Decrease in Circulating Concentrations of Soluble Receptors for Advanced Glycation End Products at the Time of Seroconversion to Autoantibody Positivity in Children With Prediabetes

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OBJECTIVE

Dietary advanced glycation end products (AGEs) and their interactions with the receptor for AGEs (RAGE) may play a role in the pathogenesis of type 1 diabetes. This study set out to assess whether there is any association of circulating concentrations of soluble RAGE (sRAGE), AGEs, and their ratio with the appearance of diabetes-associated autoantibodies in children progressing to clinical diabetes.

RESEARCH DESIGN AND METHODS

Serum concentrations of sRAGE, *N*-ε(carboxymethyl)lysine (CML) adducts, and the sRAGE/CML ratio were analyzed in children who progressed to type 1 diabetes. The samples were taken at four time points: before seroconversion, at the time of the first autoantibody-positive sample, at the time of the first sample positive for multiple (>2) autoantibodies, and close to the disease diagnosis. Samples of autoantibody-negative controls matched for age, sex, and HLA-conferred diabetes risk were analyzed at corresponding time points.

RESULTS

The prediabetic children had higher sRAGE concentrations before seroconversion (P_c = 0.03), at the appearance of multiple autoantibodies (P_c = 0.008), and close to diagnosis (P_c = 0.04). Close to diagnosis, the cases had lower CML concentrations than the controls (P_c = 0.004). Prediabetic children had a higher sRAGE/CML ratio than the controls before seroconversion (P_c = 0.008) and at diagnosis (P_c < 0.001).

CONCLUSIONS

Prediabetic children have higher concentrations of sRAGE and a higher sRAGE/CML ratio than healthy controls. Circulating sRAGE concentrations seem to decline with the appearance of diabetes-predictive autoantibodies in children progressing to type 1 diabetes. The higher sRAGE/CML ratio in prediabetic children may reflect a higher AGE scavenger capacity.

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Type 1 diabetes is an autoimmune disease causing increased morbidity and mortality worldwide (1). The incidence has increased rapidly during the past decades in Finland and most other developed countries, and the increase has been most conspicuous among children younger than 5 years of age (2,3). The reason for this is not known, although interactions between genetic and environmental factors are likely to be involved (4).

Advanced glycation end products (AGEs) and their interactions with the receptor for AGEs (RAGE) are postulated as potential nutritional modulators of the risk for type 1 diabetes (5). The AGE-RAGE axis is quite well established as a pathological pathway contributing to the complications of diabetes (6) and the metabolic changes leading to type 2 diabetes, including insulin resistance (7–9). More recent evidence suggests a role for AGEs and RAGE in the pathogenesis of type 1 diabetes (5,10,11). Further, excess dietary AGE intake can induce pancreatic β-cell dysfunction in animal models (12), while AGE-lowering strategies such as a low-AGE diet or pharmacotherapy decrease the incidence of autoimmune diabetes in NOD*L*t mice (12,13). *N*-ε(carboxymethyl)lysine (CML) is one of the most prominent AGEs physiologically. It is formed through various pathways and is often used as a marker of AGE accumulation in human studies (5). We have previously identified an association between three polymorphisms of the *AGER* gene encoding RAGE on a high-risk HLA background (10). We also reported recently in a population-based study that two of the risk-associated *AGER* polymorphisms also predicted reduced sRAGE concentrations in children with newly diagnosed type 1 diabetes (14).

The circulating pool of soluble RAGE (sRAGE) represents a combination of endogenous secretory RAGE (esRAGE) produced via alternative splicing of the RAGE gene transcript (15) and cleaved membrane-derived sRAGE. Both appear to act as decoys for AGE and other RAGE ligands and are considered cytoprotective (5). However, the complex equilibrium of membrane-bound RAGE, sRAGE, esRAGE, and RAGE ligands is anything but deciphered. Some investigators have reported a direct correlation between sRAGE concentrations and risk of diabetic and cardiovascular complications (16,17), while in a somewhat different population, decreased concentrations were associated with increased risk (18).

There are numerous reports of a decline in sRAGE concentrations in acute inflammation and acute phases of autoimmune diseases (19). Interestingly, patients with acute Kawasaki disease had very low sRAGE concentrations and a high ratio of the proinflammatory RAGE ligand S100A12 to sRAGE when compared with controls. After successful treatment with intravenous Ig, both of these markers returned close to normal (20). Similarly, our previous study showed that sRAGE concentrations tend to decline at seroconversion to autoantibody positivity both in an animal model of autoimmune diabetes and in a small group of prediabetic children (10). However, there was no difference in the sRAGE concentrations between newly diagnosed children and adolescents with type 1 diabetes and controls in our recent study (14). These observations created a need for a more specific study of this phenomenon in a larger population of prediabetic children.

RESEARCH DESIGN AND METHODS

Subjects

The study subjects were derived from the Diabetes Prediction and Prevention (DIPP) study, the protocol of which has been described previously (21). We included 114 children who progressed to overt type 1 diabetes during prospective follow-up and 114 controls matched for time and city of birth, sex, and HLA-genotype-based risk group and analyzed their serum samples. We selected one sample taken before seroconversion to positivity for diabetes-associated autoantibodies (islet cell antibodies [ICA], insulin autoantibodies [IAA], GAD antibodies [GADA], islet antigen 2 antibodies [IA-2A], or zinc transporter 8 antibodies [ZnT8A]), the first autoantibody-positive sample, the first sample positive for ≥2 autoantibodies (which, in 76 of the 114 cases, was the same as the second sample), and a sample taken close to the diagnosis from the children who progressed to type 1 diabetes. The diagnosis of diabetes was based on the World Health Organization criteria. Sample 4 was drawn the earliest, 12 days prior to and at the latest 13 days after the diagnosis (mean −2.7 days). The sRAGE concentrations of the matched controls were analyzed at corresponding time points. We also analyzed the serum concentrations of CML from samples with sufficient volume remaining after the analysis of sRAGE. Of the progressors, 113 children had at least one sample available, whereas there were one or more samples available for 93 controls. Number of available samples at time points 1, 2, 3, and 4 were 99, 106, 95, and 100 for the cases and 81, 84, 66, and 82 for the controls, respectively.

Serum Concentrations of sRAGE

Serum samples were analyzed undiluted according to the manufacturer's instructions (Human RAGE ELISA, R&D Systems, Minneapolis, MN). The interassay coefficient of variation was 7.6%, while the intra-assay coefficient of variation was 3.5%. The analysis covers the whole pool of circulating sRAGE, both esRAGE and sRAGE components.

Serum Concentrations of CML

Serum concentrations of CML were determined using an indirect enzyme immunoassay specific to CML-modified human serum albumin (650.8 μmol/mol lysine) as described previously (22), with the modification that 739 μg/mL of rabbit polyclonal anti-CML antibody was used and reactions were terminated after 5 min with 1.8 mol/L H_2SO_4. Serum samples were diluted either 1:48,000 or 1:96,000. The intra- and interassay coefficients of variation were 13 and 28%, respectively. The linearity of dilution of the assay was $r^2 = 0.96$.

HLA Typing

HLA typing of the major predisposing and protective DR-DQ haplotypes was performed with a PCR-based lanthanide-labeled hybridization method using time-resolved fluorometry for detection. The presence of the *(DR3)-DQA1*05-DQB1*02* haplotype is shortened to *DR3* and that of HLA-*DRB1*04-DQB1*0302* to *DR4*, according to convention.

Diabetes-Associated Autoantibodies

IAA, GADA, IA-2A, and ZnT8A were analyzed with specific radiobinding assays as described earlier (23). The cutoff limits for antibody positivity were defined as the 99th percentiles in 354 Finnish nondiabetic children and adolescents and were 3.48 RU for IAA, 5.36 RU for GADA, 0.43 RU for IA-2A, and 0.61 RU

for ZnT8A. Disease sensitivities and specificities of the assays in our laboratory according to the 2002–2013 Diabetes Antibody Standardization Program workshops were 44–50 and 96–99% for IAA, 82–92 and 94–97% for GADA, 64–72 and 97–100% for IA-2A, and 60–62 and 99–100% for ZnT8A, respectively. Islet cell antibodies were analyzed with indirect immunofluorescence on human group 0 donor pancreas with 2.5 JDFU as the detection limit.

Statistical Analysis

All statistical analyses were performed with the IBM SPSS Statistics 21.0 (SPSS Inc., Chicago, IL). Comparisons between groups were made using the Student *t* test for paired samples or Wilcoxon signed rank test, when comparing the subsequent samples from the patients or samples from the patients and matched controls. The mean levels and SD of sRAGE concentrations in paired comparisons are reported only in individuals with paired sampling, when appropriate. Correlations were analyzed using the Spearman rank correlation test (r_s). Bonferroni correction for multiple comparisons (P_c) was applied to the analyses between groups under the conditions of four tests comparing the difference between cases and controls and 12 tests comparing the subsequent samples of the two groups. For all analyses, *P* values less than 0.05 were considered significant.

RESULTS

The children who progressed to type 1 diabetes had higher sRAGE concentrations than the controls at all four time points, although this difference was not significant at the time of the second sample, i.e., the first autoantibody-positive sample from the progressors (Table 1). There was no difference in the sRAGE concentrations between girls and boys (mean sRAGE 1,240 vs. 1,197 pg/mL; $P = 0.20$). There was an inverse correlation between age and sRAGE concentration in the control group ($r_s = -0.23$; $P < 0.001$) when all four samples from each patient were included in the analysis (mean age 2.9 years in the progressors and 2.8 years in the controls, respectively; if the second and the third sample were the same, it was included only once), but not in the children who progressed to diabetes ($r_s = 0.03$; $P = 0.55$). In fact, there was a statistically significant inverse correlation between age and sRAGE in the controls in all samples but the first one. In the prediabetic children, the sRAGE concentrations were lower in the first autoantibody-positive sample (sample 2) when compared with sample 1, taken before seroconversion (1,232 [SD 460] vs. 1,384 [SD 563] pg/mL; $P = 0.001$; $P_c = 0.01$). In the controls, the difference in the sRAGE concentrations between the first and the second sample was not significant ($P = 0.22$). The sRAGE concentrations were further reduced in the prediabetic children when we compared the third sample positive for multiple autoantibodies to the first sample, taken before seroconversion (1,218 [SD 438] vs. 1,380 [SD 576] pg/mL; $P < 0.001$; Bonferroni corrected $P_c = 0.005$). The reduction between the second and the third sample was not significant ($P = 0.72$) in the prediabetic group. However, a decline in sRAGE concentration between the second and the third sample (1,167 [SD 456] vs. 1,111 [SD 455] pg/mL; $P = 0.008$; $P_c = 0.10$) and also between the first and the third sample of the controls (1,236 [SD 468] vs. 1,128 [SD 454] pg/mL; $P = 0.02$; $P_c = 0.24$) was observed. These differences were not significant after the Bonferroni correction. The variations in the sRAGE concentrations in the cases and the controls are shown in Fig. 1.

There was no significant correlation between sRAGE concentrations in any of the samples and the interval from seroconversion to diagnosis of type 1 diabetes ($r_s = -0.07$ to 0.07; $P = 0.48$–0.68). No significant correlations could be observed either between sRAGE concentrations on one hand and the titers or number of detectable autoantibodies on the other hand at any time point (data not shown).

Serum concentrations of CML were lower in the prediabetic children than in the controls at all time points, reaching statistical significance only in the last sample (2,699 [SD 1,260] vs. 3,424 [SD 998] μmol/mol lysine; $P = 0.001$; $P_c = 0.004$). The CML concentrations remained stable both in the cases and the controls, with the exception of the last sample taken close to the diagnosis of type 1 diabetes, in the progressors. At diagnosis, the CML concentrations were significantly lower than in the sample taken before seroconversion (2,606 [SD 1,286] vs. 3,095 [SD 1,143] μmol/mol lysine; $P < 0.001$; $P_c = 0.003$), at the detection of the first autoantibody (2,709 [SD 1,286] vs. 3,225 [SD 1,160] μmol/mol lysine; $P < 0.001$; $P_c < 0.001$), and the first sample positive for multiple autoantibodies (2,664 [SD 1,259] vs. 3,422 [SD 1,146] μmol/mol lysine; $P < 0.001$; $P_c = 0.001$). The sRAGE/CML ratio was higher among the prediabetic children than in the controls before seroconversion and close to the diagnosis of type 1 diabetes (Table 2).

CONCLUSIONS

The current study indicates that children carrying a risk HLA genotype and progressing to type 1 diabetes have higher sRAGE concentrations than the matched controls, prior to the emergence of the first autoantibody. We observed a decline in the sRAGE concentrations at

Table 1—Number, mean age, and mean sRAGE concentrations in the cases and controls at each sampling point

Sample	*n* progressor/control	Mean age years (SD) progressor	Mean age years (SD) control	Mean sRAGE pg/mL (SD) progressors/controls	*P*	P_c
1	107/105	1.4 (1.4)	1.4 (1.4)	1,403 (562)/1,228 (465)	0.008	0.03
2	110/110	2.1 (1.6)	2.1 (1.6)	1,235 (456)/1,136 (412)	0.10	0.40
3	100/107	2.5 (1.8)	2.5 (1.9)	1,216 (435)/1,042 (380)	0.002	0.008
4	108/109	5.6 (2.9)	5.3 (2.9)	1,270 (454)/1,106 (471)	0.01	0.04

The first sample was taken before seroconversion, the second sample at the time of seroconversion, the third sample at the time of seroconversion to multiple (≥2) autoantibodies, and the fourth sample close to the diagnosis of diabetes in the prediabetic children and at corresponding ages in the control children. The *P* values are derived from Student *t* test for paired samples, and the P_c represents the Bonferroni corrected value.

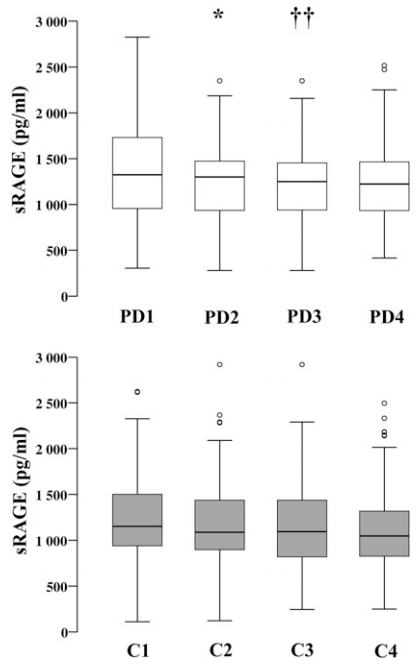

Figure 1—Box plots of the sRAGE concentrations in prediabetic children and in the control children. The line within the boxes represents the median, the bottom of each box the 25th percentile, and the top of the box the 75th percentile. The whiskers represent the 5th and 95th percentiles, and the small circles represent outliers. The first sample was taken before seroconversion, the second sample at the time of seroconversion, the third sample at the time of seroconversion to multiple (≥2) autoantibodies, and the fourth sample close to the diagnosis of diabetes in the prediabetic children and at corresponding ages in the control children. There was a decline in the sRAGE concentrations in the prediabetic children after the initial sample. There was also a trend for decreasing concentrations in the control children, but the changes became nonsignificant after correction or multiple comparisons. *P_c = 0.01 vs. the first sample; ††P_c = 0.005 vs. the first sample. C, control children; PD, prediabetic children.

seroconversion to autoantibody positivity in the progressors, and the concentrations reached a nadir in the first sample positive for multiple autoantibodies. There was an inverse correlation between age and sRAGE concentrations in the control group but not among the prediabetic children. The reduction in the sRAGE concentrations occurred later in the controls and became nonsignificant after correction for multiple comparisons.

In this work, we confirm the results from our previous study in an animal model of autoimmune diabetes and in a smaller group of prediabetic children (10). Since the current samples were derived from a unique prospective collection, we were able to study the variation in the sRAGE concentrations in relation to signs of humoral autoimmunity and compare the results to those of the controls, matched for most of the possible confounding factors. Since the study visits in the DIPP study occur with an interval of 3–12 months (21), the exact time of the seroconversion to autoantibody positivity was not known. This may affect the results, as earlier studies have suggested that the decline in sRAGE concentrations often seen during the acute phase of the inflammation is, at least in some cases, rapidly transformed into an increase as the inflammatory response becomes chronic (20). In stable, chronic inflammation, sRAGE concentrations have been reported to be normal or even higher than normal (20,24), which might represent compensatory mechanisms. Therefore, the difference seen in the samples taken before and at seroconversion to autoantibody positivity likely represents an underestimation of the change in sRAGE at this time since the latter sample may not correspond exactly to the actual time of seroconversion, and the compensatory mechanisms might have already started to increase the sRAGE concentration. There seems to be a trend of increasing sRAGE concentrations from seroconversion toward the time of the diagnosis (Fig. 1), but a larger study would be needed to evaluate whether this elevation is significant.

Serum CML concentrations were similar in the case and control children in the first three time points, but at diagnosis, the CML concentrations were significantly lower in the progressors. Since serum CML is affected by the renal function (25), this is most likely due to hyperfiltration seen close to the diagnosis of type 1 diabetes. However, there appears to be incongruity in the sRAGE/CML ratio between the cases and the controls. Serum CML concentration is affected by intrinsic regulatory mechanisms, and increased dietary intake of AGEs does not directly affect the CML concentration in the serum (26). Therefore, it is not possible to reliably assess whether there are differences in the dietary AGEs between cases and controls in this study, although this is an interesting issue, as it has been shown that dietary AGES can induce inflammatory diseases (15,27). Higher levels of sRAGE and elevated sRAGE/CML ratio could reflect a dysregulated AGE-RAGE interaction in the prediabetic children. On the other hand, the increased ratio provides an enhanced scavenger capacity that may be involved in the defense against ongoing β-cell destruction.

In the current study, there was an inverse correlation between age and circulating sRAGE concentrations in the control group not progressing to type 1 diabetes. In our previous study, children <2 years of age had the highest sRAGE concentrations in a series of nondiabetic children aged 0–15 years, although we did not see a linear correlation between age and circulating sRAGE concentrations in that group (14). The number of very young children was small in that population, and the relationship between sRAGE and age seemed to be relatively stable after the age of 5 years (14). The results of the current study and the earlier study suggest that there is a physiological drop in the sRAGE concentration around 2 years of age. The weakness of these studies is related to the generalizability of the results due to the overrepresentation of HLA genotypes associated with increased type 1 diabetes risk, as

Table 2—Number of progressor-control pairs included in the analysis, mean sRAGE/CML ratio in the cases and controls at each sampling point

Sample	*n* progressor-control pairs	Ratio of sRAGE/CML (SD) in progressors/controls	*P*	P_c
1	74	0.83 (2.1)/0.40 (0.15)	0.002	0.008
2	76	0.47 (0.35)/0.35 (0.16)	0.02	0.08
3	53	0.50 (0.40)/0.35 (0.18)	0.06	0.22
4	71	0.61 (0.37)/0.35 (0.15)	<0.001	<0.001

The first sample was taken before seroconversion, the second sample at the time of seroconversion, the third sample at the time of seroconversion to multiple (≥2) autoantibodies, and the fourth sample close to the diagnosis of diabetes in the prediabetic children and at corresponding ages in the control children. The *P* values are derived from Wilcoxon signed rank tests, and the P_c represents the Bonferroni corrected values.

there is a strong linkage disequilibrium between *AGER* and HLA class II genes (28). This apparent physiological decrease also complicates the interpretation of the observed decline in the sRAGE concentrations in the prediabetic children. However, there are differences in the natural course of sRAGE concentrations between cases and controls, and the decline in the sRAGE concentrations occurs earlier in the prediabetic group.

The higher sRAGE concentrations in the children progressing to type 1 diabetes when compared with matched controls prior to seroconversion suggest that the dynamics of the AGE/RAGE interaction can differ early in life. Recent studies have shown that events before or at birth are associated with sRAGE concentrations later in life (29,30). Unlike the current study, our previous study on a large population of children with newly diagnosed type 1 diabetes did not show any significant difference in the sRAGE concentrations between cases and healthy controls (14). However, in the previous study, there was a significant difference in the number of children in the diabetic and control groups, and the matching between cases and controls was conducted at the group and not the individual level. There are not many reports comparing sRAGE concentrations between children and adolescents with type 1 diabetes and healthy controls. Dettoraki et al. (31) reported higher concentrations in the diabetic patients, whereas Giannini et al. (32) observed the opposite. The groups were not matched for the HLA genotype in those studies, and the investigators in the latter study reported differences in the kidney function between the cases and the controls (31), which could influence the results. Zorena et al. (33) discovered that diabetic children and adolescents with signs of microangiopathy had lower sRAGE concentrations than controls, but the results in patients with type 1 diabetes without microangiopathy and controls were similar. Studies in adult populations have more consistently reported higher sRAGE concentrations in diabetic patients than healthy controls (34,35), and recently Lam et al. (35) suggested that insulin could affect the sRAGE formation. Results in patients affected by adult or juvenile rheumatoid arthritis (20,24,36) are equally controversial. Although the sRAGE levels in the general population seem to be quite stable (37), more evidence has started to build up favoring a possible role of sRAGE as a one of the acute phase proteins (19). From that perspective, it is possible to understand why the results of various studies may be so inconsistent depending on small differences in the study populations and study design. The real challenge is to draw together all the conflicting results and to be able to make definitive conclusions about the role of sRAGE concentration in acute and chronic inflammation and consider possible mechanisms mediating such an effect.

To conclude, prediabetic children have higher circulating concentrations of sRAGE when compared with controls, and a reduction in the circulating sRAGE concentrations coincides with the appearance of diabetes-predictive autoantibodies in children progressing to overt type 1 diabetes. Concentrations of circulating sRAGE correlate inversely with age in the controls but not in the cases in this study, when the mean age at sample collection is close to 3 years. The increased circulating concentrations of sRAGE in prediabetic children might reflect an attempt to protect against β-cell damage as sRAGE binds excessive harmful AGEs, while the decrease seen in serum RAGE close to the time of seroconversion to autoantibody positivity may represent a failing protection mechanism, which is supported by the simultaneous decrease in the sRAGE/CML ratio.

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References

1. Borchers AT, Uibo R, Gershwin ME. The geoepidemiology of type 1 diabetes. Autoimmun Rev 2010;9:A355–A365
2. Harjutsalo V, Sjöberg L, Tuomilehto J. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. Lancet 2008; 371:1777–1782
3. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltész G; EURODIAB Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-20: a multicentre prospective registration study. Lancet 2009;373:2027–2033
4. Knip M, Simell O. Environmental triggers of type 1 diabetes. Cold Spring Harb Perspect Med 2012;2:a007690
5. Yap FY, Kantharidis P, Coughlan MT, Slattery R, Forbes JM. Advanced glycation end products as environmental risk factors for the development of type 1 diabetes. Curr Drug Targets 2012;13:526–540
6. Yan SF, Ramasamy R, Schmidt AM. Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response. J Mol Med (Berl) 2009;87:235–247
7. Piarulli F, Sartore G, Lapolla A. Glyco-oxidation and cardiovascular complications in type 2 diabetes: a clinical update. Acta Diabetol 2013; 50:101–110
8. Cai W, Ramdas M, Zhu L, Chen X, Striker GE, Vlassara H. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. Proc Natl Acad Sci U S A 2012;109:15888–15893
9. Mark AB, Poulsen MW, Andersen S, et al. Consumption of a diet low in advanced glycation end products for 4 weeks improves insulin sensitivity in overweight women. Diabetes Care 2014;37:88–95
10. Forbes JM, Söderlund J, Yap FY, et al. Receptor for advanced glycation end-products (RAGE) provides a link between genetic susceptibility and environmental factors in type 1 diabetes. Diabetologia 2011;54:1032–1042
11. Beyan H, Riese H, Hawa MI, et al. Glycotoxin and autoantibodies are additive environmentally determined predictors of type 1 diabetes: a twin and population study. Diabetes 2012;61: 1192–1198

12. Coughlan MT, Yap FY, Tong DC, et al. Advanced glycation end products are direct modulators of β-cell function. Diabetes 2011;60:2523–2532
13. Peppa M, He C, Hattori M, McEvoy R, Zheng F, Vlassara H. Fetal or neonatal low-glycotoxin environment prevents autoimmune diabetes in NOD mice. Diabetes 2003;52:1441–1448
14. Salonen KM, Ryhänen SJ, Forbes JM, et al.; Finnish Pediatric Diabetes Register. Circulating concentrations of soluble receptor for AGE are associated with age and AGER gene polymorphisms in children with newly diagnosed type 1 diabetes. Diabetes Care 2014;37:1975–1981
15. Yonekura H, Yamamoto Y, Sakurai S, et al. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. Biochem J 2003;370:1097–1109
16. Nin JW, Ferreira I, Schalkwijk CG, et al.; EURODIAB Prospective Complications Study Group. Levels of soluble receptor for AGE are cross-sectionally associated with cardiovascular disease in type 1 diabetes, and this association is partially mediated by endothelial and renal dysfunction and by low-grade inflammation: the EURODIAB Prospective Complications Study. Diabetologia 2009;52:705–714
17. Thomas MC, Söderlund J, Lehto M, et al.; FinnDiane Study Group. Soluble receptor for AGE (RAGE) is a novel independent predictor of all-cause and cardiovascular mortality in type 1 diabetes. Diabetologia 2011;54:2669–2677
18. Selvin E, Halushka MK, Rawlings AM, et al. sRAGE and risk of diabetes, cardiovascular disease, and death. Diabetes 2013;62:2116–2121
19. Maillard-Lefebvre H, Boulanger E, Daroux M, Gaxatte C, Hudson BI, Lambert M. Soluble receptor for advanced glycation end products: a new biomarker in diagnosis and prognosis of chronic inflammatory diseases. Rheumatology (Oxford) 2009;48:1190–1196
20. Wittkowski H, Hirono K, Ichida F, et al. Acute Kawasaki disease is associated with reverse regulation of soluble receptor for advance glycation end products and its proinflammatory ligand S100A12. Arthritis Rheum 2007;56:4174–4181
21. Kupila A, Muona P, Simell T, et al.; Juvenile Diabetes Research Foundation Centre for the Prevention of Type I Diabetes in Finland. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. Diabetologia 2001;44:290–297
22. Coughlan MT, Patel SK, Jerums G, et al. Advanced glycation urinary protein-bound biomarkers and severity of diabetic nephropathy in man. Am J Nephrol 2011;34:347–355
23. Salonen KM, Ryhänen S, Härkönen T, Ilonen J, Knip M; Finnish Pediatric Diabetes Register. Autoantibodies against zinc transporter 8 are related to age, metabolic state and HLA DR genotype in children with newly diagnosed type 1 diabetes. Diabetes Metab Res Rev 2013;29:646–654
24. Chen YS, Yan W, Geczy CL, Brown MA, Thomas R. Serum levels of soluble receptor for advanced glycation end products and of S100 proteins are associated with inflammatory, autoantibody, and classical risk markers of joint and vascular damage in rheumatoid arthritis. Arthritis Res Ther 2009;11:R39
25. Semba RD, Fink JC, Sun K, Bandinelli S, Guralnik JM, Ferrucci L. Carboxymethyl-lysine, an advanced glycation end product, and decline of renal function in older community-dwelling adults. Eur J Nutr 2009;48:38–44
26. Semba RD, Gebauer SK, Baer DJ, et al. Dietary intake of advanced glycation end products did not affect endothelial function and inflammation in healthy adults in a randomized controlled trial. J Nutr 2014;144:1037–1042
27. Cai W, Uribarri J, Zhu L, et al. Oral glycotoxins are a modifiable cause of dementia and the metabolic syndrome in mice and humans. Proc Natl Acad Sci U S A 2014;111:4940–4945
28. Prevost G, Fajardy I, Fontaine P, Danze PM, Besmond C. Human RAGE GLY82SER dimorphism and HLA class II DRB1-DQA1-DQB1 haplotypes in type 1 diabetes. Eur J Immunogenet 1999;26:343–348
29. Chiavaroli V, D'Adamo E, Giannini C, et al. Serum levels of receptors for advanced glycation end products in normal-weight and obese children born small and large for gestational age. Diabetes Care 2012;35:1361–1363
30. Rogers LK, Graf AE, Bhatia A, Leonhart KL, Oza-Frank R. Associations between maternal and infant morbidities and sRAGE within the first week of life in extremely preterm infants. PLoS One 2013;8:e82537
31. Dettoraki A, Gil AP, Spiliotis BE. Association between serum levels of the soluble receptor (sRAGE) for advanced glycation endproducts (AGEs) and their receptor (RAGE) in peripheral blood mononuclear cells of children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2009;22:895–904
32. Giannini C, D'Adamo E, de Giorgis T, et al. The possible role of esRAGE and sRAGE in the natural history of diabetic nephropathy in childhood. Pediatr Nephrol 2012;27:269–275
33. Zorena K, Kula M, Malinowska E, Raczyńska D, Myśliwiec M, Raczyńska K. Threshold serum concentrations of tumour necrosis factor alpha (TNFα) as a potential marker of the presence of microangiopathy in children and adolescents with type 1 diabetes mellitus (T1DM). Hum Immunol 2013;74:75–81
34. Skrha J Jr, Kalousová M, Svarcová J, et al. Relationship of soluble RAGE and RAGE ligands HMGB1 and EN-RAGE to endothelial dysfunction in type 1 and type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2012;120:277–281
35. Lam JK, Wang Y, Shiu SW, Wong Y, Betteridge DJ, Tan KC. Effect of insulin on the soluble receptor for advanced glycation end products (RAGE). Diabet Med 2013;30:702–709
36. Myles A, Viswanath V, Singh YP, Aggarwal A. Soluble receptor for advanced glycation end-products is decreased in patients with juvenile idiopathic arthritis (ERA category) and inversely correlates with disease activity and S100A12 levels. J Rheumatol 2011;38:1994–1999
37. Bower JK, Pankow JS, Lazo M, et al. Three-year variability in plasma concentrations of the soluble receptor for advanced glycation end products (sRAGE). Clin Biochem 2014;47:132–134