CLINICAL STUDY

Erythropoietin and vascular endothelial growth factor as risk markers for severe hypoglycaemia in type 1 diabetes

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Abstract

Objective: Circulating erythropoietin (EPO) and vascular endothelial growth factor (VEGF) increase during hypoglycaemia and may represent protective hormonal counter-regulatory responses. We tested the hypothesis that low levels of EPO and VEGF are associated with a higher frequency of severe hypoglycaemia in a cohort of patients with type 1 diabetes.

Design: Prospective observational follow-up study.

Methods: Totally 219 patients with type 1 diabetes (41% females, age 46 ± 13 years (mean $\pm$ s.d.), duration of diabetes 21 ± 12 years, and HbAlc $8.5 \pm 1.1\%$) were followed in a 1-year observational study. Plasma EPO and serum VEGF levels were measured at baseline with ELISA. Events of severe hypoglycaemia defined by third party assistance were recorded and validated in telephone interviews within 24 h.

Results: Totally 235 episodes of severe hypoglycaemia (1.1 episodes per patient-year) were reported by 82 patients (37%). At baseline, plasma EPO was 8.6 (3.1–34.3) U/l (median (range)), and serum VEGF was 52.2 (6.6–337) pg/ml. The levels of EPO and VEGF were not associated with frequency of severe and mild hypoglycaemia. The levels of EPO were not associated with age, sex, duration of diabetes, body mass index, HbAlc, C-peptide level or hypoglycaemia awareness status. The levels of VEGF were positively associated with age and female sex.

Conclusions: Although several studies suggest that VEGF and EPO may affect brain function during hypoglycaemia, this study does not support random VEGF or EPO levels to determine future risk of severe hypoglycaemia in people with type 1 diabetes.

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Introduction

In type 1 diabetes, the unavoidable and frequent episodes of mild hypoglycaemia may develop into severe hypoglycaemia with profound cognitive dysfunction. The primary protective mechanisms include hormonal counter-regulation to mobilise endogenous glucose. The classic hormonal counter-regulation consists of a rise in glucagon, adrenaline and noradrenaline to be followed by cortisol and GH. New data show that other circulating proteins are also stimulated by mild hypoglycaemia. Two such substances are erythropoietin (EPO) (1) and vascular endothelial growth factor (VEGF) (2–5), both of which are induced via the transcription factor hypoxia-inducible factor (HIF).

HIFs are proteins that mediate a protective response to hypoxia. Certain HIF subunits are also activated during hypoglycaemia (6). When activated, HIF promotes the formation of EPO and VEGF, but also the glucose transporter-1 (GLUT1; reviewed in (7)). This latter mechanism may increase cellular uptake of glucose and thereby provide protection against hypoglycaemia.

In vitro studies suggest that EPO may preserve cellular function during hypoglycaemia (8–10). Low EPO levels may be associated with more pronounced cerebral dysfunction during hypoglycaemia than higher levels in people with type 1 diabetes (1). Hypoglycaemia induces stabilisation of *VEGF* mRNA (11), and *GLUT1* (or SLC2A1 as listed in the HUGO Database) mRNA is up-regulated by VEGF (12). A high VEGF rise during hypoglycaemia is reportedly associated with preservation of cognitive function during hypoglycaemia in healthy adults (4). Thus, high levels of circulating EPO or VEGF may preserve cognitive function during hypoglycaemia and thereby help the patient to prevent episodes of severe hypoglycaemia. Therefore, we tested the hypothesis that low levels of circulating EPO and VEGF are associated with a higher frequency of severe hypoglycaemia in a cohort of patients with type 1 diabetes.

Subjects and methods

Subjects and design

The present study is a *post hoc* study of blood samples gathered during a prospective study designed to evaluate risk markers for severe hypoglycaemia in type 1 diabetes (13). Briefly, adults with type 1 diabetes for more than 2 years and not treated with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) were recruited from our outpatient clinic. The reason for excluding subjects treated with ACEIs and ARBs is that such agents may lead to changes in concentrations of EPO and VEGF, since angiotensin II affects HIF – the common transcription factor of EPO and VEGF (14). A total of 269 eligible subjects gave informed consent to participate. Thirty-nine subjects dropped out during the study period (38 withdrew their consent and 1 died). Furthermore, 11 subjects were excluded as they commenced treatment with ACEIs or ARBs. Subjects medicated with β-receptor antagonists (*n*=5) or psychopharmacological agents (*n*=3) were not excluded. A total of 219 subjects completed the study. Clinical characteristics of the participating subjects appear from Table 1. The study was approved by the regional ethics committee. Results regarding the association between renin–angiotensin system components and risk of severe hypoglycaemia have been published elsewhere (13, 15).

Baseline data

At baseline, the participants completed a questionnaire assessing previous experience of hypoglycaemia and self-estimated state of hypoglycaemic awareness. State of awareness was classified according to a validated method based on the patients' ability to recognise symptoms during a hypoglycaemic episode (16). Subjects always recognising their symptoms were categorised as having normal awareness, those usually recognising their symptoms as having impaired awareness, and those occasionally or never recognising their symptoms as having unawareness. Data on patients' history of diabetes were extracted from medical records.

Laboratory analyses

At baseline, blood was sampled during daytime with subjects in the sitting position after resting in the clinic. Thereafter, blood was centrifuged and stored at $-80\,°C$

Table 1 Clinical characteristics of 219 patients with type 1 diabetes. Data of two subgroups defined by the number of episodes of SH in the 1-year study period are also shown. Numbers are means (S.D.) or percentage, unless otherwise stated.

	All patients *n*=219	Patients with no episodes of SH *n*=137	Patients with one or more episodes of SH *n*=82	*P*
Age (years)	46 (13)	44 (13)	49 (12)	0.009
Sex (% female)	41	39	43	0.63
Body mass index (kg/m^2)	25 (3.6)	26.5 (3.8)	24.1 (3.2)	0.008
Age of onset of diabetes (years)	25 (14)	24 (14)	26 (14)	0.38
Duration of diabetes (years)	21 (12)	20 (12)	22 (11)	0.08
Detectable C-peptide[a] (%)	57.5	59.9	53.7	0.37
HbA1c (%)	8.5 (1.1)	8.6 (1.2)	8.2 (1.0)	0.01
Retinopathy[b] None, simplex, proliferative (%)	46/39/15	45/41/14	49/36/15	0.82
Nephropathy[c] None/microalbuminuria/albuminuria/ elevated serum creatinine (%)	78/15/6/1	76/15/7/2	81/14/4/1	0.76
Peripheral neuropathy (%)	33	31	35	0.54
Autonomic neuropathy[d] (%)	14	14	14	0.97
Myocardial infarction (%)	5.5	4.4	7.3	0.37
Stroke (%)	1.8	1.5	2.4	0.63
Amputation (%)	0.9	1.5	0	0.53
Hypertension (%)	17	18	16	0.73
Daily insulin dose/kg (units/kg)	0.66 (0.19)	0.67 (0.19)	0.64 (0.18)	0.27
Awareness of hypoglycaemia Normal/impaired/unaware (%)	41/46/13	53/38/9	20/60/20	<0.00001
Plasma EPO (U/l) Median (range)	8.6 (3.1–34.3)	8.5 (3.1–34.3)	8.6 (3.3–25.6)	0.45
Serum VEGF (pg/ml) Median (range)	52.2 (6.6–337)	52.4 (6.6–337)	49.9 (6.8–242)	0.45

SH, severe hypoglycaemia; EPO, erythropoietin; VEGF, vascular endothelial growth factor. *P* values refer to comparison between the two groups.
[a]Detection limit=10 pmol/l.
[b]*n*=213.
[c]*n*=207.
[d]*n*=137.

until analysis. Plasma EPO concentrations were measured using ELISA technique (R&D Systems' Colorimetric Sandwich ELISA kit: Quantikine IVD, R&D Systems, Europe, UK); detection limit is ∼0.1 U/l; normal range is 6–18 U/l. Serum VEGF (VEGF-165) was measured using Elisa (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). The intra- and inter-assay coefficients of variation in VEGF, as indicated by the manufacturer, are 4.5 and 7.3% respectively. C-peptide was measured by RIA (Autodelfia, Wallac Oy, Turku, Finland). Subjects were classified as being C-peptide negative if the value was below the detection limit of 10 pmol/l. Serum concentration of ACE was measured by a kinetics-based assay (Sigma Diagnostics). HbAlc was measured spectrophotometrically (DCA-2000, Bayer; normal range 4.1–6.4%).

Reporting and classification of hypoglycaemia

Episodes of hypoglycaemia were reported monthly on questionnaires mailed to the participants. Events of severe hypoglycaemia defined as episodes with symptoms of hypoglycaemia with need for assistance from another person were reported by telephone within 24 h after the event. These episodes were the primary end point. Structured telephone interviews were carried out by two trained study nurses to establish the level of documentation, classify severity and explore circumstances of the incidents. Additionally, at the end of the follow-up period, severe hypoglycaemia was reported retrospectively by questionnaire, and in the case of missed reporting, telephone interviews were carried out. Episodes of severe hypoglycaemia were validated as previously described in detail (13). Mild hypoglycaemia was defined as episodes with symptoms familiar to the patient as hypoglycaemia, and managed solely by the patient. These episodes were reported for the week preceding the return of the monthly questionnaires.

Statistical analysis

Calculations were performed with SPSS software package (Version 17.0; Chicago, IL, USA). Since the distribution of episodes of severe hypoglycaemia (primary end point) is very skewed (17), a log-linear negative binomial model was applied (SPSS: Analyse > Generalised Linear Models > Negative binomial >). Events of severe hypoglycaemia were dependent variable. First, univariate analyses with serum VEGF or plasma EPO as explanatory variables were done. Secondly, a multivariate analysis with adjustment for C-peptide status, hypoglycaemia awareness status, duration of diabetes, baseline HbAlc, serum ACE, body mass index (BMI) and age was performed. BMI and age were included in the model since these factors may influence the concentration of circulating VEGF (18, 19). Regression coefficients were transformed into percentage by means of the natural logarithm ($e^{coefficient}$).

Any associations between mild hypoglycaemia and levels of EPO and VEGF were calculated using univariate and multivariate linear regression (before analysis, mean episodes of mild hypoglycaemia were logarithmically transformed ($\log_{10}$), and regression coefficients were back-transformed to percentage). In the multivariate analysis adjustment for C-peptide status, hypoglycaemia awareness status, duration of diabetes, baseline HbAlc, serum ACE, BMI and age were performed. Associations between the levels of EPO and VEGF and demographic and diabetes-related variables were calculated using univariate and multivariate linear regression (the levels of EPO and VEGF were logarithmically transformed ($\log_{10}$), and regression coefficients were back-transformed to percentage). Comparisons between the subgroups in Table 1 were done by independent *t*-tests for continuous variables (logarithmised values were used in case of non-normally distributed values) or Pearson's χ^2-test for categorical variables (or Fisher's exact test, when relevant). A two-tailed *P* value of <0.05 was considered statistically significant.

Results

Reported episodes of hypoglycaemia

A total of 82 of the 219 patients (37%) contributed to 235 episodes of severe hypoglycaemia during the 1-year study period, corresponding to 1.1 episodes per patient-year. Patients who experienced one or more episodes of severe hypoglycaemia and reported a mean of 2.9 episodes per patient-year were older with a longer duration of diabetes, lower HbAlc and BMI, and more frequently had impaired hypoglycaemia awareness (Table 1). The frequency of mild hypoglycaemia was 1.7 episodes per patient-week.

Plasma EPO and serum VEGF concentrations

The plasma concentration of EPO was 8.6 (3.1–34.3) U/l (median (range)), and the serum concentration of VEGF was 52.2 (6.6–337) pg/ml. Eighteen patients had a blood glucose value below 3.1 mmol/l (mean 2.24 (s.d. 0.7) mmol/l), but no symptoms of hypoglycaemia were observed when blood samples were taken. The EPO and VEGF values in these patients did not differ from those with blood glucose values higher than 3.1 mmol/l (*P*=0.47 and *P*=0.63 respectively). There was no association between the levels of EPO and VEGF (data not shown).

Association between EPO and VEGF and episodes of severe hypoglycaemia

There was neither association between concentrations of plasma EPO nor serum VEGF and the number of episodes of severe hypoglycaemia in the study period

(Table 2 and Fig. 1a and b). Adjustment for duration of diabetes, HbAlc, serum ACE, hypoglycaemia awareness status (normal awareness, impaired awareness and unawareness), C-peptide status (detectable and undetectable), BMI and age did not change the results. Analyses excluding the 18 subjects with hypoglycaemia during blood sampling did not change the results. Neither did removal of 123 subjects with late diabetic complications, i.e. albuminuria, retinopathy or previous stroke/myocardial infarction, change the results. Finally, removing subjects treated with β-receptor antagonists ($n = 5$) or psychopharmacologic agents ($n = 3$) from the analysis did not change the results.

Association between EPO and VEGF and episodes of mild hypoglycaemia

The frequency of mild hypoglycaemia was not associated with the levels of EPO and VEGF either in univariate or multivariate analyses with adjustment for duration of diabetes, HbAlc, serum ACE, awareness status, C-peptide status, age and BMI (Table 2).

Demographic and diabetes-related characteristics and concentrations of EPO and VEGF

No demographic and diabetes-related characteristics (age, sex, diabetes duration, BMI, HbAlc at baseline, C-peptide or hypoglycaemia awareness category) were significantly associated with the levels of plasma EPO in a multivariate analysis (Table 3). Age was positively associated with serum VEGF levels ($P = 0.03$) with an increase in VEGF of 1.2% (95% confidence interval (CI): 0.2–2.3%) for every 1-year increase in age (Table 3) in a multivariate analysis. Female sex was associated with a 26% (95% CI: 1.4–44%) higher concentration of VEGF ($P = 0.04$). No other variables were associated with the level of VEGF.

Discussion

As opposed to our hypothesis, we found in this study that the concentrations of circulating EPO and VEGF are not associated with the number of prospectively recorded episodes of severe hypoglycaemia. Nor did we find an association between circulating EPO and VEGF and the frequency of mild hypoglycaemia. This is surprising, since both EPO and VEGF are probably involved in glucose metabolism and may also affect brain metabolism. Excluding subjects with hypoglycaemia during blood sampling, subjects with late diabetic complications which may alter EPO or VEGF levels and subjects treated with β-receptor antagonists or psycho-pharmacological agents did not change the results.

In recent years, a neuroprotective role has emerged for EPO in conditions with impaired substrate supply (20). EPO and its receptor are produced in the brain, especially in response to intracerebral metabolic stress such as acute brain hypoxia (21, 22). EPO receptors are also located in rat and mouse brain endothelial cells (23). A number of *in vitro* studies suggest that EPO may preserve cellular function during hypoglycaemia (8–10). A study by Miskowiak *et al.* (24, 25) has demonstrated that high-dose EPO treatment modulates neural processing in healthy adults and may improve cognitive function. We recently demonstrated in patients with type 1 diabetes that the concentration of EPO increases during hypoglycaemia, and that low baseline EPO levels may be associated with more pronounced cerebral dysfunction during hypoglycaemia (nadir plasma glucose concentration 2.2 mmol/l) than higher levels of EPO (1). VEGF, a potent regulator of normal and pathological angiogenesis (26), enhances glucose transport across the blood–retina barrier, most likely via translocation of the insulin-independent GLUT1 to the plasma membrane (27). *GLUT1* mRNA is also up-regulated by VEGF in an endothelial cellular model (12). Under normal conditions, GLUT1-mediated

Table 2 Regression coefficients (95% CIs) for concentrations of serum VEGF and plasma EPO (explanatory variables) in a generalised linear model (negative binomial distribution) with episodes of severe hypoglycaemia as dependent variable. The coefficients have been transformed into percentage by means of the natural logarithm ($e^{coefficient}$). In the analysis of mild hypoglycaemia, a regression analysis has been done. The regression coefficients (95% CIs) have been back-transformed to percentage since the episodes of mild hypoglycaemia were logarithmically transformed (log_{10}). Two-hundred and nineteen patients with type 1 diabetes are included in the analysis. The multivariate analyses are adjusted for HbAlc, duration of diabetes, serum angiotensin-converting enzyme (ACE), hypoglycaemia awareness status, C-peptide status, body mass index and age.

	Regression coefficients			
Variable	Univariate	*P*	Multivariate	*P*
	Severe hypoglycaemia (episodes/year), percentage change per unit change in explanatory variable			
EPO (U/l)	0.6 ($-3.9 \rightarrow 5.1$)	0.79	-0.7 ($-5.6 \rightarrow 4.5$)	0.79
VEGF (pg/ml)	-0.1 ($-0.4 \rightarrow 0.2$)	0.50	0 ($-0.4 \rightarrow 0.3$)	0.75
	Mild hypoglycaemia (episodes/week), percentage change per unit change in explanatory variable			
EPO (U/l)	1.6 ($-0.69 \rightarrow 4.2$)	0.18	1.4 ($-1.1 \rightarrow 3.8$)	0.28
VEGF (pg/ml)	0 ($-0.3 \rightarrow 0.2$)	0.58	0 ($-0.2 \rightarrow 0.2$)	0.64

EPO, erythropoietin; VEGF, vascular endothelial growth factor.
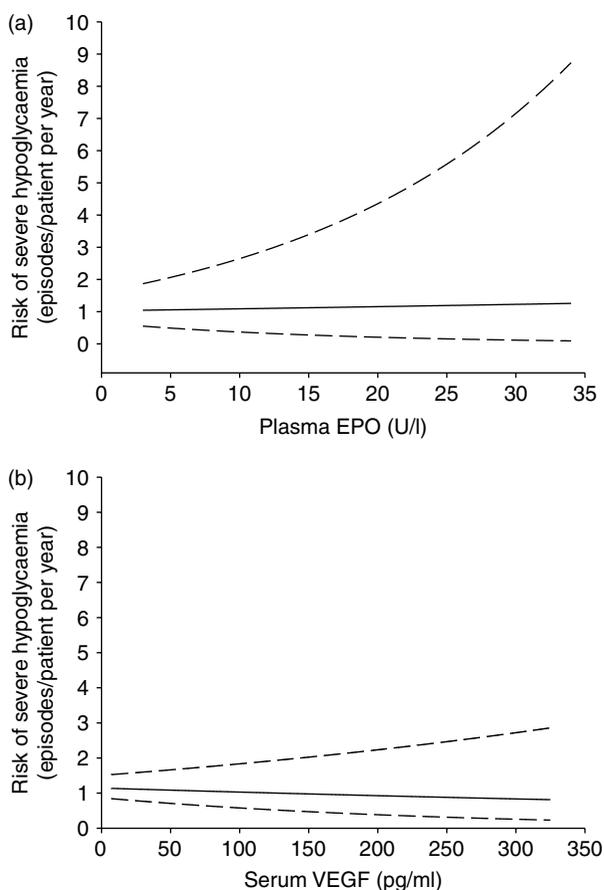
Figure 1 Relationship between concentration of plasma EPO (a) or serum VEGF (b) and rate of severe hypoglycaemia in 219 subjects with type 1 diabetes. Since the distribution of episodes of severe hypoglycaemia (dependent variable) is very skewed, a log-linear negative binomial model was applied. Dashed lines show 95% confidence limits.

glucose transport is not rate limiting for glucose metabolism in the brain. However, when blood glucose levels are reduced to 2–3 mmol/l, the transport of glucose through GLUT1 becomes rate limiting to glycolysis in neurons (28). VEGF concentrations have been shown to increase during hypoglycaemia in cells (human monocytic cell line) (29), in healthy adults (3–5) and in people with type 1 diabetes (2). Accordingly, in cell models, hypoglycaemia induces stabilisation of *VEGF* mRNA (11). Moreover, a high VEGF rise during hypoglycaemia in one study was associated with preservation of cognitive function during hypoglycaemia in healthy adults (4). In contrast, brief hyperglycaemia seems to down-regulate VEGF (3). Because hypothalamic cells involved in neuroendocrine regulation with afferent projections to blood glucose-responsive cells can synthesise VEGF, VEGF may play a central role in regulating glucose transport across the blood–brain barrier (30). Hence, if EPO and VEGF play important roles in transport of glucose from blood-stream to neurons and intracerebral glucose

metabolism, our results indicate that this role is primarily exerted in a paracrine or autocrine manner.

We found no differences in VEGF and EPO when comparing the 18 subjects who were hypoglycaemic during blood collection (but without symptoms of hypoglycaemia) at baseline and those subjects who were either eu- or hyperglycaemic. This was rather unexpected since VEGF (repeatedly) and EPO (once) have been found to increase during experimental hypoglycaemia (1–5). The unchanged VEGF and EPO level may be a consequence of (long-standing) diabetes as indicated by a comparison of the magnitude of the VEGF response during hypoglycaemia in people without and with diabetes (2, 4). We do not know for how long the subjects with asymptomatic hypoglycaemia had been exposed to hypoglycaemia when the non-arterialised venous blood was collected. Short-term hypoglycaemia probably has lesser stimulating impact on VEGF levels than a more robust hypoglycaemic stimulus. Furthermore, antecedent hypoglycaemia leads to a clearly diminished VEGF response, which has been demonstrated by Merl *et al.* (5). Since many of the patients with plasma glucose below 3.1 mmol/l suffer from impaired hypoglycaemia awareness, it is plausible that they have been exposed to antecedent low plasma glucose values recently. It is also possible that collection of blood samples and recruitment of subjects to hypoglycaemic clamp experiments were standardised to a further extent than in the present study, leading to lower variation in VEGF and EPO during experimental hypoglycaemia.

No significant associations were found between circulating EPO and any demographic or diabetes-related characteristics. This is in contrast to studies reporting that plasma EPO is either positively (31) or negatively associated with age (32), negatively associated with duration of diabetes (31) and negatively correlated with HbAlc (33). Unfortunately, information about EPO levels in unselected patients with type 1 diabetes is very limited, and most studies have focused on anaemia or diabetic neuropathy and nephropathy. New cross-sectional and longitudinal studies elucidating possible roles of endogenous EPO in other aspects of diabetes are therefore warranted. The positive association between age and serum VEGF levels in our study is in accordance with a study by Chiarelli *et al.* (34) and partly with a study by Sandhofer *et al.* (18), but in contrast to a study by Chaturvedi *et al.* (35). In accordance with our results, Chaturvedi *et al.* (35) did not find any associations between VEGF level and duration of diabetes or HbAlc. This is, however, opposed by a study by Lim *et al.* (36) who found a positive association between VEGF and HbAlc in people with type 1 diabetes. The reduction in VEGF associated with male sex is in accordance with a study by Malamitsi-Puchner *et al.* (37). It should, however, be kept in mind that age and gender only account for a small proportion of variance in VEGF in the present study. Lack of

Table 3 Multiple regression analyses of associations between logarithmic values ($\log_{10}$) of plasma EPO and serum VEGF and demographic and diabetes-related characteristics in 219 patients with type 1 diabetes. The logarithmic regression coefficients and CIs have been back-transformed to percentage.

	Percentage change in EPO/VEGF per one unit of explanatory variable							
	EPO	CI 95%		P	VEGF	CI 95%		P
Age (years)	0	−0.7	0.5	0.73	1.2	0.2	2.3	0.03
Sex (female versus male)	−9.4	−20.6	3.5	0.14	−25.8	−44.3	−1.4	0.04
Duration of diabetes (years)	0	−0.7	0.5	0.90	1.2	0	2.3	0.10
Body mass index (kg/m^2)	0	−2.1	1.6	0.84	1.4	−2.7	5.4	0.53
HbA1c at baseline (%)	1.2	−4.3	6.7	0.69	5.2	−6.5	18.3	0.40
C-peptide (pmol/l)	0	0	0	0.72	0	0	0.2	0.69
Categorised hypoglycaemia awareness (aware→impaired→unaware)	−1.1	−10.3	8.6	0.80	−13.7	−29.7	5.9	0.16
R^2	0.02				0.04			

R^2 is the proportion of variance in EPO and VEGF explained by the model. EPO, erythropoietin; VEGF, vascular endothelial growth factor; CI, confidence interval.

consensus between different studies probably reflects a combination of different methodology and different populations in the studies. However, to improve quality of future cross-sectional studies of circulating VEGF in patients with type 1 diabetes, credible data are warranted to identify clinical factors affecting VEGF levels.

The strength of our study is its prospective design with thorough gathering of data regarding the primary end point, i.e. episodes of severe hypoglycaemia. Moreover, we have used a statistical model that deals with the very skewed distribution of episodes of severe hypoglycaemia. Therefore, we have found that the results regarding the primary end point are reliable. Our study has limitations that may influence the results. First, severe hypoglycaemia may be underreported, at least in subjects with recurrent events (16). Especially, nocturnal episodes may not be registered, since these episodes may occur without notice of the patients or relatives. Secondly, the levels of VEGF and EPO may be influenced by several factors potentially biasing the study: the level of VEGF may change during the menstrual cycle (38), antecedent hypoglycaemia attenuates VEGF response to subsequent hypoglycaemia (5), and VEGF levels seem to affect carbohydrate intake (39). EPO measurements may show diurnal variations, although consensus on this topic has not been established (40, 41). This may be of importance since blood sampling was performed within a rather broad time period of about 6 h. More standardised blood collection may lead to lower variation in EPO and VEGF, and unmask a statistically significant relationship between EPO/VEGF and the frequency of severe hypoglycaemia. However, the clinical goal of the present study is to determine the risk of future severe hypoglycaemic episodes by random measuring of VEGF and EPO, as it would be done in a clinical and practical context. In that situation, a relatively straightforward blood sample procedure is needed. Thirdly, it is a well-known clinical observation that severe hypoglycaemia occurs due to poor self-care and may be affected by other confounders, such as severe liver problems or hormonal

diseases. Owing to lack of robust measures of such confounders, we have had to include only broadly accepted risk factor in the analysis. Finally, the hypothesis that low VEGF levels are associated with increased risk of severe hypoglycaemia is based on clamped hypoglycaemic studies and cellular studies. The relationship – if any – between spontaneous circulating VEGF levels and stimulated hypoglycaemic levels of VEGF is unclear, and it is possible that a protective mechanism of brain function depends on raised hypoglycaemic VEGF levels and does not on spontaneously high euglycaemic concentrations of VEGF. This may explain the negative findings of the present study and challenges the hypothesis of the study.

The ability to predict severe hypoglycaemia would be a major advantage in daily diabetes management. To date, the only consistently reported biomarkers of severe hypoglycaemia in type 1 diabetes are undetectable C-peptide levels (42–44), low HbA1c (44–46) and high serum ACE activity (13, 43, 47, 48). However, these markers only explain a minor proportion of the risk variation between subjects. We conclude that random measurements of serum VEGF or plasma EPO levels do not add to prediction of risk of severe hypoglycaemia in type 1 diabetes. Further efforts should be directed towards the identification of new biomarkers for severe hypoglycaemia, enabling improved understanding, prediction and prevention of this major clinical problem in type 1 diabetes.

Declaration of interest

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