Glycemic Variability Is Associated with Markers of Vascular Stress in Adolescents

Paul S. Dasari, MD[1], Benjamin S. Gandomani, BS[2], April M. Teague, MS[1], Ameya Pitale, PhD[3], Michael Otto, PhD[4], and Kevin R. Short, PhD[1]

Objectives We used continuous glucose monitoring to test the hypothesis that mean amplitude of glycemic excursions (MAGE) is associated with circulating markers of oxidative and vascular stress in adolescents with habitually low physical activity classified as healthy weight, healthy obese, or obese with type 2 diabetes mellitus (T2DM).

Study design A group of 13- to 21-year-olds (healthy weight = 12, healthy obese = 10, T2DM = 12) wore a continuous glucose monitor and step activity monitor for 5 days.

Results Physical activity was similar among groups (6551 ± 401 steps/d), but aerobic fitness (peak rate of oxygen consumption) was lower ($P < .05$) in T2DM (15.6 ± 1.8 mL/kg/min) than either healthy weight (26.2 ± 2.2) or healthy obese (24.4 ± 2.5). MAGE (mg/dL) was higher ($P < .01$) in T2DM (82 ± 10) vs healthy obese (33 ± 3) and healthy weight (30 ± 3). Average glucose followed a similar pattern as MAGE. Oxidized low density lipoprotein was higher ($P < .05$) in T2DM (70.3 ± 5.0 U/L) and healthy obese (58.1 ± 3.8) than healthy weight (48.4 ± 2) and positively correlated with MAGE (r = 0.77). Other stress markers that were both elevated in T2DM and correlated with MAGE included E-selectin (r = 0.50), intercellular adhesion molecule 1 (r = 0.35), and C-reactive protein (r = 0.52); soluble receptor for advanced glycosylation end product was lower in T2DM and inversely correlated with MAGE (r = −0.38).

Conclusions MAGE is highest in obese youth with T2DM. The associations between MAGE and oxidative stress markers support the proposed contribution of glycemic variability to risk for future cardiovascular disease. *(J Pediatr 2016;172:47-55)*.

Obesity is a major risk factor for prediabetes and type 2 diabetes mellitus (T2DM) in children and adults.[1,2] The progression from insulin resistance to T2DM tends to be faster in adolescents compared with adults.[3] Among the many factors that influence this progression, plasma glucose concentration, both fasting and post prandial, is the best predictor for the development of T2DM.[4] Glycemic control also determines the future risk of micro- and macrovascular complications, including cardiovascular disease.[5-7]

Optimal glycemic control is important for prevention of long-term complications in diabetes.[8,9] Although keeping the average blood glucose tightly controlled is intuitively valuable, recent studies of adults with diabetes showed that it may be equally, or even more important to avoid large daily fluctuations in blood glucose in order to maintain healthy vascular function.[10-12] Those studies showed that an increase in glycemic variability, whether measured in free-living adults with T2DM[11] or experimentally induced for 2 days in adults with or without T2DM[10] is positively correlated with urinary concentration of 8-iso prostaglandin F2α, a marker of oxidative stress. However, to our knowledge, the relationship between glycemic variability and measures of oxidative stress in adolescents with T2DM or their nondiabetic healthy weight or obese peers has not been previously published. Because evidence has demonstrated the difficulty of preventing the progression of T2DM and related complications in youth,[13] it is important to increase our understanding of how glycemic control and its association with vascular health is regulated in children and adolescents, especially in those with elevated risk for cardiometabolic diseases.

AUC	Area under the curve	MAGE	Mean amplitude of glycemic excursions
BMI	Body mass index		
CGM	Continuous glucose monitoring	NEFA	Nonesterified fatty acids
CRP	C-reactive protein	OGTT	Oral glucose tolerance test
HbA1c	Hemoglobin A1c	oxLDL	Oxidized low density lipoprotein
HDL-C	High density lipoprotein-cholesterol	sRAGE	Soluble receptor of advanced glycation end-products
ICAM-1	Intercellular adhesion molecule 1	T2DM	Type 2 diabetes mellitus
iHOMA2	Interactive homeostatic model of assessment 2	VCAM-1	Vascular cellular adhesion molecule 1
LDL-C	Low density lipoprotein-cholesterol	VO_2peak	Peak rate of oxygen consumption

From the [1]Department of Pediatrics, Section of Diabetes and Endocrinology, [2]College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [3]Mathematics, University of Oklahoma, Norman, OK; and [4]SAP SE, Walldorf, Germany

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Hemoglobin A1c (HbA1c) is the most commonly used measure of glycemic control but does not reflect daily glucose variability[14] and may not be a reliable predictor for cardiovascular events.[15] Continuous glucose monitoring (CGM) has become a useful tool to measure glucose fluctuations at high frequency resolution over several days. CGM systems measure interstitial glucose but have been shown to be a valid and reliable surrogate for blood glucose concentration during glycemic variations.[16] A recent report demonstrated that CGM could be used in overweight or obese adolescents without diabetes and that average glucose over 2-3 days, and the time spent above selected glycemic thresholds (120 or 140 mg/dL) was positively correlated with HbA1c and 2-hour glucose during an oral glucose tolerance test (OGTT).[17] However, that study did not assess the relationship between glycemic variability and markers of oxidative stress or vascular risk in adolescents. Thus, the goal of the current study was to use CGM to measure glycemic variability in adolescents and to test the hypothesis that the mean amplitude of glycemic excursions (MAGE), a summary measure of glycemic variability,[18,19] is significantly correlated with circulating markers of oxidative stress in adolescents with T2DM vs age-matched, healthy-weight, or obese peers without diabetes and similarly low habitual physical activity.

Methods

Boys and girls between 13 and 21 years old with Tanner pubertal staging ≥2 were enrolled into 1 of 3 study groups. The healthy weight (N = 12) group had body mass index (BMI) between the 25th and 75th percentile for age and sex on the Centers for Disease Control standard growth curves. The obese (N = 10) group had BMI ≥95th percentile. The T2DM group (N = 12) was obese and met the criteria for T2DM defined by the American Diabetes Association. The recruitment strategy was to enroll similar numbers of boys and girls into each group. In addition, all participants had low habitual physical activity, defined as <30 minutes of moderate-to-vigorous intensity physical activity on ≤2 d/wk. We did not attempt to match the groups for other characteristics, such as body size or body composition of the healthy obese and T2DM groups, for example. Participants were excluded if they had endocrine causes of obesity, or metabolic, cardiovascular, or other medical conditions, or were using medications that were expected to impact the study outcomes. The exception for medications was the use of metformin by 9 of the 12 participants with T2DM because this compound is extensively used in clinical practice and excluding participants who use metformin would impair recruitment and generalizability of the results. None of the participants with T2DM used exogenous insulin.

Participants (and parents of participants <18 years of age) provided oral and written consent/assent in accordance with the policies of the University of Oklahoma Health Sciences Center Institutional Review Board. During the initial visit, a pediatric endocrinologist performed a medical history and physical examination. Total body and regional fat and lean tissue were measured using dual energy X-ray absorptiometry (GE/Lunar iDXA; GE Healthcare, Fairfield, Connecticut). Exercise fitness was measured as described below. On a separate morning at least 2 days after the fitness test, participants returned following a 10-hour overnight fast for collection of 2 venous blood samples, separated by 5 minutes. These samples were used for measurement of all analytes described below. The average concentration measured in the 2 separate samples from each person was used for data analyses. Healthy weight and healthy obese participants then completed a standard 2-hour OGTT with a 75-g glucose load to confirm that they did not have T2DM. The T2DM group did not perform the OGTT because of concerns that the test would exacerbate their hyperglycemia. During the OGTT, blood was collected at −8, −3, 30, 60, 90, and 120 minutes before and after glucose ingestion, respectively. The concentrations of glucose, insulin, and nonesterified fatty acids (NEFA) were measured at each collection time.

CGM was performed for 5 consecutive days using the iPro2 from Medtronic MiniMed (Northridge, California). During the second visit, the device was installed on the abdomen. Participants were given a finger stick glucose analyzer and instructed to check their glucose at least 3 times per day in order to synchronize the readings with the CGM. Glucose analyzers were calibrated according to the manufacturer. Each participant was also instructed to wear a step activity monitor, as described below, and to maintain their normal patterns of physical activity, particularly avoiding novel vigorous activities prior to, and during the 5-day monitoring period. They were asked to keep a diary of their physical activity, timing of food intake with approximate portion sizes, and the use of medications, to confirm consistency of behavior during the measurement period.

Measurements

MAGE. MAGE is the arithmetic average of all increases (or decreases) in glucose concentrations that exceed 1 SD of the total set of glucose values. MAGE can be computed manually using the data downloaded from the CGM, or using computer software as previously described.[19,20] We developed a program to calculate the number of excursions and MAGE using the free software environment *R*.[21,22] The program algorithm was designed to calculate the mean and SD for each set of glucose values, define the inflection points at which glucose concentration changed from either increasing or decreasing, count the number of excursions (upward or downward changes in glucose concentration that exceeded 1 SD), and determine the amplitude of each excursion. Separate MAGE values for each participant were calculated for each day and for the entire measurement period using the corresponding SD for glucose concentration within the specific time interval. For each participant, all of the available glucose concentration values from the CGM, without filtering or smoothing, were used to calculate MAGE. The iPro2 device records glucose concentration every 5 minutes (288 data points per day). We did not attempt to interpolate

missing values in the event of missing data due to technical error (eg, the unit may stop recording if there is excessive movement of the subcutaneous catheter). For 13 participants who completed the protocol but had less than 4 full days of CGM data, the 5-day monitoring period was repeated to ensure that a representative data set was acquired for each person.

Physical Activity Assessment. Free-living daily ambulatory activity was measured with an accelerometer (StepWatch 3, Modus Health LLC, Washington, DC) worn above the ankle during waking hours. Step count was recorded each minute. This monitor has high reliability and validity.[23]

Fitness Test. A bicycle ergometer test with increasing workloads was used to measure peak aerobic work output, peak rate of oxygen consumption (VO_2peak), and heart rate. Continuous measurements of expired gases were performed with a facemask and metabolic measurement system (TrueOne 2400; ParvoMedics, Sandy, Utah) and heart rate was measured with a chest-strap monitor (Polar Electro USA, Lake Success, New York).

Plasma/Serum Analysis. All venous blood samples were centrifuged to separate plasma and serum and aliquots were stored at −80°C until analysis. Plasma glucose concentration was measured by the glucose oxidase method (2300STAT Plus; Yellow Springs Instruments, Yellow Springs, Ohio). Serum insulin was measured using an ELISA from Millipore (#EZHIASF-14K; Millipore, St. Louis, Missouri). C-reactive protein (CRP), serum triglycerides, and total-cholesterol, high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) were measured at the Clinical Chemistry Laboratory of the Oklahoma Veteran's Administration Hospital (Oklahoma City, Oklahoma) using validated enzymatic assays (Synchron Systems, Beckman Coulter, Brea, California). NEFA were measured using an enzymatic colorimetric assay from Wako Chemicals (NEFA-HR2; Wako Chemicals, Richmond, Virginia). The following measurements were made using ELISAs (assay kit number, manufacturer): oxidized high density lipoprotein (#MBS706079; MyBioSource, Inc, San Diego, California), oxidized low density lipoprotein ([oxLDL] #10-1143-01; Mercodia, Uppsala, Sweden), intercellular adhesion molecule 1 ([ICAM-1] #DCD540; R&D Systems, Minneapolis, Minnesota), vascular cellular adhesion molecule 1 ([VCAM-1] #DSLE00; R&D Systems), E-selectin (#DSLE00; R&D Systems), soluble receptor of advanced glycation end-products ([sRAGE] #RD191116200R, BioVendor, Candler, North Carolina), and fetuin-A (#DFTA00; R&D Systems).

Insulin Sensitivity. Fasting insulin sensitivity and pancreatic beta cell function were calculated using the interactive homeostatic model of assessment 2 (iHOMA2) model (v 8.2.2).[24] The concentrations of glucose and insulin and from 0-120 minutes during the OGTT were used to calculate the total area under the curve (AUC) for the healthy weight and healthy obese participants. The Matsuda insulin sensitivity index was calculated as previously described.[25]

Statistical Analyses

Descriptive statistics were computed for all variables and presented as mean ± SEM. Comparisons among groups were performed with 1-way ANOVA and Bonferroni post-hoc tests were used to determine pairwise differences. Pearson correlations were used to measure the univariate association between selected variables. The set of variables that best predicted MAGE was determined using multiple linear regression modeling. A P value of $<.05$ was considered statistically significant for all tests.

Results

The distribution of boys and girls, age, and Tanner stage were similar among groups, by design (**Table I**; available at www.jpeds.com). The T2DM group had the highest values for body mass, BMI, body fat, percent body fat, and trunk fat, although their BMI percentile and lean body mass were similar to the healthy obese group. There were no differences among groups for daily physical activity (step counts) or for aerobic fitness (peak power, VO_2peak, or heart rate achieved on the bicycle test), although the VO_2peak relative to body mass (mL/kg/min) was lower in the T2DM group than either the healthy weight or healthy obese groups. Although we instructed participants to follow their usual diet and record their daily food intake, several dietary records were incomplete so we did not use those results in the data analysis.

Values for blood biochemistry analytes measured following overnight fast are shown in **Table II** (available at www.jpeds.com). Fasting glucose concentration was within the normal range for all members of the healthy weight and healthy obese groups, but significantly higher in the T2DM, as expected. Insulin concentration was higher and the iHOMA2 insulin sensitivity estimate was lower in the healthy obese and T2DM groups compared with healthy weight, but the iHOMA2 estimate of pancreatic beta cell function was significantly elevated only in the healthy obese group; the iHOMA beta cell function in the T2DM group did not differ from the healthy weight group. In comparison with the healthy weight group the healthy obese group had higher oxLDL and lower HDL-C and sRAGE, and the group with T2DM had higher values for LDL-C, triglycerides, oxLDL, CRP, and ICAM-1, and lower HDL-C and sRAGE. The T2DM group also had the highest concentration of E-selectin, significantly higher than healthy weight and approaching statistical significance vs healthy obese (P = .086). There were no significant differences among groups for total cholesterol, oxidized high density lipoprotein, NEFA, VCAM-1, or fetuin A.

During the OGTT, the concentrations of glucose, which increased to a peak value of 134 ± 5 mg/dL at 30 minutes,

and NEFA, which rapidly and continuously declined to 0.070 mEq/L at 120 minutes, were similar at all measurement times for the healthy weight and healthy obese groups and the integrated 2-hour AUCs did not differ. Glucose AUC was 17.1 ± 0.7 for healthy weight and 17.8 ± 1.0 mL-min/dL/1000 for healthy obese groups, respectively. NEFA AUC was 23.8 ± 1.7 and 26.9 ± 2.6 mEq-min/L for healthy weight and healthy obese groups, respectively. Insulin was 1- to 3-fold higher in the healthy obese group (peak: 216 ± 52 μIU/mL at 60 minutes) vs the healthy weight group (peak: 85 ± 17 μIU/mL at 90 minutes), and the AUC was 1.5-fold higher ($P < .01$) in the healthy obese group (10.3 ± 1.5 and 26.5 ± 5.6 μIU-min/mL/1000 for healthy weight and healthy obese groups, respectively). The Matsuda insulin sensitivity index was higher ($P = .010$) in the healthy weight group (8.3 ± 1.6 arbitrary units) than the healthy obese group (3.0 ± 0.9).

Daily and total values for MAGE are shown in Figure 1. CGM data were available for 89% of the 5-day monitoring period, with no difference in the number of glucose readings (1279 ± 26 data points per person) or the amount of missing data among groups. The average glucose concentration during the monitoring period was 40% higher ($P < .01$) in the T2DM group (160 ± 16 mg/dL) than both the healthy weight (96 ± 1) and healthy obese (102 ± 3) groups. The SD values, which were used to determine excursions, were 14, 16, and 37 mg/dL for the healthy weight, healthy obese, and T2DM groups, respectively, for the 5-day measurement period. The corresponding SD values for individual days were 20% smaller than for the total measurement period ($P < .01$, data not shown) with similar inter-day variability among groups (coefficient of variation = 33, 30, and 37% for healthy weight, healthy obese, and T2DM, respectively). The number of excursions for the 5-day monitoring period was lower in the T2DM group (35 ± 5) than both the healthy weight group (68 ± 7, $P < .01$ vs T2DM) and healthy obese group (53 ± 6, $P = .047$ vs T2DM, not different from healthy weight). The standard data management program available with the CGM includes a calculation for the number of glucose excursions above 140 mg/dL. For that variable, the T2DM group (9.7 ± 1.7 for 5 days) was higher than the healthy weight group (1.8 ± 0.9, $P < .01$ vs T2DM) and the healthy obese group (3.7 ± 1.5, $P = .015$ vs T2DM, not different from healthy weight).

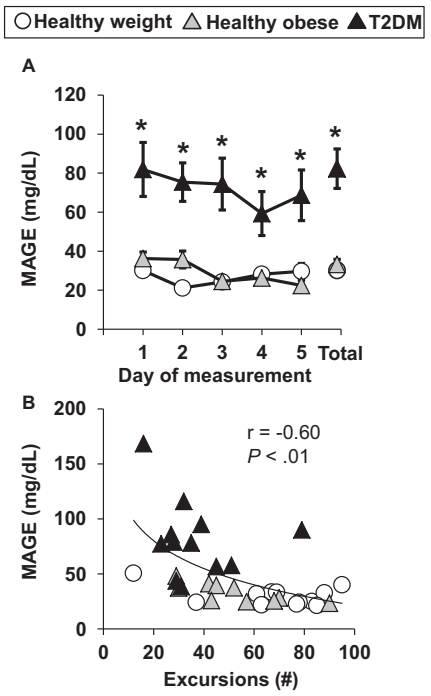

Figure 1. **A,** MAGE calculated separately for each day and for the total 5-day period and for the total measurement period. Values shown as mean ± SEM. *Greater than healthy weight and healthy obese groups, $P < .01$. **B,** The correlation between MAGE and number of excursions. The line of best fit was a logarithmic curve.

Total MAGE was ~60% higher ($P < .01$) in the T2DM group vs the healthy obese group and healthy weight group but the healthy weight and healthy obese groups did not differ (Figure 1). Like glucose SD, there was high variability in MAGE values between days, particularly in the T2DM group (Figure 1). The interday coefficient of variation for MAGE was higher ($P < .05$) in the T2DM group (43 ± 5%) vs either the healthy obese group (29 ± 4) or the healthy weight group (29 ± 4). Figure 2 (available at www.jpeds.com) shows the individual values for average glucose, glucose SD, number of excursions, and MAGE, and reveals the range of values across all participants and the overlap between groups. There was an inverse relationship between the number of excursions and MAGE (Figure 1). Average glucose was positively correlated with MAGE (r = 0.92, $P < .01$).

Because we were interested in exploring whether glycemic variability was related to markers of inflammation or vascular stress, univariate correlations were calculated between MAGE, average glucose, and the number of glycemic excursions, and the descriptive and biochemical measurements. Several variables were significantly correlated with MAGE. The variable that had the highest (positive) correlation with MAGE was oxLDL, a biomarker of atherosclerotic risk (Figure 3). That relationship was driven largely by the values within the T2DM group (r = 0.85), but there was also a positive correlation between MAGE and oxLDL within the healthy weight group (r = 0.47); within the healthy obese group the correlation did not reach statistical significance (Table III). Other traditional cardiovascular disease risk factors that were moderately- to strongly-correlated with MAGE included BMI, body fat, HDL-C, LDL-C, CRP, and NEFA (data not shown). Among the inflammatory and vascular stress biomarkers measured, E-selectin and ICAM-1 were positively correlated, and sRAGE was negatively correlated with MAGE (Figure 3). Table III

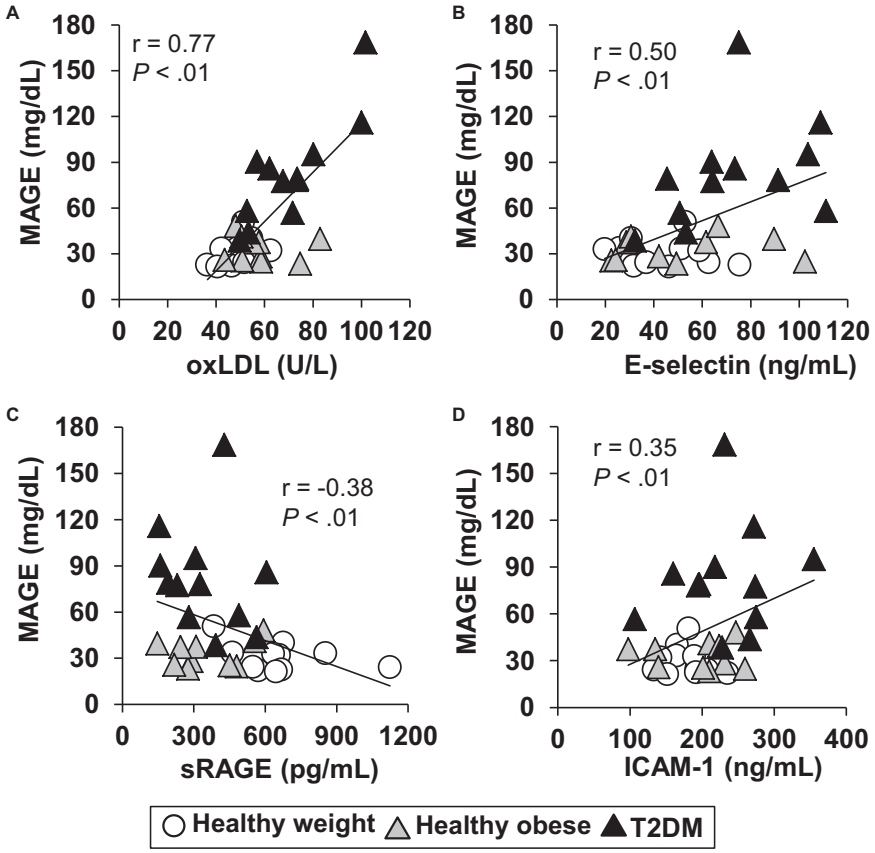

Figure 3. Correlations between MAGE and selected markers of vascular and oxidative stress. **A,** oxLDL; **B,** E-selectin; **C,** sRAGE; **D,** ICAM-1. Linear trend lines were determined to provide the line of best fit for each correlation shown.

shows the correlation coefficients for the combined participants and within the healthy weight, healthy obese, and T2DM groups for the relationships between oxLDL, E-selectin, sRAGE, and ICAM-1, and 4 measures of glycemic control: MAGE, average glucose, glucose SD, and the number of glucose excursions above 140 mg/dL. When the entire study cohort was included, the highest correlation coefficients were with MAGE, although they were not statistically different compared with the corresponding values for average glucose. The relatively small sample size may have prevented the detection of differences in the strength of those correlations for MAGE vs average glucose. Similarly, for the within-group correlations, the smaller number of participants and range of values resulted in smaller correlation coefficients, most of which did not reach statistical significance. Because glucose SD is used to define MAGE, the correlations between either MAGE or glucose SD and selected outcomes were confirmed to be similar. The number of excursions above 140 mg/dL, a standard variable provided in the CGM software, was generally less strongly correlated with markers of oxidative and vascular stress than MAGE, glucose SD, or average glucose.

Using multivariate modeling the best set of predictor variables for MAGE was a 3-variable set comprised of oxLDL, CRP, and NEFA, with an adjusted $r^2 = 0.70$. For average glucose the strongest model was comprised of 4 predictor variables: NEFA, E-selectin, Tanner stage, and fetuin A (adjusted $r^2 = 0.72$).

Discussion

Our results demonstrate that glycemic variability is elevated in adolescents with T2DM and that glycemic variability is associated with markers of oxidative and vascular stress. In a cross-sectional study of adults with different levels of glucose tolerance, it was shown that MAGE progressively increased from normal glucose tolerance to impaired glucose tolerance to T2DM.[26] Intra-day glucose variability in adults also increases during the early stage of abnormal glucose tolerance.[26,27] To date, CGM has been used to measure average glucose concentration and the number and duration of fluctuations above or below defined values in children with type 1 diabetes,[16] and in overweight/obese children with HbA1c in either the normal or prediabetic range.[17] In a study of children with poorly controlled type 1 diabetes, CGM data

Table III. Univariate correlations between indices of glycemic variability and selected markers of oxidative and vascular stress

Variables	Group	MAGE (mg/dL)	Average glucose (mg/dL)	Glucose SD (mg/dL)	Excursions (number above 140 mg/dL)
oxLDL (U/L)	All	0.77*	0.72*	0.73*	0.32
	T2DM	0.85*	0.73*	0.77*	−0.27
	Healthy obese	−0.08	−0.11	−0.21	0.01
	Healthy weight	0.47*	0.46*	0.52*	0.75*
ICAM-1 (ng/mL)	All	0.35†	0.21	0.36†	0.20
	T2DM	0.14	−0.16	0.18	−0.17
	Healthy obese	−0.02	0.28	−0.04	0.29
	Healthy weight	−0.12	−0.16	−0.25	−0.38†
E-selectin (ng/mL)	All	0.50*	0.41†	0.48*	0.37†
	T2DM	0.39†	0.23	0.32	0.10
	Healthy obese	0.14	0.01	0.12	0.19
	Healthy weight	−0.11	−0.25	0.03	−0.02
sRAGE (pg/mL)	All	−0.38†	−0.33	−0.35†	−0.21
	T2DM	−0.19	−0.20	−0.22	0.06
	Healthy obese	0.29	0.46	0.29	0.48†
	Healthy weight	−0.38†	0.24	−0.01	−0.01

Values shown as Pearson correlation coefficients.
*$P < .01$.
†$P < .05$.

was collected for 2 days before and after the participants switched from multiple daily insulin injections to a continuous subcutaneous insulin infusion for 3 weeks.[28] In that study, the intervention resulted in an improvement in arterial flow-mediated dilation, a measure of vascular endothelial function, which was significantly correlated with improvement in glycemic variability but not HbA1c. However, values for MAGE in adolescents with T2DM, or at risk for developing T2DM because of obesity and/or sedentary lifestyle have not been published.

We found that healthy obese and healthy weight adolescents had similar MAGE even though the healthy obese group had greater insulin resistance than the healthy weight group, based on their lower fasting homeostatic model of assessment of insulin sensitivity (%S) and lower Matsuda insulin sensitivity index. The group with obesity maintained normal glycemia because of a compensatory increase in insulin production, as shown during the OGTT and higher calculated iHOMA2 beta cell function. The MAGE and inter-day variability of MAGE was higher in the T2DM group vs the healthy weight group, but the healthy weight group had more excursions than the T2DM group. The inverse relationship between the number and magnitude of glycemic excursions may reflect the relative loss of feedback control on glucose regulation in the adolescents with T2DM. In adults with T2DM, the magnitude and duration of postprandial glycemic excursions are increased as a result of delayed insulin release, blunted suppression of glucagon and hepatic glucose output, and reduced hepatic and peripheral glucose uptake.[29] It has not yet been determined whether those same underlying changes account for the larger glycemic excursions in adolescents with or at high risk for T2DM.

MAGE values were correlated with several markers of oxidative and vascular stress, supporting the premise that glycemic variability is potentially more harmful for vascular health than static hyperglycemia.[7] OxLDL, a proatherogenic particle, was highest in the T2DM group and the vascular stress marker most closely related to MAGE. Our group and others have previously reported that circulating oxLDL is increased in obese adolescents with T2DM.[30,31] We did not have sufficient statistical power, because of small sample size, to definitively determine whether glycemic variability had significantly larger correlation than average glucose with oxLDL or other oxidative stress markers. In contrast, Monnier et al[11] reported that in 21 adults with T2DM urinary concentration of 8-iso prostaglandin F2α, a marker of oxidative stress, was strongly correlated with MAGE measured over three days with CGM ($r = 0.86$), but not with the average glucose concentration recorded over that same time ($r = 0.22$). In addition, studies of endothelial cells in culture and studies employing short-term manipulation of circulating glucose in adults have demonstrated that hyperglycemia results in increased signs of oxidative stress (and apoptosis in endothelial cells), but oscillations in glucose concentration can further increase the magnitude of those outcomes.[10,32-34]

In addition to oxLDL, we found that MAGE was significantly correlated with other markers of inflammation and vascular oxidative stress, including ICAM-1, E-selectin, sRAGE, and CRP, though not with VCAM-1 or fetuin A. ICAM-1, VCAM-1, and E-selectin are adhesion molecules involved in atherogenesis, regulating the interaction between the endothelial cells and leukocytes.[32] In vitro work has shown that all 3 of those molecules increase in human endothelial cells exposed to high glucose for 14 days, and further increase when the media glucose concentration is switched between high (20 mM) and normal (5 mM) every 24 hours.[32] We measured sRAGE because advanced glycation end products have been implicated in chronic diseases like diabetes, through binding to their receptor (RAGE) on cell surfaces and activating intracellular signaling.[35,36] sRAGE is the truncated form of the receptor in circulation and prevents the

binding of Advanced Glycosylation Endproducts to the receptor, thus protecting against vascular damage and long-term microvascular complications.[35,36] We found that the concentration of sRAGE was significantly lower in the healthy obese and T2DM groups compared with the healthy weight group, which may reflect depletion of sRAGE as a result of binding excess Advanced Glycosylation Endproducts. In some cardiometabolic disease states, sRAGE was reported to be reduced in adults,[36] but the impact of T2DM or glycemic variability on sRAGE concentration in adolescents has not been reported. A recent study of adolescents without diabetes in Taiwan showed that sRAGE was inversely related to waist circumference and components of the metabolic syndrome, which is consistent with the current findings.[37] CRP is an acute phase reactant that is a marker of endothelial cell dysfunction in patients with diabetes.[38] CRP was significantly increased in the T2DM group and had a stronger association with MAGE than average glucose, which supports an interaction among glycemic variability and inflammation. We measured fetuin A because it was reported to be elevated in adults with obesity and/or T2DM, may regulate glycemic control through several mechanisms, and may be involved in vascular calcification.[39] However, fetuin A did not differ among groups in this study and was not associated with MAGE.

The participants in the current study had low cardiorespiratory fitness and physical activity relative to recommended levels for adolescents.[40,41] We expected the adolescents with T2DM would have low fitness and physical activity and, therefore, included healthy weight and healthy obese participants with low habitual activity. The low values for VO_2peak and similarity in daily step counts among groups may explain why those variables were not correlated with MAGE or average glucose. Consideration of physical activity is important because we have shown that when adolescents with habitually low activity complete a single exercise session, the improvement in insulin sensitivity and postprandial glycemia may last for up to 17 hours.[42] In addition, recent studies that used CGM showed that glycemic variability increased in healthy adults who reduced their daily physical activity for 1 week,[43] and a daily walking program reduced glycemic excursions in adults with T2DM.[44] The impact of varying levels of physical activity on glycemic excursions in adolescents has not yet been reported.

A potential limitation of this study was that diet and physical activity were not strictly controlled, although participants were asked to maintain their typical diet and activity patterns. This may have contributed to higher intra- and/or intersubject variability in MAGE than if prescribed meals and exercise patterns were used. However, the value of the approach used was that estimates of glycemic variability in adolescents were obtained during normal lifestyle conditions. We reasoned that the results would, therefore, be generalizable to clinical applications, especially for adolescents with, or at risk for developing diabetes. MAGE is not typically calculated in the standard CGM software and has primarily been used as a research tool in clinical investigations. Within clinical settings, a useful surrogate for MAGE is the SD of glucose concentration over the measurement period.[45] Because glucose SD is used to define the threshold for a glycemic excursion, it is inherently associated with MAGE. Glucose SD may be a useful tool for clinicians to monitor glucose control over several days or weeks because glycated hemoglobin is less likely to reflect changes over such a short time. In addition, we found that although there were differences among groups for the number of glucose excursions above 140 mg/dL, this variable was a weaker predictor of oxidative and vascular stress markers than MAGE, glucose SD, or average glucose. Importantly, though, our results suggest that when CGM is used to measure glycemic variability in clinical practice or to determine the response to interventions, it is advisable to collect data for several days to obtain a representative sample. We found that the inter-day coefficient of variation in glucose SD was similar among groups (30%-37%) but was higher for MAGE, particularly in the T2DM group (43%).

Another potential limitation of the current study is that we did not account for the timing of menses in the female participants, which could affect circulating biomarkers of cardiovascular risk.[46] We included participants who were classified as Tanner stages II-V, and some girls had not started menarche. Because of the small sample size, we did not explore potential differences between boys and girls or the impact of the female hormone cycle. It is also unclear whether cofactors associated with T2DM (eg, lower VO_2peak, greater body fat than the healthy obese group) exacerbated the differences in MAGE between T2DM and healthy obese groups. It would be valuable to determine the strength of the relationships between MAGE and measures of vascular health in groups of adolescents specifically matched for, or differing in body fat, cardiorespiratory fitness, or glycemic control. As noted, the small sample size may have limited the number of subgroup comparisons and the strength of some associations among variables. However, MAGE was clearly highest in the T2DM group, with no trend for difference between the healthy weight and healthy obese groups. Additional participants would not change the mean value for MAGE but may alter the correlations between MAGE and measures of vascular and oxidative stress, especially if youth with impaired fasting glucose and/or impaired glucose tolerance were included.

In this study, youth with T2DM had higher glycemic variability than their healthy weight and obese peers, and obese youth with normal glucose tolerance had similar MAGE as healthy weight, normal glucose tolerant youth. Adolescents with T2DM also had elevated biomarkers of oxidative stress and inflammation compared with healthy weight and obese peers. Across the range of adolescents in this study, glycemic variability was significantly associated with oxidative and vascular stress. Additional studies in adolescents are needed to determine whether MAGE is a better predictor of oxidative stress than average glucose and what types of interventions are needed to reduce glycemic variability. Because it has been shown that oxidative stress is a predictor of future

microvascular complications, minimizing glycemic variability, as well as lowering HbA1c, should be a priority in treating children with T2DM. ■

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Reprint requests: Kevin R. Short, PhD, Department of Pediatrics, Section of Diabetes and Endocrinology, University of Oklahoma Health Sciences Center, 1200 Children's Ave Suite 4500, Oklahoma City, OK 73104. E-mail: kevin-short@ouhsc.edu

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50 Years Ago in *The Journal of Pediatrics*

Fetal Cardiac Failure Resulting from Congenital Anomalies of the Heart

Moller J, Lynch RP, Edwards JE. J Pediatr 1966;68:699-703

In an effort to understand the etiology of heart failure in the fetus with congenital cardiac defects, Moller et al present a series of necropsy cases. All 3 patients were identified in the immediate postnatal period to have signs of "heart failure." The authors noted that prior reports identified restricted flow across the patent foramen ovale as the most common etiology. The first case, a stillbirth with anasarca, had right ventricular endocardial fibroelastosis with pulmonary valve stenosis, abnormal mitral valve morphology, and a patent foramen ovale. The third case, a stillbirth with hepatomegaly, had Ebstein malformation of the tricuspid valve, pulmonary valve stenosis. Case 2 was actually identified prenatally with polyhydramnios and a murmur heard from the maternal abdomen. This fetus delivered alive with anasarca, hepatomegaly, cyanosis, and a murmur consistent with mitral regurgitation, but died at 3 hours of life. Evaluation of the heart revealed left ventricular endocardial fibroelastosis and abnormal mitral valve. The authors conclude that the cases provide evidence that cardiac failure results from in utero hemodynamic burdens on the right ventricle.

Prior to the 1980s, there was no "prenatal diagnosis." Physicians were presented with a sick or deceased baby, and then studied a pathology specimen in order to extrapolate backwards what physiologic perturbations may have contributed to the ultimate demise. In short, they were solving a puzzle.

The field of pediatric cardiology grew by linking careful pathologic observations to physiology and outcomes. These historic retrospective observations identified the physiologic perturbations associated with intrauterine heart failure and thus informed the modern era where routine prenatal ultrasound prospectively identifies fetuses either at risk for or with cardiovascular dysfunction. When structural and functional cardiac defects are identified, progression of physiologic disturbances may be monitored. We utilize serial observations in order to understand and predict outcomes and, importantly, to intervene prior to a fetal or neonatal demise. The field of fetal cardiology began with the inquisitive minds of pediatric cardiologists defining the cardiac pathology and physiology associated with intrauterine heart failure; now inquisitive minds go beyond ultrasound-based diagnosis to develop fetal therapies that improve the longer term functional outcomes of survivors.

Amanda J. Shillingford, MD
Samuel S. Gidding, MD
Nemours Cardiac Center
Alfred I. duPont Hospital for Children
Wilmington, Delaware
http://dx.doi.org/10.1016/j.jpeds.2015.12.011

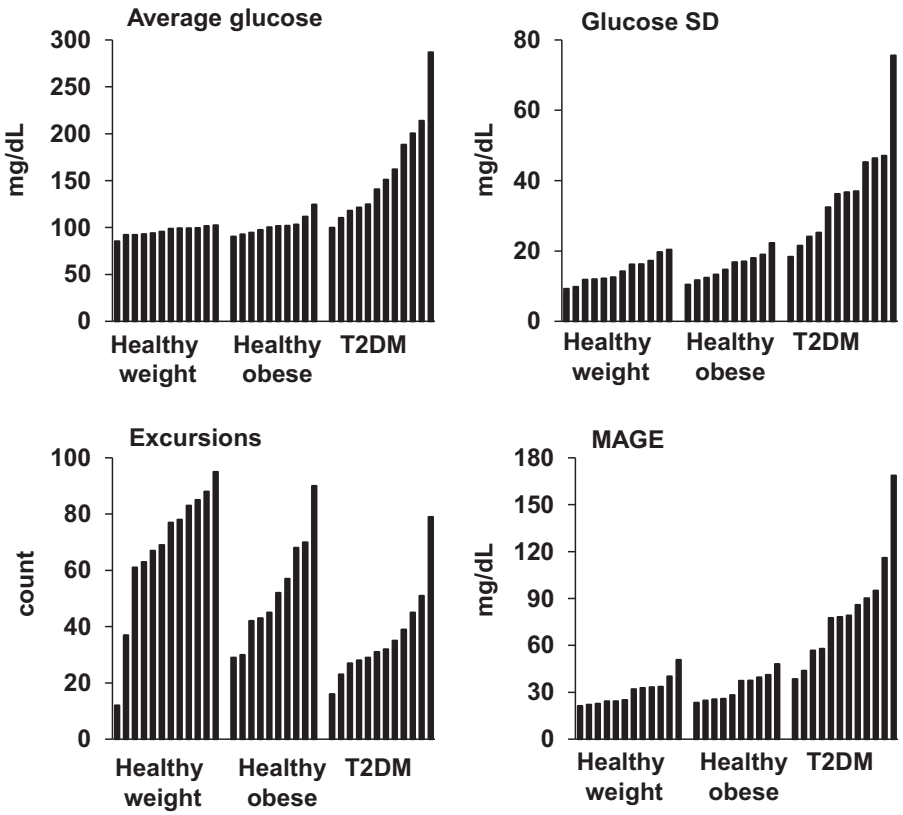

Figure 2. Individual values for average glucose, glucose SD, glucose excursions, and MAGE. These data were calculated for each participant from the data collected on the continuous glucose monitor for 5 days. The values are clustered from low to high within each of the study groups. Each *bar* represents a single participant but the position of that participant is not same in each panel. The purpose of this figure is to demonstrate the overall range of each variable and the overlap between groups.

Table I. Descriptive characteristics

Variables	Healthy weight	Healthy obese	T2DM
Age (y)	17 ± 1	16 ± 1	16 ± 1
Sex	6 Male 6 Female	6 Male 4 Female	6 Male 6 Female
Tanner stage	4 ± 1	4 ± 1	4 ± 1
Body mass (kg)	58.1 ± 2.1	94.2 ± 4.7*	122.3 ± 8.5*,†
BMI (kg/m^2)	20.6 ± 0.5	32.5 ± 0.9*	42.5 ± 2.9*,†
BMI (percentile)	48 ± 6	98 ± 1*	99 ± 1*
Total body fat (%)	25.5 ± 2.5	41.2 ± 2.6*	48.4 ± 2.3*,†
Total fat mass (kg)	14.1 ± 1.5	37.7 ± 2.9*	58.0 ± 5.5*,†
Total lean mass (kg)	39.8 ± 2.3	52.9 ± 3.9*	58.3 ± 3.6*
Trunk fat mass (kg)	6.3 ± 0.6	19.4 ± 2.0*	33.7 ± 3.6*,†
Systolic BP (mm Hg)	113 ± 2	122 ± 2*	126 ± 2*,†
Diastolic BP (mm Hg)	70 ± 2	74 ± 2	77 ± 2*
Physical activity (steps/d)	6763 ± 692	7175 ± 717	5820 ± 675
Peak exercise power (Watts)	123 ± 12	161 ± 22	129 ± 8
VO_2peak (L/min)	1.56 ± 0.15	2.29 ± 0.32‡	1.81 ± 0.14
VO_2peak (mL/kg body mass/min)	26.6 ± 2.1	24.3 ± 2.6	15.7 ± 1.8*,†
VO_2peak (mL/kg lean mass/min)	38.7 ± 2.6	41.5 ± 3.0	31.8 ± 2.7†,‡
HR peak (beats/min)	183 ± 4	180 ± 1	175 ± 5

BP, blood pressure; *HR*, heart rate.
Values shown as mean ± SEM.
*Different from healthy weight, $P < .05$.
†Different from obese, $P < .05$.
‡$P = .081$ vs healthy weight.

Table II. Biochemical variables in fasting serum

Variables	Healthy weight	Healthy obese	T2DM
Glucose (mg/dL)	85 ± 3	85 ± 2	135 ± 16*,†
Insulin (μIU/mL)	5.8 ± 1.7	26.2 ± 5.9*	36.6 ± 7.5*
iHOMA2-IS (%S)	346 ± 79	58 ± 14*	43 ± 12*
iHOMA2-Beta (%B)	76 ± 19	209 ± 28*	153 ± 37
Total cholesterol (mg/dL)	146 ± 7	154 ± 6	165 ± 8
HDL-C (mg/dL)	48.3 ± 2.4	40.9 ± 2.8*	35.6 ± 1.8*
LDL-C (mg/dL)	80.6 ± 6.7	89.8 ± 5.4	106.6 ± 8.2*
Triglycerides (mg/dL)	63 ± 8	116 ± 26	128 ± 12*
oxHDL (ng/mL)	462 ± 38	481 ± 69	567 ± 53
oxLDL (U/L)	48.4 ± 2.0	58.1 ± 3.8*	70.3 ± 5.0*
NEFA (mmol/L)	0.48 ± 0.05	0.49 ± 0.03	0.60 ± 0.06
CRP (mg/L)	0.28 ± 0.03	2.13 ± 0.67	6.34 ± 1.65*,†
ICAM-1 (ng/mL)	181 ± 9	195 ± 17	231 ± 19*
VCAM-1 (ng/mL)	578 ± 36	581 ± 39	543 ± 40
E-selectin (ng/mL)	43.5 ± 4.9	51.8 ± 8.8	72.8 ± 7.5*,‡
sRAGE (pg/mL)	655 ± 54	356 ± 48*	344 ± 44*
Fetuin A (μg/mL)	570 ± 49	613 ± 38	604 ± 43

iHOMA2-Beta, iHOMA2 beta cell function; *iHOMA2-IS*, iHOMA2 insulin sensitivity; *oxHDL*, oxidized high density lipoprotein.
Values shown as mean ± SEM.
*Different from healthy weight, $P < .05$.
†Different from obese, $P < .05$.
‡$P = .086$ vs healthy obese.