Early Formation of Serum Advanced Glycation End-Products in Children with Type 1 Diabetes Mellitus: Relationship with Glycemic Control

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Objectives To quantify serum advanced glycation end-products (AGEs) at the onset of type 1 diabetes mellitus and to determine their potential usefulness as retrospective indicators of glycemic balance.

Study design Carboxymethyllysine (CML) and pentosidine concentrations were determined by liquid chromatography–tandem mass spectrometry in 3 groups of children with type 1 diabetes mellitus: group (Gr) 1, subjects included at disease onset (n = 36); Gr2, subjects with diabetes of 5 years duration (n = 48); Gr3, subjects with diabetes of 10 years duration and in control subjects (n = 33). Hemoglobin A1c (HbA1c) values were recorded over the entire course of treatment for assessing long-term glycemic balance.

Results Serum AGE concentrations were increased in all groups of subjects with diabetes compared with control subjects, but were highest in Gr1 (for CML: 0.155, 0.306, 0.219, and 0.224 mmol/mol Lys in control, Gr1, Gr2, and Gr3 subjects, respectively; for pentosidine: 312, 492, 365, and 403 nmol/mol Lys, respectively). AGE concentrations were closely correlated with HbA1c values (r = 0.78 for CML; r = 0.49 for pentosidine). In Gr2 and Gr3, the overall glycemic balance estimated by average HbA1c values was positively correlated with CML and pentosidine concentrations, especially in the first year of follow-up.

Conclusion Our results indicate that AGE concentrations are elevated in serum at the time of diabetes mellitus diagnosis, suggesting that the deleterious role of AGEs in the development of long-term complications should be taken into account even at the initial stages of the disease. Moreover, in some circumstances, AGEs could serve as surrogate markers of HbA1c for monitoring glycemic control. *(J Pediatr 2016;172:56-62)*.

Diabetes mellitus is characterized by a chronic hyperglycemia that accelerates the rate of protein glycation. This reaction is due to the nonenzymatic binding of reducing sugars, including glucose, to amino groups of proteins. This binding leads to formation of a labile Schiff base, which then undergoes a molecular rearrangement resulting in a stable ketoamine bond.[1,2] The compounds formed during this initial step are called Amadori products, among which glycated hemoglobin A1c (HbA1c) and fructosamine are the most commonly measured. Amadori products are further subjected to a set of irreversible reactions associated with oxidative processes, known as glycoxidation, which generate a wide group of complex by-products known as advanced glycation end-products (AGEs). AGEs that have been structurally identified include N-ε-carboxymethyllysine (CML) and pentosidine.

Because the glycation reaction is a cumulative and irreversible process, glycation-derived products have long been considered valuable biomarkers for the management of patients with diabetes mellitus. For instance, HbA1c provides retrospective information on glycemic control over the 6-8 weeks preceding the test,[3] and is also being increasingly used as a diagnostic test in many countries.[4] Moreover, HbA1c values are correlated with the development of long-term complications in both type 1 and type 2 diabetes.[5,6] Similar observations have been reported for serum AGEs, which are increased in patients with diabetes and are also associated with long-term complications.[7-10]

To our knowledge, no previous studies have evaluated AGE concentrations in serum at the time of diagnosis of type 1 diabetes mellitus, probably because it is expected that AGEs, which are formed during the later steps of the glycation process, would be increased in serum only long after disease onset. Indeed, the most plausible hypothesis explaining AGE accumulation in serum is that AGEs are present at low concentrations at the onset of diabetes mellitus and increase progressively based on the duration of diabetes and level of glycemic control. Thus, AGEs could be considered interesting biomarkers for assessing glycemic control over the duration of the disease. Nonetheless, to date few investigations have been carried out to really determine the usefulness of AGEs as long-term indicators of glycemic

AGE	Advanced glycation end-product
CML	Carboxymethyllysine
Gr	Group
HbA1c	Hemoglobin A1c

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balance, other than some studies showing decreased AGE concentrations in patients with good metabolic control.[11-13] This decrease was slower than that of HbA1c, suggesting that AGEs could complement HbA1c values in assessing the quality of glycemic control over longer periods.

Consequently, the aim of the present study was to quantify serum AGEs (CML and pentosidine) in children with noncomplicated type 1 diabetes, classified into 3 groups: children included at the diagnosis of the disease and children with either 5 years or 10 years of average diabetes duration. In the latter 2 groups, HbA1c values retrospectively collected over the follow-up period served as long-term indicators of glycemic control. Thus, our study results were expected to provide new information about: (1) the kinetics of AGE accumulation in serum of subjects with type 1 diabetes, by comparing AGE concentrations in subjects with different diabetes durations; and (2) the potential added value of AGEs to HbA1c for assessing glycemic control in these subjects, by establishing correlations between AGE concentrations and variations in HbA1c values.

Methods

This Advanced Glycation End-Products in Type 1 DIAbetes study was approved by a French Ethics Committee (2010-A00737-32), and informed consent was obtained from all subjects. A total of 111 young subjects with type 1 diabetes who were referred to the pediatrics unit of the University Hospital of Reims (France) and 33 sibling control subjects were included in this cross-sectional study. The subjects with type 1 diabetes were divided into 3 groups according to diabetes duration: subjects included in the study at the time of diabetes diagnosis (group [Gr] 1; n = 36), subjects with an average diabetes duration of 5 years (Gr2; n = 48), and subjects with an average diabetes duration of 10 years (Gr3; n = 27). The subjects in Gr1 were included in the protocol at the time of admission to the hospital, with occasional subjects included on the second day of hospitalization. No subject from Gr2 or Gr3 exhibited diabetes-related complications. Glycemic control was assessed over the course of treatment by HbA1c values (at least 1 value per year) collected in a shared database (Champagne-Ardenne Réseau DIABète database).

Biological Measurements

HbA1c values were determined using a VARIANT II NU Kit analyzer (Bio-Rad, Hercules, California). Fructosamine, creatinine, triglycerides, cholesterol (total and high-density lipoprotein), glucose, total protein, and albumin were quantified by automated assays using a COBAS analyzer (Roche Diagnostics, Indianapolis, Indiana). Low-density lipoprotein-cholesterol was calculated using the Friedewald formula. For AGE assays, total (free and protein-bound) serum pentosidine and CML concentrations were assayed by liquid chromatography–tandem mass spectrometry as described previously.[14]

Statistical Analyses

Continuous variables are expressed as mean (95% CI) or as mean ± SD. The unpaired Student *t* test was used to compare normally distributed variables. Correlations between continuous variables were assessed by Pearson rank correlation analysis. A P value <.05 was considered statistically significant.

Results

The main biological characteristics used for characterizing the subjects with type 1 diabetes and the control subjects included in this study are shown in the **Table**. The mean age of subjects was identical across the study groups (approximately 10 years); however, subjects from Gr1 were younger (mean age, 8.2 years) and those from Gr3 were older (mean age, 13.7 years) than control subjects. The average duration of diabetes was 4.7 years in Gr2 and 9.8 years in Gr3. Creatinine, urinary proteins, and urinary albumin values were similar in subjects with type 1 diabetes and control subjects, suggesting the absence of renal complications. Several metabolic variables (ie, HbA1c, fructosamine, total cholesterol, and urinary glucose) were significantly increased in the subjects with type 1 diabetes. HbA1c values were significantly higher in all subjects with type 1 diabetes compared with control subjects (mean, 81 mmol/mol [9.6%] vs 31 mmol/mol [5.0%]; $P < .01$). In the groups of subjects with type 1 diabetes, Gr1 had the highest mean HbA1c value (112 mmol/mol; 12.4%) compared with Gr2 and Gr3, which had identical HbA1c values (66 mmol/mol [8.2%] and 67 mmol/mol [8.2%], respectively) (**Figure 1**, A). The same pattern was observed for fructosamine concentrations (mean, 422 μmol/L in all subjects with type 1 diabetes vs 216 μmol/L in control subjects) (**Figure 1**, B). Gr1 had the highest mean fructosamine concentration (551 μmol/L), and Gr2 and Gr3 had identical values (358 μmol/L and 364 μmol/L). These results demonstrate that subjects from Gr2 and Gr3 had similar glycemic status.

Serum AGE Concentrations by Duration of Diabetes and Correlations with HbA1c Values

Both CML and pentosidine concentrations were increased in subjects with type 1 diabetes compared with control subjects, and the values in the 3 groups showed the same patterns as those obtained for Amadori products HbA1c and fructosamine (**Figure 1**, C and D). For instance, CML concentrations in Gr1 were double those of the control group (mean, 0.306 mmol/mol vs 0.155 mmol/mol Lys; $P < .001$), whereas the increases in Gr2 and Gr3, although significant, were less strong (0.219 and 0.224 mmol/mol Lys, respectively). A 1.6-fold increase in pentosidine was found in Gr1 compared with controls (492 nmol/mol vs 312 nmol/mol Lys). As with the other glycation products, the concentrations were comparatively lower in Gr2 and Gr3 (365 nmol/mol Lys [$P < .05$] and 403 nmol/mol Lys [$P < .01$], respectively), but still higher than in controls.