ONLINE APPENDIX—Supplemental Material:

Rationale for Using the Chosen Datasets

The datasets were chosen to increase the number of subjects by combining a prospectively collected dataset with two cross-sectional studies on American populations that included both A1c and OGTT in subjects without known diabetes and were enriched in black subjects. We have previously demonstrated comparability of the three studies, including similar mean A1c values (average A1c was 5.4% in SIGT, 5.4% in NHANES III, and 5.3% in NHANES 2005-2006) (1). Further rationale for choosing these datasets includes increasing the generalizability since the results will derive from more than one time period, in populations with different age-groups, and utilize different, though standardized, A1c assays.

Inclusion criteria for accepting NHANES data

In NHANES III, a subset of adults over 40 years old had an OGTT. We included subjects with no known history of diabetes, that completed the OGTT before 11 am after an overnight fast of at least 9 hours, and had the 2-hour post-challenge glucose level measured between 100 and 135 minutes after the glucose load. We excluded subjects considered "non-responders" due to lack of data or "ineligible" due to other factors who received a survey weight value of zero. In NHANES 2005-2006, all subjects 12 years and older seen in the morning were asked to have an OGTT. Subjects were eligible for the OGTT if they had fasted overnight for at least 9 hours, reported no oral medications or insulin for diabetes, were not pregnant, did not have hemophilia, and did not receive cancer chemotherapy in the previous three weeks. We accepted subjects if blood samples for the 2-hour glucose measurement were obtained between 100 and 135 minutes after the glucose load, they were18 years and older, with no known history of diabetes, and a survey weight value greater than zero. Age, body mass index, blood pressure, lipids, and family history were categorized using conventional criteria. After excluding subjects with missing data, we included only nonHispanic black or white subjects to match the SIGT study population There were 2014 subjects in NHANES III and 1111 subjects in NHANES 2005-2006, as described previously (1).

Comparison of A1c Assays from the Different Datasets

The SIGT study used an immunoassay (Beckman Coulter Synchron LX Hemoglodin A1c assay), while ion-exchange HPLC assays were used in NHANES III (Bio-Rad Diamat Analyzer performed at University of Missouri-Columbia Diabetes Diagnostic Laboratory) and NHANES 2005-2006 (Tosoh 2.2 Plus Glycohemoglobin Analyzer performed at Fairview University Collaborative Studies Central Laboratory, Minneapolis). The CV of the ion-exchange HPLC for the NHANES III study was 1.73% at A1c=4.6 and 1.52% at A1c 10.2. The CV of the ion-exchange HPLC for the NHANES 2005-2006 study was 1.5% at A1c=5.37, 1.7% at A1c=5.48, and 1.0% at A1c=10.63 and 10.75. The CV of the immunoassay used in the SIGT study was calibrated according to the manufactures instructions to have CV <5% within runs, and less than 7.5% total at A1c=8.0. Bias is not provided for the assays in the technical manuals fpr the SIGT assay nor the procedure manual from the NHANES assay. Bias can be expected and has been independently tested over time across the relevant A1c ranges

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for for the assays included here(2), All 3 assays had overlapping bias between 2004-2006 between -0.1 to 0.1 measured at A1c=5.2-5.3%, while there was slightly different bias measured at A1c=7.0-9.3% for the Bio-Rad assay (0.0 to 0.4), Synchron assay (-0.1 to -0.5), and the Tosoh assay (0.2 to 0.7). Laboratory practice standards may limit any bias by calibrating the assays on more than one point, but the chance that bias compared to reference measurements differs between assays must be considered. The NHANES III assay was calibrated with 2 points by the DCCT reference method, the NHANES 2005-2006 was calibrated to 2 points using NGSP standards (ZS8001 at A1c=6.1 and 10.7). The SIGT assay was calibrated to 4 points using NGSP standards (M812163 0.49, 0.98, 1.48, and 2.25g/dL A1c in 17.8g/dL Hemoglobin), and adjustments are calculated between the calibration standards included with the kit and the NGSP standard. Additional comparisons of the bias between the assays are found in two reports from the Beckman Coulter product information directly comparing the SYNCHRON LX to the Diamat HPLC and the Tosoh HPLC. Using over 100 samples they determined the difference between means, and linear regressions between the immunoassay and the ion-exchange assays:

Synchron LX vs. Diamat - $y = 1.02 \, x + 0.24$, R=0.97, Mean 7.08 vs 7.17, n= 112

Synchron LX vs. Tosoh - $y = 1.055x - 0.50$, R=0.975, mean 7.61 vs. 7.69 , n= 111

The SIGT immunoassay is not known to have any interference from hemoglobinopathies. The NHANES III and NHANES 2005-2006 HPLC assays can be prone to errors with abnormal hemoglobin, and each study had pre-determined methods for double-checking samples with known hemoglobinopathies or values out of range with a second test that overcame these problems (3; 4).

Analysis of A1c against higher FBG cutoff for prediabetes
Alternate cutoffs for "prediabetes" above FPG of 100 mg/dl or "high risk prediabetes" above FPG of 110 mg/dl could be considered. The analysis included in the manuscript is reported for the lower FPG cutoff. The analyses with the higher fasting plasma glucose cutoff for normal glucose tolerance were repeated using the SIGT datasets for this paper, and have been also been considered in other publications with this data (5). Comparisons between the SIGT results for the two different cutoffs are included in this Supplementary Material (Tables 2, 3, and 4, and Figure 1). Using the higher FPG cutoff modestly increased the sensitivity for A1c criteria to identify prediabetes compared to the higher FPG cutoff (Supplementary Table 2), due to an increased number of normal glucose tolerance subjects (Supplementary Table 3), resulting in a small increase in the areas under the curve for the ROC's (Supplementary Figure 1). However, this reduced the rate of correct classification of normal glucose tolerant subjects, despite a small increase in correct classification of those with prediabetes and dysglycemia (Supplementary Table 4). In summary, the observation is unchanged that that the IEC and ADA A1c diagnostic criteria have: (i) high specificity but low sensitivity, and (ii) intrinsic inaccuracy even with alternative cutoffs.

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Supplemental Table 1A: SIGT Demographics and distribution of OGTT and A1c Diagnoses.

SIGT		OGTT			A1c				
					IEC Criteria		ADA Criteria		IEC and ADA
		NGT	Prediabetes	Diabetes	Normal (<6.0%)	High Risk (6.0-6.4%)	Normal (<5.7%)	High Risk (5.7-6.4%)	Diabetes (6.5%)
All Subjects (n)	1581	982	527	72	1427	120	1181	366	34
% (95% CI)		62.1 (59.7 64.5)	33.3 (31.1-35.7)	4.6 (3.6-5.7)	90.3 (88.7-91.6)	7.6 (6.4-9.0)	74.7 (72.5-76.8)	23.1 (21.1-25.3)	2.2 (1.5-3.0)
Age - ave.	48	45	52	54	47	53	46	52	53
BMI - ave.	30.2	29.2	31.7	34.4	29.7	35.2	29.2	33.0	36.6
Male – % (95% CI)	42 % n=665	52.9 (49.1-56.7)	41.5 (37.8-45.3)	5.6 (4.1-7.6)	92.6 (90.4-94.4)	5.3 (3.8-7.2)	72.3 (69.3-75.1)	25.5 (22.8-28.5)	2.1 (1.3-3.5)
Female - % (95% CI)	58% n=916	68.8 (65.7-71.7)	27.4 (24.6-30.4)	3.8 (2.8-5.3)	88.5 (86.3-90.4)	9.3 (7.6-11.3)	78.0 (74.7-81.0)	19.8 (17.0-23.1)	2.2 (1.4-3.3)
White – % (95% CI)	42% n=662	61.3 (57.6-65.0)	35.0 (31.5-38.8)	3.6 (2.4-5.3)	97.3 (95.7-98.3)	2.0 (1.2-3.3)	89.3 (86.7-91.4)	10.0 (7.9-12.5)	0.8 (0.3-1.8)
Black - % (95% CI)	58% n=919	62.7 (59.5-65.7)	32.1 (29.2-35.2)	5.2 (4.0-6.9)	85.2 (82.8-87.4)	11.6 (9.7-13.9)	64.2 (61.0-67.2)	32.6 (29.7-35.7)	3.2 (2.2-4.5)

Supplemental Table 1B: NHANES III Demographics and distribution of OGTT and A1c Diagnoses. All values of n are unweighted, while all averages, and percentages are from the weighted estimates.

NHANES III		OGTT			A1c				
					IEC Criteria		ADA Criteria		IEC and ADA
		NGT	Prediabetes	Diabetes	Normal (<6.0%)	High Risk (6.0-6.4%)	Normal (<5.7%)	High Risk (5.7-6.4%)	Diabetes (6.5%)
All Subjects (n)	2014	1004	820	190	1689	225	1336	578	100
% (95% CI)		54.3 (51.3 57.3)	38.1 (35.4-40.9)	7.6 (6.4-8.9)	90.2 (88.5-91.7)	6.9 (5.8-8.3)	75.8 (72.3-78.9)	21.4 (18.5-24.7)	2.8 (2.1-3.7)
Age- ave.	54.7	52.8	56.2	60.8	54.3	58.7	53.7	57.9	59.9
BMI- ave.	27.3	26.2	28.1	30.7	27.0	28.8	26.8	28.4	31.1
Male- % (95% CI)	47.3% n=957	45.4 (40.2-50.7)	46.3 (41.6-51.1)	8.3 (6.4-10.7)	89.6 (86.8-91.9)	7.0 (5.2-9.4)	73.9 (69.8-77.7)	22.7 (19.3-26.4)	3.4 (2.4-4.8)
Female- % (95% CI)	52.7% n=1057	62.3 (57.7-66.7)	30.7 (27.1-34.7)	7.0 (5.4-8.9)	90.8 (88.5-92.6)	6.9 (5.4-8.8)	77.4 (72.7-81.5)	20.3 (16.5-24.6)	2.3 (1.6-3.5)
White- % (95% CI)	90.4% n=1341	54.4 (51.0-57.7)	38.2 (35.2-41.3)	7.5 (6.2-9.0)	91.9 (90.0-93.4)	5.8 (4.6-7.3)	78.3 (74.3-81.9)	19.4 (16.1-23.1)	2.3 (1.7-3.3)
Black- % (95% CI)	9.6% n=673	53.6 (49.2-57.8)	37.7 (33.6-41.9)	8.8 (7.0-10.9)	74.7 (70.9-78.3)	17.8 (15.0-21.0)	52.0 (47.6-56.3)	40.6 (36.3-45.0)	7.5 (5.6-9.9)

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Supplemental Table 1C: NHANES 2005-2006 Demographics and distribution of OGTT and A1c Diagnoses. All values of n are unweighted, while all averages, and percentages are from the weighted estimates.

NHANES 2005-2006		OGTT			A1c				
					IEC Criteria		ADA Criteria		IEC and ADA
		NGT	Prediabetes	Diabetes	Normal (<6.0%)	High Risk (6.0-6.4%)	Normal (<5.7%)	High Risk (5.7-6.4%)	Diabetes (6.5%)
All Subjects (n)	1111	625	421	65	1026	62	886	202	23
% (95% CI)		59.1 (52.1-65.7)	35.8 (30.2-41.8)	5.2 (3.8-7.0)	94.2 (92.6-95.5)	4.1 (3.0-5.6)	84.2 (82.2-86.0)	14.0 (12.7-15.5)	1.8 (0.9-3.4)
Age- ave.	45.6	40.7	51.0	63.8	44.6	60.5	43.3	57.4	61.5
BMI- ave.	28.5	26.7	30.8	32.3	28.1	33.8	27.8	31.1	37.2
Male- % (95% CI)	49.2% n=606	51.4 (42.9-59.9)	43.3 (36.3-50.6)	5.3 (3.5-7.8)	94.8 (92.1-96.5)	3.1 (1.7-5.7)	84.1 (81.2-86.7)	13.7 (11.5-16.3)	2.2 (0.9-5.4)
Female- % (95% CI)	50.8% n=505	66.5 (60.6-71.9)	28.5 (23.6-33.9)	5.0 (3.2-7.8)	93.7 (90.8-95.7)	5.0 (3.4-7.3)	84.1 (80.2-87.7)	14.3 (11.7-17.5)	1.3 (0.6-3.1)
White- % (95% CI)	87.1% n=752	58.7 (51.0-65.9)	35.9 (29.9-42.4)	5.4 (3.9-7.6)	94.9 (93.3-96.2)	3.4 (2.3-4.9)	86.4 (83.7-88.7)	11.9 (10.1-14.0)	1.7 (0.8-3.6)
Black- % (95% CI)	12.9% n=359	61.9 (55.0-68.4)	34.9 (29.0-41.2)	3.2 (1.7-6.0)	89.3 (85.4-92.3)	8.6 (5.9-12.5)	69.5 (65.6-73.3)	28.4 (24.5-32.6)	2.1 (1.1-3.8)

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Supplemental Table 2: Characteristics of a screening test in each Dataset.

% A1C	SIGT				NHANES III				NHANES 2005-2006				SIGT (repeated with normal FPG <110 mg/dl)			
	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
Diabetes																
4.5	100.0	0.3	4.6	100.0	98.4	2.5	9.5	93.8	98.5	2.0	5.9	95.5				
5.0	95.8	9.9	4.8	98.0	94.7	16.8	10.6	96.8	95.4	20.1	6.9	98.6				
5.7	73.6	77.0	13.3	98.4	74.2	70.6	20.8	96.3	72.3	83.0	20.9	98.0				
6.0	56.9	92.5	26.6	97.8	58.9	88.3	34.5	95.4	50.8	95.0	38.8	96.9				
6.5	33.3	99.3	70.6	96.9	37.9	98.5	72.0	93.8	29.2	99.6	82.6	95.8				
7.0	13.9	99.9	83.3	96.0	26.3	99.8	92.6	92.9	13.8	100.0	100.0	94.9				
Dysglycemia																
4.5	100.0	0.5	38.0	100.0	98.6	3.4	50.7	70.8	99.0	2.7	44.2	77.3	100.0	0.4	23.3	100.0
5.0	95.0	12.5	39.8	80.4	90.0	21.4	53.5	68.0	90.1	26.4	48.8	77.5	95.1	11.1	24.4	88.2
5.7	41.6	84.6	62.3	70.4	44.6	77.3	66.4	58.1	34.4	90.7	74.2	64.0	49.3	82.0	45.3	84.3
6.0	19.5	96.2	76.0	66.2	24.9	92.6	77.2	55.1	14.4	97.6	82.4	59.5	24.3	94.6	57.8	80.5
6.5	4.8	99.5	85.3	63.2	9.4	99.5	95.0	52.2	4.5	99.8	95.7	57.4	7.9	99.6	85.3	78.2
7.0	1.8	99.9	91.7	62.5	5.2	99.9	98.1	51.2	1.9	100.0	100.0	56.7	3.0	99.9	91.7	77.3
Prediabetes																
4.5	100.0	0.5	35.0	100.0	98.7	3.4	45.5	75.6	99.0	2.7	40.7	81.0	100.0	0.4	19.6	100.0
5.0	94.9	12.5	36.8	82.0	88.9	21.4	48.0	70.3	89.3	26.4	45.0	78.6	94.9	11.1	20.6	90.0
5.7	37.2	84.6	56.5	71.5	37.7	77.3	57.5	60.3	28.5	90.7	67.4	65.3	43.4	82.0	36.9	85.6
6.0	14.4	96.2	67.3	67.7	17.0	92.6	65.3	57.7	8.8	97.6	71.2	61.4	16.3	94.6	42.5	82.3
6.5	0.9	99.5	50.0	65.2	2.8	99.5	82.1	55.6	0.7	99.8	75.0	59.9	1.7	99.6	50.0	80.7
7.0	0.2	99.9	50.0	65.1	0.4	99.9	75.0	55.1	0.0	99.8	0.0	59.7	0.3	99.9	50.0	80.5

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Supplemental Figure 1: ROC's from SIGT Data comparing different values for fasting plasma glucose cutoffs to define "prediabetes". Normal plasma glucose cutoff 100 vs 110 mg/dl. A. Prediabetes, B. Dysglycemia (prediabetes or diabetes)

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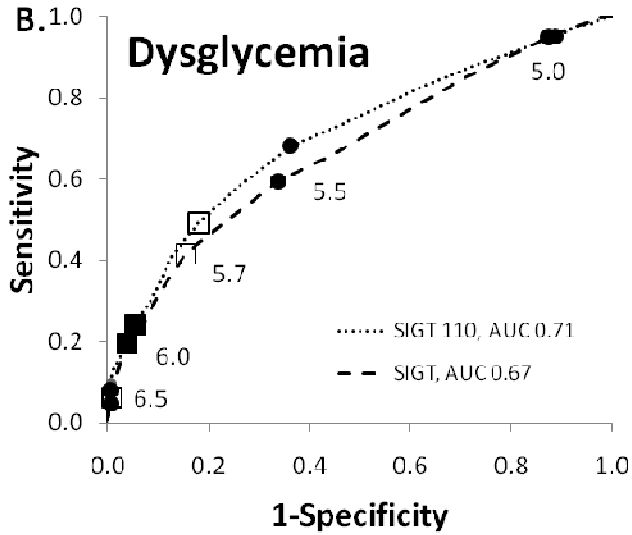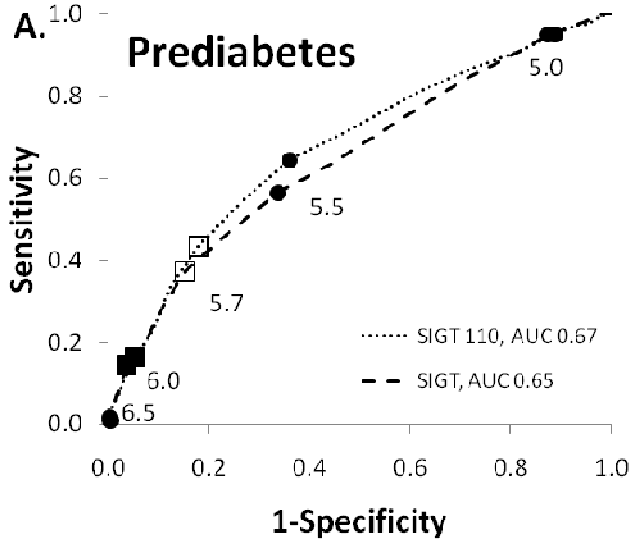
Supplemental Table 3: Comparison of different levels of fasting plasma glucose to define "prediabetes" at FBG≥100 mg/dl or FBG≥110 mg/dl.

SIGT		A1c categories						
		IEC				ADA		
% of Total	OGTT Categories	Normal A1c < 6.0%	High Risk A1c = 6.0-6.4%	Diabetes A1c ≥ 6.5% (a)		Normal A1c < 5.7%	High Risk A1c = 5.7-6.4%	Diabetes A1c ≥ 6.5% (a)
(b)	NGT	59.8%	2.02%	0.32%		52.6%	9.23%	0.32%
	Prediabetes	28.5%	4.49%	0.32%		20.9%	12.1%	0.32%
(c)	NGT	72.7%	3.80%	0.32%		62.9%	13.5%	0.32%
	Prediabetes	15.6%	2.72%	0.32%		10.6%	7.78%	0.32%
(d)	Diabetes	1.96%	1.08%	1.52%		1.20%	1.83%	1.52%

(a) Diabetes rates are the same for either IEC or ADA criteria
(b) Prediabetes defined as FBG of 100-125, and/or 2 hour BG of 140-199
(c) Prediabetes defined as FBG of 110-125, and/or 2 hour BG of 140-199
(d) Diabetes definitions and rates are the same for either cutoff of prediabetes. These are included in the table to account for 100% of values

Supplemental Table 4: Rates of incorrect classification at different FPG cutoffs for prediabetes or dysglycemia in SIGT Dataset

SIGT Data		IEC Criteria (a, d)		ADA Criteria (a, d)	
OGTT Criteria		FPG 100 mg/dl (b)	FPG 110 mg/dl (c)	FPG 100 mg/dl (b)	FPG 110 mg/dl (c)
Normal	%Correct	96.2%	94.7%	84.6%	82.0%
	FP High Risk	3.26%	4.94%	14.9%	17.6%
	FP Diabetes	0.51%	0.41%	0.51%	0.41%
Prediabetes	%Correct	13.5%	14.6%	36.2%	41.7%
	FN Normal	85.6%	83.7%	62.8%	56.6%
	FP Diabetes	0.95%	1.69%	0.95%	1.69%
Dysglycemia	%Correct	19.5%	24.2%	41.6%	49.3%
	FN Normal	80.5%	75.8%	58.4%	50.7%

(a) Diabetes rates are the same for either IEC or ADA criteria, at both FPG cutoffs
(b) Prediabetes defined as FPG of 100-125, and/or 2 hour BG of 140-199
(c) Prediabetes defined as FPG of 110-125, and/or 2 hour BG of 140-199
(d) Diabetes definitions and rates are the same for either cutoff of prediabetes

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