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Circulating Soluble Receptor for Advanced Glycation End Products Is Inversely Associated with Glycemic Control and S100A12 Protein

Giuseppina Basta, Anna Maria Sironi, Guido Lazzerini, Serena Del Turco, Emma Buzzigoli, Arturo Casolaro, Andrea Natali, Ele Ferrannini, and Amalia Gastaldelli

Consiglio Nazionale delle Richerche Institute of Clinical Physiology (G.B., A.M.S., G.L., S.D.T., E.B., E.F., A.G.) and Department of Internal Medicine (A.C., A.N., E.F.), University of Pisa School of Medicine, 56124 Pisa, Italy

Context: The interaction of advanced glycation end products, including *N*ε-(carboxymethyl)lysine-protein adducts (CML) and S100A12 protein, with their cellular receptor (RAGE) is implicated in the pathogenesis of diabetic vascular complications. RAGE has a circulating secretory receptor form, soluble RAGE (sRAGE), which, by neutralizing the action of advanced glycation end products, might exert a protective role against the development of cardiovascular disease.

Objective: The objective of the study was to investigate whether plasma sRAGE levels are associated with glycemic control, proinflammatory factors, or circulating ligands of RAGE such as plasma CML and S100A12 protein.

Study Design: We studied 160 subjects, 84 subjects with type 2 diabetes (aged 60 ± 7 yr) and 76 nondiabetic controls (aged 45 ± 10 yr).

Results: Plasma sRAGE was lower in diabetic patients than controls [141 (53–345) *vs.* 735 (519–1001) pg/ml, median (interquartile range), $P < 0.0001$], whereas CML levels were higher in diabetic patients than controls [67.9 (46.0–84.7) *vs.* 43.4 (28.0–65.0) μg/ml, $P < 0.0001$]. In stepwise regression analysis of the whole data set, hemoglobin A_{1c}, insulin resistance (as homeostasis model assessment), and C-reactive protein were independently associated with plasma sRAGE, whereas age was not. In a subgroup of 26 diabetic and 24 nondiabetic subjects of similar age (54 ± 3 yr), plasma S100A12 levels were higher in diabetic subjects [49 (39–126) *vs.* 28 (21–39) ng/ml]. Moreover, low sRAGE and high S100A12 were strongly associated with increased risk for cardiovascular disease (Framingham score). In this subgroup, the plasma S100A12 level was the only determinant of plasma sRAGE concentration.

Conclusion: Plasma level of sRAGE is down-regulated in chronic hyperglycemia; among its ligands, S100A12 protein, but not CML, appears to be associated with this effect. (***J Clin Endocrinol Metab* 91: 4628–4634, 2006**)

DRIVEN BY HYPERGLYCEMIA and oxidant stress, advanced glycation end products (AGEs), including *N*ε-(carboxymethyl)lysine-protein adducts (CML), are formed in a greatly accelerated degree in diabetes (1, 2). Accumulation of AGEs and the enhanced tissue expression of the main receptor for AGEs (RAGE) may contribute to vascular damage (3, 4). Two classes of RAGE ligands, AGEs and S100A12 proteins, appear to drive receptor-mediated cellular activation and, potentially, perturbation of a variety of homeostatic functions of the vasculature (5, 6). S100A12, also called ENRAGE (extracellular newly identified receptor for AGE binding protein), is a proinflammatory cytokine expressed mainly by granulocytes and is involved in Ca^{+}-dependent signal transduction events (7). Recently it has been reported that human vascular cells express several RAGE variant proteins, including three novel human RAGE transcripts (8), all encoding truncated soluble forms of RAGE (sRAGE), consisting of only the extracellular ligand binding domain and lacking the cytosolic and transmembrane domains. Due to these characteristics, the sRAGE could represent a naturally occurring competitive inhibitor of the signaling pathways induced by the interaction of AGEs with its cellular receptor; by functioning as a decoy, sRAGE may well contribute to the removal/neutralization of circulating RAGE-ligands (8).

In animal models of streptozotocin-induced diabetes or genetically manipulated mice, it has been demonstrated that treatment with sRAGE, produced by recombinant technology, prevents cell-bound RAGE signaling (9–11). Administration of sRAGE to experimental animals predisposed to exaggerated neointimal expansion significantly limited atherosclerosis and restenosis (12). In humans, clinical studies in nondiabetic subjects have shown that higher levels of endogenous sRAGE may be associated with a lower incidence of coronary artery disease (13), arthritis (14), and hypertension (15). Also, it has been suggested that in type 1 diabetic subjects, higher sRAGE levels confer resistance to AGEs (16) and that plasma sRAGE levels were inversely associated with components of the metabolic syndrome (17).

The aim of the present study was to investigate whether plasma sRAGE levels are associated with glycemic control, proinflammatory factors, or circulating ligands of RAGE such as plasma CML and S100A12 protein.

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Abbreviations: AGE, Advanced glycation end products; CML, *N*ε-(carboxymethyl)lysine-protein adducts; CRP, C-reactive protein; CVD, cardiovascular disease; HbA_{1c}, glycosylated hemoglobin; HOMA, homeostasis model assessment; Ln, natural logarithm; RAGE, AGE receptor; RAS, renin-angiotensin-system; RT, room temperature; sRAGE, soluble RAGE; WBC, white blood cell.

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Subjects and Methods

Subjects

A total of 160 subjects, 84 with type 2 diabetes and 76 nondiabetic subjects, were studied (Table 1). Nondiabetic subjects were recruited according to the following criteria: 1) absence of diabetes on the oral glucose tolerance test (18), 2) absence of metabolic or other diseases, and 3) no treatment with drugs known to affect glucose tolerance. Diabetic patients were in good general health without evidence of cardiac, hepatic, renal, or chronic inflammatory diseases or other chronic diseases, as determined by history, examination, and routine blood chemistry. Patients had normal serum creatinine (*i.e.* <1.2 mg/dl) and were not taking any medication known to affect glucose tolerance; 21 diabetic subjects were on renin-angiotensin-system (RAS) blockade. Hypertension was defined as blood pressure greater than 140/90 mm Hg or the use of antihypertensive medications. Twenty-three nondiabetic subjects were newly diagnosed with essential hypertension, and none was on antihypertensive treatment. Forty-four diabetic subjects were also hypertensive, of whom 29 were on antihypertensive treatment. No subject was involved in strenuous physical activity, and body weight was stable (±2 kg) for at least 3 months before the study.

The study protocol was approved by the Institutional Review Board of the University of Pisa, and all subjects gave written informed consent to the study.

Laboratory methods

Blood samples were drawn in tubes without additives, containing heparin or citrate (for routine biochemistry). Fasting plasma glucose, glycosylated hemoglobin (HbA_{1c}), fasting plasma insulin, total cholesterol, triglycerides, white blood cells (WBCs), C-reactive protein (CRP), and creatinine were determined as described previously (18, 19). For CML, sRAGE, and S100A12 determination, blood samples were collected in tubes containing Na_2EDTA and aprotinin, centrifuged at 4 C and immediately divided in aliquots. Plasma and serum samples were stored at −80 C until analysis.

Determination of plasma CML levels

Plasma CML levels were measured in triplicate by an in-house competitive ELISA using the mouse F(ab')2 fragment anti-AGE monoclonal antibody (clone 6D12) (ICN Biochemical Division, Aurora, OH), as described previously (20). The lower limit of detection of CML was 0.5 μg/ml.

Determination of plasma sRAGE levels

Plasma sRAGE levels were determined using an ELISA kit (DuoSet ELISA development kit; R&D Systems, Minneapolis, MN) containing the basic components required for the development of double-sandwich ELISAs. Briefly, 96-well microplates (Nunc-Immuno Plates Maxisorp, Nunk, Roskilde, Denmark) were coated with monoclonal antihuman RAGE (1 μg/ml) in coating buffer [10 mmol/liter PBS (pH 7.2)] and incubated overnight at room temperature (RT). Four rinses with washing buffer followed each incubation step. After blocking with reagent buffer (1% BSA in PBS) at RT for 1 h, 100 μl of sample diluted in reagent diluent was added and incubated at RT for 2 h. Recombinant human RAGE was used as standard in a concentration range of 31.2–4000 pg/ml. Then 100 μl of biotinylated goat antihuman RAGE (200 ng/ml) was added and incubated at RT for 2 h. Next, 100 μl of streptavidin-horseradish peroxidase (1:200) was added and incubated for 20 min at RT. Finally, 100 μl of tetramethybenzidine substrate (Sigma, St. Louis, MO) was added. After 5–30 min, 50 μl of 2 mol/liter sulfuric acid was added to stop the reaction and OD at 450 nm determined by an ELISA plate reader.

The intraassay and interassay coefficients of variation were less than 5.9% and less than 8.2%, respectively. The lower limit of detection of sRAGE was 21.5 pg/ml. According to the manufacturer, no significant cross-reactivity with EN-RAGE, high-mobility group box 1, S100A10 or S100Baa, and CML was observed.

Determination of plasma S100A12 levels

Concentrations of S100A12 were determined using a double-sandwich ELISA system established in our laboratory. The 96-well microplates (Nunc-Immuno Plates) were coated with 50 ng/ml antihuman S100A12 antibody (R&D Systems) in 50 mmol/liter sodium bicarbonate buffer (pH 9.6) and incubated for 16 h at 4 C. Four rinses with washing buffer followed each incubation step. After blocking with 100 μl blocking buffer [1% BSA in 20 mmol/liter HEPES (pH 7.4)] at RT for 1 h, 100 μl of sample in at least three dilutions using blocking buffer containing 1 mmol/liter $CaCL_2$ and 0.05% Tween 20 was added and incubated at RT for 2 h. Recombinant S100A12 protein (21, 22) was kindly supplied by Dr. Olga Moroz and used as standard in a concentration range of 0.1–50 ng/ml. Then 100 μl of biotinylated goat antihuman S100A12 (R&D Systems) (100 ng/ml) was added and incubated at RT for 2 h. Next, 100 μl of streptavidin-horseradish peroxidase was added and incubated for 20 min at RT. Finally, 100 μl of tetramethybenzidine substrate (Sigma) was added. After 5–30 min, 50 μl of 2 mol/liter sulfuric acid was added to stop the reaction and OD at 450 nm determined by an ELISA plate reader. The lower limit of detection of S100A12 was 0.05 ng/ml. The intraassay and interassay coefficients of variation were less than 7.9% and less than 8.0%, respectively.

Statistical analysis

Data are given as the mean ± SD. Variables with a skewed distribution are expressed as median and interquartile range. Group differences were analyzed by Student *t* test, Mann Whitney *U* test, and χ^2 test for normally distributed, nonnormally distributed, and noncontinuous variables, respectively.

Relationships between natural logarithm of plasma sRAGE, Ln(sRAGE), and other factors were first assessed by Pearson correlation analysis; for these, variables with a skewed distribution were log transformed. Normal distribution was checked using the Z test. All variables significantly associated with Ln(sRAGE) were included in the multivariate analysis plus gender and BMI as confounding factors. We then considered, one at a time, the explanatory variables by stepwise regression analysis by adding to the model at each step the variable that explained most variation in Ln(sRAGE) and stopping when the addition of an extra variable made no significant improvement in the amount of variation explained. In Tables 2 and 4, we reported only significant associations as simple or partial (in stepwise analysis) correlation coefficients and amount of variation explained (R^2 adjusted). In Table 4, we used a hierarchical approach by performing the multivariate analysis with all variables that were significant in simple correlation analysis (model 1) and sequentially excluding the variables that resulted as significant (models 2 and 3) in the multivariate approach. The 10-yr cardiovascular disease (CVD) risk was calculated by the Framingham score (23).

By *post hoc* power analysis, the current number of controls and patients was sufficient to detect a significant correlation between variables. In the entire group (n = 160), assuming a type I error of 5% and a power of 90%, the minimum association that could be detected was r = 0.23. In the age-selected subgroup, specifying a minimum correlation of 0.3 and assuming a type I error of 5%, we obtained a power greater than 90%, in the association of Ln(sRAGE) with HbA1c and Ln(S100A12).

Results

Diabetic patients were older than the controls but were similar for gender, BMI, and blood pressure (Table 1). Patients with diabetes had higher values of fasting plasma glucose, HbA_{1c}, insulin resistance [homeostasis model assessment (HOMA)], triglycerides, and white blood cell count. Circulating plasma sRAGE levels were lower, and CML levels were higher, in diabetic subjects than controls (Table 1). Use of RAS-blocking agents had no effect on plasma sRAGE levels; treated (n = 21) and untreated (n = 23) diabetic patients had similar values [182 (318) *vs.* 125 (284) pg/ml, respectively, p = ns].

In the whole data set, in simple correlation analysis,

TABLE 1. Clinical characteristics

	Control subjects (n = 76)	Diabetic patients (n = 84)	*P* value
Gender (female/male)	23/53	25/59	ns
Age (yr)	45 ± 10	60 ± 7	<0.0001
HT (yes/no)	23/53	44/40	<0.005
BMI ($kg \cdot m^{-2}$)	28.0 ± 3.9	28.8 ± 3.5	ns
SBP (mm Hg)	131 ± 17	133 ± 15	ns
DBP (mm Hg)	76 ± 14	78 ± 8	ns
sRAGE (pg/ml)	735 (519–1001)	141 (53–345)	<0.0001
CML (μg/ml)	43.4 (28.0–65.0)	67.9 (46.0–84.7)	<0.0001
CRP (mg/dl)	0.1 (0–0.2)	0.4 (0.3–0.4)	<0.0001
Fasting plasma glucose (mg/dl)	98 ± 12	158 ± 38	<0.0001
HbA_{1c} (%)	4.9 ± 0.4	7.3 ± 1.0	<0.0001
Fasting plasma insulin (mU/liter)	12.2 (9.9–17.1)	12.7 (9.4–16.1)	ns
HOMA (mM·pM)	21.0 ± 11.4	32.8 ± 18.2	<0.0001
Cholesterol (mg/dl)	194 ± 34	198 ± 39	ns
Triglycerides (mg/dl)	84 (56–123)	113 (93–152)	<0.0001
WBC ($10^3/\mu l$)	5.9 ± 1.1	6.4 ± 1.6	<0.02

HT, Hypertension; BMI, body mass index; SBP/DBP, systolic/diastolic blood pressure; ns, not significant.

sRAGE levels were inversely associated with age, fasting plasma glucose, HbA_{1c}, HOMA, triglycerides, and CRP levels (Table 2 and Fig. 1). No correlation was found with BMI, total cholesterol, blood pressure, white blood cell count, creatinine, or CML levels (Table 2). The reciprocal relationship between sRAGE and HbA_{1c} was the strongest, with patients and controls falling on the same regression line (Fig. 1). When the diabetic patients and controls were analyzed separately, fasting plasma glucose, HbA_{1c}, and HOMA remained significantly associated with sRAGE in diabetic patients, whereas CRP levels remained inversely correlated with sRAGE levels in controls (Table 2). In stepwise multiple regression analysis, only HbA_{1c}, HOMA, and CRP remained independent determinants of plasma sRAGE concentrations. When analyses were performed separately in the two groups, HbA_{1c} and CRP in nondiabetic subjects, and HbA_{1c} in diabetic patients, were identified as independent predictors of plasma sRAGE (Table 2).

Because the age difference between controls and diabetic patients could bias the results, we analyzed a subgroup of subjects between 47 and 61 yr of age (24 controls and 26 diabetic subjects), in whom we also determined the plasma levels of the proinflammatory RAGE ligand, S100A12. In this subgroup as in the whole population, fasting plasma glucose, HbA_{1c}, HOMA, and CRP, but not triglycerides, leukocytes, or CML, were higher in diabetic subjects, compared with age-selected controls; moreover, sRAGE levels were lower in diabetic subjects, whereas S100A12 levels were lower in controls (Table 3). Correlation analysis of these data showed that sRAGE levels were inversely associated with fasting plasma glucose, HbA_{1c}, HOMA, CRP, and S100A12 (Table 4). In addition, decreased sRAGE and increased S100A12 were strongly associated with an increased CVD risk (Fig. 2). In multivariate analysis, only S100A12 was significantly associated with plasma sRAGE (Table 4, model 1). When S100A12 was omitted, HbA_{1c} and CRP were then selected as independent determinants of sRAGE (Table 4, model 2). By removing these variables from the model, the only factor that

TABLE 2. Simple correlation and stepwise multivariate analysis of the factors associated with plasma sRAGE levels

	Ln(sRAGE)		
	All subjects, r (*P* value)	Control subjects, r (*P* value)	Diabetic patients, r (*P* value)
Simple correlation			
Age	−0.37 (<0.0001)	ns	ns
Fasting plasma glucose	−0.66 (<0.0001)	ns	−0.46 (<0.0001)
HbA_{1c}	−0.66 (<0.0001)	ns	−0.35 (0.0014)
Ln(triglycerides)	−0.25 (0.0017)	ns	ns
Ln(insulin)	ns	ns	ns
Ln(creatinine)	ns	ns	ns
HOMA	−0.39 (<0.0001)	ns	−0.25 (0.026)
Ln(CRP)	−0.48 (<0.0001)	−0.61 (0.0026)	ns
Multivariate analysis			
Gender	ns	ns	ns
BMI	ns	ns	ns
Age	ns	ns	ns
HbA_{1c}	−0.43	−0.33	−0.29
Ln(triglycerides)	Ns	ns	ns
HOMA	−0.14	ns	ns
Ln(CRP)	−0.19	−0.76	ns
Adjusted R^2	0.42 (<0.0001)	0.48 (0.001)	0.11 (0.002)

In the simple correlation analysis, we reported the correlation coefficients of statistically significant variables. In the multivariate analysis, we included all variables significantly associated with sRAGE in simple analysis plus gender and BMI as possible confounding factors; partial correlation coefficients of statistically significant variables were reported. BMI, Body mass index; ns, not significant.

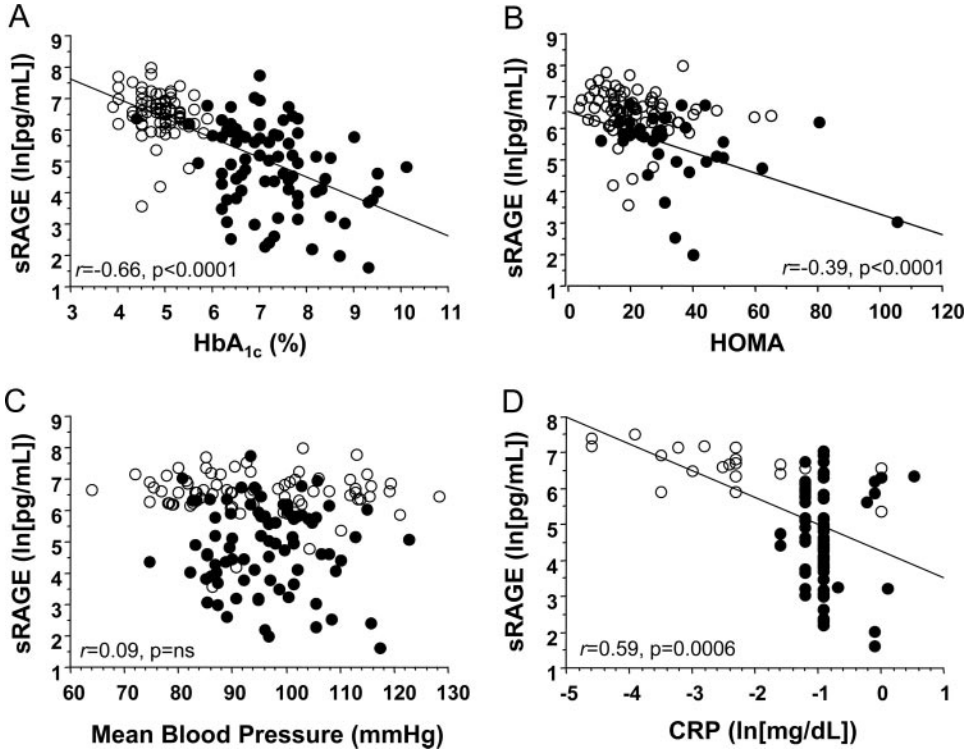

FIG. 1. Correlations between plasma sRAGE concentrations and HbA_{1c} (A), HOMA (B), mean arterial blood pressure (C), and CRP (D) in the entire group of study subjects (n = 160). *Open* and *closed circles* represent nondiabetic and diabetic subjects, respectively.

remained independently associated was HOMA (Table 4, model 3).

Discussion

A number of studies have suggested that administration of sRAGE is protective against diabetic complications (10, 24, 25), and low levels of sRAGE have been indicated as a possible new marker in the predisposition to CVD (26). In the present report, we provide evidence that plasma levels of sRAGE are decreased in diabetic patients, and, more importantly, they are tightly correlated with glycemic control, HOMA, and the inflammatory marker CRP. In addition, in a subgroup of subjects between 47 and 61 yr of age, we document an inverse association between plasma sRAGE and its ligand S100A12.

We found HbA_{1c} to be a significant independent factor inversely associated with plasma sRAGE levels in both diabetic and nondiabetic subjects. CRP levels, on the other hand, were inversely associated only in the nondiabetic group, possibly because in diabetic subjects CRP had a narrow distribution (Fig 1). Recently another study has described HbA_{1c} and CRP as major factors determining plasma sRAGE but only in nondiabetic subjects (17). However, both studies confirm that sRAGE levels are associated with glycemic control and inflammation; the discrepancy may be due to the fact that we measured total sRAGE levels, whereas Koyama *et al.* (17) measured a specific variant of sRAGE. Importantly, we have shown the inverse relationship between plasma sRAGE and S100A12, a relevant proinflammatory molecule strongly linked with RAGE signaling. This

TABLE 3. Clinical characteristics of the age-selected subgroup

	Control subjects (n = 24)	Diabetic patients (n = 26)	*P* value
Gender (female/male)	7/17	5/21	ns
Age (yr)	54 ± 3	54 ± 3	ns
BMI ($kg \cdot m^{-2}$)	29.2 ± 4.1	29.9 ± 4.2	ns
SBP (mm Hg)	140 ± 18	134 ± 15	ns
DBP (mm Hg)	83 ± 10	80 ± 7	ns
sRAGE (pg/ml)	816 (539–1138)	82 (25–378)	<0.0001
CML (μg /ml)	44.4 (26.9–64.4)	56.8 (44.6–77.1)	ns
S100A12 (ng/ml)	28 (21–39)	49 (39–126)	<0.0001
CRP (mg/dl)	0.1 (0–0.1)	0.4 (0.3–0.4)	<0.003
Fasting plasma glucose (mg/dl)	99 ± 19	151 ± 38	<0.0001
HbA_{1c} (%)	5.0 ± 0.4	7.2 ± 1.1	<0.0001
Fasting plasma insulin (mU/liter)	12.0 (9.6–17.6)	14.6 (10–19.7)	ns
HOMA (mM·pM)	21.6 ± 13.0	38.1 ± 22.4	0.003
Cholesterol {mg/dl)	207 ± 31	203 ± 33	ns
Triglycerides (mg/dl)	112 (83–164)	111 (93–199)	ns
WBC ($10^3/\mu l$)	5.9 ± 1.1	6.3 ± 1.3	ns

BMI, Body mass index; SBP/DBP, systolic/diastolic blood pressure; ns, not significant.

TABLE 4. Simple correlation and stepwise multivariate analysis of the factors associated with sRAGE levels in the age-selected subgroup

	Ln(sRAGE)		
	All subjects, r (*P* value)	Control subjects, r (*P* value)	Diabetic patients, r (*P* value)
Simple correlation			
Fasting plasma glucose	−0.64 (<0.0001)	ns	−0.43 (0.027)
HbA_{1c}	−0.67 (<0.0001)	ns	−0.39 (<0.05)
HOMA	−0.41 (0.003)	ns	ns
Ln(CRP)	−0.59 (<0.0004)	−0.78 (0.037)	ns
Ln(S100A12)	−0.64 (<0.0001)	ns	−0.69 (<0.0001)
Multivariate analysis	Model 1	Model 2	Model 3
Gender	ns	ns	ns
BMI	ns	ns	ns
HbA_{1c}	ns	−0.37	—
HOMA	ns	ns	−0.38
Ln(CRP)	ns	−0.30	—
Ln(S100A12)	−0.55	—	—
Adjusted R^2	0.58 (0.0001)	0.40 (0.0004)	0.23 (0.001)

In the simple correlation analysis, we reported the correlation coefficients of statistically significant variables. Partial correlation coefficients of statistically significant variables were reported for the multivariate analysis. Models 1, 2, and 3 are described in *Subjects and Methods*. Dashes correspond to the absence in the model of indicated variable. BMI, Body mass index; ns, not significant.

finding suggests that S100A12 could be involved in a negative-feedback mechanism in the RAGE signaling pathway. Further studies will be needed to elucidate the cellular sources of sRAGE and confirm that S100A12 and other cytokines are involved in its regulation/production.

A role of AGEs and oxidative stress in the development of insulin resistance has been hypothesized (27, 28). Several epidemiological studies have shown a relationship between hyperglycemia and CVD in the general population (27); additionally, postprandial hyperglycemia has been indicated as an independent predictor of increased intima-media thickness in both nondiabetic (29) and diabetic subjects (30). The lower levels of sRAGE detected in diabetic patients might indicate an enhanced propensity to diabetic vascular complications, given that RAGE ligands can interact freely with cell-bound RAGE signaling (4). In support of this possibility, we found that subjects between 47 and 61 yr of age with low sRAGE levels carried a higher CVD risk (by Framingham score). These data together with the inverse association between sRAGE and atherosclerosis previously published (13, 17) suggest a role for sRAGE as a novel marker in the development of CVD.

Recent studies have shown an inverse relationship between sRAGE and blood pressure (15, 17). In the present series, no relationship was found between sRAGE and any measure of blood pressure (Fig. 1), in either the whole group or subgroups regardless of antihypertensive treatments. In the study by Geroldi *et al.* (15), the relationship was present

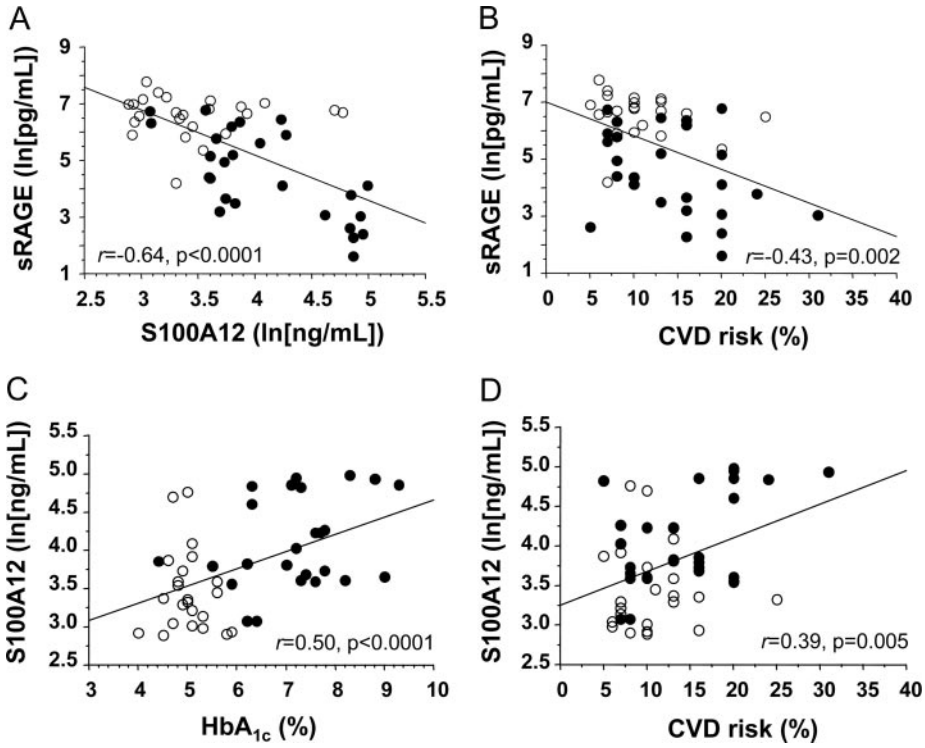

FIG. 2. Correlations between plasma sRAGE levels and S100A12 (A) and CVD risk calculated as Framingham score (B); the correlation between S100A12 and HbA_{1c} (C) or CVD risk (D) refers to a subgroup of age-selected subjects (n = 50). *Open* and *closed circles* represent nondiabetic and diabetic subjects, respectively.

only with pulse pressure, and in the study by Koyama *et al.* (17), only diastolic pressure correlated in the diabetic group, suggesting that subject selection may account for the differences. We also investigated the possible effect of RAS blockade on sRAGE levels because it has been postulated that angiotensin-converting enzyme inhibition reduces the accumulation of AGE products in diabetes partly by increasing production and secretion of plasma sRAGE (31). In our diabetic patients, the sRAGE values of those treated with angiotensin-converting enzyme inhibitors tended to be higher than those of untreated patients, but only a prospective trial can definitively prove this predicament.

The mechanism by which plasma sRAGE levels are decreased in diabetic patients is unknown. One possibility is that hyperglycemia inhibits sRAGE production directly or via increased cytokines and/or hyperglycemia-induced AGEs. Alternatively, lower sRAGE levels could be due to an increased clearance of AGE ligand/sRAGE complexes (32). However, plasma CML levels were found to not correlate with sRAGE levels, despite being higher in diabetic patients than controls, suggesting that CML may not be involved in sRAGE modulation. On the other hand, non-CML AGEs, representing the extent of glycation, could be inversely associated to sRAGE; in fact, it has been reported that non-CML AGE levels are associated with both fasting glucose and HbA_{1c} levels in diabetic patients (33), and we have found a strong inverse association between sRAGE and HbA_{1c}.

In the subgroup of subjects between 47 and 61 yr of age, we also determined the plasma levels of the RAGE-ligand, S100A12, a molecule closely linked with RAGE signaling, which has been reported to be elevated in many chronic inflammatory diseases (34–36). As previously described (37), plasma S100A12 levels were related to subclinical inflammation (CRP) and glycemic control. Thus, it can be hypothesized that hyperglycemia itself might stimulate S100A12 protein production, either directly in granulocytes, monocytes, or other cells or indirectly via hyperglycemia-produced cytokines, *e.g.* IL-6, because recently it has been reported in human macrophages (38). The inverse correlation between S100A12 and sRAGE suggests that insulin resistance, by promoting hyperglycemia and inflammation, may up-regulate S100A12 release, which in turn decreases plasma sRAGE levels.

This study has some limitations. First, the system so far developed for sRAGE determination cannot distinguish among diverse sRAGE splice variants. Because the proportion of diverse isoforms of sRAGE could differ among individuals, the development of new specific assays will improve the evaluation of sRAGE levels. Second, because S100A12 protein has been measured in a small subgroup of subjects, our study also had insufficient power to evaluate, in multivariate analysis, the relation with sRAGE and the other parameters; this important caveat limits the generalizability of the conclusions, and further studies need to be done to confirm in larger clinical studies these important relationships. Third, whether lower sRAGE levels in diabetic patients are the consequence of diabetes or a causal factor of the disease needs to be elucidated. Prospective and intervention studies are necessary to clarify the cause-effect relationships among sRAGE, hyperglycemia, and S100A12 protein.

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Address all correspondence and requests for reprints to: Giuseppina Basta, DBiol, Consiglio Nazionale delle Richerche Institute of Clinical Physiology, Via Moruzzi, 1, 56124 Pisa, Italy. E-mail: lapina@ifc.cnr.it.

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