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Divergent effects of hyper- and hypoglycemia on circulating vascular endothelial growth factor in humans

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Abstract

Vascular endothelial growth factor (VEGF) is known to be up-regulated by hypoxia, hyperglycemia, and hypoglycemia in vitro. In contrast, it has been found in healthy humans that plasma concentrations of VEGF decrease upon hypoxia under in vivo conditions, indicating that systemic VEGF concentration may be differently regulated than cellular expression. To test the effect of blood glucose levels on VEGF concentrations in vivo, we examined plasma VEGF changes upon brief hyper- and hypoglycemia in healthy male subjects. We rapidly induced in a crossover design hypoglycemia by insulin bolus application of 0.1 U/kg or hyperglycemia by dextrose infusion in 24 healthy young men. Plasma VEGF concentrations were measured at baseline, at the target glucose concentration of <2.2 mmol/L or >10 mmol/L, and after further 5 and 10 minutes. Plasma VEGF concentrations decreased upon hyperglycemia as compared with euglycemic baseline (P = .027), whereas during hypoglycemic condition, there was a trend for an increase (P = .091). Analysis for repeated measurements including both conditions revealed a differential regulation of plasma VEGF levels upon glycemic condition (P = .035). Our results are consistent with the hypothesis that systemic VEGF concentration may be differentially regulated than expression on cellular basis. Because VEGF is a candidate hormone for regulating glucose passage across the blood-brain barrier under critical conditions, it possibly acts as a neuroprotective controller for constant cerebral glucose supply. This may be of relevance for the understanding of VEGF alterations in different pathological states such as diabetes mellitus.

1. Introduction

Vascular endothelial growth factor (VEGF), a potent mediator of vascular permeability, has been the object of various studies investigating its expression, regulation, and function in metabolism in vitro [1]. On cellular level, it is well established that VEGF expression is induced by hypoxia [1-3], hypoglycemia [3-6], and hyperglycemia [4,7-10].

On systemic level, however, there is evidence that VEGF concentrations are differently regulated than the expression on a cellular basis. Although it was previously found in accordance with in vitro data that hypoglycemia induces a rapid increase in circulating VEGF concentrations in humans [11-13], our group could also demonstrate that, contrasting to in vitro investigations, acute hypoxia decreases plasma VEGF concentrations in healthy men [13]. It has been postulated that high glucose concentrations induce a pseudo-hypoxic state because of a high $NADH^+/NAD^+$ ratio in cells even when the oxygen tension is normal [14]. Based on the theory that hyperglycemia-induced pseudohypoxia mimics the effects of true hypoxia on vascular and neural function [14], we hypothesized that high glucose concentrations, in analogy to our previously found hypoxic effects, would decrease circulating VEGF concentrations in vivo. To control for the known VEGF rise upon hypoglycemia, we performed a randomized crossover study investigating the effects of brief hypo- and hyperglycemia on plasma VEGF

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concentrations in healthy young men. Simultaneously, we measured circulating catecholamine and cortisol concentrations as known parameters influencing VEGF concentrations in vivo.

2. Subjects and methods

2.1. Subjects

Twenty-four healthy and lean white men (baseline characteristics shown in Table 1) participated in the study. Exclusion criteria were chronic or acute physical and mental illness, alcohol or drug abuse, smoking, competitive sports, exceptional physical or psychological stress, and current medication of any kind. Each volunteer gave written informed consent, and the study was approved by the ethics committee of the University of Luebeck.

2.2. Experimental design

Each subject participated in a hypoglycemic and a hyperglycemic intervention. Subjects were requested to abstain from alcohol, not to perform any kind of exhausting physical activity, and to go to bed no later than 11:00 PM on the day preceding the study. On the days of experimental testing, subjects reported to the medical research unit after an overnight fast of at least 12 hours. A cannula was inserted into a vein on the back of the hand and a second cannula into an antecubital vein of the contralateral arm. Subsequently, baseline blood samples for determining plasma glucose, VEGF, insulin, catecholamine, and cortisol concentrations were collected. After the baseline period, an intravenous insulin bolus (H-insulin; Hoechst, Frankfurt, Germany) of 0.1 U/(min kg) was administered or a 20% dextrose infusion at a rate of 325 mL/h was started in a randomized manner to induce hypo- or hyperglycemia according to a crossover design. There was an interval of at least 4 weeks between the 2 interventions. Blood glucose concentrations were monitored at 5-minute intervals during the entire experimental epoch (B-Glucose Data Management; HemoCue, Grossostheim, Germany). When blood glucose concentration reached the target glucose levels, that is, less than 2.2 mmol/L (hypoglycemia) or greater than 10.0 mmol/L (hyperglycemia), the second blood sampling for VEGF, insulin, catecholamine, and cortisol determination occurred, which was followed by 2 further samplings of VEGF after 5 and after 10 minutes. Subsequently, blood glucose was normalized again by infusion of a 20% dextrose solution or by physiological glucose degradation. Hormone probes were sampled and measurements were performed as previously described [13].

Table 1
Mean values (±SEM) of baseline characteristics for the participating subjects (N = 24)

Parameter	Values ± SEM
Age (y)	25 ± 0.61
Body height (m)	1.81 ± 0.02
Body weight (kg)	77.42 ± 2.08
BMI (kg/m^2)	20.65 ± 1.69
Fasting glucose concentration (mmol/L)	4.57 ± 0.10

BMI indicates body mass index.

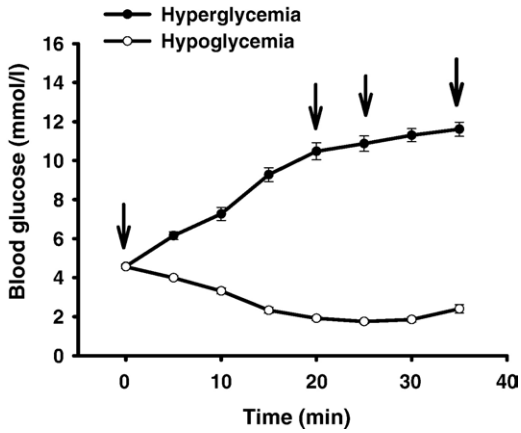

Fig. 1. Blood glucose concentrations (mean values ± SEM) at baseline and during the hypo- (white circles) and hyperglycemic (black circles) intervention (N = 24). Arrows mark the time points of VEGF measurements.

2.3. Statistical analysis

Values are presented as mean values ± SEM. Statistical analysis was based on analysis of variance (ANOVA) for repeated measurements, including the factors *time* (time points of data collection) and *session* (hypoglycemia vs hyperglycemia). The interaction of these 2 factors was termed *session* × *time*. Paired *t* tests were also performed to assess differences between both conditions at baseline measurements as well as to confirm changes between baseline and final values of VEGF measurements. A P value < .05 was considered significant.

3. Results

3.1. Plasma glucose concentrations

After a 10-minute period with basal glucose levels of 4.58 ± 0.11 mmol/L during hyperglycemia and 4.56 ± 0.09 mmol/L during hypoglycemia, blood glucose concentrations were altered by dextrose infusion or insulin bolus to levels of 10.48 ± 0.44 and 1.92 ± 0.12 mmol/L in the 2 conditions (Fig. 1). Levels were maintained for another 15 minutes at 11.07 ± 0.39 and 1.98 ± 0.14 mmol/L during hyper- and hypoglycemia, respectively.

3.2. Plasma VEGF concentrations

Hypoglycemia induced a trend for an increase of VEGF levels at the target glucose concentration in comparison with baseline levels (time effect P = .061, Fig. 2A). Similarly, VEGF concentrations measured during the hypoglycemic

plateau showed an increase, although it failed to reach significance (time effect of final value as compared with baseline $P = .091$, N = 24). During hyperglycemia, there was a trend for a decrease in plasma VEGF concentrations from baseline to the target glucose value (time effect $P = .061$), a drop that reached significance at the end of the intervention (time effect of final value as compared with baseline $P = .027$, Fig. 2B).

The small insert in Fig. 2 illustrates the divergent effects of glycemic levels on plasma VEGF concentrations comparing baseline values with the final time point of measurements. Statistical analysis of original data revealed a significant interaction effect of the glycemic intervention (effect of session by time $P = .035$). Because VEGF baseline values were different (26.99 ± 5.84 vs 47.84 ± 9.54 pg/mL in the hypoglycemic and the hyperglycemic condition, respectively; $P = .034$), with higher concentrations in the hyperglycemic session, values in the insert of Fig. 2 are baseline adjusted for illustrative reasons.

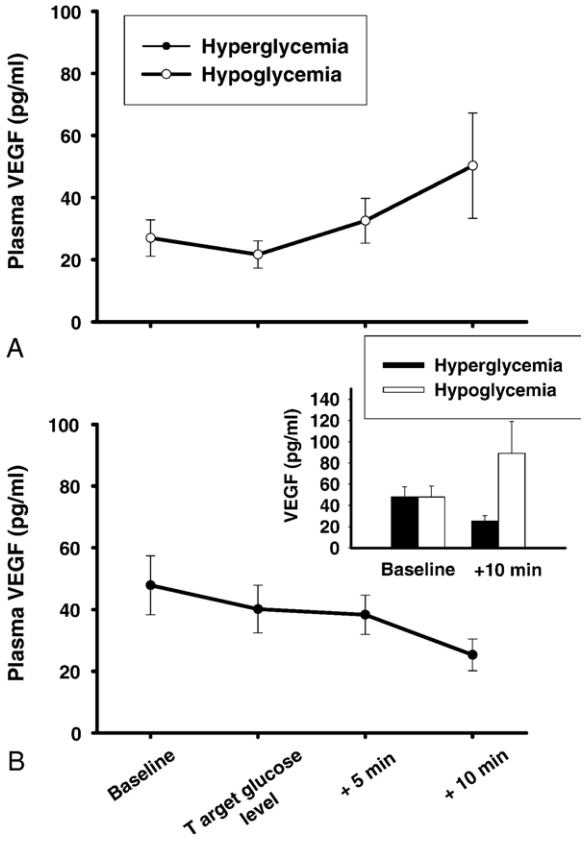

Fig. 2. Plasma VEGF concentrations (mean values ± SEM) at baseline and during the hypo- (A) and the hyperglycemic (B) intervention at target glucose level, and after 5 and 10 min thereafter (N = 24). The ANOVA analysis revealed a decrease of VEGF concentrations during hyperglycemia, whereas there was a trend for an increase upon hypoglycemia. Small insert: Plasma VEGF concentrations (mean values ± SEM) at baseline and at the end of the interventions after baseline adjustment to illustrate the divergence in hormonal response upon the 2 glycemic conditions.

Table 2
Mean values (± SEM) of circulating catecholamines, cortisol, and insulin concentrations at baseline measurements and after having reached the target glucose concentrations during hypo- and hyperglycemia

	Baseline values (mean ± SEM)	Values at target glucose level (mean ± SEM)	*P*
Epinephrine (pg/mL)			
Hypoglycemia	54.48 ± 11.72	130.22 ± 50.82	.165
Hyperglycemia	63.36 ± 14.82	20.09 ± 1.97	.008**
Norepinephrine (pg/mL)			
Hypoglycemia	259.50 ± 20.36	213.87 ± 20.73	.028*
Hyperglycemia	293.95 ± 18.01	186.10 ± 20.26	<.001***
Cortisol (μg/dL)			
Hypoglycemia	17.71 ± 1.41	14.50 ± 1.35	.019*
Hyperglycemia	16.24 ± 1.31	11.75 ± 1.00	<.001***
Insulin (μU/mL)			
Hypoglycemia	6.03 ± 0.85	267.45 ± 30.83	<.001***
Hyperglycemia	5.74 ± 0.66	23.86 ± 2.88	<.001***

P values refer to ANOVA analyses (asterisks mark the level of significance).

3.3. Serum insulin, plasma catecholamine, and serum cortisol concentrations

Stress hormonal and insulin concentrations are summarized in Table 2. Serum insulin levels increased under both conditions ($P < .001$), whereas there was a general decrease in overall stress hormonal response only upon hyperglycemia ($P < .01$ for all). Although epinephrine concentrations were not altered after reaching the hypoglycemic target glucose level as compared with baseline measurements, norepinephrine and cortisol levels surprisingly decreased significantly upon hypoglycemia ($P < .05$).

4. Discussion

Our data show divergent effects of high and low plasma glucose concentrations on circulating VEGF levels in healthy humans. Results from the hypoglycemic intervention are consistent with previous investigations demonstrating an increase of circulating VEGF concentrations upon blood glucose drop in humans [11-13], although data from the present study failed to reach significance probably because of the short period of blood sampling after induction of hypoglycemia. In contrast, brief hyperglycemia clearly decreases circulating VEGF concentrations. This result conflicts with previous investigations in human or animal vascular smooth muscle cells, mesangial cells, or podocytes in vitro [7-9] as well as data from vitreous and aqueous fluids of diabetic patients [15] reporting stimulatory effects of high glucose on VEGF expression or concentrations. However, systemically elevated VEGF levels have not been found in diabetic patients yet [16,17]. On the contrary, one study even found an impairment in fibroblast VEGF production resulting from the diabetic state in db/db mice [18], which is compatible with our results.

To date, the function and potential clinical relevance of circulating VEGF remain unclear. Alterations in various

pathological conditions have been reported [19-21], particularly in diabetes mellitus [10]. Although these data hint at a role of VEGF in the pathogenesis of this disease and diabetes-specific complications, the exact mechanistic role of VEGF has not been unraveled so far. However, one of the known effects of VEGF is the enhancement and translocation of the glucose transporter 1 to the cell surface at the blood-brain barrier [11,22,23], increasing glucose transport rate into the brain. If VEGF plays a central role in regulating glucose transport across the blood-brain barrier, one could speculate that the decrease of VEGF concentrations upon hyperglycemia as seen here in our study may serve a neuroprotective purpose to prevent the brain from glucose overload during this state. In this context, a failure of such mechanisms could have extensive relevance for diabetic features such as hypoglycemia unawareness or body weight regulation [24]. Moreover, VEGF also seems to play a crucial role in terms of diseases not directly related to diabetes mellitus. The contribution of VEGF to cardiovascular disease and atherogenesis has gained much attention in recent years, but the exact mechanisms still remain unclear [25]. In summary, it appears that the effects of VEGF are strongly dependent on its concentration: Low physiological amounts seem to be required for blood vascular homeostasis, endothelial cell survival, and production of nitric oxide, resulting in vasodilatation and antithrombosis, that is, are vasculoprotective, whereas much higher concentrations are required for angiogenic and vasculogenic effects [25]. Taken together, it is possible that glucose-mediated VEGF regulation as focused in our study may be a crucial factor underlying cardiovascular complications in diseases with known alterations in glucose metabolism such as the metabolic syndrome even before diabetes occurs. However, until confirmation by future studies, these hypotheses remain speculative.

Because of our experimental approach, our study has some limitations. Our study design inducing brief changes in blood glucose concentrations does not allow for drawing any conclusions about long-term regulation of VEGF levels. Furthermore, our study cannot unravel the underlying mechanism of the observed effects. Plasma VEGF regulation is sensitive to influences of various parameters such as norepinephrine, cortisol, or insulin in circulating blood, probably confounding our data [13]. However, in our study, serum insulin concentrations increased in both glycemic conditions, which does not argue for a central involvement of insulin in plasma VEGF regulation. Furthermore, a previous study investigating VEGF regulation under the condition of a hyperinsulinemic glucose clamp, that is, under constant high insulin concentrations, did not indicate an influence of insulin on plasma VEGF [13]. Rather, norepinephrine, which is known to induce VEGF expression in adipose cells, may play a role in this context because we found pronounced decreases in catecholamine concentrations upon hyperglycemia in the present study. On the other hand, although it is well known that hypoglycemia causes a rise in all stress hormonal concentrations, our present data do not reach significance in terms of an epinephrine increase and even show a decrease in norepinephrine and cortisol concentrations probably due to the initial insertion of the vein catheter and the relatively short period between the first and second blood sampling. Taken together, circulating VEGF concentrations do not seem to be influenced by stress hormonal alterations in our study. However, the precise mechanisms leading to the differential regulation of VEGF upon high glucose concentrations on cellular and systemic levels have to be unraveled in future studies.

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