ORIGINAL ARTICLE

Hypoglycemia, but Not Glucose Variability, Relates to Vascular Function in Children with Type 1 Diabetes

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Abstract

Background: Chronic sustained hyperglycemia unequivocally predicts vascular disease in diabetes. However, the vascular risk of glucose variability, including hypoglycemia, is uncertain. Vascular dysfunction is present in children with type 1 diabetes and is a critical precursor of atherosclerosis. We aimed to evaluate the relationship between glucose variability and vascular function in children with type 1 diabetes.

Subjects and Methods: Fifty-two type 1 diabetes subjects (14 [SD 2.7] years old, 25 males) had continuous glucose monitoring that included 48 h of data used to evaluate glucose variability (mean amplitude of glycemic excursions [MAGE] and other measurements) and hypoglycemia indices (glycemic risk assessment diabetes equation [GRADE] hypoglycemia, Low Blood Glucose Index [LBGI], and observed duration of hypoglycemia). Children with type 1 diabetes and 50 age- and gender-matched controls had assessments of vascular function (flow-mediated dilatation [FMD] and glyceryl trinitrate-mediated dilatation [GTN]).

Results: Children with type 1 diabetes had lower FMD and GTN than controls ($P=0.02$ and $P<0.001$, respectively). GRADE hypoglycemia and LBGI were inversely related to FMD ($r=-0.36$, $P=0.009$ and $r=-0.302$, $P=0.03$, respectively) but did not relate to GTN. GRADE hypoglycemia was independently related to FMD (regression coefficient $=-0.25\pm0.09$, $P=0.006$). MAGE and other measurements of glucose variability measurements did not relate to FMD or GTN.

Conclusions: Hypoglycemia, but not glucose variability, during continuous glucose monitoring relates to impaired vascular endothelial function in children with type 1 diabetes. Hypoglycemia may be an additional risk factor for early cardiovascular disease, but the effect of glucose variability, independent of glycosylated hemoglobin, on vascular function remains uncertain.

Introduction

CHRONIC SUSTAINED HYPERGLYCEMIA unequivocally increases the risk of vascular complications of type 1 diabetes (T1D), and its improvement reduces the risk.¹ However, the effect of glucose fluctuations throughout the day and night (glucose variability), including hypoglycemic episodes, on vascular complications, in addition to chronic sustained hyperglycemia, is still controversial.²⁻⁷ Glycosylated hemoglobin (HbA1c) is the accepted method to measure chronic hyperglycemia, but there is no gold standard method to measure glucose variability. Glucose variability can be calculated from either isolated blood glucose measurements done throughout the day using glucose test strips or from a continuous glucose monitoring system (CGMS) worn over 3

days. CGMS measures subcutaneous interstitial glucose levels every 5 min and allows calculation of intraday and day-to-day glucose variability using different mathematical formulas such as mean amplitude of glycemic excursions (MAGE), SD of mean blood glucose, mean of daily differences (MODD), and continuous overall net glycemic action (CONGA).^{8,9} CGMS allows calculation of the glycemic risk assessment diabetes equation (GRADE) score and the percentages of the GRADE score attributed to hypoglycemia, euglycemia, and hyperglycemia.¹⁰ CGMS also allows calculation of a low glucose index, a statistical measurement, that increases when the frequency and/or the duration of low glucose readings increase.^{11,12}

In adults with T1D, glucose variability measurements obtained from seven isolated blood glucose levels a day is not an

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additional risk factor for development of retinopathy or nephropathy⁵ but may be important in the development of peripheral neuropathy.⁴ There are few data on glucose variability measured using CGMS in T1D and vascular health, but it does not relate to arterial stiffness, an early marker of atherosclerosis, in adults with T1D.⁶

In adults with metabolic syndrome and type 2 diabetes, glucose variability measured by CGMS relates to endothelial dysfunction.¹³ In healthy adults and adults with type 2 diabetes, fluctuations in blood glucose levels during a hyperglycemic-euinsulinemic clamp have more deleterious effects than sustained high glucose on endothelial dysfunction and oxidative stress.¹⁴ In vitro studies have shown that fluctuating blood glucose levels increase markers of endothelial dysfunction, including vascular cell adhesion molecules, intracellular adhesion molecules, and E-selectin.¹⁵

There are few data evaluating hypoglycemia and vascular function. These data are limited to adults with T1D and use either acutely induced hypoglycemia or a history of severe hypoglycemia to evaluate the association with vascular function.^{7,16,17} Acutely induced hypoglycemia increases markers of endothelial dysfunction (vascular cell adhesion molecules, intracellular adhesion molecules, and E-selectin), inflammation, and platelet activation^{16,18} and causes arterial stiffness.¹⁷ Repeated and severe episodes of hypoglycemia cause impaired endothelial function measured by flow-mediated dilatation (FMD).⁷ There are no studies evaluating mild to moderate hypoglycemia, which was not induced, and endothelial function in adults or children.

Children with T1D have higher glucose variability and a higher number of hypoglycemic episodes compared with adults with type 1 or type 2 diabetes.^{19,20} After a short duration of T1D they have vascular endothelial and smooth muscle dysfunction as measured by FMD and glyceryl trinitrate-induced dilatation (GTN), respectively.²¹

The relationship between glucose variability or hypoglycemic episodes and vascular function as an early marker of cardiovascular disease has not been evaluated in children with T1D. Therefore, we aimed to determine the role of glucose variability and hypoglycemia, assessed using CGMS, on vascular function in these children. Our primary outcome was the association between glucose variability and vascular function.

Subjects and Methods

Subjects

Fifty-two children with T1D and 50 age- and gender-matched healthy children were enrolled in the study. Both groups had assessment of vascular function (FMD and GTN). Children with T1D who were referred from the diabetes outpatient clinics at Women's and Children's Hospital (Adelaide, Australia) to have a CGMS were recruited consecutively. The three main reasons for referral were optimization of therapy, concerns about nocturnal hypoglycemia, and resistance to change in insulin schedule. Only six of 58 consecutive subjects referred to CGMS did not participate in the study (four were not interested, one was more than 100 km distance from the investigation center, and one did not have CGMS done). Healthy children were recruited from two sources: siblings or friends of children with T1D and relatives of staff members. Exclusion criteria were subjects younger

than 8 years to ensure cooperation with ultrasound tests, diabetes duration of less than 1 year, retinopathy on direct fundoscopy, microalbuminuria measured by early morning albumin/creatinine ratio, smoking, hypertension (defined as blood pressure at rest above the 95th percentile for age, gender, and height), antihypertensives, lipid-lowering treatment, and/or multivitamins. The Women's and Children's Hospital's Human Research Ethics Committee approved the study. Written informed consent was obtained from parents/guardians and children if older than 16 years of age.

Glucose variability and hypoglycemia evaluation

Subjects with T1D wore a CGMS (CGMS[®] System Gold[™], Medtronic Minimed, Northridge, CA) for assessment of glucose variability and hypoglycemia. A CGMS was inserted by a diabetes educator on Day 0, and children and families were instructed to perform at least four measurements of blood glucose levels a day and to enter them into the CGMS for calibration of the system. CGMS data were downloaded on Day 3. Glucose measurements on Day 0 and Day 3 were not used in the calculations to avoid bias related to anxiety in relation to insertion or removal of the sensor and to include a consistent 48 h for all subjects (from 12 a.m. on Day 1 to 11:59 p.m. on Day 2). Glucose measurements obtained from Days 1 and 2 were exported into an Excel[®] (Microsoft, Redmond, WA) database and entered into a computer algorithm developed by P.B. as previously described.²² This computer algorithm allows calculations of the following measurements of glucose variability: MAGE, SD of mean blood glucose (MBG), CONGA, MODD, and J Index. MAGE was calculated by an automated algorithm designed to locate all the peaks and nadirs in each CGMS data set (and its subsets) according to the rules defined by Service et al.⁸ The SD required to determine whether a glycemic excursion was eligible to be included in MAGE was estimated from each subject's 48 h of CGMS usage (not recalculated for each 24-h period), and only the magnitudes of upward excursions were averaged. Downward excursions were also calculated and averaged; as this MAGE calculation was not different from MAGE calculated using upward excursions, we will be referring to MAGE calculated from upwards excursions.²² CONGA was calculated after different hour intervals of observations called *n* (*n*=1, 2, 3, 4, 5, 6, 7, and 8). For each observation or glucose value after *n* h of observations, the difference between the current observation and the observation *n* h (*n*=1, 2, 3, 4, 5, 6, 7, and 8) previously was calculated.⁹ MODD was calculated from the absolute differences between paired sensor glucose values during two successive 24-h periods of CGMS. The J Index was calculated from the formula $J = 0.324 (MBG + SD)$.²³

Using the data from CGMS hypoglycemia was evaluated using GRADE, Low Blood Glucose Index (LBGI), and duration of hypoglycemia. GRADE scores are an empirical representation of the "risk" (on a scale of 0 to 50) associated with a specified glucose concentration.¹⁰ A GRADE score is assigned to each glucose observation in an individual's CGMS profile according to the formula $GRADE\ score = 425[\log[\log(\text{glucose})] + 0.16]^2$, and scores are averaged over the entire CGMS profile. The relative contributions (as a percentage) to the overall GRADE score from glucose observations in the ranges <3.9, 3.9–7.8, and >7.8 mmol/L form the hypoglycemic, euglycemic, and hyperglycemic GRADE scores, respectively.¹⁰

LBG1 was calculated and adapted for CGMS as described by McCall et al.¹² LBG1 combines, in a single number, the percentage of low glucose readings and their magnitude, in the lower glucose range.¹¹ Duration of hypoglycemia was calculated as percentage of time with glucose levels under 3.5 mmol/L using the data obtained from CGMS.

History of severe hypoglycemia since T1D diagnosis, defined as a hypoglycemic event that resulted in seizure or collapse and required assistance from others, was obtained in addition to diabetes duration, insulin dose, and insulin regimen. These data were verified with outpatient medical records. There were no changes in insulin regimen during CGMS.

Measurements and laboratory tests

Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm. Weight with minimal clothing was taken on an electronic digital scale to the nearest 0.1 kg. Body mass index (BMI) and BMI z-score were calculated using EpiInfo database version 3.2.2 (www.cdc.gov/epiinfo). Blood pressure was measured with appropriate size cuff, and an average of three readings was recorded.

Fasting venous blood samples were collected. HbA1c was measured using a latex immunoagglutination inhibition methodology (DCA 2000 HbA1c reagent kit, Bayer, Toronto, ON, Canada). Glucose, lipid profile, and high-sensitivity C-reactive protein were measured as previously described.²¹

Vascular function assessment

Vascular function (FMD and GTN) assessment was performed on Day 3 of wearing the CGMS before the download and was assessed as previously reported.^{21,24} Brachial artery diameter was measured in a longitudinal section, with a L17-5 MHz linear array transducer (Phillips, Bothel, WA), using an iU22 ultrasound system. Each study included four scans. The first one was taken at rest. FMD was then induced by occluding arterial blood flow for 4 min using a sphygmomanometer inflated to 250 mm Hg. The second scan (FMD) was measured between 45 and 75 s after cuff deflation. The third (re-control) scan was taken after 10 min, allowing for vessel recovery. The last scan was taken 4 min after sublingual administration of glyceryl trinitrate spray (400 µg, Nitrolingual Spray; G. Pohl-Boskamp, Hohenlockstedt, Germany).

Images were recorded and analyzed by a blinded observer. For each scan, measurements were made incident with the electrocardiogram R wave using ultrasonic calipers over four cardiac cycles, and the measurements were averaged. There were four final average measurements (resting vessel diameter, FMD, re-control, and GTN) that were expressed as percentages of the first control (resting) scan. Our coefficient of variance between 20 subjects is 3.9% for FMD and 4.0% for GTN.²⁴

Statistics

The data were analyzed using S plus version 8.0 for Windows software. Natural logarithmic transformation was applied where appropriate. Independent-samples *t* tests and χ^2 tests were used to assess differences between T1D and controls. Pearson correlation coefficients and Spearman correlation were used to evaluate the associations between glucose

TABLE 1. SUBJECT CHARACTERISTICS

	Children with type 1 diabetes (n=52)	Controls (n=50)	P
Age (years)	14 (2.7)	14.8 (3.3)	0.19
Sex (male/female)	25/27	20/30	0.41
Body mass index z-score	0.42 (0.7)	0.23 (0.8)	0.21
Waist circumference (cm)	70.2 (8.9)	70.1 (8.1)	0.99
Blood pressure (mm Hg)			
Systolic	114.6 (9.9)	109 (8.9)	0.008
Diastolic	62.7 (5.6)	61 (6)	0.27
FMD (%)	5 (4.2)	7.4 (5.6)	0.02
GTN (%)	22.7 (6.7)	29 (7.1)	<0.001
Vessel diameter (cm)	0.27 (0.04)	0.26 (0.03)	0.15
hsCRP (mg/L)	0.9 (0.1–31)	0.6 (0.1–11)	0.07
LDL cholesterol (mmol/L)	2.5 (0.8)	2.7 (0.9)	0.29
HDL cholesterol (nmol/L)	1.5 (0.3)	1.4 (0.3)	0.08
Fasting glucose (mmol/L)	11.1 (4.3)	4.8 (0.4)	<0.001
HbA1c (%)	8.9 (6.7–14)	5 (4.4–5.8)	<0.001

Data are mean (SD) or geometric mean (range) values.

FMD, flow-mediated dilatation; GTN, glyceryl trinitrate-mediated dilatation; hsCRP, high-sensitivity C-reactive protein; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

variability measurements, hypoglycemia measurements (GRADE hypoglycemia, LBG1, and duration of hypoglycemia), vascular function, and other variables. Associations of FMD with measurements of hypoglycemia were further investigated using multiple regression with BMI z-score, diabetes duration, and glucose predictors. Statistical significance was inferred with a *P* value (type I error) of <0.05.

A sample size of 44 subjects provide a power of 80% at a significance of 0.05 to detect an association between glucose variability and vascular function ($r=0.4$).

Results

Fifty-two children with T1D (14 [SD 2.7] years old and mean diabetes duration of 5.5 ± 4 years) and 50 age- and gender-matched healthy children participated in the study. Subjects with T1D had significantly lower FMD and GTN compared with controls (Table 1).

Fifty-two subjects with T1D wore the CGMS for 72 h, but two CGMS datasets had to be excluded from the analysis as there were missing interstitial glucose levels that did not allow glucose variability or hypoglycemia calculations. Glucose variability and hypoglycemic measurements in the 50 subjects are included in Table 2. Their mean insulin dose was 0.9 ± 0.3 units/kg/day. Five subjects were using a continuous subcutaneous insulin infusion, and 47 received multiple daily injections (two or more). Two subjects had celiac disease and were on gluten-free diet.

Forty-one children had one or more mild episodes of hypoglycemia during the whole CGMS recording. No subjects had a moderate or severe episode of hypoglycemia during the

TABLE 2. GLUCOSE VARIABILITY AND HYPOGLYCEMIA MEASUREMENTS IN CHILDREN WITH TYPE 1 DIABETES

	Children with type 1 diabetes (n=50)
MBG (mmol/L)	11.6 (2.9)
SD (mmol/L)	4.1 (1.1)
MAGE (mmol/L)	11.6 (2.8)
CONGA 1 (mmol/L)	2.7 (0.6)
CONGA 4 (mmol/L)	5.2 (1.3)
CONGA 8 (mmol/L)	5.9 (1.8)
MODD (mmol/L)	4.4 (1.6)
J Index	83.4 (33.7)
GRADE	
Mean score	15 (5.8)
Hyperglycemic (%)	90 (41.93–100)
Euglycemic (%)	4.9 (0–42.17)
Hypoglycemic (%)	5.2 (0–53.43)
LBGI	1.04 (0–7.77)
Duration of hypoglycemia (% of time with glucose levels below 3.5 mmol/L)	2.87 (0–22.2)

Data are mean (SD) or geometric mean (range) values.

CONGA, continuous overall net glycemic action; GRADE, glycemic risk assessment diabetes equation; LBGI, Low Blood Glucose Index; MAGE, mean amplitude of glycemic excursions; MBG, mean blood glucose; MODD, mean of daily differences.

whole CGMS period. Eleven of 52 subjects had a history of at least one severe hypoglycemic episode since diagnosis.

The mild hypoglycemic episodes during CGMS and measured by GRADE hypoglycemia significantly and inversely related to FMD ($r = -0.36$, $P = 0.009$) but did not relate to GTN ($r = -0.14$, $P = 0.32$) or vessel diameter ($r = 0.06$, $P = 0.65$). LBGI and duration of hypoglycemia evaluated during CGMS inversely related to FMD ($r = -0.30$, $P = 0.03$ and $r = -0.26$, $P = 0.06$, respectively). The association between GRADE hypoglycemia and FMD remained significant after controlling for covariates affecting FMD (Table 3), and none of the predictors was actually found to be statistically significant predictors.

There were no significant differences in FMD or GTN when comparing children with a positive or negative history of severe hypoglycemia ($P = 0.22$ and $P = 0.98$, respectively).

GRADE hypoglycemia inversely related to MBG and J Index but did not relate to other measurements of glucose variability or HbA1c. GRADE hypoglycemia did not relate to diabetes duration ($r = 0.09$, $P = 0.52$), insulin dose measured by units of insulin/kg/day ($r = -0.04$, $P = 0.73$), or high-sensitivity C-reactive protein ($r = -0.08$, $P = 0.58$). GRADE hypoglycemia

related to LBGI and duration of hypoglycemia ($r = 0.94$, $P < 0.001$ and $r = 0.88$, $P < 0.001$, respectively).

Univariate regression analysis showed that none of the measurements of glucose variability used (MAGE, SD of MBG, CONGA 1, 4, and 8, MODD, or J Index) related to endothelial function evaluated by FMD ($r = -0.06$, $r = 0.16$, $r = -0.04$, $r = 0.04$, $r = -0.05$, $r = 0.24$, and $r = 0.17$, respectively, and all P values > 0.05). FMD related to GTN ($r = 0.4$, $P < 0.05$).

The mean (SD) glucose level measured using CGMS at the time of assessment of FMD was 10.01 (4.0) mmol/L and at the time of assessment of GTN was 9.97 (3.7) mmol/L. FMD and GTN did not relate to glucose measured at the time of FMD or GTN assessment ($r = 0.05$ or $r = -0.08$, $P > 0.05$), fasting glucose ($r = -0.05$, $P = 0.7$ and $r = -0.07$, $P = 0.6$, respectively), or HbA1c ($r = 0.06$, $P = 0.6$ and $r = 0.05$, $P = 0.7$, respectively). None of the measurements of glucose variability related to diabetes duration, fasting glucose, BMI z-score, waist circumference, or gender.

Discussion

Hypoglycemia, but not glucose variability, related to vascular endothelial dysfunction in children with T1D. To our knowledge this is the first study evaluating hypoglycemic episodes or glucose variability and early markers of cardiovascular disease in children with T1D.

Hypoglycemia measured over 48 hours using CGMS data independently related to impaired vascular endothelial function (FMD). This has been shown in a smaller cohort of adults with T1D but with a history of severe and repeated episodes of hypoglycemia.⁷ In addition, in adults with T1D acutely induced hypoglycemia by intravenous insulin infusion causes arterial stiffness¹⁷ and changes in serum markers of endothelial dysfunction (vascular cell adhesion molecules, intracellular adhesion molecules, etc.) and inflammation.^{16,18} In a large cohort of adults with type 2 diabetes followed for 5 years in the ADVANCE trial, a history of severe hypoglycemia is strongly associated with major cardiovascular events (nonfatal myocardial infarction and nonfatal stroke) and deaths from cardiovascular disease.²⁵

The underlying mechanism for vascular changes during hypoglycemia is not completely understood but may relate to the compensatory physiological, hematological, and inflammatory changes that occur in blood vessels that are already affected by diabetes and oxidative stress.^{1,16,18} Hypoglycemia briefly increases sympathetic neural activation by increasing the release of epinephrine, which causes physiological changes in the heart such as an increase in heart rate, cardiac output, myocardial contractility, and systolic blood pressure. Moderate hypoglycemia also increases circulating levels of

TABLE 3. MULTIPLE REGRESSION ANALYSES OF FLOW-MEDIATED DILATATION AND HYPOGLYCEMIC VARIABLES

Predictor	Regression coefficient	
	Ignoring other covariates	Adjusting for covariates
GRADE hypoglycemia	-0.16 ± 0.06 ($P = 0.009$)	-0.25 ± 0.09 ($P = 0.006$)
LBGI	-0.86 ± 0.39 ($P = 0.03$)	-1.04 ± 0.56 ($P = 0.07$)
Percentage of time under 3.5 mmol/L	-0.24 ± 0.13 ($P = 0.06$)	-0.25 ± 0.17 ($P = 0.15$)

Units of all the regression coefficients are percentage flow-mediated dilatation per unit of the corresponding predictor.

GRADE, glycemic risk assessment diabetes equation; LBGI, Low Blood Glucose Index.

plasminogen activator inhibitor and markers of platelet activation and inflammation (platelet monocyte activation, P-selectin, and high-sensitivity C-reactive protein) in healthy adults and in adults with T1D.^{1,16,18}

The main outcome of this study was to evaluate the association between glucose variability and vascular function in children with T1D. Despite measuring a comprehensive range of markers of glucose variability, none related to vascular function in our subjects with T1D. This is consistent with the study of Gordin et al.,⁶ who showed that glucose variability assessed by MAGE does not relate to arterial stiffness in a smaller cohort of adults with T1D. In addition, glucose variability measured by isolated seven-point blood glucose levels a day does not relate to the development of retinopathy or nephropathy independent of HbA1c in adults with T1D in the Diabetes Control and Complications Trial.⁵ However, glucose variability measured by SD of 70 isolated blood glucose measurements over a 4-week period predicts the development of peripheral neuropathy but not retinopathy or nephropathy in adults with T1D.⁴ Glucose variability assessed using CGMS also relates to vascular function in healthy adults and adults with metabolic syndrome with or without type 2 diabetes.¹³ In addition, fluctuations in blood glucose levels during a hyperglycemic-euglycemic clamp relate to vascular endothelial function in healthy adults and adults with type 2 diabetes only treated with diet.¹⁴ This highlights possible differences in the role of glucose variability on cardiovascular disease in T1D versus type 2 diabetes.

The lack of association between glucose variability and vascular function was confirmed for all the measurements of glucose variability we assessed (MAGE, SD of MBG, CONGA [$n=1-8$], MODD, or J Index) and occurred despite the fact that children with T1D have substantially higher glucose variability in comparison with adults with T1D or type 2 diabetes.^{3,19,26} All glucose variability measurements were done by algorithms developed by one of the authors (P.B.)²² and were comparable with previously reported glucose variability measurements in children and adolescents.^{9,19} The lack of association between glucose variability and vascular function is unlikely to be related to sample size. Our sample size provided 80% power at a 0.05 significance level to detect a correlation of $r=-0.4$, and a previous study found a strong association between glucose variability and oxidative stress with a smaller sample size ($n=21$).³

A limitation of the study is that the subjects with T1D included in the study were referred from the clinic for CGMS, but these subjects had age, gender, diabetes duration, and HbA1c similar to those of our diabetes clinic attendees as a whole. In addition, we had a high participation rate in the study (90%), and the history of severe hypoglycemia in these subjects was comparable to previous studies of children with T1D.¹⁹ Another limitation of this study is that it was cross-sectional, precluding a cause-effect relationship evaluation between hypoglycemia and vascular function. Additionally, this study's primary aim was to evaluate the association of glucose variability and vascular function, not hypoglycemia and vascular function.

In conclusion, we have shown that hypoglycemia, but not glucose variability, relates to impaired vascular endothelial function in children with T1D. This study shows some evidence that hypoglycemia even in childhood can be an aggravating factor in early vascular changes in T1D. The

vascular benefit of reducing glucose variability independent of HbA1c remains unresolved. Studies including interventions known to reduce glucose variability and hypoglycemic episodes—for example, continuous subcutaneous insulin infusion²⁷—are required to further evaluate the relationship among glucose variability, hypoglycemia, and early markers of cardiovascular disease.

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Author Disclosure Statement

No competing financial interests exist.

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