GLYCATED CD59 AS A NOVEL BIOMARKER FOR GLUCOSE HANDLING IN DIABETES

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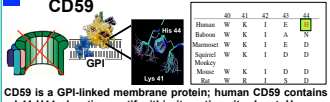
1 CD59 is an inhibitor of MAC formation

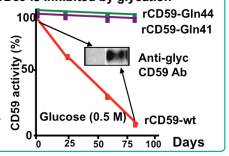


	40	41	42	43	44
Human	W	K	I	E	N
Baboon	W	K	I	A	N
Marmoset	W	K	I	E	D
Squirrel Monkey	W	K	I	D	D
Mouse	W	K	I	D	D
Rat	W	R	I	S	D

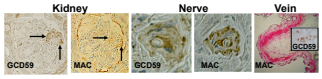
CD59 is a GPI-linked membrane protein; human CD59 contains a L41-H44 glycation motif within its active site. *Inset:* Humans are uniquely propense to develop diabetes complications: H44 is present in human CD59 but not in CD59 from other species

2 Human CD59 is inhibited by glycation

Recombinant wild type CD59 is inactivated by glucose but Gln41- or Gln44- mutants are not. Glycation was confirmed with an anti-glyCD59 Ab. *(PNAS, 2000)*

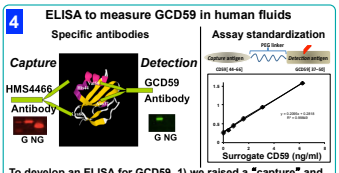


3 GCD59 co-localized with MAC is present in target organs of diabetic complications



Consistently with a role of complement in the pathogenesis of vascular diabetic complications, MAC deposits are present in kidneys, nerve micro-vessels and veins from diabetic but not from non-diabetic subjects. *(Diabetes, 2004)*

4 ELISA to measure GCD59 in human fluids



To develop an ELISA for GCD59, 1) we raised a "capture" and a "detection" mAb using synthetic peptides (the detection antigen carried a pre-formed Lys[N^{ε}-glucitol]lysine), 2) synthesized a surrogate CD59 for assay standardization *(Am J Hematol, 2013)*

OVERVIEW

The mechanism by which hyperglycemia causes vascular complications in diabetes is poorly understood. We have advanced the concept that *glycation-inactivation of the complement regulatory protein CD59* promotes complement mediated tissue damage, a novel mechanism contributing to the pathogenesis of diabetes complications.

1. Human CD59, a key inhibitor of MAC formation contains a glycation-prone motif formed by residues K41 and H44 that is unique to humans *(PNAS 2000).*
2. The K41-H44 motif makes human CD59 sensitive to inactivation by glycation, as demonstrated by site directed mutagenesis.
3. GCD59 co-localized with MAC is seen in kidneys, nerves and blood vessels from diabetic subjects *(Diabetes 2004).*
4. We developed a specific ELISA for GCD59 and used it to measure plasma GCD59 in samples from human studies evaluating acute and chronic glucose handling, and glucose responses to insulin therapy *(AJH, 2013; JCEM 2014)*

Objective We hypothesized that blood levels of glycated CD59 (GCD59) represent a novel biomarker in diabetes and tested this hypothesis in 4 human studies.

Methods Plasma levels of GCD59 were measured in different populations of individuals with and without diabetes in 4 human studies described below *(JCEM 2014)*

Study 1 Evaluated the cross-sectional association between GCD59 and HbA1c in 400 subjects with and without T2 diabetes.
Results: GCD59 was a) significantly higher in individuals with than in individuals without diabetes and b) independently and positively associated with HbA1c in individuals with and without diabetes (p<0.0001, p<0.001, respectively).

Study 2 Evaluated whether fasting GCD59 independently predicted the 2-hour glucose response to an oral glucose tolerance test (OGTT) in 109 subjects without a diagnosis of diabetes. ***Results***: a single GCD59 measurement independently predicted the results of 2hOGTT (β=19.8, p<0.05).

Study 3 Evaluated the effect on GCD59 of improved glycemic control with intensified insulin therapy in 21 poorly controlled individuals with diabetes.
Results: intensification of glucose control with insulin resulted in a significant reduction of average weekly glucose (28 measurements/week) with a parallel reduction of GCD59 within 2 weeks.

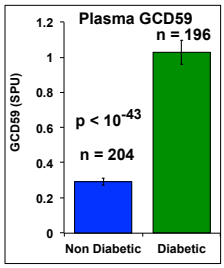
Study 4 Evaluated GCD59 in 423 pregnant women undergoing screening for gestational diabetes (GDM).
Results: GCD59 at 24 weeks gestation, when standard of care screening for GDM takes place, predicted GDM with high sensitivity and specificity ($p < 3 \times 10^{-32}$; ROC AUC 0.93).

Study 1

GCD59 was measured in 400 consecutive individuals with or without T2D.

Individuals with T2D had significantly higher GCD59

Non-diabetic: HbA1c:5.6 ± 0.03
Diabetic: HbA1c: 8.7 ± 0.1



GCD59 was independently associated with HbA1c

	Adjusted β	STβ	Model R^2	p-value
Diabetes	-36.5	-0.34	0.34	<0.0001
No Diabetes	1.19	0.31	0.29	<0.001
Combined	19.8	0.21	0.65	<0.0001

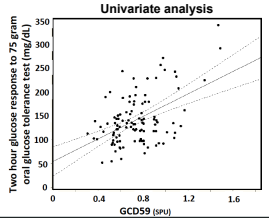
JAH is a founder and has financial interest in Mellitus LLC. Mellitus had licensed IP used in this research and has interests in developing diagnostic tools for diabetes. These interests were reviewed and are managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies.

Study 2

GCD59 was measured in 109 individuals with no history of T2D who underwent an OGTT

GCD59 was an independent predictor of 2-h plasma glucose in OGTT

Univariate analysis



Multivariate analysis

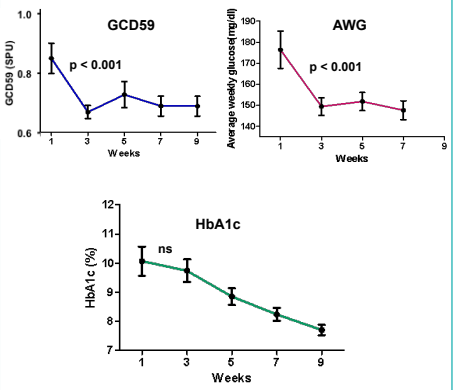
Variable	β	STβ	p-value
Gender (female)	-36.5	-0.34	<0.0001
Fasting Glucose (mg/dL)	1.19	0.31	<0.01
GCD59 (SPU)	19.8	0.21	0.027
Age (years)	0.66	0.16	0.04
HbA1c (%)	11.8	0.13	0.26*
BMI (kg/m²)	-0.50	-0.07	0.43
Race (white)	4.52	0.04	0.64

** HbA1c was also positively associated with 2h-h plasma glucose in univariate but not in multivariate analysis that included known univariate predictors of OGTT results*

Study 3

A cohort of 21 individuals with poorly controlled diabetes were subjected to intensified insulin treatment for 8 weeks; blood glucose was measured 4 times/day (28 weekly) and other parameters weekly.

GCD59 paralleled the decline in weekly average glucose



Study 4

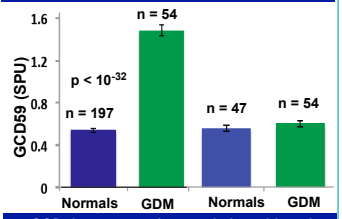
BACKGROUND: Gestational Diabetes Mellitus (GDM) is a major risk factor for adverse pregnancy outcomes. Screening for GDM with glucose load tests (1 or 2 steps) is now standard of care for all pregnant women. There is agreement that a simpler, shorter, patient-friendlier, sensitive and specific test would be a much better screen for GDM.

Frozen archival plasma samples from the POPs study were assayed for GCD59.

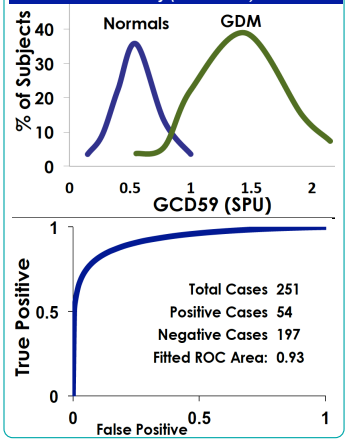
POPs Study Participants tested (Mean ±SEM)

	Normals	GDM
Age (y)	31 ± 5	34 ± 5
Weight (lb)	140 ± 22	152 ± 35
BMI	24 ± 4	28 ± 6

GCD59 is significantly higher in women with GDM than those without (week 24); and normalizes with treatment (week 35)



GCD59 separates the population with and without GDM with high specificity and sensitivity (ROC > 0.93)



SUMMARY & CONCLUSIONS

We observed robust relationships between single blood levels of GCD59 and both acute (OGTT) and chronic measures of glucose handling (HbA1c) in non-pregnant individuals.

Single GCD59 measurements predicted GDM with high sensitivity and specificity and also paralleled glucose trends during active insulin therapy. We conclude that GCD59 may represent a promising new biomarker for the diagnosis and monitoring of pre-diabetes, diabetes and GDM.