

A Pre-Pregnancy Biomarker Risk Score Improves Prediction of Future Gestational Diabetes

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Context: Previous studies have not examined the ability of multiple preconception biomarkers, considered together, to improve prediction of gestational diabetes mellitus (GDM).

Objective: To develop a preconception biomarker risk score and assess its association with subsequent GDM.

Design: A nested case-control study among a cohort of women with serum collected as part of a health examination (1984 to 1996) and subsequent pregnancy (1984 to 2009). Biomarkers associated with GDM were dichotomized into high/low risk.

Setting: Integrated health care system.

Participants: Two controls were matched to each GDM case (n = 256 cases) on year and age at examination, age at pregnancy, and number of pregnancies between examination and index pregnancy.

Main Outcome Measure: GDM.

Results: High-risk levels of sex hormone-binding globulin (SHBG; <44.2 nM), glucose (>90 mg/dL), total adiponectin (<7.2 μg/mL), and homeostasis model assessment-estimated insulin resistance (>3.9) were independently associated with 2.34 [95% confidence interval (CI): 1.50, 3.63], 2.03 (95% CI: 1.29, 3.19), 1.83 (95% CI: 1.16, 2.90), and 1.67 (95% CI: 1.07, 2.62) times the odds of GDM and included in the biomarker risk score. For each unit increase in the biomarker risk score, odds of GDM were 1.94 times greater (95% CI: 1.59, 2.36). A biomarker risk score including only SHBG and glucose was sufficient to improve prediction beyond established risk factors (age, race/ethnicity, body mass index, family history of diabetes, previous GDM; area under the curve = 0.73 vs 0.67, *P* = 0.002).

Conclusions: The improved, predictive ability of the biomarker risk score beyond established risk factors suggests clinical use of the biomarker risk score in identifying women at risk for GDM before conception for targeted prevention strategies.

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Freeform/Key Words: biomarker, gestational diabetes, preconception, pre-pregnancy, risk score

Abbreviations: AUC, area under the curve; BMI, body mass index; CARDIA, Coronary Artery Risk Development in Young Adults; CI, confidence interval; GDM, gestational diabetes mellitus; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; KPNC, Kaiser Permanente Northern California; LDL, low-density lipoprotein; MHC, multiphasic health checkup; OR, odds ratio; SHBG, sex hormone-binding globulin.

Gestational diabetes mellitus (GDM) is a common pregnancy complication, affecting 5% to 9% of pregnancies [1], with important short- and long-term health consequences for a mother and her child. In the short term, GDM is associated with an increased risk of hypertensive disorders during pregnancy, cesarean delivery, and macrosomia [2]. In the long term, GDM is associated with an increased risk of diabetes and cardiovascular disease in the mother [3, 4] and obesity, glucose intolerance, and diabetes in her child [5, 6].

Lifestyle interventions starting in early pregnancy for prevention of GDM have had limited success [7], potentially because the pathogenic processes leading to GDM have already begun before pregnancy [8]. Pregnancy-induced insulin resistance acts as a metabolic stress test to unmask underlying metabolic dysfunction [9]. Women with GDM have pancreatic beta cell dysfunction, which contributes to the inability to increase insulin secretion adequately to compensate for pregnancy-induced insulin resistance [10]. The majority of women who develop GDM also enter pregnancy with chronic insulin resistance, which further limits the insulin response to pregnancy-induced insulin resistance [11]. Preconception care, involving optimization of a woman's health before planning and conceiving a pregnancy, as recommended by the American Congress of Obstetricians and Gynecologists [12], provides the potential for identification of women at high risk of GDM before pregnancy. Preconception interventions in these women may be more successful than interventions beginning in early pregnancy [8]; therefore, a clear definition of the phenotype at high risk may increase the success of prevention strategies.

Whereas several biomarkers [*i.e.*, total adiponectin [13], sex hormone-binding globulin (SHBG) [14], total high-density lipoprotein (HDL) [15, 16], low-density lipoprotein (LDL) peak diameter [15], and gamma-glutamyltransferase (GGT) [17]] measured before pregnancy have been individually associated with risk of GDM in studies by our group, previous studies have not examined the ability of multiple preconception biomarkers considered together, beyond established risk factors, to improve prediction of GDM. To the best of our knowledge, a preconception biomarker risk score has not yet been developed and assessed. The objective of this study was to create a composite preconception biomarker risk score using biomarkers that have previously been associated with GDM risk and assess the association of this risk score with subsequent GDM, above and beyond established risk factors.

1. Methods

A. Study Setting

This study was conducted within Kaiser Permanente Northern California (KPNC), an integrated health care delivery system that provides medical care for approximately one-third of the population in the San Francisco Bay area. KPNC members are representative of the underlying population of this region [18]. From 1984 to 1996, female KPNC members were invited to complete a comprehensive health examination [multiphasic health checkup (MHC)] upon enrollment. The MHC consisted of a clinic visit for the completion of questionnaires and clinical measurements, including a random blood draw [19]. An extra serum sample was collected and stored at -40°C for future use.

B. Study Design and Population

We conducted a nested case-control study within the larger cohort of women who participated in the MHC. All subsequent pregnancies and births to MHC women were identified in the KPNC hospitalization database and the Pregnancy Glucose Tolerance and GDM Registry, an active surveillance registry that annually identifies all pregnancies resulting in a livebirth or stillbirth among KPNC members [20]. Women diagnosed with diabetes before the index pregnancy were then identified in the KPNC Diabetes Registry [21] and excluded from the current study. Of the 27,743 women, 15 to 45 year olds who participated in the MHC from 1984 to 1996, 4098 subsequently became pregnant and gave birth before 31 December 2010;

had questionnaire and clinical data, including serum samples, available; and were free of recognized diabetes. Women diagnosed with GDM were considered cases. Two controls were selected for each case from among women who did not meet the GDM case definition during the study period. This study was approved by the Institutional Review Board of the Kaiser Foundation Research Institute. We have previously published results on individual pre-conception biomarker associations with GDM risk from this study [13–15, 17].

C. GDM Case Definition

GDM cases ($n = 267$) met the following criteria: glucose values obtained during a standard 100-gram, 3-hour oral glucose tolerance test that met the Carpenter-Coustan plasma glucose thresholds for GDM in the laboratory database ($n = 228$) or a hospital discharge diagnosis of GDM in the electronic hospital discharge database for pregnancies occurring before 1994, when the electronic laboratory data became available ($n = 39$). Trained abstractors conducted a standardized medical chart review to confirm that all 267 identified cases had 100-gram, 3-hour oral glucose tolerance test glucose values that met the Carpenter-Coustan criteria for GDM [two or more values exceeding plasma glucose thresholds: fasting, 5.3 mM (95 mg/dL), 1 hour, 10.0 mM (180 mg/dL), 2 hour, 8.6 mM (155 mg/dL), 3 hour, 7.8 mM (140 mg/dL)]. After chart review, cases were excluded for random glucose >200 mg/dL at the time of the MHC examination (indicating prepregnancy diabetes, $n = 6$) or no indication of GDM during the index pregnancy among women identified using a hospital discharge diagnosis of GDM ($n = 5$). A total of 256 confirmed cases of GDM with valid biomarker measurements were included in this study.

D. Control Selection and Matching Criteria

Controls were randomly selected among women without an indication of GDM. Two controls were individually matched to each case on year of MHC serum collection date (± 3 months), age at MHC serum collection (± 2 years), age at delivery of the index pregnancy (± 2 years), and number of pregnancies between serum collection and the index pregnancy (0, 1, ≥ 2). Cases and controls were matched on year of MHC serum collection to assure approximately equal sample storage time to account for potential degradation in the quality of the serum over time. Age at MHC serum collection, age at delivery of the index pregnancy, and number of pregnancies between the MHC serum collection and the index pregnancy were included as matching variables to address potential differences in age and parity between cases and controls, as GDM is more common in older women and women with greater parity [22, 23]. Controls were excluded from the analysis if they had glucose values that met the Carpenter-Coustan criteria for GDM found during medical chart review ($n = 5$), had an abnormal screening glucose but no follow-up diagnostic glucose test ($n = 5$), or had one abnormal glucose value on the diagnostic glucose test, suggestive of mild GDM ($n = 5$). Of the 508 identified matched controls, 497 were included in this study.

E. Biomarkers

Serum glucose was measured using the hexokinase method by the KPNC regional laboratory at the time of the MHC. For all other biomarkers, stored serum samples were thawed, aliquoted, and transported in batches on dry ice to laboratories for biomarker assays. Dr. Peter Havel's laboratory at the University of California, Davis, performed the measurement of insulin, total adiponectin, SHBG, and GGT. Insulin was measured by radioimmunoassay (Millipore, Burlington, MA), SHBG was measured by ELISA (Alpco, Salem, NH), and GGT was measured on a Polychem analyzer (MedTest DX, Cortlandt Manor, NY). The intra-assay and interassay coefficients of variability were $<4.0\%$ and $<10\%$ (insulin), $<6.0\%$ and $<9.0\%$ (total adiponectin), and 4.7% and 5.6% (GGT), respectively. Dr. Ronald Krauss's laboratory at the Children's Hospital Oakland Research Institute performed the measurement of LDL peak diameter and HDL.

Insulin resistance was calculated using the homeostasis model assessment-estimated insulin resistance [HOMA-IR; = (fasting glucose \times fasting insulin)/22.5], where glucose was measured in millimoles per liter, and insulin was measured in microunits per milliliter.

F. Covariates

All covariates were assessed at the MHC examination. Body mass index (BMI; in kilograms per square meter) was calculated using measured height (measured by stadiometer) and weight (measured by balance beam scale) at the MHC examination. Race/ethnicity, family history of diabetes, alcohol consumption, smoking, and education were assessed by self-administered questionnaires.

G. Statistical Analyses

Descriptive statistics were calculated separately for cases and controls. Means and SDs were used to describe continuous variables. Frequencies and percentages were used to describe categorical variables.

Selected biomarkers were dichotomized into high/low risk based on the distribution of each biomarker in the controls. For total adiponectin, SHBG, LDL peak diameter, and total HDL, values, less than the lowest quartile cutpoint (<7.2 $\mu\text{g/mL}$ for total adiponectin, <44.2 nM for SHBG, <228.8 Å for LDL peak diameter, <3403.6 nM for total HDL), were considered high risk. For GGT, glucose, and HOMA-IR, values greater than the highest quartile cutpoint (>24.5 U/L for GGT, >90 mg/dL for glucose, >3.9 for HOMA-IR) were considered high risk. To determine which biomarkers to include in the biomarker risk score, high-risk indicator variables for each biomarker were included in a single, mutually adjusted, conditional logistic regression model. The model was also run adjusted for *a priori*-identified potential confounders: BMI at MHC examination (kilograms per square meter), race/ethnicity (non-Hispanic white, non-Hispanic black, Asian/Pacific Islander, Hispanic), family history of diabetes (yes, no), alcohol use at MHC examination (yes, no, unknown), smoking at MHC examination (never, former, current, unknown), and education at MHC examination (≤ 12 years, 13 to <16 years, ≥ 16 years, unknown). All statistically significant biomarkers in this model were included in the biomarker risk score.

To create an unweighted biomarker risk score, the number of high-risk indicators that were significantly associated with GDM in the multivariable model was summed for each participant. To create a weighted biomarker risk score, a conditional logistic regression model, including all statistically significant biomarkers and all potential confounders listed above, was fit. Each high-risk indicator variable was multiplied by the coefficient for that biomarker from this model and summed for each participant. The formula for the weighted risk biomarker score for each participant was the following: high-risk SHBG \times 0.7844 + high-risk glucose indicator \times 0.7379 + high-risk adiponectin indicator \times 0.6804 + high-risk HOMA-IR indicator \times 0.4860.

Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for each unit increase in the unweighted and weighted biomarker risk score. The unweighted and weighted biomarker risk score was categorized into four categories, according to the distributions of the risk scores in the controls. Scores of zero were grouped into one reference category, and nonzero scores were categorized according to tertiles. Models were adjusted for *a priori*-identified potential confounders: BMI at MHC examination (kilograms per square meter), race/ethnicity (Non-Hispanic white, non-Hispanic black, Asian/Pacific Islander, Hispanic), family history of diabetes (yes, no), alcohol use at MHC examination (yes, no, unknown), smoking at MHC examination (never, former, current, unknown), and education at MHC examination (≤ 12 years, 13 to <16 years, ≥ 16 years, unknown). A model without the biomarker score, including established risk factors and potential confounders, was run to compare associations of biomarker scores with established risk factors for GDM. This model was adjusted for age at MHC ≥ 35 years, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander),

overweight/obesity at MHC, family history of diabetes, alcohol use at preconception examination (yes, no, unknown), smoking at preconception examination (never, former, current, unknown), and education at preconception examination (≤ 12 years, 13 to < 16 years, ≥ 16 years, unknown). Previous GDM was omitted from this model, as no controls had a history of GDM.

The relative incremental-predictive value of the unweighted and weighted continuous biomarker risk scores beyond established risk factors for GDM recommended by the American College of Obstetricians and Gynecologists [24] and the American Diabetes Association [25] [age at MHC examination ≥ 35 years old, high-risk race/ethnicity (non-Hispanic black, Hispanic, or Asian/Pacific Islander), prepregnancy (as assessed at MHC examination) overweight/obesity ($\text{BMI} \geq 25 \text{ kg/m}^2$), family history of diabetes, and previous GDM (as assessed through review of the medical record)] was assessed using receiver-operating-characteristic curve analysis for matched case-control studies [26]. The relative incremental-predictive value of sequential unweighted biomarker scores, created starting with the biomarker most strongly associated with GDM and adding one biomarker based on decreasing strength of association, was also assessed. The area under the curve (AUC) for GDM risk prediction models, including unweighted and weighted biomarker risk scores, in addition to established risk factors for GDM, was compared by leave-one-out cross-validation using DeLong's test. Select models were also compared using net reclassification improvement to measure improvement in classification of participants into risk categories based on predicted probabilities from the models (low risk = 0% to 30%, moderate risk = 31% to 60%, high risk = 61% to 100%) [27]. All analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC).

2. Results

On average, women who developed GDM had a higher BMI at MHC examination (26.0 vs 23.7 kg/m^2) and gained more weight from the MHC examination to their index pregnancy (8.9 vs 4.4 kg) than women who did not develop GDM (Table 1). Women who developed GDM were also more likely to be Asian/Pacific Islanders or Hispanic, to be multiparous at the time of the MHC, have a family history of diabetes, and not report alcohol use at the MHC examination than women who did not develop GDM.

From the mutually adjusted model, high-risk levels of SHBG, glucose, total adiponectin, and HOMA-IR were each associated with greater odds of GDM (Table 2). Women in the lowest quartile (high risk) of SHBG had 2.34 times greater odds of GDM (95% CI: 1.50, 3.63) compared with women in the highest three quartiles (low risk) of SHBG. Women in the highest quartile (high risk) of glucose had 2.03 times greater odds of GDM (95% CI: 1.29, 3.19) compared with women in the lowest three quartiles (low risk) of glucose. Women in the lowest quartile (high risk) of total adiponectin had 1.83 times greater odds of GDM (95% CI: 1.16, 2.90) compared with women in the three highest quartiles (low risk) of total adiponectin. Women in the highest quartile (high risk) of HOMA-IR had 1.67 times greater odds of GDM (95% CI: 1.07, 3.62) compared with women in the lowest three quartiles (low risk) of HOMA-IR. High-risk levels of LDL peak diameter, GGT, and total HDL were not associated with odds of GDM (OR = 1.33, 95% CI: 0.84, 2.11; OR = 1.24, 95% CI: 0.80, 1.93; and OR = 1.04, 95% CI: 0.67, 1.60, respectively). SHBG, glucose, total adiponectin, and HOMA-IR were included in the unweighted and weighted biomarker risk scores.

For each one unit increase in the unweighted biomarker risk score (having high-risk levels of one additional biomarker), odds of GDM were 1.94 times greater (95% CI: 1.59, 2.36) after adjustment for potential confounders (Table 3). Women with an unweighted biomarker risk score of one had a 1.87 times greater odds of GDM (95% CI: 1.13, 3.10) compared with women with an unweighted biomarker risk score of zero. Women with an unweighted biomarker risk score of three or four had a 9.84 times greater odds of GDM (95% CI: 4.88, 19.9) compared with women with an unweighted biomarker risk score of zero.

Table 1. Characteristics of Case and Control Participants at the Preconception Examination

	GDM Cases (n = 256)	Controls (n = 497)
	Means (SD)	Means (SD)
Age, y	28.2 (5.5)	28.4 (5.2)
Age at delivery, y	35.4 (5.1)	35.1 (4.9)
Time between preconception examination and delivery, y	7.1 (4.4)	6.7 (4.4)
BMI, kg/m ²	26.0 (6.5)	23.7 (4.6)
Weight change from preconception examination to pregnancy, kg	8.9 (9.9)	4.4 (8.2)
HOMA-IR index	4.1 (3.5)	2.9 (2.9)
Glucose, mg/dL	89.6 (13.5)	83.6 (8.3)
Insulin, μ U/mL	25.8 (28.6)	17.5 (16.7)
Total adiponectin, μ g/mL	7.7 (3.5)	10.6 (4.4)
SHBG, nM	57.7 (45.1)	79.7 (58.5)
GGT, U/L	28.0 (21.7)	22.4 (16.6)
LDL peak diameter, Å	230.6 (5.6)	232.0 (4.7)
Total HDL, nM	4180.4 (1524.9)	4650.8 (1605.5)
	n (%)	n (%)
Race/ethnicity		
Non-Hispanic white	50 (20)	186 (37)
Non-Hispanic black	91 (36)	184 (37)
Asian/Pacific Islander	80 (31)	84 (17)
Hispanic	35 (14)	43 (9)
Nulliparous	142 (34)	278 (66)
Number of pregnancies between preconception examination and delivery		
0	182 (71)	349 (70)
1	61 (24)	124 (25)
2	11 (4)	23 (5)
3	2 (1)	1 (0)
Family history of diabetes	151 (59)	192 (39)
History of GDM	12 (5)	0 (0)
Alcohol use		
Yes	149 (58)	346 (70)
No	74 (29)	81 (16)
Unknown	33 (13)	70 (14.1)
Smoking		
Current	38 (15)	61 (12)
Former	37 (15)	92 (19)
Never	150 (59)	277 (56)
Unknown	31 (12)	67 (14)
Education		
\leq 12 y	74 (29)	119 (24)
13 to <16 y	85 (33)	157 (32)
\geq 16 y	92 (36)	214 (43)
Unknown	5 (2)	7 (1)

Each additional unit of the weighted biomarker risk score was associated with 2.70 times greater odds of GDM (95% CI: 2.02, 3.62). Women with a weighted biomarker risk score in the highest quartile had 7.78 times greater odds of GDM (95% CI: 4.30, 14.1) compared with women with a weighted biomarker risk score of zero. Established GDM risk factors were associated with up to three times greater odds of GDM (OR range = 0.38 to 3.00), with the strongest established risk factor Asian race/ethnicity.

In receiver-operating-characteristic curve analyses, the continuous unweighted biomarker risk score had a relative incremental-predictive value beyond established risk factors for GDM (AUC = 0.74 vs 0.67; *P* value for comparison <0.0001)(Table 4). The weighted biomarker risk score did not have a relative incremental-predictive value over the unweighted

Table 2. Mutually Adjusted Associations of Biomarkers Assessed at the Preconception Examination with GDM

Biomarker	High Risk	Model 1 ^a	Model 2 ^b
		OR (95% CI)	OR (95% CI)
SHBG	<44.2 nM	2.74 (1.84, 4.09)	2.34 (1.50, 3.63)
Glucose	>90 mg/dL	2.05 (1.37, 3.08)	2.03 (1.29, 3.19)
Total adiponectin	<7.2 µg/mL	2.01 (1.34, 3.01)	1.83 (1.16, 2.90)
HOMA-IR index	>3.9	1.76 (1.17, 2.64)	1.67 (1.07, 2.62)
LDL peak diameter	<228.8 Å	1.27 (0.84, 1.94)	1.33 (0.84, 2.11)
GGT	>24.5 U/L	1.43 (0.97, 2.13)	1.24 (0.80, 1.93)
Total HDL	<3403.6 nM	1.15 (0.77, 1.72)	1.04 (0.67, 1.60)

^aModel 1 is mutually adjusted for all biomarkers (high risk/low risk) listed.

^bModel 2 is additionally adjusted for BMI at preconception examination (kilograms per square meter), race/ethnicity (Non-Hispanic white, non-Hispanic black, Asian/Pacific Islander, Hispanic), family history of diabetes (yes, no), alcohol use at preconception examination (yes, no, unknown), smoking at preconception examination (never, former, current, unknown), and education at preconception examination (≤ 12 y, 13 to < 16 y, ≥ 16 y, unknown).

risk score (AUC = 0.74 vs 0.74; P value for comparison = 0.09). An unweighted biomarker score, including only SHBG, increased relative incremental-predictive value beyond established risk factors for GDM (Fig. 1; AUC = 0.71 vs 0.67; P value for comparison = 0.02). An unweighted biomarker score, including only glucose, increased relative incremental-predictive value beyond established risk factors for GDM by a similar amount (AUC = 0.70 vs 0.67; P value for comparison = 0.02). An unweighted score, including SHBG and glucose, further increased the relative incremental-predictive value (AUC = 0.73; P value for comparison = 0.01). Addition of adiponectin and HOMA-IR to the unweighted biomarker score did not statistically significantly increase the relative incremental-predictive value (AUC = 0.74 for both; P for comparison = 0.14 and 0.98, respectively).

3. Discussion

In this study, a preconception biomarker risk score, consisting of SHBG, glucose, total adiponectin, and HOMA-IR—measured, on average, 7 years before pregnancy—was associated with greater risk of GDM. A preconception biomarker risk score, including only SHBG and glucose, improved predictive ability for GDM beyond established risk factors, resulting in 18% of GDM cases (47 cases) being reclassified into a higher-risk category and 5% of controls (25 controls) being reclassified into a lower-risk category (net reclassification index = 0.23). In total, including a biomarker score with SHBG and glucose, this resulted in improved prediction for 72 women in our data. Addition of adiponectin and HOMA-IR to the preconception risk score did not further increase the predictive ability. This finding is of clinical relevance, as it suggests that a preconception biomarker risk score may identify a group of high-risk women who may otherwise not be identified as high risk for GDM using established risk factors. Identification of high-risk women before pregnancy may allow for preconception interventions to reduce the risk of GDM in future pregnancies.

Previous studies by our group have identified preconception biomarkers that were independently associated with increased GDM risk, including total adiponectin [13], SHBG [14], GGT [17], and total HDL [15] and lower LDL peak diameter [15]. When we included these biomarkers in a multivariable model in the current study, only SHBG, glucose, total adiponectin, and HOMA-IR were independently associated with GDM and thus, included in the risk score. The consideration of more than one biomarker in a risk score is more strongly associated with risk of future GDM than individual biomarkers or established GDM risk factors, with women with three or four biomarkers with high-risk levels at almost 10 times greater odds of GDM than women with no biomarkers with high-risk levels. Only the Coronary Artery Risk Development in Young Adults (CARDIA) study has also examined

Table 3. Associations of the Preconception Biomarker Score and Established Risk Factors With GDM

	n Cases	n Controls	OR (95% CI)
Established risk factors			
Age at preconception examination ≥ 35 y old ^a	26	60	0.38 (0.12, 1.18)
Race/ethnicity ^a			
Non-Hispanic white	50	186	Reference
Non-Hispanic black	91	184	1.46 (0.93, 2.29)
Asian/Pacific Islander	80	84	3.00 (1.81, 4.97)
Hispanic	35	43	2.68 (1.41, 5.07)
Overweight/obesity (BMI ≥ 25 kg/m ²) at preconception examination ^a	123	140	2.81 (1.90, 4.14)
Family history of diabetes ^a	151	192	2.12 (1.51, 2.97)
Preconception biomarker scores			
Unweighted score ^{b,c} (continuous)	256	497	1.94 (1.59, 2.36)
Unweighted score ^{b,c} (categorical)			
Quartile 1 (0)	42	209	Reference
Quartile 2 (1)	66	170	1.87 (1.13, 3.10)
Quartile 3 (2)	77	78	5.04 (2.85, 8.90)
Quartile 4 (3–4)	71	40	9.84 (4.88, 19.9)
P for trend			<0.0001
Weighted score ^c (continuous)	256	497	2.70 (2.02, 3.62)
Weighted score ^c (categorical)			
Quartile 1 (0)	55	209	Reference
Quartile 2 (0.49–0.74)	37	123	1.61 (0.91, 2.84)
Quartile 3 (0.78–1.22)	58	80	2.93 (1.69, 5.08)
Quartile 4 (1.27–2.69)	119	85	7.78 (4.30, 14.1)
P for trend			<0.0001

^aModel is adjusted for age at preconception examination ≥ 35 y, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander), overweight/obesity at preconception examination, family history of diabetes, alcohol use at preconception examination (yes, no, unknown), smoking at preconception examination (never, former, current, unknown), and education at preconception examination (≤ 12 y, 13 to <16 y, ≥ 16 y, unknown). Previous GDM was omitted from the model, as no controls had a history of GDM.

^bScore includes adiponectin, SHBG, HOMA-IR, and glucose.

^cModel is adjusted for BMI at preconception examination (kilograms per square meter), race/ethnicity (Non-Hispanic white, non-Hispanic black, Asian/Pacific Islander, Hispanic), family history of diabetes (yes, no), alcohol use at preconception examination (yes, no, unknown), smoking at preconception examination (never, former, current, unknown), and education at preconception examination (≤ 12 y, 13 to <16 y, ≥ 16 y, unknown). A separate model was used for each biomarker score.

individual preconception biomarkers for GDM. The CARDIA study found that higher glucose and insulin levels and lower HDL levels (measurements of SHBG and adiponectin were not available in CARDIA), measured 3 years before pregnancy, were independently associated with 2.4 to 4.7 times greater odds of subsequent GDM after adjusting for established GDM risk factors [16]; however, a risk score was not evaluated, and the predictive ability of the biomarkers beyond established risk factors for GDM (receiver operating characteristic curves) was not assessed.

Few studies have considered multiple biomarkers together for GDM prediction, and these studies are limited to biomarkers assessed during pregnancy. A study by White *et al.* [28] assessed pregnancy biomarkers for GDM prediction in a high-risk cohort of 1303 obese, pregnant women. Addition of adiponectin, SHBG, glucose, hemoglobin A1c, triglycerides, and fructosamine, measured in the second trimester to a GDM prediction model, including age, previous GDM, family history of diabetes, systolic blood pressure, and anthropometric measures, improved the predictive value of the model (AUC = 0.77 vs 0.71). In a nested case-control study of 80 GDM cases and 300 controls attending hospital-based clinics in the United Kingdom, adiponectin and SHBG, measured at 11 to 13 weeks gestation, improved prediction of GDM beyond maternal risk factors, including age, BMI, race/ethnicity, family history of diabetes, history of GDM, and history of delivery of a macrosomic neonate when added to a predictive model (AUC = 0.84 vs 0.79) [29].

Table 4. AUC for Predictive Value for GDM Risk

	Leave-One-Out, Cross-Validated AUC	P Value for AUC Comparison
Model 1: established risk factors ^a	0.67	
Model 2: established risk factors ^a + SHBG	0.71	Model 2 vs 1: 0.02
Model 3: established risk factors ^a + glucose	0.70	Model 3 vs 1: 0.02
Model 4: established risk factors ^a + score with SHBG and glucose	0.73	Model 4 vs 1: 0.002 Model 4 vs 2: 0.01
Model 5: established risk factors ^a + score with SHBG, glucose, and adiponectin	0.74	Model 5 vs 1: <0.0001 Model 5 vs 4: 0.14
Model 6: established risk factors ^a + score with SHBG, glucose, adiponectin, and HOMA-IR	0.74	Model 6 vs 1: <0.0001 Model 6 vs 5: 0.98

^aAge at preconception examination ≥ 35 y, high-risk race/ethnicity (non-Hispanic black, Hispanic, Asian/Pacific Islander), overweight/obesity at preconception examination, family history of diabetes, and previous GDM.

In contrast to the results of these previous studies that measured biomarkers during pregnancy, our use of a preconception risk score establishes temporality, and our results suggest that pathophysiological changes related to glucose homeostasis, adipocyte function, and sex hormone balance, which may lead to GDM, are also present years before pregnancies and are not a consequence of pregnancy-induced physiologic and hormonal changes in women

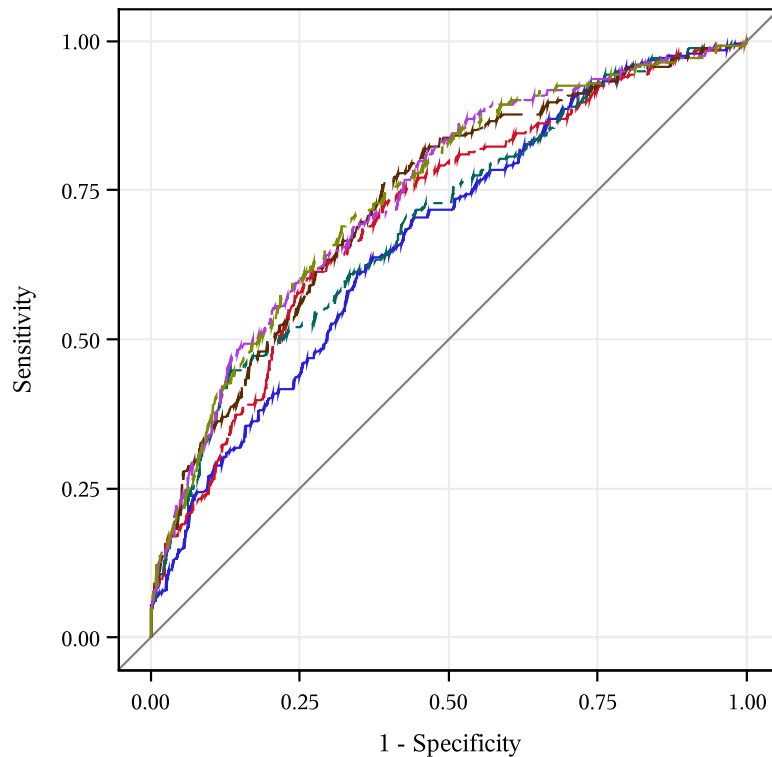


Figure 1. Leave-one-out, cross-validated, incremental-predictive value of incremental preconception biomarker score for prediction of GDM (blue, Model 1: established risk factors; red, Model 2: established risk factors + SHBG; green, Model 3: established risk factors + score with glucose; brown, Model 4: established risk factors + score with SHBG and glucose; purple, Model 5: established risk factors + score with SHBG, glucose, and adiponectin; yellow-green, Model 6: established risk factors + score with SHBG, glucose, adiponectin, and HOMA-IR).

who develop GDM. This suggests that women at high risk for GDM can be identified before pregnancy (during preconception care) for preconception interventions, which may be more effective than interventions beginning in early pregnancy for GDM prevention [8].

Our results suggest that a high-risk level of preconception SHBG may be a stronger predictor of future GDM than glucose and insulin resistance, as measured by HOMA-IR. An unweighted biomarker score, including SHBG and glucose, increased AUC by 3% and resulted in 13% of GDM cases (33 cases) being reclassified into a higher-risk category and 3% of controls (14 controls) being reclassified into a lower-risk category compared with a model, including glucose only (net reclassification index = 0.16). In total, including SHBG, in addition to glucose, in the biomarker score resulted in improved prediction for 47 women in our data. In women, lower levels of SHBG are a marker of a more androgenic hormonal profile that has been associated with increased risk of type 2 diabetes and insulin resistance [30–32], suggesting that SHBG may have functions contributing to glucose homeostasis and insulin resistance, in addition to its primary role of regulating and transporting sex steroids [31]. The role of SHBG in glucose homeostasis and GDM risk should be explored further.

Our results also suggest that high-risk levels of adiponectin before pregnancy are associated with future GDM but do not improve prediction of GDM beyond glucose and SHBG. Adiponectin is an adipokine with insulin-sensitizing effects [33, 34].

Our study has several strengths, including measurement of serum biomarkers before pregnancy to identify biological changes associated with GDM that precede pregnancy, complete GDM case ascertainment within our cohort, and consideration of multiple candidate biomarkers that have previously been individually associated with GDM in our study population. In addition, the population in our study was representative of the KPNC population and distributions of biomarkers in controls and in the general population were similar [35]. However, several limitations should be considered when interpreting our results. We did not have information available on other potential GDM risk factors, including measures of body fat mass, diet, and physical activity, which may impact associations. We were not able to validate the incremental-predictive value of our preconception biomarker score in a separate cohort. However, in the absence of a separate validation cohort, we used a computational approach (leave-one-out cross-validation) to replicate a validation dataset. Approximately one-half of the serum samples in our study were nonfasting (<6 hours since last food intake; 43% of cases, 47% of controls). Results of sensitivity analyses limited to women who were fasting at serum collection were similar to the main results [36], although independent associations of several biomarkers were no longer statistically significant. Associations of the preconception biomarker score with GDM were generally stronger in women who were fasting at serum collection; however, AUC values were similar to main results.

In conclusion, a preconception biomarker risk score, including SHBG, glucose, adiponectin, and HOMA-IR—measured, on average, 7 years before pregnancy—was associated with future GDM risk; however, a biomarker score, including only SHBG and glucose, may be sufficient to improve substantially the predictive ability for GDM beyond established GDM risk factors. Lifestyle interventions in early pregnancy to prevent GDM have had limited success [7], potentially because the pathogenic processes leading to GDM have already begun before pregnancy [8], as suggested by our finding. Future studies are needed to validate our preconception biomarker risk score in a separate cohort to support use of this risk score to identify women with high risk for future GDM. Future research should also include cost-benefit analyses, as SHBG is not a routinely measured biomarker in primary care. Identification of women at high risk for future GDM before pregnancy would allow for preconception interventions to attempt to alter the modifiable pathophysiologic pathways underlying the altered biomarker levels that we identified here.

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