Validation of Hemoglobin A1c as a Biomarker of Long-Term Glycemic Control in Mouse Models of Obesity and Diabetes

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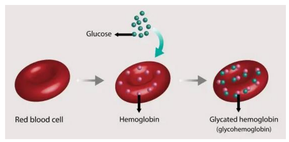
BACKGROUND

- Hemoglobin A1c (HbA1c) is a primary clinical measure of glycemic control in patients with diabetes, but is rarely measured in rodents. Glycation of hemoglobin is a non-enzymatic and irreversible process, occurs continuously, and is only dependent on the concentration of glucose in the blood.
- HbA1c assays provide an estimated integrative measure of blood glucose homeostasis over the previous 5-6 weeks in mice, and 11-12 weeks in humans.
- Single sample blood glucose measures are sensitive to stress and acute environmental stimuli, as observed during continuous glucose monitoring studies.
- Measurement of HbA1c in rodents may provide more information, and less stress than repeat measures of single point and GTT glucose assays in chronic studies of obesity and glucose homeostasis.
- Diet Induced Obesity (DIO) mouse models are the most commonly used pre-clinical models of obesity, displaying increased body weight, elevated glucose, and insulin resistance.
- Non-obese diabetic (NOD) mice are an inbred laboratory strain used extensively to study Type 1 Diabetes (T1D) and other autoimmune diseases, and serves as a positive control for elevated HbA1c in this study.

OBJECTIVES

- To validate the use of the A1CNow®+ assay in young pre-diabetic and diabetic NOD female mice, an immune deficiency and type1 diabetes model, as a positive assay control.
- To survey a commercially available colony of age matched DIO and chow fed male mice to characterize HbA1c levels and their correlation to body weight and single point glucose measures in a commonly used DIO model.

Hemoglobin A1c (HbA1c)



MATERIALS AND METHODS

Animals:

[3]Non-Obese Diabetic Mice
NOD/MrkTac female mice were maintained ad libitum on NIH 31 chow and water at a cage density of 5/cage until either 8 weeks (n=5) or 22 weeks (n=7) of age. Mice of these ages were selected randomly from a commercial breeding/supply colony, and studies performed, by Taconic Biosciences, Rensselaer, NY.

Diet Induced Obese Mice
70 C57Bl/6NTac male mice were maintained ad libitum on NIH 31 chow and water at a cage density of 10/cage until 6 weeks of age (Taconic Biosciences) as part of a commercially available colony. At 6 weeks of age, animals are randomized for either continuation on NIH31 chow, or being fed a 60 kcal% fat diet, D12492 (Research Diets, Inc., New Brunswick, NJ) to induce DIO as a subset of a commercially available colony.

Whole Blood Assays
[5]Fasting blood glucose using Accu-Chek blood glucose meter, in duplicate.
[4]HbA1c using PTS Diagnostics test (A1CNow®+) in duplicate.

Procedure:
A subset of the DIO production lot was chosen, with age matched diet cohorts selected to within a week of age, and sampled over two days. Half were assayed on the same day following a 2-4 hour fast. C57BL/6NTac mice fed either a high-fat diet (D12492, 60 kcal% fat) to induce diet-induced obesity or NIH-31 chow. Animals were assayed for blood glucose, followed by HbA1c via the same tail nick, and weighed, at 6, 14, 22, and 30 weeks of age (D12492 or chow fed for an additional 0, 8, 16, or 24 weeks).
Eight week old NOD female mice and the age matched male chow or DIO animals were assayed as above on day one after a 2-4 hour fast. The 22 week old NOD female animals were assayed on a separate day following a 2-4 hour fast. Animals were euthanized following the study.

Statistics:
Body weight, glucose, and HbA1c measures were assessed with a repeated measures two-way ANOVA with time and diet as variables. Scatterplots analyzed using Pearson correlations.

DIET COMPOSITION

D12492 Purified Diet Induced Obesity 60 kcal% Fat Diet and NIH 31 Grain Based Chow

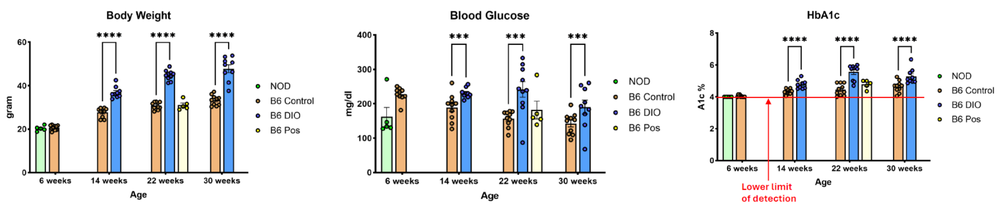

D12492

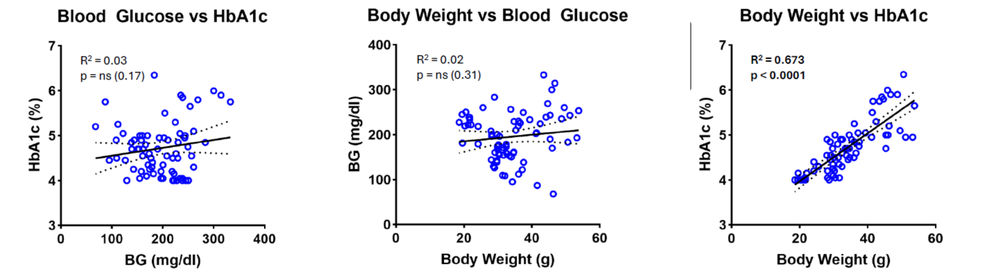

NIH-31 CHOW

	D12492 60 kcal% Fat Purified Ingredient Diet		NIH 31 M Chow Grain Based Diet	
Macronutrients				
	gm%	kcal%	gm%	kcal%
Protein	26.2	20	NR	21
Carbohydrate	25.4	19	NR	65
Fat	34.9	60	NR	14
Total		100		100
kcal/gm	5.26			
Ingredients	**gm**	**kcal**	**gm**	**kcal**
Casein	200	800	NR	NR
L-Cystine	3	12	NR	NR
			NR	NR
Sucrose	71.21	285	NR	NR
Maltodextrin 10	125	500	NR	NR
Corn Starch	0	0	NR	NR
Fructose	0	0	NR	NR
			NR	NR
Cellulose, BW200	50	0	NR	NR
			NR	NR
Soybean Oil	25	225	NR	NR
Lard	245	2205	NR	NR
Coconut Oil, 101	0	0	NR	NR
			NR	NR
Mineral Mix S100264A	5	0	NR	NR
Dicalcium Phosphate	13	0	NR	NR
Calcium Carbonate	5.5	0	NR	NR
Potassium Citrate, 1 H20	16.5	0	NR	NR
Potassium Bicarbonate	0	0	NR	NR
Sodium Chloride	2.59	0	NR	NR
Sodium Bicarbonate	0	0	NR	NR
			NR	NR
Vitamin Mix V10001	10	40	NR	NR
Vitamin Mix V10001C	0	0	NR	NR
Choline Bitartrate	2	0	NR	NR
			NR	NR
Yellow Dye #5, FD&C	0	0	NR	NR
Red Dye #40, FD&C	0	0	NR	NR
Blue Dye #1, FD&C	0.0.5	0	NR	NR

DIET INDUCED OBESITY RESULTS

HbA1c and Body Weight are Correlated

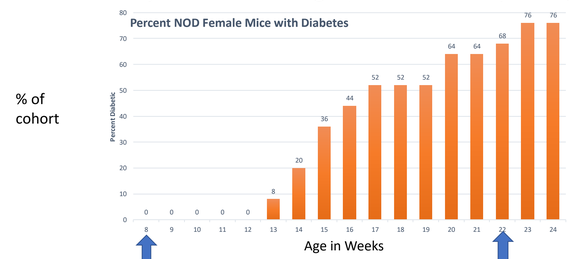




Body weight, glucose, and HbA1c measures were assessed with a repeated measures two-way ANOVA with time and diet as variables.
*** and **** are significantly different, p< 0.05. Scatterplots analyzed using Pearson correlations.

NOD COLONY SURVEY RESULTS

76% of NOD Female Mice Spontaneously Develop Type 1 Diabetes by 24 Weeks of Age (Taconic Colony 2025,n=25)



Animal	BW	glucose	HbA1c
	grams	mg/dl	%
NOD #1	18.8	271	≤ 4
NOD #2	20.2	134	≤ 4
NOD #3	20.6	147	≤ 4
NOD #4	22.1	131	≤ 4
NOD #5	19.8	128	≤ 4

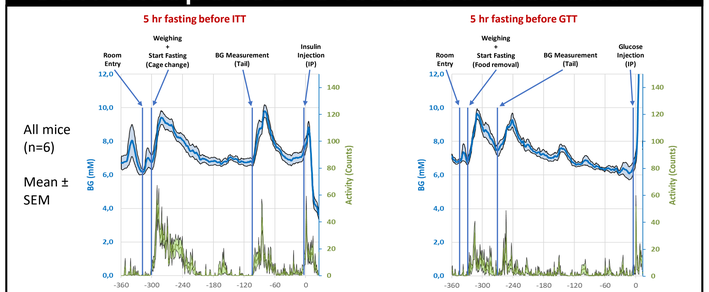
N=5 mice were selected at 8 weeks of age

N=7 mice were selected at 22 weeks of age

Animal	BW	glucose	HbA1c
	grams	mg/dl	%
NOD #6	27.5	≥ 600	7.15
NOD #7	17.4	≥ 600	11.45
NOD #8	32.3	258	≤ 4
NOD #9	20	≥ 600	9.45
NOD #10	31.4	285	4.7
NOD #11	29.6	229	≤ 4
NOD #12	31.4	235	≤ 4

Two cohorts of NOD mice were selected from a breeding colony at Taconic Biosciences.

CGM Response to Routine Procedures



CGM stress response characterization provided by Thomas Svava-Nielsen[c], separate study.

SUMMARY AND FOLLOWUP

[1]Obesity is a global health emergency, and is on the rise based on worldwide health data. Diet Induced Obesity (DIO) mouse models (D12492, 60kcal% fat and D12451,45 kcal% fat, Research Diets, Inc. New Brunswick, NJ) are the most commonly used pre-clinical models of obesity, displaying increased body weight, elevated glucose, and insulin resistance. [2]In addition to obesity, these diets and other defined modifications are used in research in the comorbid diseases of pre-diabetes, cardiovascular disease, metabolic disease, MASH, cancer risk, immunology, neuroscience, and inflammation research. Diet compositions vary based on target focus.

Glucose exposure causes biochemical changes at the proteome level as reflected in accumulation of glycated proteins. A prominent example is HbA1c. HbA1c is a superior measure of long-term glycemic control than intermittent blood glucose measurements. However, very little literature exist on the use of HbA1c in mice, and most of the studies reporting HbA1c results do so without addressing the temporal dynamics, sensitivity, and the physiological range of HbA1c in this species. This study does not address these details.

The use of the [4]A1CNOW+ assay was validated using prediabetic NOD female mice as a negative control (8 weeks old) and emergent [3]NOD female diabetic mice as a test cohort (22 weeks old). 8 week old animals did not register above the 4% lower limit if the HbA1c assay. 3/7 animals were positive for frank diabetes with greater than 600 mg/dl glucose and elevated HbA1c, with an average HbA1c of 9.35 +/- 1.2 (SEM), compared to non- NOD-diabetic but aged animals with HbA1c levels of 4.2 +/- 0.2 (SEM).

In the second study, the DIO group exhibited significantly elevated blood glucose, HbA1c, and body weight following 8 weeks of D12492 feeding from baseline (14 weeks old) compared to animals maintained on NIH 31 for 8 weeks from baseline (14 weeks old) These parameters' significance continued after 16 weeks and 24 weeks of post baseline feeding. HbA1c showed a strong correlation with body weight ($r^2 = 0.672$; $p < 0.0001$). However, correlations between single point fasted blood glucose and both HbA1c ($r^2 = 0.3$) and body weight ($r^2 = 0.02$) were not significant.

We demonstrate that the A1CNow®+ assay provides an accurate and cost-effective integrative measure of HbA1c reflecting glycemic control in a commonly used model of DIO and mild hyperglycemia. These findings suggest that HbA1c is a reliable measurement of chronic hyperglycemia in mice, and its measurement could enhance studies of glycemic control through diet and therapeutic interventions in mice, and reduce the amount of handling and blood sampling needed for chronic monitoring of glycemic status.

The incorporation of periodic measures of HbA1c every 5-6 weeks during chronic DIO and therapeutic treatment studies may lessen the stress on animals during chronic treatment compared to monitoring via weekly glucose measures.

REFERENCES

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[2] de Moura e Dias, M., dos Reis, S.A., da Conceição, L.L. et al. Diet-induced obesity in animal models: points to consider and influence on metabolic markers. Diabetol Metab Syndr 13, 32 (2021). https://doi.org/10.1186/s13098-021-00647-2

[3] Mouse Models of Autoimmune Diabetes: The Nonobese Diabetic (NOD) Mouse Methods Mol Biol. 2020 2128: 87–92. doi:10.1007/978-1-0716-0385-7_6. NOD Mice Chen et al, 2021: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8253669

[4] HbA1c Assay: A1CNow®+ (PTS Diagnostics, www.ptsdiagnostics.com)

[5] Fasting Blood Glucose (Accu-Chek, www.accu-chek.com)

POSTER PRESENTATION DAY: Friday, April 24 **POSTER PRESENTATION TIME:** 4:30 – 7:00 p.m.