE signaling.31 Importantly, sRAGE has been previously administered in several animal models of RAGE-mediated disorders, in which it successfully prevented or reversed RAGE effects such as diabetic atherosclerosis,11 altered wound healing,32 and tumor growth and invasion.33 Despite this, the significance of endogenous levels of sRAGE in human disease has been poorly investigated, particularly among nondiabetic subjects. To date, only preliminary data by Yonekura et al reported that diabetic subjects with higher sRAGE levels may be more resistant to AGEs-mediated damage with respect to those with lower concentrations.22

Although the role of AGEs–RAGE interaction is deemed of great importance in diabetic vasculopathy, a growing body of evidence indicates that this signaling pathway can also play a role in nondiabetic atherosclerosis.17,18 Accordingly, deposits of AGEs have been detected not only in patients with CAD and type 2 diabetes34 but also in normoglycemic subjects.35 In addition, Kanauchi et al reported that AGEs concentrations are significantly associated with the presence and severity of CAD in nondiabetic Japanese subjects.19 More recently, we reported a significant association between a functional polymorphism of the RAGE gene promoter with angiographically proven CAD in nondiabetic Italian persons.20 It should be also noted that RAGE may interact not only with AGEs but also with a diverse repertoire of ligands, including S100/calgranulins,36 amphoterin,37 and amyloid-β peptide.38 Thus, it seems reasonable to hypothesize that the effect of RAGE on euglycemic atherosclerosis may be dependent, at least in part, on molecular interactions with proinflammatory ligands other than AGEs.

Because the results of the present study are consistent with a role of RAGE in nondiabetic CAD, future studies are needed to further examine how levels of the soluble form of RAGE could influence atherosclerosis and vascular inflammation. Specifically, studies on the interrelationships be-

### TABLE 2. Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Term</th>
<th>Simple</th>
<th></th>
<th></th>
<th>Multiple</th>
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<tr>
<td></td>
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<td>SE</td>
<td>P</td>
<td>Estimate</td>
<td>SE</td>
<td>P</td>
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<td>0.010</td>
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<td>BMI</td>
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<td>0.756</td>
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<td>Log sRAGE</td>
<td>−3.147</td>
<td>0.387</td>
<td>&lt;0.0001</td>
<td>−3.124</td>
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<td>Log FPG</td>
<td>1.546</td>
<td>1.416</td>
<td>0.275</td>
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<tr>
<td>Total cholesterol</td>
<td>0.179</td>
<td>0.404</td>
<td>0.022</td>
<td>1.781</td>
<td>1.618</td>
<td>0.271</td>
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<tr>
<td>Log triglycerides</td>
<td>1.786</td>
<td>0.396</td>
<td>&lt;0.0001</td>
<td>0.002</td>
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<td>0.998</td>
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<td>HDL cholesterol</td>
<td>−2.154</td>
<td>0.245</td>
<td>&lt;0.0001</td>
<td>−3.840</td>
<td>1.653</td>
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<td>LDL cholesterol</td>
<td>0.335</td>
<td>0.728</td>
<td>&lt;0.0001</td>
<td>−1.655</td>
<td>1.616</td>
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<td>Log Lp(a)</td>
<td>0.273</td>
<td>0.131</td>
<td>0.037</td>
<td>0.174</td>
<td>0.164</td>
<td>0.287</td>
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<td>Hypertension</td>
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<td>0.182</td>
<td>&lt;0.0001</td>
<td>1.830</td>
<td>0.214</td>
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<td>Ever-smoking</td>
<td>0.759</td>
<td>0.163</td>
<td>&lt;0.0001</td>
<td>0.769</td>
<td>0.201</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Bold text indicates significant parameters in simple logistic regression analysis.

FPG indicates fasting plasma glucose; SE, standard error.

### TABLE 3. Crude and Multivariate-Adjusted Odds Ratios for Prevalence of CAD in Each Quartile of sRAGE Concentration Relative to the Fourth

<table>
<thead>
<tr>
<th>Category</th>
<th>Simple Logistic Regression Analysis</th>
<th>Multivariate Logistic Regression Analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR</td>
<td>95% CI</td>
</tr>
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<td>Category 1 vs 4</td>
<td>7.211</td>
<td>4.432–11.733</td>
</tr>
<tr>
<td>Category 2 vs 4</td>
<td>3.103</td>
<td>1.951–4.933</td>
</tr>
<tr>
<td>Category 3 vs 4</td>
<td>2.680</td>
<td>1.686–4.260</td>
</tr>
</tbody>
</table>

*Adjusted for triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, Lp(a), hypertension, and smoking habit.

CI indicates confidence interval; OR, odds ratio.
tween plasma sRAGE concentration and levels of S100 proteins as well as C-reactive protein are warranted.

Several caveats should be considered in the interpretation of our findings. First, an important limitation of our study is measurement of total sRAGE, because the detection system used cannot discriminate between specific sRAGE splice variants. Therefore, it should be kept in mind that reduced sRAGE levels measured by this assay may be caused by a reduction of distinct circulating sRAGE isoforms. Development of assays that detect specifically the different variants of sRAGE will substantially improve assessment of the sRAGE concentrations. Second, our results share the limitations of cross-sectional, observational studies. We evaluated association, not prospective prediction or causation. Although sRAGE was a strong and independent discriminator between CAD cases and the control group, there was considerable overlap in sRAGE levels in the 2 groups. Third, the presence of occult ischemic heart disease in the control group cannot be ruled out and may have attenuated the differences among the 2 groups. Finally, because male sex is an important risk factor for CAD, in the current study we specifically focused on a representative group of white Italian men. The present data therefore cannot be generalized to other ethnic groups, women, or other populations.

Despite these limitations, our present report is the first investigation to our knowledge on the clinical significance of plasma sRAGE levels in any human disease. Specifically, our results strongly suggest that the soluble decoy receptor sRAGE is one of the clinically important molecules associated with atherosclerosis and that measurement of its plasma level will be helpful to evaluate CAD risk. Our data of the reduced sRAGE levels in CAD subjects with normal levels of LDL cholesterol could also raise the possibility that the measurement of sRAGE level may improve risk assessment among normolipidemic subjects. Future studies should examine whether these differences in sRAGE levels have an implication for asymptomatic subjects as well as for acute coronary syndromes.

Acknowledgments

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