

Glycation of the Complement Regulatory Protein CD59 Is a Novel Biomarker for Glucose Handling in Humans

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Context: Human CD59, an inhibitor of the membrane attack complex of complement, is inactivated by glycation. Glycation inactivation of CD59 enhances complement-mediated injury in target organs of diabetes complications.

Objective: We hypothesized that circulating soluble glycated CD59 (GCD59) represents a novel biomarker of blood glucose handling and aimed to conduct human study protocols to test this hypothesis.

Design, Setting, Participants, and Outcome Measures: Using a newly developed ELISA, we measured circulating soluble GCD59 in samples from 3 separate human studies evaluating acute and chronic glucose handling and glucose responses to insulin therapy. Study 1 (normal vs diabetic subjects) evaluated the cross-sectional association between GCD59 and glycated hemoglobin (HbA1c) in 400 subjects with and without type 2 diabetes. Study 2 (oral glucose tolerance test [OGTT] in nondiabetics) evaluated whether fasting GCD59 independently predicted the 2-hour glucose response to an OGTT in 109 subjects without a diagnosis of diabetes. Study 3 (intensified insulin treatment) evaluated the effect of intensification of glycemic control with insulin on GCD59 in 21 poorly controlled individuals with diabetes.

Results: In study 1 (normal vs diabetic subjects), GCD59 was independently and positively associated with HbA1c in individuals with and without diabetes ($\beta = 1.1$, $P < .0001$ and $\beta = 1.1$ $P < .001$, respectively). In study 2 (OGTT in nondiabetics), a single GCD59 measurement independently predicted the results of the 2-hour OGTT ($\beta = 19.8$, $P < .05$) after multivariate modeling. In study 3 (intensified insulin treatment), intensification of glucose control with insulin resulted in a concomitant and parallel reduction of average weekly glucose and GCD59 within 2 weeks.

Conclusions: We observed robust relationships between a single measurement of blood levels of GCD59 and both acute (2-hour OGTT) and chronic (HbA1c) measures of glucose handling. Lowering of GCD59 levels closely reflected lowering of average weekly glucose within 2 weeks. The role of GCD59 in the diagnosis, management, and vascular risk stratification in diabetes warrants further investigation. (*J Clin Endocrinol Metab* 99: E999–E1006, 2014)

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Abbreviations: AWG, average weekly glucose; BMI, body mass index; BP, blood pressure; CV, coefficient of variation; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; MAC, membrane attack complex; OGTT, oral glucose tolerance test; SPU, standard peptide units.

Macrovacular and microvascular complications associated with poor glycemic control contribute significantly to morbidity and mortality in patients with diabetes. Recent experimental and clinical evidence support a strong link between the complement system, activity of the complement regulatory protein CD59, and the pathogenesis of vascular complications of diabetes (1–8). Complement activation ultimately leads to formation of the cytotoxic, pore-forming membrane attack complex (MAC), the main effector of complement-mediated tissue damage. Insertion of the MAC into cell membranes induces the release of cytokines and growth factors that promote inflammation, thrombosis, and cell proliferation (1, 9–11).

CD59 is a complement regulatory protein ubiquitously expressed on mammalian cell surfaces; it specifically inhibits MAC formation and thereby protects self-cells from complement-mediated damage (12). Functional inhibition of CD59 with antibodies (13), targeted deletion of the CD59 genes in mice (14, 15), or the acquired deficiency of the protein that occurs in paroxysmal nocturnal hemoglobinuria (16) results in a marked increase of MAC deposition associated with MAC-induced pathological responses. We have reported that the complement regulatory function of CD59 is inhibited by the nonenzymatic glycation of Lys⁴¹ (K41), a residue within CD59's active site (1). Glycation-inactivation of CD59 and the consequent increase in MAC deposition explain colocalization of MAC deposits and GCD59 that we have described in kidneys, nerves, and the vasculature of patients with diabetes (10). Increased MAC deposition likely contributes to the inflammatory, thrombotic, and cell proliferative pathologies (1, 9, 11, 17) characteristically seen in target organs of diabetic complications.

Although CD59 is a cell membrane-bound protein, a soluble form of CD59 that is shed from cell membranes by phospholipases can be measured in the blood (18). Because soluble CD59 in blood derives from cell membranes, we hypothesized that circulating concentrations of soluble GCD59 may have utility in the diagnosis and management of diabetes as a novel biomarker that reflects both the glycation process at the cellular level and integrated blood glucose handling over time.

Glycated hemoglobin (HbA1c) is routinely measured for the diagnosis and management of patients with diabetes because it reflects mean glucose concentrations over the preceding 6 to 8 weeks; however, HbA1c is an intracellular bystander that is not pathogenically involved in the tissue damage caused by sustained hyperglycemia and is sensitive to variations in red cell survival (19). The availability of a biomarker that reflects the glycation process at the cellular and tissue levels and that is also involved in the

pathogenesis of diabetes complications may improve the diagnosis and clinical monitoring of glucose control and the risk assessment for vascular complications of diabetes. We recently reported the development and optimization of an ELISA that measures GCD59 in human serum/plasma with high sensitivity and specificity (20). In the work reported here, we apply this novel assay to evaluate the relationship between GCD59 and clinical parameters of glucose metabolism in 3 distinct human studies: 1) chronic glucose handling as reflected by HbA1c (study 1), 2) acute glucose handling as reflected by the glucose response to a 2-hour oral glucose tolerance test (OGTT) (study 2), and 3) the glycemic response to intensification of insulin therapy (study 3).

Subjects and Methods

ELISA for GCD59

In all studies, circulating levels of soluble GCD59 (serum or plasma) were measured by the GCD59 ELISA recently reported (20). Briefly, the assay uses a human CD59-specific mouse monoclonal antibody coated on the wells of ELISA plates (capture antibody), human plasma or serum samples reduced by incubation with sodium borohydride, an anti-GCD59 primary detection rabbit monoclonal antibody plus a goat antirabbit IgG-horseradish peroxidase secondary antibody and 3,3',5,5'-tetramethylbenzidine as horseradish peroxidase-substrate (all as in Ref. 20). For assay calibration, we used the synthetic GCD59 surrogate as described previously (20). Results are expressed in standard peptide units (SPU; 1 SPU is the OD reading corresponding to 1 ng/mL of the synthetic GCD59 surrogate in the calibration curve, as previously defined in Ref. 20). Analytical characteristics of the assay were as described previously: coefficient of variation (CV) of 5 determinations of the same samples tested over a period of 5 days was <10.0% for each of the 3 GCD59 concentrations tested (CV at low GCD59 concentration, 2.9%; CV at medium GCD59 concentration, 8.3%; CV at high GCD59 concentration, 7.0%) (20).

Human clinical studies

Informed consent was obtained, when appropriate, from all participating human subjects, and local institutional review committees approved all 3 study protocols.

Study 1 (normal vs diabetic subjects), GCD59 and chronic glucose handling: the association between GCD59 and HbA1c in subjects with and without type 2 diabetes

We prospectively collected plasma samples from 400 consecutive individuals with (n = 226) and without (n = 174) type 2 diabetes who sought care at either the Brigham and Women's Hospital or Massachusetts General Hospital (affiliates of Partners Healthcare). In all cases, blood was drawn for routine clinical indications that included an HbA1c measurement. Extra plasma samples, which otherwise would be discarded, were anonymously collected and saved by the Partners' Crimson Bio-

specimen Repository Core (21) when the following criteria were met: age 18 to 65 years, estimated glomerular filtration rate ≥ 60 mL/min in the last 2 years, and serum creatinine < 1.4 mg/dL in the last 2 years. Patients with type 2 diabetes had to have an International Classification of Diseases (ICD) diagnosis of 250.xx and at least 1 HbA1c value that was $> 6.5\%$ in the previous 2 years. Patients without type 2 diabetes did not have an ICD diagnosis indicative of diabetes and had to have at least 1 HbA1c value of $< 6.0\%$ in the previous 2 years. Additional information on subjects' age and gender was also available. Information regarding other demographic variables or fasting or random blood glucose was not available. These collected plasma samples were used to measure GCD59.

Study 2 (OGTT in nondiabetics), GCD59 and acute glucose handling: the association between GCD59 and 2-hour OGTT

We recruited a cohort of 109 individuals without any history of diabetes but considered to be at high risk for developing type 2 diabetes. High-risk factors included 1 or more of the following: obesity (body mass index [BMI] ≥ 30 kg/m²), family history of type 2 diabetes in a first-degree relative, personal history of gestational diabetes, history of abnormally elevated random plasma glucose, and at-risk ethnicity (Hispanic, African American, or Native American). Each participant completed personal history forms, had relevant demographic and anthropometric characteristics measured, and underwent a 75-g 2-hour OGTT. Participants were categorized for glucose tolerance based on the 2-hour glucose determination on the 2-hour OGTT following standard clinical definitions: normal, < 140 mg/dL; impaired glucose tolerance (IGT), 140 to 200 mg/dL; and type 2 diabetes mellitus ≥ 200 mg/dL. Fasting serum GCD59 was measured on samples stored at -80°C .

Study 3 (intensified insulin treatment), changes in GCD59 during intensified insulin therapy in diabetes

A cohort of 21 subjects with poorly controlled diabetes (8 with type 2 diabetes and 13 with type 1 diabetes) participated in this intervention study designed to evaluate the relationship between GCD59 and glucose responses to intensified insulin treatment. Inclusion criteria were between 18 and 65 years of age, either type 1 diabetes or type 2 diabetes, and HbA1c levels above 8% at the time of enrollment. Subjects with hypertension or hyperlipidemia requiring a change in medical therapy or known liver, kidney, inflammatory, or malignant diseases were excluded. Participants were treated with daily insulin for 8 weeks with the aim of achieving near-normal glycemia, defined as a preprandial glucose level of 80 to 120 mg/dL. Participants were instructed in home glucose monitoring 4 to 8 times daily (One Touch; Lifescan) and seen weekly over 2 months for interim health assessment, review of glucose logs, insulin adjustment, and lifestyle (diet and exercise) counseling. At each visit, self-measured daily home glucose values were downloaded from the meter and the average weekly glucose (AWG) was calculated as the mean of all daily measurements for the preceding 7 days. Fasting blood was collected at weekly visits to assess HbA1c and GCD59 measurements.

Statistical methods

Normally distributed variables are represented as mean values with SD and were compared using paired *t* tests or one-way ANOVA. The homeostatic model assessment index was not normally distributed and is therefore presented with median values and interquartile ranges and was analyzed using the Kruskal-Wallis test. Group frequencies are presented as percentages, with intergroup differences determined using χ^2 tests. Pearson coefficients (*r*) are presented for correlations between normally distributed variables. Results for linear regression analyses are reported with effect estimates (β) and standardized effect estimates, the total model R^2 , and the corresponding *P* value. The level for significance for all tests conducted was set at $\alpha = .05$, with all reported *P* values as two-tailed. Data analyses were performed using SAS statistical software version 9.1.

Study 1

All sample collection was performed prospectively with the primary objective to evaluate the independent association between GCD59 and HbA1c. Analyses were performed using multivariable linear regression with adjustments for available covariates: age, gender, and diabetes status (yes or no). Dichotomized subgroup analyses were also performed to evaluate the association between GCD59 and HbA1c in just those with diabetes and those without.

Study 2

The relationship between GCD59 and the 2-hour OGTT was evaluated using univariate and multivariate linear regression to test continuous associations between GCD59 and the 2-hour OGTT. We used 4 separate multivariable models to adjust for covariates that either exhibited univariate associations with the 2-hour OGTT response or were of relevant clinical significance. Model 1 included adjustment for age, gender, race, and BMI. Model 2 included variables in model 1 plus high-density lipoprotein (HDL) and systolic blood pressure (BP). Model 3 includes the variables in model 2 plus fasting blood glucose measured before ingestion of 75 g glucose. Model 4 included all variables in model 3 plus HbA1c.

Study 3

The relationship between insulin-induced blood glucose lowering and GCD59 was evaluated. The AWG was calculated based on the 28 weekly (4 times per day) home glucose measurements and the change in AWG, as well as GCD59 and HbA1c measurements, were compared.

Results

Study 1 (normal vs diabetic subjects), GCD59 and chronic glucose handling: the association between GCD59 and HbA1c in subjects with and without type 2 diabetes

The plasma samples of 400 consecutive subjects undergoing HbA1c assessment for clinical indications were obtained. Patients with known type 2 diabetes were older and had higher HbA1c levels when compared with individuals without diabetes (Table 1). Levels of GCD59 were

Table 1. Basic Characteristics of Individuals in Study 1 (Normal vs Diabetic Subjects)^a

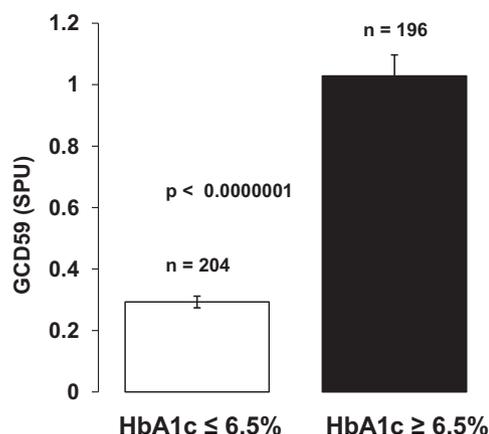
	Diabetes	No Diabetes	Combined
n	226	174	400
Age, y	53.4 (10.7)	47.4 (11.0) ^b	50.8 (11.2)
Gender, % female	24.3	36.0	29.4
HbA1c, %	8.35 (1.81)	5.58 (0.53) ^b	7.15 (1.96)
GCD59 (SPU)	0.95 (0.93)	0.27 (0.22) ^c	0.65 (0.79)

^a Results are shown as mean (SD).^b $P < .001$ vs diabetes^c $P < 6 \times 10^{-19}$ vs diabetes.

significantly increased in the individuals with diabetes (Table 1) and associated with HbA1c in univariate analysis (Supplemental Figure 1) as well as after adjusting for age, gender, and diabetes status (Table 2). Notably, this independent positive association between GCD59 and HbA1c was apparent when evaluated in just the subgroup of individuals with diabetes, and likewise in the subgroup without diabetes that had HbA1c levels well below 6.5%. Among the 226 individuals that met the criteria for diabetes as described in Subjects and Methods, there were 30 individuals whose HbA1c value measured in the sample drawn for GCD59 were $\leq 6.5\%$. Figure 1 shows the highly significant difference in the GCD59 values of the study subjects with HbA1c values $\leq 6.5\%$ as compared with those with HbA1c $\geq 6.5\%$ ($P < 3.7 \times 10^{-23}$).

Study 2 (OGTT in nondiabetics), GCD59 and acute glucose handling: the association between GCD59 and 2-hour OGTT

To further investigate GCD59 as a biomarker of glucose handling, we evaluated the association between GCD59 levels in the fasting blood samples and the 2-hour OGTT results in a population of medication-naive subjects who had never been diagnosed with but were at high risk of type 2 diabetes, as described in Subjects and Methods. The study population comprised 109 consecutively recruited individuals of which 39% were male, 78% were Caucasian, 10% were Asian, 6% were black, and 6% were Hispanic. The mean age was 42.8 years (SD, 12.4; range, 18–68) and the mean BMI was 29.6 kg/m² (SD, 7.3; range, 18.8–59.2). Characteristics of the study par-

**Figure 1.** Blood levels of GCD59 in study 1 (normal vs diabetic subjects) participants with HbA1c values either $\leq 6.5\%$ or $\geq 6.5\%$. Bars represent means \pm SEM. Student's t test $P = 3.7 \times 10^{-23}$.

ticipants are described in Table 3 based on their clinical response to the 2-hour OGTT. Although most subjects had a normal 2-hour OGTT, we observed that 13.8% of the population had undiagnosed diabetes, whereas another 26.7% exhibited IGT. As expected, a worse 2-hour OGTT status was associated with older age, higher BMI, poorer markers of glycemic index, and higher BP.

GCD59 concentrations were highly correlated with 2-hour OGTT glucose values ($\beta = 78.5$, $R^2 = 0.25$, $P < .0001$) (Supplemental Figure 2) in a similar manner to HbA1c ($\beta = 50.1$, $R^2 = 0.29$, $P < .0001$) (Supplemental Figure 3). The association between GCD59 and 2-hour OGTT remained significant and independent of adjustments made in models 1 to 3 (model 1: $\beta = 47.7$, $R^2 = 0.39$, $P < .0001$; model 2: $\beta = 41.2$, $R^2 = 0.45$, $P < .0001$; model 3, $\beta = 22.2$, $R^2 = 0.53$, $P < .05$). Our fully adjusted model 4 (including age, gender, race, BMI, systolic BP, HDL, fasting blood glucose, and HbA1c) explained more than half of the variance in 2-hour OGTT values ($R^2 = 0.54$) and continued to demonstrate a strong and independent association between GCD59 and 2-hour OGTT values ($\beta = 19.8$, $P < .05$) (Table 4). Of note, several covariates (including BMI, systolic BP, HbA1c, and race) did not demonstrate independent associations with the 2-hour OGTT values in model 4.

Table 2. Univariate and Multivariable Adjusted Associations Between GCD59 and HbA1c in Subjects With and Without Type 2 Diabetes (Study 1, Normal vs Diabetic Subjects)

	r	Univariate β	Adjusted β	Adjusted ST β	Adjusted Model R^2	Adjusted P for GCD59
Diabetes	0.58	1.13	1.10	0.56	0.34	$< .0001$
No diabetes	0.45	1.08	1.10	0.46	0.29	$< .001$
Combined	0.67	1.66	1.12	0.45	0.65	$< .0001$

Abbreviation: ST β , standardized effect estimate.

Table 3. Characteristics of the 109 Subjects of Unknown Diabetes Status in Study 2 (OGTT in Nondiabetics)^a

Characteristics	Normal	IGT	Diabetes	ANOVA <i>P</i> Value	Normal vs IGT <i>P</i> Value	Normal vs Diabetes <i>P</i> Value
n	65	29	15			
Age, y	40.6 (12.2)	44.0 (12.5)	50.2 (10.2)	<.05	.08	<.01
Gender, % male	48	24	33	.09	.04	.39
Race, % white	72	86	87	.22	.19	.33
BMI, kg/m ²	28.5 (5.7)	31.6 (9.4)	30.9 (8.3)	.12	.19	.31
HOMA-IR	1.75 (1.31,2.74)	2.59 (1.33,5.61)	5.54 (1.79,7.68)	<.05	.07	<.01
FPG, mg/dL	93.5 (7.8)	96.4 (9.1)	117.2 (22.2)	<.0001	.09	<.001
2-h OGTT PG, mg/dL	109.8 (21.3)	162.1 (19.0)	244.1 (36.0)	<.0001	<.0001	<.0001
HbA1c, %	5.38 (0.41)	5.47 (0.31)	6.31 (0.84)	<.0001	<.05	<.001
GCD59, SPU	0.61 (0.17)	0.65 (0.12)	0.88 (0.26)	<.0001	.09	<.001
Systolic BP, mm Hg	116.3 (13.3)	124.3 (14.0)	130.9 (17.1)	<.001	<.01	<.01
Diastolic BP, mm Hg	73.7 (9.7)	78.0 (7.5)	82.3 (9.5)	<.01	<.05	<.01
Total cholesterol, mg/dL	178.6 (36.5)	184.1 (30.3)	162.8 (31.1)	.15	.36	.16
LDL, mg/dL	117.6 (37.7)	119.9 (34.1)	111.8 (36.6)	.78	.80	.68
HDL, mg/dL	45.9 (11.2)	47.7 (11.7)	37.4 (8.1)	<.05	.38	<.01
Triglycerides, mg/dL	92.4 (56.7)	116.0 (72.4)	115.1 (59.3)	.16	.24	.32

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.

^a Results are shown as mean (SD).

Study 3 (intensified insulin treatment), GCD59 response to intensified insulin therapy in individuals with diabetes

To further evaluate the relationships between GCD59 and acute and chronic glucose handling described above, we investigated the effect of intensification of glycemic control with insulin therapy on GCD59 levels. A total of

21 patients with poorly controlled diabetes underwent 8 weeks of supervised insulin therapy to achieve near-normal fasting glucose levels (characteristics of participants in this study are shown in Supplemental Table 1). After the first 2 weeks of insulin therapy, AWG declined from 176.4 ± 33.2 to 149.4 ± 16.8 mg/dL ($P < .02$) in parallel with a decline in GCD59 from 0.94 ± 0.3 to 0.73 ± 0.16 SPU ($P < .008$) (Figure 2). In contrast, HbA1c values did not change significantly during these first 2 weeks ($P = .62$). After the initial 2 weeks of treatment, AWG and GCD59 levels had essentially stabilized, whereas HbA1c values continued to decline over the following 6 weeks. In addition, we evaluated fructosamine levels as another biomarker of glycemic control and found that although fructosamine levels declined with 8 weeks of insulin therapy, the decline did not reach significance in the initial 2 weeks of treatment and was overall slower than that of GCD59 (Supplemental Figure 4).

Table 4. The Independent Association Between GCD59 and 2-Hour OGTT in the 109 Subjects in Study 2 (OGTT in Nondiabetics)^a

Variable	β	ST β	<i>P</i> Value
Gender (female)	-36.5	-0.34	<.0001
FPG	1.19	0.31	<.01
GCD59	19.8	0.21	.027
Systolic BP	0.56	0.16	.06
Age	0.66	0.16	.04
HbA1c	11.8	0.13	.26
HDL	-0.43	-0.09	.29
BMI	-0.50	-0.07	.43
Race (white)	4.52	0.04	.64

Abbreviations: FPG, fasting plasma glucose; ST β , standardized effect estimate.

^a Values represent the results of multivariable linear regression for the association between GCD59 and the 2-hour OGTT, adjusted for most clinically relevant and univariate predictors of the 2-hour OGTT. Variables are ordered by the magnitude of standardized effect estimates.

Discussion

Previous work has established a pathophysiologic link between the complement system, glycation-inactivation of the complement regulatory protein CD59, and the vascular complications of human diabetes (1–4, 22, 23). Using a newly developed specific and sensitive ELISA GCD59

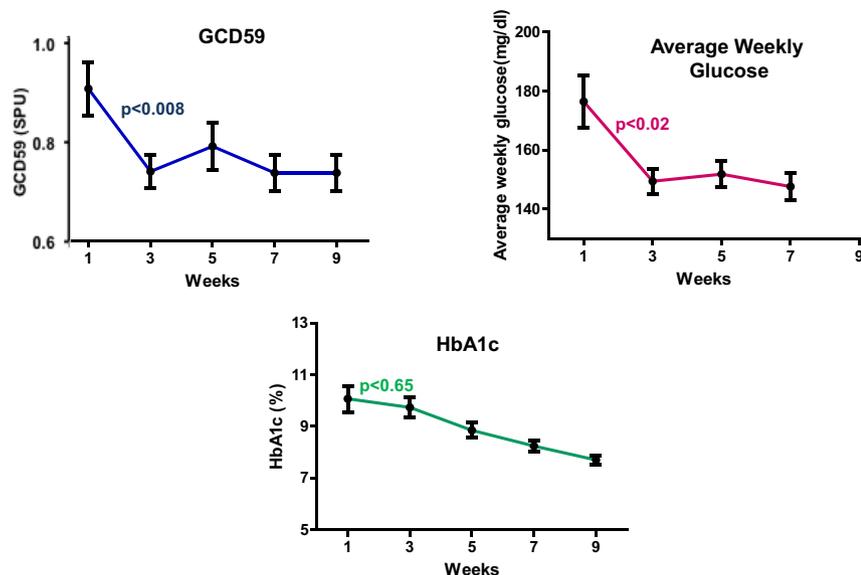


Figure 2. Time course of blood levels of GCD59, AWG, and HbA1c in 21 individuals with poorly controlled diabetes (13 with type 1 and 8 with type 2) who underwent intensified insulin therapy during 2 months (study 3). GCD59 and HbA1c were measured every other week in blood drawn during home monitoring visits. Weekly points in all graphs represent means \pm SEM values. Each AWG data point represents the mean of all daily measurements of all participants (4 measurements for each individual) in the preceding 7 days. The *P* values in the figures represent Student's *t* test applied to the values in week 1 (study entry) vs values in week 3.

assay we evaluated the role of GCD59 as a pathogenically relevant biomarker for human blood glucose handling. To this end, we conducted 3 human studies to assess the relationship between GCD59, chronic glucose control as reflected by HbA1c, acute glucose control as reflected by the 2-hour OGTT, and glycemic changes during intensification of insulin treatment. Our findings support strong positive and independent associations between GCD59 and HbA1c in patients with and without diabetes and the 2-hour OGTT plasma glucose level in patients at risk for diabetes but without established disease. Furthermore, subacute insulin-induced reductions in blood glucose were tightly paralleled by reductions in blood levels of GCD59. These observations suggest that GCD59 may represent a novel biomarker of intermediate average blood glucose handling and glycemic status that may have utility in the diagnosis and monitoring of diabetes.

In this report, we have extended the preliminary observation reported previously (20) to a significantly larger number of subjects (from 190 to 400). The comparable results strengthen the validity of the experimental observation that blood levels of GCD59 are significantly higher in individuals with diabetes compared with individuals without diabetes.

Monitoring of chronic glucose control and predicting the risk for developing end-organ complications of chronic hyperglycemia is paramount to curbing the impact of diabetes on human health (24). The use of established biomarkers that reflect subacute and chronic hyperglyce-

mia, such as HbA1c, has improved our ability to manage diabetes and predict risk of future adverse outcomes (25–28). Although HbA1c is an established and reliable marker that reflects integrated blood glucose handling over a window of 6 to 8 weeks, its role in diagnosing diabetes or predicting the risk of diabetes complications is not as reliable (29, 30). These limitations may be related to the fact that HbA1c is an intracellular bystander, namely a protein not involved in the pathogenesis of hyperglycemia-induced diabetic complications. Similarly, other known biomarkers that reflect glycemic variability (such as fructosamine, glycated albumin, and 1,5-anhydroglucitol) are limited in that they are not directly involved in the mechanism of hyperglycemia-induced disease. In that regard, novel biomarkers such as GCD59 that not only

reflect intermediate integrated blood glucose handling but are also involved in the pathogenesis of hyperglycemia-mediated end-organ complications may significantly improve our ability to detect and monitor abnormal glycemic control and assess risk for complications of diabetes.

Our cross-sectional findings suggest that GCD59 may be such a novel biomarker. Our findings indicate that the blood levels of GCD59 were highly correlated with HbA1c values even in individuals without a diagnosis of diabetes or hyperglycemia. We identified significant correlations between values of GCD59 and HbA1c even when HbA1c values were $<6.5\%$, implicating GCD59 as a potential tool for assessing subacute to chronic blood glucose trends. More importantly, acute insulin-mediated reductions in blood glucose levels over a span of 2 weeks were closely reflected by serum GCD59 concentrations. Changes in GCD59 values closely paralleled acute declines in AWG, whereas changes in fructosamine and HbA1c were delayed, as would be expected given the known turnover times for each of these established biomarkers. Together, these findings may suggest not only that GCD59 reflects integrated blood glucose handling over time but also that it is capable of reflecting shorter-term changes in blood glucose than those reflected by HbA1c and other biomarkers such as fructosamine.

Although the OGTT is not a convenient or practical diagnostic tool for routine testing, it has been shown to predict micro- and macrovascular complications and mor-

tality in diabetes (31–36). In this regard, our observation that a single blood measurement of GCD59 was independently associated with the 2-hour OGTT glucose values, even in individuals within the normal 2-hour OGTT range, support GCD59 as a potentially practical diagnostic marker for detecting IGT or early diabetes. Although our OGTT in nondiabetics study did not have sufficient power or sample size to evaluate whether GCD59 could distinguish normal from impaired glycemic control, the results did suggest a strong favorable trend that should be further investigated in larger studies. The 65 high-risk individuals classified as normal by OGTT criteria in study 2 showed a higher mean GCD59 value than normal individuals in study 1 who were selected among the general population. This difference likely reflects the high risk of diabetes in the study 2 population and possibly blunted the difference between individuals classified as either normal or IGT by OGTT criteria.

Ultimately, the value of GCD59 measurements may exceed simply diagnosing and monitoring blood glucose handling; the role of GCD59 in hyperglycemia-mediated complement injury suggests that GCD59 may also serve as a prognosticator for future vascular complications in diabetes. As an inhibitor of MAC formation that is universally expressed on the surface of mammalian cells, CD59 plays a crucial role in maintaining the delicate balance between complement activation and restriction, and specifically protects self-cells from the harmful effects of complement activation (37). In vitro and in vivo glycation of human CD59 results in the loss of its MAC-inhibitory function and a subsequent increase in MAC-mediated cell and tissue damage (1, 10, 29, 38). Although we did not directly evaluate the relationship between GCD59 concentrations and the risk of developing complications, future case-control or prospective interventional studies may be able to evaluate the potential value of GCD59 for stratification of patients at risk of developing diabetic complications.

Our current findings should be interpreted in the context of our study design. We performed cross-sectional analyses to identify independent associations between GCD59 and measures of glucose handling; although we attempted to account for multiple known confounders in these relationships, other unmeasured or unaccounted confounding variables may exist. At the same time, the parallel decline of GCD59 levels and average blood glucose after insulin intervention supports the findings of our cross-sectional studies. We did not evaluate long-term glucose monitoring or outcomes of diabetes complications; however, we demonstrated independent associations between GCD59 concentrations and the 2-hour OGTT, a surrogate of long-term outcomes, reportedly associated

with micro- and macrovascular complications as well as death. Prospective long-term studies to evaluate more thoroughly the predictive value of GCD59 in 1) diagnosing IGT or diabetes and 2) end-organ complications of diabetes are required to validate our observations. Despite the relatively small sample size of our studies, they were sufficient to observe robust relationships and as such will serve as the foundation for the design of future larger clinical trials.

In summary, a single measurement of circulating GCD59 may independently predict the likelihood of abnormal blood glucose handling (2-hour OGTT), long-term integrated blood glucose handling (HbA1c), and the short-term glucose response to therapy with insulin. Because GCD59 is pathogenically involved in mediating end-organ diabetes complications, it may present a novel biomarker capable of simultaneously assessing glycemic control and the risk for developing complications in diabetes. Our findings provide support for future studies to investigate whether single measurements of serum GCD59 may be useful in the diagnosis of IGT or diabetes, the assessment of integrated blood glucose values over time, and risk stratification for end-organ complications of diabetes.

Acknowledgments

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