Increased Serum Concentrations of Soluble Receptor for Advanced Glycation Endproducts in Patients with Type 1 Diabetes

To the Editor:

The hyperglycemic state occurring in diabetes mellitus is responsible for formation and accumulation of advanced glycation endproducts (AGEs) that participate in the pathogenesis of vascular complications of diabetes mellitus *(1)*. There is considerable interest in the receptors for AGEs (RAGE) found in many cell types, particularly those affected in diabetes. RAGE are members of the immunoglobulin family of cell surface molecules with a diverse repertoire of ligands. Interaction of AGEs with RAGE alters several cell functions through modulation of intracellular signaling, gene expression, and release of proinflammatory molecules and free radicals that contribute to the pathology of diabetic complications *(2, 3)*. A soluble form of RAGE (sRAGE) can be measured in peripheral blood, which could result from the expression of a RAGE splice gene variant that encodes an amino-terminally truncated form of the receptor and/or from the cleavage of the native membranous receptor *(4)*. This form may be released by several cell types, such as endothelial cells and circulating leukocytes. Because sRAGE is presumed to be a decoy for RAGE ligands and thus to have cytoprotective properties against AGE actions, we measured circulating sRAGE concentrations in patients with type 1 diabetes and compared it with values for nondiabetic controls.

We collected blood samples from 45 patients with type 1 diabetes [mean (SD) age, 40 (5) years; range, 23–75 years]. Patients with macrovascular complications of diabetes mellitus (clinical evidence or history of coronary artery disease), nephropathy (urinary albumin excretion >30 mg/24 h), or peripheral neuropathy, as well as those with known hepatic or rheumatoid disease, recent acute illness, alcoholism, or treatment with antiinflammatory medications were not included. Nine diabetic patients presented with moderate to severe retinopathy [classification of the French Association for The Study of Diabetes and Metabolic Diseases (ALFEDIAM) *(5)*], as assessed by direct ophthalmoscopy through dilated pupils. A control group of healthy persons [n = 35; mean (SD) age, 43 (10) years; range, 25–63 years] was also recruited. On the day of the clinical examination, blood samples were taken by venipuncture after a 12-h overnight fast. Glycohemoglobin (Hb A_{1c}) was measured by an HPLC method (Tosoh); the reference interval for this assay was 4.0%–5.6%. High-sensitivity C-reactive protein (hsCRP) was measured with an immunonephelometric assay on a BNII analyzer (Dade-Behring). Serum concentrations of sRAGE were measured by the Quantikine RAGE enzyme-linked immunoassay (R&D Systems). Intraassay CVs were <7%, and between-assay CVs were <9%. Statistical analysis was carried out with the nonparametric Mann–Whitney test; P values <0.05 were considered statistically significant.

The results of these analyses are shown in Table 1. Mean (SD) values for serum RAGE were significantly higher in patients with type 1 diabetes than in nondiabetic controls [1320 (459) and 1041 (392) ng/L, respectively; P <0.001]. Median values were similarly different between the two groups: 1343 and 953 ng/L, respectively, for patients with type 1 diabetes and controls (P <0.001). Circulating concentrations of sRAGE were also significantly higher in patients with type 1 diabetes with moderate/severe retinopathy than in controls (Table 1); however, values for diabetic patients with ocular complications did not differ significantly from the values for patients free of retinopathy. Finally, we found no associations between circulating concentrations of sRAGE and duration of diabetes (r = −0.31), Hb A_{1c} concentrations (r = −0.169), or hsCRP concentrations (r = −0.228; not significant for all three).

Substantial evidence supports a role for AGE/RAGE interactions in the pathophysiology of diabetes. Accumulation of RAGE ligands, as occurs in the diabetic state, leads to enhancement of cellular expression of the receptor, thereby triggering cell dysfunction. Little is known about the regulatory aspects of RAGE and sRAGE gene expression; we can presume that in diabetes mellitus, increased formation of AGEs, which are positive regulators of cell expression of RAGE, enhances secretion of sRAGE in a similar manner. On the other hand, the lack of association between sRAGE concentrations and Hb A_{1c} values or diabetes duration is unexpected because these two variables have been shown to correlate with AGEs *(6)*. The observed increase in sRAGE in diabetic patients, however, can be viewed as a protective reaction to counterbalance, at least partly, the increase in AGE formation in these patients. Recently, Falcone et al. *(7)* reported low concentrations of sRAGE in plasma in nondiabetic patients with coronary artery disease. This result does not disagree with ours, if we consider that the soluble isoform of RAGE could play an antagonistic role by competing with the cell-surface receptor. However, the pathophysiologic mechanisms that are responsible for the increase in circulating concentrations of sRAGE in diabetes or its decrease in coronary artery disease remain to be clarified.

Finally, although our cohort of patients with retinopathy was limited, we failed to observe a significant difference between serum sRAGE concentrations in diabetic patients with retinopathy and concentrations in diabetic patients free of ocular complications. An unpublished observation of Yonekura et al. *(2)* suggests that patients with retinopathy have significantly lower circulating sRAGE concentrations than patients without retinopathy. We did not observe such a decrease; further studies are therefore required to elucidate the potential relevance of sRAGE

Table 1. Blood concentrations of RAGE and of biological variables in patients with type 1 diabetes and healthy controls.[a]

	Patients with diabetes			
	All patients	Without retinopathy	With retinopathy	Controls
n	45	36	9	35
Age, years	40 (15)	39 (15)	47 (13)	43 (10)
Diabetes duration, years	14 (11)	11 (10)	23 (13)[b]	
Hb A_{1c}, %	8.5 (1.7)	8.6 (1.8)	8.4 (1.4)	
hsCRP, mg/L	1.66 (1.79)	1.31 (1.47)	3.04 (2.33)	1.45 (0.77)
sRAGE, ng/L	1320 (459)[c]	1317 (430)[c]	1332 (590)[b]	1041 (392)

[a] All values are the mean (SD).
[b,c] Compared with control group: [b] $P < 0.05$; [c] $P < 0.01$.

measurements in the diagnosis of complications of diabetes mellitus.

[2] *Service de Diabétologie*
Groupe Hospitalier Pitié-Salpêtrière
Paris, France

References

1. Ahmed N. Advanced glycation endproducts. Role in pathology of diabetic complications. Diabetes Res Clin Pract 2005;67:3–21.
2. Yonekura H, Yamamoto Y, Sakurai S, Watanabe T, Yamamoto H. Roles of the receptor for advanced glycation endproducts in diabetes-induced vascular injury. J Pharmacol Sci 2005;97:305–11.
3. Stern DM, Yan SD, Yan SF, Schmidt AM. Receptor for advanced glycation endproducts (RAGE) and the complications of diabetes. Ageing Res Rev 2002;1:1–15.
4. Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, Abedin MJ, Li H, et al. Novel splice variants of the receptor for advanced glycation endproducts expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem J 2003;370:1097–109.
5. Massin P, Angioi-Duprez K, Bacin F, Cathelineau B, Cathelineau G, Chaine G, et al. Recommendations of the ALFEDIAM (French Association for the Study of Diabetes and Metabolic Diseases) for the screening and surveillance of diabetic retinopathy. J Fr Ophtalmol 1997;20:302–10.
6. Aso Y, Inukai T, Tayama K, Takemura Y. Serum concentrations of advanced glycation endproducts are associated with the development of atherosclerosis as well as diabetic microangiopathy in patients with type 2 diabetes. Acta Diabetol 2000;37:87–92.
7. Falcone C, Emanuele E, D'Angelo A, Buzzi MP, Belvito C, Cuccia M, et al. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. Arterioscler Thromb Vasc Biol 2005;25:1–6.

Marion Challier[1]
Sophie Jacqueminet[2]
Ouardia Benabdesselam[1]
André Grimaldi[2]
Jean-Louis Beaudeux[1*]

[1] *Fédération de Biochimie and*

*Address correspondence to this author at: Fédération de Biochimie, Groupe Hospitalier Pitié-Salpêtrière, 47-83 Boulevard de l'Hôpital, F75651 Paris Cedex 13, France. Fax 33-1-42-16-21-75; e-mail jean-louis.beaudeux@psl.aphp.fr.

DOI: 10.1373/clinchem.2005.051961

High γ-Glutamyltransferase (GGT) Activity in Human Breast Milk Confounds Interpretation of High Serum GGT Activity in a Nursing Infant with Liver Disease

To the Editor:

We are writing to reiterate to the medical community that γ-glutamyltransferase (GGT) activity is high in human breast milk for at least 4 weeks postpartum *(1, 2)* and to suggest that GGT from milk may increase circulating GGT activity in breastfed newborns. An infant was diagnosed with biliary atresia and was surgically managed by a Roux-en-Y hepatoportojejunostomy. The subsequent persistence of high serum GGT activity in the circulation, despite a gradual decrease in the serum bilirubin concentration, prompted concern of an incomplete response to treatment. Because the infant was being breast fed, an alternative explanation for the persistence of the high circulating GGT activity was proposed.

To affirm that breast milk is high in GGT activity and to determine the duration of the increased activity, we evaluated 17 women (16 Caucasian and 1 Hispanic; age range, 25–38 years) during their lactation periods (from 1 day to 17 months postpartum). Procedures were approved by the Institutional Review Board, and informed consent was given by all patients. Breast milk was collected by artificial pump and stored frozen (approximately −20 °C) before analysis. GGT was measured on the Vitros™ 250 Chemistry System (Ortho-Clinical Diagnostics) by a modification of the method of Szasz *(3)*. GGT was detectable in all milk samples. The GGT activity in breast milk was high during the first 4 weeks postpartum with values ranging from 1231 to 16 060 U/L with a decreasing trend at week 4 (Table 1). The GGT activity of the breast milk of the patient's mother was 1138 U/L at 3 months, the first obtained measurement. The GGT activity in breast milk continued to decrease in all milk samples with an ~10-fold decrease in activity from 1 week postpartum to 6 months postpartum (Table 1). The mean values for 2 samples collected 1 and 3 days after delivery in this study was 12 613 U/L, between the mean (SD) value of 22 990 (7263) U/L for colostrum and 4090 (2069) U/L for early breast milk collected on postpartum day 4 in a previous study *(2)*.

High GGT activity in breast milk is not unique to humans. In animals, the circulating GGT concentration is increased in canine pups after the ingestion of colostrum, and the concentration correlates with the passive transfer of immunoglobulin into the circulation of newborn lambs *(4, 5)*. The high GGT activity in human breast milk may reflect activity in the terminal and lactiferous ducts of the breast, which may facilitate the transport of essential proteins into milk *(6, 7)*. Whether GGT facilitates protein absorption by the developing mucosa of the small intestine is not known.

In conclusion, we suggest that the