Serum ACE Predicts Severe Hypoglycemia in Children and Adolescents With Type 1 Diabetes

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OBJECTIVE — To investigate whether risk of severe hypoglycemia is related to serum (S) ACE level during intensive treatment in type 1 diabetic children.

RESEARCH DESIGN AND METHODS — A cohort of 86 intensively treated type 1 diabetic patients was studied during 1999–2000. In 1999, the age range was 7–19 years (median 12.8), diabetes duration was 1.2–14.7 years (5.3), insulin dose was 0.4–1.7 units · kg^{-1} · $24\ h^{-1}$ (1.0), and the HbA_{1c} year mean was 4.7–10.2% (6.8). HbA_{1c}, insulin doses, and events of severe hypoglycemia (needing assistance from another person) were prospectively registered at regular visits, scheduled quarterly. S-ACE was determined once.

RESULTS — Severe hypoglycemia was correlated to S-ACE ($r = 0.22$, 95% CI 0.01–0.41, $P = 0.0093$). The square root of severe hypoglycemia was correlated to S-ACE ($r = 0.27$, 95% CI 0.06–0.45, $P = 0.0093$). Patients with S-ACE at the median level or above ($n = 44$) reported a mean of 3.0 yearly events of severe hypoglycemia compared with 0.5 events in patients with S-ACE lower than the median ($n = 42$) ($P = 0.0079$). Of the patients with an S-ACE at the median level or above, 27 (61%) reported severe hypoglycemia, compared with 17 (40%) patients with an S-ACE lower than the median ($P = 0.0527$). Insulin dose, HbA_{1c}, age, onset age, duration, C-peptide, and sex did not differ between these two groups. S-ACE was negatively correlated with age ($r = -0.27$, 95% CI −0.46 to 0.07, $P = 0.0265$) but not with HbA_{1c}, duration, or blood pressure.

CONCLUSIONS — The elevated rate of severe hypoglycemia among patients with higher S-ACE suggests, among other factors, that a genetic determinant for severe hypoglycemia exists. Further evaluation is needed before the clinical usefulness of this test can be elucidated.

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Prediction and prevention of severe hypoglycemia have become increasingly important, with modern intensive treatment aimed at near-normal blood glucose levels (1). The treatment may otherwise increase the risk of severe hypoglycemia (2). Organs may be damaged and early deaths may even occur from severe hypoglycemia (3). Patients and their families may experience fear, worry, and disturbances (4). Quality of life is compromised (5). Treatment control may be worsened, resulting in long-term complications (1,6). Patients and third parties may suffer from physical injuries and property damage, e.g., traffic accidents (7). On the whole, severe hypoglycemia is likely to result in considerable socioeconomic costs (5).

The multifactorial etiology of severe hypoglycemia is only partially known (8,9). Impaired counterregulation and loss of warning symptoms have been highlighted, but there are also patients with good metabolic control who have never previously experienced events of severe hypoglycemia. A history of severe hypoglycemia has, however, been clearly related to future risk of severe hypoglycemia (9–11), suggesting a possible genetic factor.

During events of severe hypoglycemia, insulin-treated patients suffer from cerebral glucose deficiency. The capacity to maintain cognitive function during hypoglycemia is a crucial link in a complex chain of prerequisites to ensure that severe hypoglycemia is prevented by appropriate treatment of the initial hypoglycemia (12).

Interestingly, the ACE genotype has previously been demonstrated to be a determinant for endurance performance in athletes (13). The ACE gene polymorphism consists of the two alleles I (insertion) and D (deletion), forming genotypes II, ID, and DD (13). The D allele is less common in endurance athletes (13). Athletes in peak performance situations and hypoglycemic diabetic patients share the need to maintain cognitive function during states of substrate depletion. This observation therefore suggests that the D allele is less favorable for performance during substrate depletion, although the physiological explanation for this is unknown.

The clinical routine analysis of serum (S) ACE levels has been closely related to the ACE genotype, with higher levels of S-ACE in serum and tissues related to the D allele (14). S-ACE remains rather stable over time (15), and there is no sex difference in healthy adults (16). Recently, a relationship between S-ACE and retrospectively registered severe hypoglycemia was reported in adults (14). Based on these earlier findings, we have used prospective registration to test for the first time the hypothesis that children and adolescents with higher S-ACE levels report higher rates of severe hypoglycemia.

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Abbreviations: S, serum.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of the cohort, calculated from year mean values in 1999, for all patients and for patients with S-ACE lower than median and those at or above median level*

	All patients (n = 86)			S-ACE < median (n = 42)			S-ACE ≥ median (n = 44)		
	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD
Age (years)	12.8	7.1–18.5	13.0 ± 3.1	13.6	7.1–18.3	13.3 ± 3.1	12.1	7.1–18.5	12.7 ± 3.1
Age at diabetes onset (years)	6.5	1.0–16.9	7.5 ± 3.8	6.6	1.0–15.1	7.7 ± 3.8	6.4	1.2–16.9	7.3 ± 3.9
Diabetes duration (years)	5.3	1.2–14.7	5.5 ± 3.3	4.7	1.5–14.7	5.6 ± 3.5	5.4	1.2–14.3	5.4 ± 3.0
C-peptide (nmol/l)	0.01	0.00–0.34	0.04 ± 0.07	0.02	0.00–0.24	0.05 ± 0.07	0.00	0.00–0.34	0.03 ± 0.07
Insulin (units · kg^{-1} · 24 h^{-1})	0.99	0.37–1.73	0.98 ± 0.25	1.0	0.37–1.73	1.00 ± 0.27	0.95	0.38–1.61	0.97 ± 0.24
HbA_{1c} (% year mean)	6.8	4.7–10.2	6.9 ± 1.0	7.0	4.7–10.2	7.0 ± 1.0	6.8	4.8–8.8	6.7 ± 1.0
S-ACE (μkat/l)	1.13	0.45–2.31	1.17 ± 0.37	0.91	0.45–1.09	0.88 ± 0.17	1.40	1.13–2.31	1.45 ± 0.29

There was no difference between groups (except for S-ACE, $P < 0.0001$).

RESEARCH DESIGN AND METHODS

— The study base was a geographic population of type 1 diabetic patients belonging to the catchment area of Linköping University Hospital, Östergötland county, Sweden. A cohort consisting of 86 patients (37 girls, 49 boys) aged 7–18 years, with a diabetes duration of >1 year, was studied during 1999–2000. Their clinical characteristics are shown in Table 1.

Treatment

The patients were treated with four to seven daily doses of insulin. Seven of the patients were treated with continuous subcutaneous insulin infusion (in 1999). Patient visits were scheduled at 3-month intervals, but patients were seen more often when necessary. In exceptional cases, a visit was considerably delayed. In practice, there was a median of four yearly visits. The treatment policy of combining multiple-dose insulin therapy with active self-control problem-based education and psychosocial support at the onset of type 1 diabetes has been described elsewhere (9). No patient was treated with an ACE inhibitor.

End point registration

The patients/parents were asked to prospectively register every event of severe hypoglycemia in a questionnaire distributed during each visit to the outpatient department, and the questionnaire was to be returned at the next visit. Questions regarding hypoglycemia were divided into two categories: *1*) "Number of events of severe hypoglycemia without unconsciousness but needing the assistance of another person, since last visit" and *2*) "Number of events of severe hypoglycemia with unconsciousness, since last visit: When? Date. . . Time. . . (repeated)," as described earlier (9). No other inclusion or exclusion criteria were used. Severe hypoglycemia in this study meant occurrence of an event in either or both categories during the year. HbA_{1c} values, actual insulin dose, and other data were also registered at each visit (10).

Laboratory methods

S-ACE was determined once by the Hitachi 911 kinetic method (16), standardized as fasting morning samples. The samples were taken at regular yearly checkpoints evenly distributed during 2000, except for holiday periods. The measurement unit microkatal (μkat) is a unit of catalytic activity, used particularly in the chemistry of enzymes. It was added to the Système International (SI) system in October 1999.

The HbA_{1c} method used was diagnostic chemistry analyzer 2000 (DCA 2000), calibrated to the national Mono-S standard (17). The values in our laboratory at a corresponding time were found to be comparable to the Diabetes Control and Complications Trial laboratory values after adding, on average, 1.15% (17). C-peptide was measured by radioimmunoassay according to Heding (18). Biochemical analyses other than HbA_{1c} were performed by individuals without clinical knowledge about the patients. Blood pressure was manually determined at clinical visits, and year mean values were calculated.

Statistical analysis

Primary end points were the number of prospectively self-reported events of severe hypoglycemia during 1999 and 2000 and the proportion of patients reporting this.

For statistical analysis, StatView 5.0.1 software (SAS Institute) was used. The χ^2 test was used to compare proportions of patients per year. Differences between groups in numbers of severe hypoglycemic events were tested with the nonparametric Mann-Whitney U test and the Kruskal-Wallis test, because this variable was not normally distributed. Likewise, correlations were tested with the nonparametric Spearman rank test. A transformation to the square root of severe hypoglycemia was used as a complement to reduce the variance in numbers of events. Logistic regression and multiple regression were used to test for confounding. Significant differences ≤0.05 are indicated by P values. Mean values are given with ±1 SD.

Ethics

The study was approved by the Ethics Committee for Human Research of the Faculty of Health Sciences at Linköping University, Linköping, Sweden.

RESULTS

— S-ACE levels ranged as shown in Table 1. Average S-ACE levels by quartiles are shown in Table 2. There was no sex difference in S-ACE.

The overall rate of severe hypoglycemia was 1.8 events per patient year (2.0 in 1999, 1.6 in 2000) reported by 44 of the patients (36 in 1999, 26 in 2000). Patients with S-ACE at the median level or above (n = 44) reported a mean of 3.0 yearly events of severe hypoglycemia compared with a mean of 0.5 events in patients with S-ACE levels lower than the median (n = 42) (P = 0.0079). Of the patients with S-ACE at the median level or

Table 2—*Severe hypoglycemia by S-ACE quartiles*

	Percentile			
	0–25th	26–50th	51–75th	76–100th
Patients (*n*)	22	22	21	21
S-ACE (μkat/l)*	0.75 ± 0.13	1.03 ± 0.06	1.26 ± 0.08	1.68 ± 0.25
SH patients [*n* (%)]	8 (36)	10 (45)	14 (67)	12 (57)
SH mean number of events per patient year†	0.6	0.6	3.9‡	2.3

Data for S-ACE are means ± SD. SH, severe hypoglycemia. *$P < 0.0001$; †$P = 0.0402$; ‡after exclusion of one subject, 2.2, $n = 20$, $P = 0.0613$.

above, 27 (61%) reported severe hypoglycemia compared with 17 (40%) patients with S-ACE lower than the median ($P = 0.0527$). Insulin dose, HbA_{1c}, age, onset age, duration, C-peptide, and sex did not differ significantly between these two groups (Table 1).

For hypoglycemia with unconsciousness, the rate was 0.17 per patient year (0.22 in 1999, 0.13 in 2000), reported by 17 of the patients (11 in 1999 and 8 in 2000). Patients at the S-ACE median level or above reported a mean of 0.25 yearly events of severe hypoglycemia with unconsciousness compared with 0.10 yearly events in patients with S-ACE lower than the median (NS). Of the patients with S-ACE at the median level or above, 11 (25%) reported severe hypoglycemia with unconsciousness compared with 6 (14%) of those patients with S-ACE lower than the median (NS).

The number of severe hypoglycemic events during the study was correlated to S-ACE ($r = 0.22$, 95% CI 0.01–0.41, $P = 0.0093$). The square root of the number of severe hypoglycemic events was correlated to S-ACE ($r = 0.27$, 95% CI 0.06–0.45, $P = 0.0093$), as shown in Fig. 1. Including only patients with events during the study ($n = 44$), the number of events of severe hypoglycemia was still correlated to S-ACE ($r = 0.24$, 95% CI −0.06 to 0.50, $P = 0.0190$), and the square root of the number of events of severe hypoglycemia was correlated to S-ACE ($r = 0.31$, 95% CI 0.02–0.56, $P = 0.0190$).

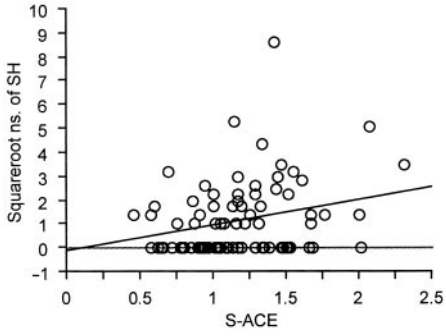

Figure 1—*S-ACE (μkat/l) related to the square root of number of severe hypoglycemia (SH) events needing assistance during a 24-month period (r = 0.27, 95% CI 0.01–0.41, P = 0.0093). Patients were aged 7–18 years. ns, numbers.*

The distribution of severe hypoglycemic events by S-ACE quartiles is shown in Table 2. There was no correlation between number of severe hypoglycemic events, or square root of number of severe hypoglycemic events, and age, diabetes duration, or HbA_{1c}.

In a polytomous logistic regression model with no events ($n = 42$) versus one to three events ($n = 24$) or more than three events ($n = 20$) as the dependent variable, more than three events of severe hypoglycemia was associated with higher S-ACE (odds ratio 1.7 for a 0.5-unit increase in S-ACE, 95% CI 1.0–3.0, $P = 0.0411$), whereas age, duration, and HbA_{1c} were not significantly related. In a multiple regression model with the square root of the number of severe hypoglycemic events as the dependent variable, S-ACE explained 7% of the variation ($P = 0.0360$), whereas age, duration, and HbA_{1c} were not significantly related.

There was no correlation between S-ACE and diabetes duration or HbA_{1c}. There was a weak negative correlation between S-ACE and age ($r = -0.27$, 95% CI −0.46 to 0.07, $P = 0.0265$). There was no correlation between S-ACE and systolic or diastolic blood pressure.

Finally, in one case, the parents of a 10-year-old girl reported 33 events needing assistance in 1999 and 42 events in 2000, but reported no events with unconsciousness. Her diabetes duration was 2.5 years, mean HbA_{1c} 6.9, insulin dose 0.7 units · kg^{-1} · 24 h^{-1}, and S-ACE 1.42 μkat/l. Her control was unstable, and she started continuous subcutaneous insulin infusion in May 2000. According to case records, a substantial proportion of the reported events were asymptomatic, with biochemical hypoglycemic events identified by frequent blood glucose monitoring. After exclusion of this subject, the overall rate of severe hypoglycemia was 1.4 events per patient-year ($n = 85$). For patients with S-ACE at the median level or above ($n = 43$), the rate after this exclusion was a mean of 2.2 yearly events compared with a mean of 0.5 yearly events in patients with S-ACE lower than the median ($n = 42$) ($P = 0.0118$). After this exclusion, the correlation between number of severe hypoglycemic events and S-ACE was greater ($r = 0.29$, 95% CI 0.08–0.47, $P = 0.0071$), and the correlation between the square root of severe hypoglycemic events and S-ACE remained the same ($r = 0.27$, 95% CI 0.06–0.46, $P = 0.0129$). In the logistic regression model with no events ($n = 42$) versus one to three events ($n = 24$) or more than three events ($n = 19$) as dependent variable, more than three events of severe hypoglycemia was still associated with higher S-ACE (odds ratio 1.7 for a 0.5-unit increase in S-ACE, 95% CI 1.0–3.0, $P = 0.0440$) and was also associated with longer duration (odds ratio 1.2 for a 1-year increase, 95% CI 1.0–1.5, $P = 0.0443$), whereas age and HbA_{1c} were not significantly related. In the multiple regression model with the square root of the number of severe hypoglycemic events as the dependent variable, S-ACE still explained 7% of the variation ($P = 0.0256$), whereas age, duration, and HbA_{1c} were not significantly related.

CONCLUSIONS — To our knowledge, this study is the first to use a prospective registration method to show that the multifactorially determined risk of severe hypoglycemia in children and adolescents is also related to S-ACE activity. To study the relationship between S-ACE and severe hypoglycemia in adults, others have previously registered severe hypoglycemia retrospectively (14) and, recently, prospectively (U. Pedersen-Bjergaard, B. Agerholm-Larsen, S. Pramming, P. Hougaard, B. Thorsteinsson, unpublished data; 19). Our prospective data suggest that S-ACE activity is an in-

dependent risk marker of an effect size comparable to other known biological variables, as was shown in the Diabetes Control and Complications Trial (11). Long duration of diabetes, history of severe hypoglycemia, and unawareness are well-established determinants for severe hypoglycemia, although these determinants and HbA_{1c} level may explain only a small proportion of the variation (11,20). The influence of HbA_{1c} level varies between studies and seems to decrease with more individualized and flexible treatment as well as with patient education (9,21).

Recent data from adults suggest that the predictive value of S-ACE is strongest in patients with a compromised defense against severe hypoglycemia (14). Therefore, it is suggested that low S-ACE activity represents a third-line defense against severe hypoglycemia, being of higher value for those patients with compromised counterregulatory hormone responses (14).

A key to preventing mild hypoglycemia from proceeding to severe hypoglycemia is to ensure that the patient recognizes the situation and takes appropriate action before cognitive dysfunction makes this impossible (22). However, this is a complex process involving several steps, some of which are probably enhanced by education and mental preparation (12). One factor of great importance is how rapidly and to what degree cognitive dysfunction develops during hypoglycemia. S-ACE level may be a marker for the ability to maintain cognitive function for a longer period of time during hypoglycemia. The mechanisms behind this hypothesis remain unknown (14).

Because our findings are reproduced in other populations where prospective registration of severe hypoglycemia has also been used, it can be assumed that the S-ACE test might enable us to identify patients with a genetically determined higher risk of severe hypoglycemia. These patients may need to be assessed more often than others for unawareness and loss of residual insulin secretion, and they may need more intensive support to maintain optimal treatment. As has been shown by many others, every improvement in metabolic control is of importance in preventing, delaying, or slowing progression of long-term complications (1,23,24). A high S-ACE level should be taken as an indication of a greater need for medical support and adequate education concerning the prevention of severe hypoglycemia without compromising metabolic control (6,9,21,25). In our study, a declining rate of severe hypoglycemia in 2000 compared with 1999 was seen after an educational intervention during 1999 aiming at the prevention of severe hypoglycemia (26,27). A lower incidence of severe hypoglycemia has also been associated with the use of educational devices targeting prevention of severe hypoglycemia in a larger controlled study (28).

The supposed explanatory factors behind the possible effect of S-ACE warrant more detailed examination. Muscle studies have suggested that low S-ACE enhances metabolic efficiency (29). Some studies have shown that bradykinin, which is degraded by the kininase activity of ACE, contributes to protecting the myocardial tissue against ischemia (30). Hence, S-ACE seems to play a less favorable role in energy metabolism. Moreover, the angiotensin deletion allele has been related to cognitive impairment in humans (31). Clearly, the physiological mechanisms behind the S-ACE hypothesis need further investigation.

Because we discovered a relationship between S-ACE and age, age-controlled reference levels for S-ACE activity in healthy children and adolescents would also be valuable. The relationship between S-ACE and the risk of hypoglycemia needs to be examined further using continuous glucose monitoring, and it might even be of interest to control S-ACE for preceding hypoglycemia as well as for ambient blood glucose level.

There have been conflicting reports regarding the effect of ACE inhibitors on risk of severe hypoglycemia (14,32,33). Some investigators found an increased risk in adults (32), possibly related to increased insulin sensitivity from certain ACE inhibitors (34). Further studies would be needed to determine whether certain young people with high ACE activity, low C-peptide levels, impaired awareness, and unstable control might benefit from well-controlled treatment with select ACE inhibitors, thereby possibly reducing the risk for severe hypoglycemia and potentially improving metabolic control.

In conclusion, these studies show a potentially useful marker for the genetic risk of severe hypoglycemia. If reproduced in other studies using prospective registration of severe hypoglycemia and also taking other variables into account, the results may have important implications for many patients when combined with other risk markers.

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