ASSOCIATIONS BETWEEN VITAMIN D BIOMARKERS AND CARDIOMETABOLIC OUTCOMES AMONG WOMEN

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Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Richard M. Fairbanks School of Public Health,
Indiana University

February 2020

Accepted by the Graduate Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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DEDICATION

To my parents, husband, and lovely daughter and in memory of my beloved and faithful dog.

ACKNOWLEDGEMENT

First, and most of all, I would like to express my deep and sincere appreciation to my research advisor and committee chair, Dr. Yiqing Song, for his dedicated, invaluable guidance and persistent help throughout my dissertation research. His insightfulness, vision, sincerity, and motivation have deeply inspired me. He has taught me how to incorporate new analytical methods and integrate perspectives in biological, statistical, and clinical sciences into epidemiologic research. It was a great privilege and honor to study and work under his research guidance.

I would take this opportunity to give special thanks to Dr. Wanzhu Tu who has taken his precious time in guiding me on applying a novel analytical method he developed in R package. I would like to extend my heartfelt gratitude to other committee members, Drs. Hongmei Nan and Jiali Han, for their insightful comments, helpful suggestions, and extraordinary support.

I also wish to express my gratitude to Dr. Jennifer Wessel, Dr. Cynthia Stone, Ms. Ann Sullivan, Ms. Shawne N. Mathis, and other faculty and staff members in our school for helping me out in many aspects during my Ph.D. study. My sincere thanks also go to Drs. Cuilin Zhang and JoAnn E. Manson, and the WHI P&P Committee, for their generous support and authorizing me to access the data from the NIH/NICHD Fetal Growth Study and the WHI-OS for my dissertation research.

Lastly, yet very importantly, I am extremely grateful to my family: my parents, Dexian Xia and Qiulian Jiang, my husband, Tao Wang, and my lovely daughter, Oria, for their support, patience, encouragement, and caring to help me complete this dissertation.

A special thanks to my beloved dog, Nina, for her faithful companion and emotional support in my stressful times during the past years.

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There is growing evidence that vitamin D endocrine system may be associated with multiple cardiometabolic outcomes, such as gestational diabetes mellitus (GDM), type 2 diabetes, and other relevant cardiometabolic comorbidities, as well as some intermediate cardiometabolic biomarkers. African Americans tend to have lower 25-hydroxyvitamin D[25(OH)D] levels and higher cardiometabolic risk than whites. However, the temporal relation between vitamin D status and cardiometabolic outcomes remains unclear due to the lack of longitudinal data. Further, whether adding information on parathyroid hormone (PTH) can explain black-white disparities in cardiometabolic health is unknown. In this dissertation, I first prospectively and longitudinally investigated vitamin D status during early to mid-pregnancy in relation to GDM risk in a multiracial cohort of women from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Fetal Growth Studies-Singleton cohort. I also analyzed the data from the Women's Health Initiative-Observational Study to 1) cross-sectionally examine race (black-white)-specific linear and non-linear relations of 25(OH)D and PTH with a panel of cardiometabolic biomarkers, including high-sensitive C-reactive protein, estimated glomerular filtration rate, and homeostatic model assessment of insulin resistance and beta-cell function, and 2) cross-sectionally and prospectively evaluate the combined associations of 25(OH)D and PTH with risk of diabetes and related cardiometabolic comorbidities (obesity, hypertension, chronic kidney disease, and cardiovascular disease) in U.S. white and black postmenopausal women. This research

provides evidence of the temporal association between vitamin D status and cardiometabolic risk among women from racially/ethnically diverse groups, and possible black-white differences in these associations. The findings enhance our understanding of the contribution of vitamin D-PTH endocrine system to racial disparities in cardiometabolic health.

Yiqing Song, M.D. Sc.D., Chair

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Chapter 1

Introduction

Maintaining and enhancing cardiometabolic health is a public health priority due to a heavy economic burden and serious health consequences in the United States and worldwide. Being historically underrepresented in cardiometabolic research, women experienced consistently higher cardiovascular disease (CVD) mortality rates than men (1). Additionally, some female-specific conditions such as pregnancy and menopause may increase women's cardiometabolic risk (2, 3). Given the increasing prevalence and incidence of cardiometabolic disease, identification of potentially modifiable risk factors could be valuable for cardiometabolic risk stratification and effective early prevention.

It is well-known that the vitamin D endocrine system acts synergistically with parathyroid hormone (PTH) to regulate calcium homeostasis. Circulating 25-hydroxyvitamin D [25(OH)D], as the major active form of vitamin D, is primarily bound to vitamin D binding protein (VDBP) and albumin (4, 5). Accumulating evidence from experimental studies strongly indicates that vitamin D endocrine system may play a role in regulating diverse physiological functions in cardiometabolic system, through binding to the nuclear vitamin D receptor in a variety of tissues (6-10). Clinical and epidemiologic studies primarily from cross-sectional studies have shown that vitamin D status is associated with multiple cardiometabolic outcomes, including gestational diabetes mellitus (GDM), type 2 diabetes, CVD, obesity, hypertension, and chronic kidney disease (CKD) as well as some intermediate cardiometabolic biomarkers (11-17). Emerging evidence has also suggested potential interactions of vitamin D and PTH on cardiometabolic outcomes (18). In addition, there is evidence that racial/ethnic disparities

exist in vitamin D status (19) and cardiometabolic risk (20-22). It has been consistently reported that African Americans had lower vitamin D levels and higher PTH levels and experienced a disproportionate burden of cardiometabolic diseases than white Americans (23-26). However, existing evidence on the associations of vitamin D levels and cardiometabolic outcomes remains inconsistent and inconclusive due to the lack of prospective data. In particular, most previous studies focused on independent associations of vitamin D or PTH with cardiometabolic risk, separately. Given the well-established interrelationship between vitamin D and PTH, further research with their combined assessments may help us better understand their comparative influence on cardiometabolic risk and racial/ethnic disparities in cardiometabolic health in relation to vitamin D-PTH endocrine system.

By analyzing prospective data from two large multiracial cohorts in the United States (U.S.), the National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies-Singleton cohort and the Women's Health Initiative Observational Study (WHI-OS), I aimed to 1) prospectively and longitudinally investigate the associations between vitamin D biomarkers during early to mid-pregnancy and GDM risk in a multiracial cohort of U.S. pregnant women; 2) cross-sectionally assess both linear and non-linear relations of vitamin D and PTH with a panel of cardiovascular biomarkers in U.S. white and black postmenopausal women; and 3) cross-sectionally and prospectively examine the combined associations of vitamin D and PTH with risk of diabetes and related cardiometabolic comorbidities (obesity, hypertension, chronic kidney disease, and CVD) in U.S. white and black postmenopausal women.

Chapter 2

Longitudinal Maternal Vitamin D Status during Pregnancy and the Risk of GDM in a Multiracial Cohort of U.S. Women[27]

2.1 Introduction

GDM is one of the most common metabolic complications of pregnancy, affecting up to 9.2% of pregnant women in U.S. (28). GDM is also a global epidemic and is thought to affect up to 12.9% of all pregnancies worldwide (29). Women with GDM have an increased risk of developing type 2 diabetes after delivery; and their offspring may be predisposed to childhood obesity and type 2 diabetes later in life (30). Therefore, identifying potentially modifiable factors that may inform the prevention of GDM may not only improve pregnant women's health, but also their children's.

Although the precise mechanisms underlying the pathophysiology of GDM remain unclear, both beta-cell dysfunction and pregnancy-induced insulin resistance are thought to be key components (31). Accumulating data indicates that vitamin D may modulate pancreatic beta-cell function, improve insulin sensitivity, and alter glucose metabolism (11,32). Vitamin D deficiency is recognized as a common health concern during pregnancy at a prevalence up to 84% worldwide, depending on the country of residence and other related factors (33,34). Emerging evidence suggests that vitamin D deficiency may contribute to the development of GDM and human studies have been summarized in two recent meta-analyses of observational studies (35,36). However, previous studies focused on a single measurement of 25(OH)D levels and did not include serial measurements to reliably reflect a time-integrated measure of vitamin D status during pregnancy (13,37,38). Limited and inconsistent findings from clinical trials to

examine the effect of vitamin D supplementation on GDM have been reported (39-42). Thus, the temporal association between maternal vitamin D status and GDM risk remains unclear. Longitudinal data on maternal vitamin D status are needed to elucidate the variation of vitamin D levels and requirements over pregnancy and better understand the role of vitamin D metabolism and function on GDM development.

In addition, accumulating evidence indicates that free and bioavailable 25(OH)D may better reflect biological activity of vitamin D than total 25(OH)D (43,44). The bioavailability of vitamin D and its metabolites is largely regulated by VDBP; however**,** the validity of monoclonal immunoassays has been criticized for lack of sensitivity to VDBP isoforms determined by genetic polymorphisms (45-48). Further, there are no standardized assays for VDBP and free or bioavailable 25(OH)D to be well-validated in ethnically diverse populations. Thus, there remains a controversy regarding the optimal markers for determining vitamin D status and action.

In a prospective, multiracial cohort of U.S. pregnant women, I focused primarily on total 25(OH)D levels [including 25(OH)D2 and 25(OH)D3] assessed by the presumed gold standard liquid chromatography-tandem mass spectrometry (LC-MS/MS), which is the most widely used and clinically accepted biomarker for vitamin D status. I aimed to investigate 1) the longitudinal trajectories of vitamin D biomarkers over pregnancy; 2) the prospective associations between levels of vitamin D biomarkers during early to mid-pregnancy and subsequent risk of GDM; and 3) whether the levels of vitamin D biomarker from early to mid-pregnancy and their prospective associations with GDM risk are modified by race/ethnicity, pre-pregnancy body mass index (BMI), physical activity, parity, or family history of diabetes.

2.2 Methods

2.2.1 Study design and population

I performed a nested case-control study using the *Eunice Kennedy Shriver* NICHD Fetal Growth Studies-Singleton cohort (2009-2013), consisting of 2,802 generally healthy multiracial women (2,334 non-obese and 468 obese women) with singleton pregnancies and aged 18-40 years at enrollment. All women were enrolled between 8 weeks 0 days and 13 weeks 6 days of gestation at 12 clinical centers throughout the U.S. and were followed up throughout their pregnancies (49). For participants to be eligible, ultrasound estimates of gestational age at enrollment were required to be consistent (±5-7 days) with gestational dating, calculated by last menstrual period. Sampling and eligibility criteria are described in detail elsewhere (49). The study was approved by all participating institutions including NICHD. All study participants gave their written informed consent prior to enrollment.

In this prospective cohort study, maternal blood samples were longitudinally collected from each participant at four targeted study visits: gestational weeks 8-13 (enrollment visit), 16-22, 24-29, and 34-37. However, the actual time ranges for blood collection were gestational weeks 10-14, 15-26, 23-31, and 33-39, respectively. All biospecimens were processed immediately and stored at -80°C before assay. All women were instructed to fast overnight for 8-14 h before their blood samples were drawn at the second visit (weeks 15-26). The screening or diagnosis of GDM was conducted according to standard clinical care, with an average gestational age of 27 weeks. A total of 107 women with incident GDM were identified as cases and matched randomly at a ratio of 1:2 to non-GDM controls on age (±2 years), race/ethnicity (non-Hispanic white,

non-Hispanic black, Hispanic, or Asian/Pacific Islander), and gestational age at blood collection (±2 weeks). Overall, this case-control study consisted of 107 women with GDM and 214 women without GDM for a total of 321 women.

2.2.2 Outcome ascertainment

Gestational diabetes was ascertained by review of medical records. Of 107 cases, the vast majority (n=95) had a confirmed diagnosis of GDM based on 100-g, 3-h oral glucose tolerance test (OGTT) results. The Carpenter and Coustan diagnostic criteria were used for GDM diagnosis (50). For those without available OGTT results, hospital discharge diagnosis after delivery was reviewed and women who received medication for GDM were considered to have GDM (n=12). Among the 214 matched controls, 195 women underwent GDM screening by a 50-g, 1-h glucose challenge test (GCT). Among the remaining women (n=19) without available GCT results, either an OGTT with the Carpenter and Coustan criteria thresholds (n=12) or review of hospital discharge diagnoses (n=7) was used to confirm non-GDM status.

2.2.3 Laboratory assessment

Biomarkers were measured at all four-time points of biospecimen collection among all cases (n=107) and one of the matched controls (n=107). In the remaining control subjects (n=107), assays were performed only for the two specimens collected prior to GDM screening (i.e., 10-14 and 15-26 gestational weeks) which are most informative for prospectively investigating biomarkers of GDM. All biospecimen samples of matched cases and controls were assayed in random order in the same analytic run, without knowledge of GDM status. Plasma levels of ergocalciferol (D2) and cholecalciferol (D3) were measured in ng/mL using liquid chromatography-tandem mass

spectrometry (LC-MS/MS). Plasma VDBP was measured in ng/mL using a quantitative sandwich enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN). Plasma albumin was measured using the bromcresol purple method (Roche Diagnostics, Indianapolis, IN). Total 25(OH)D was reflected by the summation of 25(OH)D2 and 25(OH)D3. Based on the lab data, free 25(OH)D and bioavailable 25(OH)D were derived using equations adapted from that of Vermeulen et al. (51). Plasma glucose and insulin were measured using hexokinase and immunosorbent assays (Roche Diagnostics, Indianapolis, IN), respectively. HOMA-IR, as a surrogate measure of insulin sensitivity, was computed by multiplying fasting plasma insulin (FPI) mU/L by fasting plasma glucose (FPG) mmol/L, then dividing by the constant 22.5, i.e. HOMA-IR = (FPI × FPG)/22.5 (52). Plasma levels of total cholesterol, HDL cholesterol, and triglycerides were measured using enzymatic assays (Roche Diagnostics, Indianapolis, IN). Plasma LDL cholesterol was calculated by the Friedewald's formula (53):

LDL cholesterol = total cholesterol – HDL cholesterol – triglycerides/5

The inter-assay coefficients of variation for all above-mentioned analytes were in the range of 1.3-12.9%. Plasma 25(OH)D levels were reported in nmol/L, multiply by 2.50 to convert from ng/mL to nmol/L (for 25(OH)D2, 1 ng/mL=2.42 nmol/L).

2.2.4 Covariates

Information on participant demographics, lifestyle factors, and past medical history was collected through self-reported questionnaire. A priori selection of conventional GDM risk factors, including nulliparity (yes/no), prepregnancy BMI (kg/m^2), and family history of diabetes (yes/no), was assessed at study enrollment. Based on the clinical centers where participants were enrolled, geographical regions were

categorized by latitude as Southern (≤37°N), Middle (>37°N to 40°N), and Northern (>40°N) (54). Season of blood draw (February to April, May to July, August to October, and November to January) and physical activity (quartiles) at each study visit prior to GDM screening were also considered in the current analysis. Physical activity was assessed using the Pregnancy Physical Activity Questionnaire (55). Given that cases were matched with controls within a certain range of maternal age (years) and gestational age at biospecimen collection (weeks), I also included these two matching variables as covariates to derive conservative GDM risk estimates.

2.2.5 Statistical analysis

Participant characteristics were compared according to GDM status using generalized linear mixed-effect models for continuous variables and binomial/multinomial logistic regression with generalized estimating equations for categorical variables, accounting for matched case-control pairs. To illustrate the longitudinal trends of vitamin D biomarkers throughout pregnancy in both cases and controls, median levels of each biomarker were displayed graphically by study visit; generalized linear mixed-effects regression models, accounting for matched case-control pairs, were implemented for case-control comparisons at each study visit. Spearman's partial correlation coefficients adjusting for maternal age were calculated to examine the associations of vitamin D biomarkers at either 10-24 or 15-26 gestational weeks with fasting plasma glucose during weeks 15-26, respectively. I performed the complete data analysis by excluding participants with missing measurements of vitamin D biomarkers. Additionally, one case at weeks 10-14 and five cases at weeks 15-26 were excluded from the final analysis, as their blood samples were collected after GDM diagnosis.

To evaluate the associations of maternal vitamin D levels with subsequent risk of GDM and identify the optimal timing of vitamin D assessment in relation to GDM risk, separate multivariable conditional logistic regression models were performed for each study visit prior to GDM diagnosis, i.e., gestational weeks 10-14 and 15-26. The levels of each vitamin D biomarker were parameterized as quartiles with the lowest quartile as the reference. To account for seasonal variation in vitamin D levels, season-specific quartile cutoffs of 25(OH)D were determined according to blood draw dates among the controls and applied for all the cases and controls. I also categorized total 25(OH)D levels into deficiency (<50 nmol/L) and non-deficiency (≥50 nmol/L) according to previously published criteria for vitamin D status (56). The main multivariable logistic models were adjusted for race/ethnicity, maternal age, gestational age at blood collection, geographic latitude, prepregnancy BMI, parity, and family history of diabetes. I further adjusted for physical activity in separate models for sensitivity analysis considering its potential confounding and modifying effects. Tests for linear and nonlinear trends were performed by modeling the median levels of vitamin D within each category as a continuous explanatory variable and using restricted cubic splines, respectively.

I also investigated longitudinal vitamin D profiles in relation to GDM. Between the two visits that included blood collection prior to GDM screening (i.e., the first to second trimester), the following longitudinal patterns of changes in 25(OH)D levels were identified and conditional logistic regression models was used to assess the associations between changing patterns of vitamin D status and the subsequent risk for GDM, with persistent non-deficiency being the reference group: 1) persistent non-deficiency (≥50 nmol/L); 2) persistent deficiency (<50 nmol/L); 3) non-deficiency to deficiency; or 4)

deficiency to non-deficiency. I also fitted linear mixed-effects models accounting for matched case-control pairs to compare the longitudinal trajectories of vitamin D levels during early and mid-pregnancy before the diagnosis of GDM in individuals with GDM and normal glucose levels, with adjustment for the above-listed confounders at baseline. The log-transformed levels of 25(OH)D were parameterized as a continuous variable in the models. The least-squares means were back transformed to the original scale for result presentation.

To explore possible effect modification in the relationship between vitamin D deficiency and GDM, I first performed subgroup analyses stratified by race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, or Asian/Pacific Islander) and other factors, such as pre-pregnancy weight status (normal weight or overweight/obese), maternal age (<30 or ≥30 years), physical activity (quartiles), parity (nulliparous or parous), and family history of diabetes (yes/no). I performed interaction analyses with multiplicative interaction terms to formally test their potential modifying effects on the association between vitamin D deficiency and GDM. All statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC).

2.3 Results

2.3.1 Baseline characteristics

Compared with non-GDM controls, GDM cases had a higher proportion of a family history of diabetes, lower HDL cholesterol, and higher prepregnancy BMI, triglycerides, fasting glucose, fasting insulin, and HOMA-IR (**Table 1**).

Table 1. Participant characteristics among women with GDM and their matched control subjects, the NICHD Fetal Growth Studies-Singleton cohort.

Variables		Case subjects with GDM (n = 107)	Control subjects (n = 214)	P-value†
Age (years)	mean ± SD	30.5 ± 5.7	30.4 ± 5.4	—
Race/ethnicity	n (%)			—
Non-Hispanic white		25 (23.4)	50 (23.4)	
Non-Hispanic black		15 (14.0)	30 (14.0)	
Hispanic		41 (38.3)	82 (38.3)	
Asian/Pacific Islander		26 (24.3)	52 (24.3)	
Education	n (%)			0.18
Less than high school		17 (15.9)	26 (12.1)	
High school graduate or equivalent		15 (14.0)	23 (10.7)	
More than high school		75 (70.1)	165 (77.1)	
Prepregnancy BMI (kg/m^2)	n (%)			<0.001
<25.0		37 (34.6)	123 (57.5)	
25.0-29.9		35 (32.7)	56 (26.2)	
30.0-34.9		20 (18.7)	17 (7.9)	
35.0-44.9		15 (14.0)	16 (7.5)	
Unknown/missing		0	2 (0.9)	
Nulliparity	n (%)	48 (44.9)	96 (44.9)	1.00
Smoking 6 months before pregnancy	n (%)	4 (3.7)	1 (0.5)	0.06
Alcohol consumption 3 months before pregnancy	n (%)	61 (57.0)	137 (64.0)	0.22
Family history of diabetes	n (%)	40 (37.4)	48 (22.4)	0.003
Geographical latitude (Clinical center)	n (%)			0.38
Southern (≤37°N)		40 (37.4)	77 (36.0)	
Middle (>37°N to 40°N)		22 (20.6)	37 (17.3)	
Northern (>40°N)		42 (39.3)	100 (46.7)	
Season of blood draw (weeks 10-14)	n (%)			0.01
February-April		28 (26.9)	65 (30.4)	
May-July		26 (25.0)	39 (18.2)	
August-October		32 (30.8)	50 (23.4)	
November-January		18 (17.3)	60 (28.0)	
Season of blood draw (weeks 15-26)	n (%)			0.02
February-April		24 (25.5)	65 (30.4)	
May-July		22 (23.4)	39 (18.2)	
August-October		30 (31.9)	50 (23.4)	
November-January		18 (19.2)	60 (28.0)	
Physical activity at weeks 10-14 (MET-minutes per week)	mean ± SD	419 (214, 1112)	489 (185, 1059)	0.77
Physical activity at weeks 15-26 (MET-minutes per week)	mean ± SD	288 (69, 558)	299 (117, 647)	0.34
Gestational age at blood collection (weeks 10-14)	mean ± SD	12.8 ± 0.9	12.9 ± 0.8	0.55

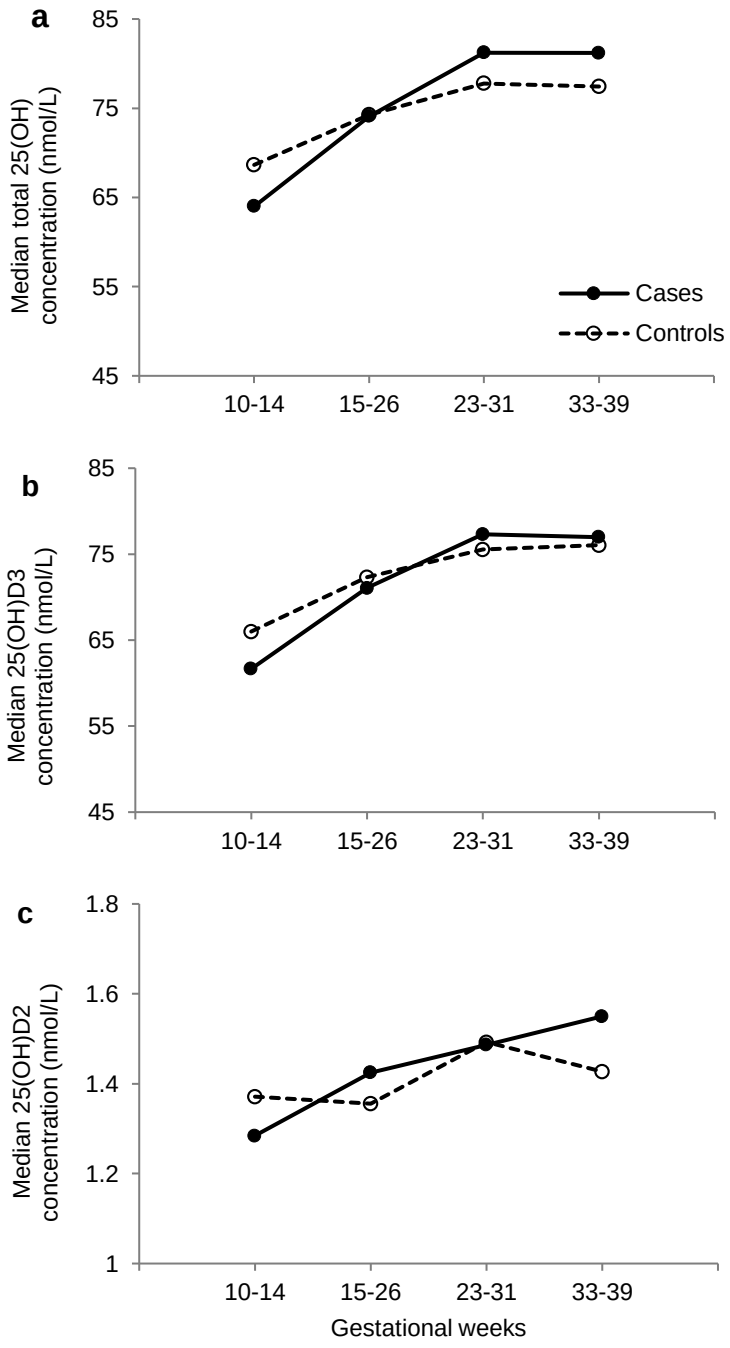
Gestational age at blood collection (weeks 15-26)	mean ± SD	19.2 ± 2.4	19.4 ± 2.2	0.27
Metabolic biomarkers at gestational weeks 10-14				
Triglycerides (mmol/L)	median (IQR)	1.7 (1.2, 2.2)	1.3 (1.1, 1.7)	<0.001
Total cholesterol (mmol/L)	median (IQR)	4.8 (4.2, 5.0)	4.6 (4.1, 5.2)	0.73
HDL-cholesterol (mmol/L)	median (IQR)	1.5 (1.3, 1.7)	1.6 (1.9, 3.2)	0.001
LDL-cholesterol (mmol/L)	median (IQR)	2.4 (1.9, 2.7)	2.3 (1.8, 2.7)	0.83
Metabolic biomarkers at gestational weeks 15-26				
Triglycerides (mmol/L)	median (IQR)	1.8 (1.5, 2.2)	1.5 (1.2, 1.9)	<0.001
Total cholesterol (mmol/L)	median (IQR)	5.1 (4.6, 5.8)	5.4 (4.6, 5.9)	0.17
HDL-cholesterol (mmol/L)	median (IQR)	1.7 (1.4, 1.9)	1.8 (1.5, 2.1)	0.005
LDL-cholesterol (mmol/L)	median (IQR)	2.5 (2.1, 3.1)	2.7 (2.1, 3.2)	0.22
Fasting glucose (mmol/L)	median (IQR)	4.9 (4.6, 5.4)	4.6 (4.3, 4.8)	<0.001
Fasting insulin (pmol/L)	median (IQR)	10.6 (6.9, 18.4)	6.7 (4.2, 10.6)	<0.001
HOMA-IR	median (IQR)	2.7 (1.8, 5.0)	1.6 (0.9, 2.6)	<0.001

†P-value for differences between case and control subjects were obtained by generalized linear mixed-effect models for continuous variables and binomial/multinomial logistic regression with generalized estimating equations for binary/multilevel categorical variables, accounting for matched case-control pairs. IQR, interquartile range; BMI, body mass index; MET, metabolic equivalent of task.

2.3.2 Longitudinal trajectories of vitamin D biomarkers

Over the entire gestational period, median levels of 25(OH)D2 and 25(OH)D3 as well as total and free 25(OH)D increased whereas median bioavailable 25(OH)D levels decreased with gestational week among both cases and controls (**Figure 1 and Supplemental Figure A-1 in Appendix A**). Median levels of VDBP increased from gestational weeks 10-14 up to weeks 15-26, with a subsequent decline until the end of pregnancy. There was no statistically significant difference in any vitamin D biomarkers between GDM cases and non-GDM controls at any study visit. After adjustment for maternal age, total, free, and bioavailable 25(OH)D were significantly and inversely correlated with fasting glucose at 15-26 gestational weeks (**Supplemental Table A-1 in Appendix A**).

Figure 1. Median levels of total 25(OH)D (**a**), 25(OH)D3 (**b**), and 25(OH)D2 (**c**) according to gestational age at blood collection among women with GDM (solid line) and their matched control subjects (dashed line).



2.3.3 Time-specific levels of vitamin D biomarkers and GDM risk

At 10-14 gestational weeks, women with higher season-specific levels of total 25(OH)D biomarkers appeared to have a 50-60% lower subsequent risk of GDM compared with women in the lowest season-specific quartile (**Supplemental Table A-2 in Appendix A**). The association was similar with 25(OH)D3. Although the significant linear trends between levels of total 25(OH)D (P=0.04) and 25(OH)D3 (P=0.03) and GDM risk were robust to adjustment for race/ethnicity, maternal age, geographical latitude, and gestational age at blood collection, I did not observe linear associations after additional adjustment for prepregnancy BMI, parity, and family history of diabetes. However, significant nonlinear associations of total 25(OH)D (P=0.025) and 25(OH)D3 (P=0.016) with GDM were observed. While increasing levels of free and bioavailable 25(OH)D were suggestive of a lower risk for GDM, none of the quartile associations or linear trends were significant. VDBP was not associated with GDM risk. No significant results were observed at 15-26 gestational weeks.

2.3.4 Longitudinal vitamin D status and GDM risk

First trimester vitamin D deficiency (10-14 gestational weeks) was significantly associated with an increased risk of developing GDM after adjusting for potential confounding factors [odds ratio (OR)=2.82, 95% confidence interval (CI): 1.15-6.93] (**Figure 2**). This association remained significant after further adjustment for physical activity (OR=3.02, 95% CI: 1.20-7.57). Furthermore, women with persistent vitamin D deficiency (10 GDM cases and 13 controls) in both the first and second trimester (10-14 and 15-26 weeks) had over a 4-fold significantly higher risk for GDM than those with persistently non-deficient vitamin D levels (68 GDM cases and 166 controls; OR=4.46,

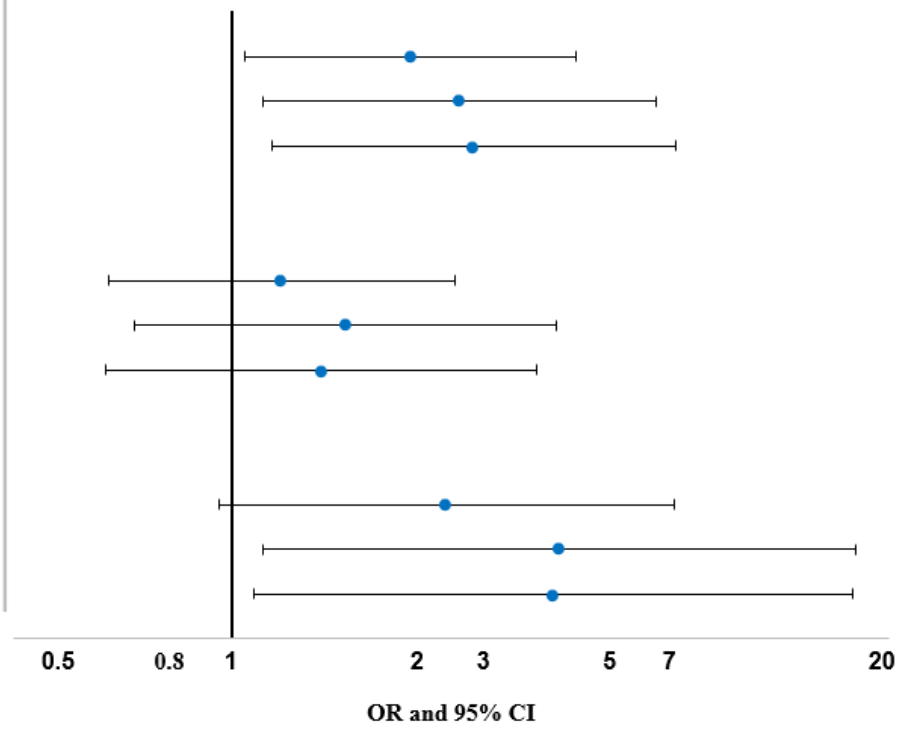
95% CI: 1.15-17.3). However, this significant association was not observed in women with a change from non-deficiency to deficiency (1 GDM cases and 11 controls; OR=0.29, 95% CI: 0.03-2.59) or vice versa (11 GDM cases and 19 controls; OR=2.59, 95% CI: 0.82-8.19).

Figure 2. ORs for GDM by vitamin D deficiency status at gestational weeks 10-14 and 15-26.

ORs for GDM in women with persistent vitamin D deficiency compared with those with persistent non-deficiency at both gestational weeks 10-14 and 15-26 were also shown. Model 1 adjusted for race/ethnicity, maternal age, gestational age at blood collection, and geographical latitude (clinical center); Model 2 (main model) further adjusted for pre-pregnancy BMI, parity, season of blood draw, and family history of diabetes; Model 3 additionally adjusted for physical activity.

Vitamin D Deficiency (< 50 nmol/L) vs. Non-deficiency (≥ 50 nmol/L)

	OR (95%CI)	P-value
Gestational weeks 10-14 (time 1):		
Model 1	2.26 (1.06, 4.81)	0.03
Model 2	2.82 (1.15, 6.93)	0.02
Model 3	3.02 (1.20, 7.57)	0.02
Gestational weeks 15-26 (time 2):		
Model 1	1.25 (0.57, 2.76)	0.58
Model 2	1.67 (0.64, 4.40)	0.30
Model 3	1.50 (0.56, 4.02)	0.42
Both time 1 and time 2:		
Model 1	2.65 (0.94, 7.52)	0.07
Model 2	4.46 (1.15, 17.3)	0.03
Model 3	4.33 (1.10, 17.0)	0.04



In the adjusted mixed-effects models, longitudinal trajectories of log-transformed total 25(OH)D and 25(OH)D3 levels during early to mid-pregnancy differed significantly between women with GDM and non-GDM controls (**Table 2 and Supplemental Table A-3 in Appendix A**). Compared to women without GDM, those who developed GDM appeared to have lower levels of total 25(OH)D at 10-14 gestational weeks (β=-0.07, 95% CI: -0.15-0.02) and had a 6% greater incremental rate of total 25(OH)D levels on a logarithmic scale from 10-14 weeks to 15-26 weeks (β=0.06, 95% CI: 0.001-0.11). Least-squares means of total 25(OH)D further illustrated this significant difference in the longitudinal increase of total 25(OH)D levels from the first to second trimester between GDM cases and non-GDM controls: least-squares means appeared to be different at 10-14 weeks between cases and controls (63.0 vs. 67.4 nmol/L; P=0.10), but were similar at 15-26 weeks (69.5 vs. 70.3 nmol/L; P=0.76) (**Figure 3 and Supplemental Figure A-2 in Appendix A**). Longitudinal change in least-squares means from 10-14 weeks to 15-26 weeks among cases was significantly greater than that among controls (P=0.046). I obtained very similar results for 25(OH)D3.

Table 2. Change of total 25(OH)D, 25(OH)D3, and 25(OH)D2 from the 1st to 2nd trimester for individuals with GDM and normal glucose levels in multivariate mixed-effects models.

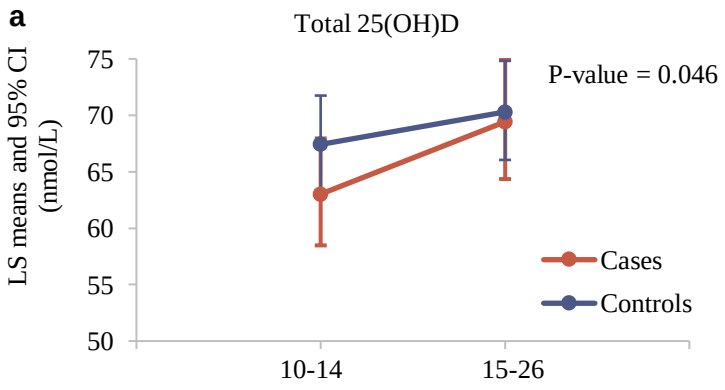
	Model 2†			Model 3‡		
	β	95% CI	P-value	β	95% CI	P-value
Total 25(OH)D						
GDM	-0.07	(-0.15, 0.02)	0.11	-0.06	(-0.14, 0.02)	0.15
Visit	0.09	(0.06, 0.13)	<0.001	0.09	(0.06, 0.13)	<0.001
GDM*Visit	0.06	(0.001, 0.11)	0.046	0.06	(0.001, 0.11)	0.046
25(OH)D3						
GDM	-0.06	(-0.15, 0.02)	0.14	-0.06	(-0.14, 0.03)	0.17
Visit	0.09	(0.06, 0.13)	<0.001	0.09	(0.06, 0.13)	<0.001

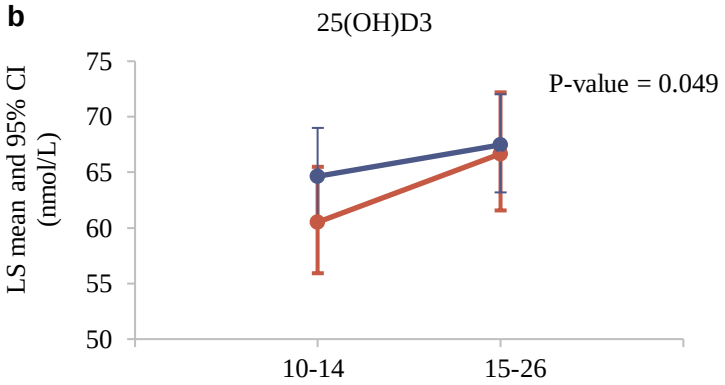
GDM*Visit	0.05	(0.00001, 0.11)	0.049	0.05	(-0.00003, 0.11)	0.05
25(OH)D2						
GDM	-0.05	(-0.23, 0.13)	0.57	-0.04	(-0.21, 0.14)	0.68
Visit	0.02	(-0.05, 0.09)	0.65	0.02	(-0.05, 0.09)	0.64
GDM*Visit	0.08	(-0.05, 0.20)	0.22	0.08	(-0.05, 0.20)	0.22

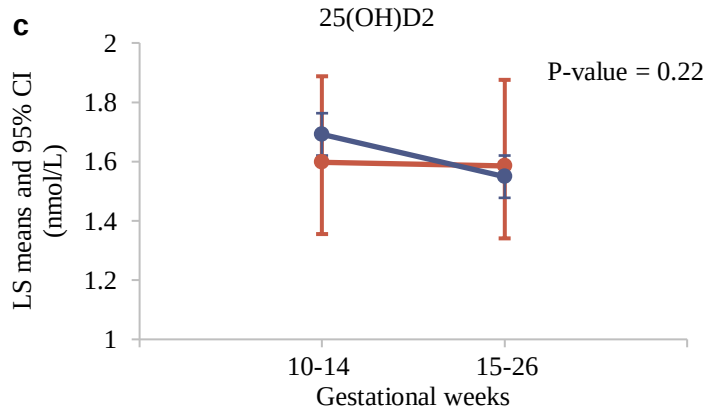
†Model 2 (main model) adjusted for race/ethnicity, maternal age, gestational age at blood collection, geographical latitude (clinical center), pre-pregnancy BMI, parity, season of blood draw, and family history of diabetes.
‡Model 3 further adjusted for physical activity.
β coefficient for "GDM" represents the difference of vitamin D biomarker levels between GDM cases and non-GDM controls when their gestational age was 10-14 weeks;
β coefficient for "Visit" represents the longitudinal change of vitamin D levels from 10-14 to 15-26 weeks of gestation among non-GDM controls;
β coefficient for "GDM*Visit" represents the difference in longitudinal change of vitamin D biomarker levels from 10-14 to 15-26 weeks of gestation between GDM cases and non-GDM controls.

Figure 3. Longitudinal change of vitamin D biomarkers, including total 25(OH)D (**a**), 25(OH)D3 (**b**), and 25(OH)D2 (**c**), for individuals with GDM (case, orange line) and normal glucose levels (control, blue line).

Back-transformed LS means and the corresponding 95% CIs of vitamin D biomarkers at gestational weeks of 10-14 and 15-26 from linear mixed-effects models, adjusting for race/ethnicity, maternal age, gestational age at blood collection, geographical latitude (clinical center), pre-pregnancy BMI, parity, season of blood draw, and family history of diabetes (Model 2).







2.3.5 Sensitivity analysis

The sensitivity analyses suggested no evidence of significant effect modification by race/ethnicity, pre-pregnancy BMI, maternal age, physical activity, parity, or family history of diabetes.

2.4 Discussion

In this ethnically diverse longitudinal study, the results showed evidence of a significant longitudinal change in the vitamin D biomarkers during pregnancy with no differences between GDM cases and controls at each visit. I found a nonlinear threshold effect of total 25(OH)D and 25(OH)D3 levels on GDM risk, and vitamin D deficiency as defined by total 25(OH)D <50nmol/L during early and mid-pregnancy was significantly

associated with risk of developing GDM among initially healthy pregnant women. The association appeared to be independent of conventional risk factors for GDM. I observed no significant effect modification by race/ethnicity, pre-pregnancy BMI, maternal age, physical activity, parity, or family history of diabetes. My findings indicate that assessment of vitamin D status during early pregnancy may be clinically valuable for developing risk stratification and intervention strategies for GDM prevention.

To our knowledge, only one study has investigated the longitudinal profiles of serum 25(OH)D during pregnancy (at 12-14, 20-22, and 32-34 weeks) and prospectively examined the associations between trimester-specific vitamin D status and GDM in a cohort of 523 Korean women (57). Similar to the present study, there was no statistically significant difference in 25(OH)D between GDM cases and controls at any time points. However, they did not examine the associations between GDM risk and longitudinal changes of 25(OH)D levels, as well as other vitamin D biomarkers. In addition, they found no association between vitamin D status in either the first or second trimester and GDM. The small case number (23 GDM cases) may limit their statistical power to identify the significant association between first-trimester vitamin D deficiency and GDM. Whereas, the present study prospectively and longitudinally examined and demonstrated, for the first time, the significant and inverse association between maternal vitamin D levels based on repeated measurements and subsequent risk of GDM. My main findings are in line with a large body of the existing literature (37,38,58), showing an inverse association between vitamin D in early gestation and subsequent risk of GDM, but also further extend these findings by showing an association between longitudinal vitamin D status during early pregnancy to mid-gestation and subsequent risk for GDM

which may be more clinically relevant for prevention of GDM. The results of the Omega Study showed that vitamin D deficiency before 20 weeks of gestation was associated with an increased risk of developing GDM (37). Another prospective study indicated that the risk of incident GDM increased by 40% with 1 SD decrease in 25(OH)D levels during gestational weeks 6-13 (38). Evidence from a recent case-control study among a cohort of Saudi pregnant women consisting of 116 GDM cases and 303 control subjects suggested that vitamin D deficiency in the first trimester was associated with a 2.87-fold greater risk of subsequent GDM (58). In the present study, I further observed that longitudinal trajectories of 25(OH)D levels during early and mid-pregnancy differed significantly between GDM cases and women with normal glucose levels. Compared to controls, GDM cases had lower 25(OH)D levels in early pregnancy, but increased to very similar levels at weeks 15-26. This might reflect the fact that pregnant women were more likely to take prenatal vitamin supplements due to counselling at their routine prenatal visits. These findings also suggest the importance of vitamin D levels during early pregnancy but not the mid-pregnancy in the development of GDM. Compared to women with non-deficient levels of vitamin D (≥50 nmol/L) from the first to second trimester of gestation, those with persistently deficient vitamin D levels (<50 nmol/L) had significantly increased risk for developing GDM. These results indicate that prenatal vitamin supplements may not be enough for GDM prevention, especially for women with deficient levels of vitamin D in early pregnancy. It is also possible that these participants with persistently deficient vitamin D levels may have not taken any vitamin D supplements or had poor adherence or response to vitamin D supplements or dietary counselling. Overall, the trajectories of total 25(OH)D levels between GDM cases and

controls were different from the first to second trimester of pregnancy and the associations of vitamin D deficiency with GDM were significant either during the first trimester or persistently through the second trimester. These findings indicate the importance of assessing trajectories of vitamin D status across gestation in relation to GDM risk.

In contrast, inconsistent findings were also reported (59-61). A cross-sectional study of a Turkish population found no association between first-trimester vitamin D deficiency and GDM risk, potentially due to small sample size (50 GDM cases and 50 controls) (59). Similar findings were reported by another nested case-control study of 180 pregnant women in North Carolina (60 GDM cases and 120 controls) (60). With more than 50% white women, the prevalence of vitamin D deficiency in this study was much lower (7.2%) compared to the present study (22.4%) as well as another study with a nationally representative sample of U.S. pregnant women (33%) (62); some important confounding effects, such as geographical latitude and family history of diabetes, were also not accounted for. Therefore, their inference may be lack of generalizability and reliability. My finding of no association between the second-trimester vitamin D deficiency and GDM was also less consistent with that from a birth cohort of 1,314 U.S. pregnant women (61). They found an inverse association between second-trimester severe vitamin D deficiency (<25 nmol/L) and GDM, although the association was attenuated to non-significance after adjusting for maternal BMI in addition to other risk factors.

A recent review pointed out that controversial findings from observational studies on vitamin D and GDM may be affected by heterogeneity in study design and insufficient

considerations of confounding factors (63). However, the results from clinical trials have also been inconsistent (39-42). Two randomized clinical trials (n=500 and n=90) in Iran found that vitamin D supplementation intake (50,000 IU every 2 weeks or 5,000 IU weekly) started in the first trimester of gestation decreased incidence of GDM (39,40), which are consistent with my findings. In contrast, the results from two randomized clinical trials conducted in Sydney (n=179) and Iran (n=210) showed that vitamin D supplementation as doses from 400 to 5,000 IU daily had no effects on incidence of GDM or maternal glucose levels (41,42). Their negative findings may be due to: 1) relatively small sample size in each treatment arm; 2) late initiation of vitamin D supplementation (gestational age of <20 weeks for Sydney's trial and 14-16 weeks for Iran's trial); 3) the control group containing low dose of vitamin D supplements; 4) extreme cutoffs used for definition of vitamin D deficiency (80 nmol/L in Sydney's trial and 10 nmol/L in Iran's trial); or 5) potentially low compliance since adherence rate was not reported in either study.

Although the exact biological mechanisms underlying vitamin D and glucose metabolism in pregnancy remain unclear, the observed associations may be explained by the influence of vitamin D on glucose homeostasis. The biological effects of vitamin D on regulation of pancreatic beta-cell function and insulin secretion involve its biologically active metabolite, $1,25(OH)_2D$, binding to the vitamin D receptor in beta-cells of the pancreas (64). Vitamin D deficiency may affect normal release of insulin by regulating the calcium pool of beta-cells intracellularly and extracellularly. Since the secretion of insulin is mediated by a calcium-dependent mechanism (65), vitamin D deficiency may decrease the insulin response to glucose. Vitamin D may also influence

insulin sensitivity through vitamin D receptors in adipose tissue and skeletal muscle through its role in activating the peroxisome proliferator activator receptor-δ, which is involved in the metabolism of fatty acids in adipose tissue and skeletal muscle (66). Thus, vitamin D deficiency may affect peripheral target tissues of insulin and thus lead to insulin resistance. Another possible indirect pathway by which vitamin D could affect glucose homeostasis is through systemic inflammation. Chronic inflammation can trigger beta-cell dysfunction or death and directly induce insulin resistance (67). Defects in insulin secretion and insulin sensitivity (insulin resistance) can contribute to GDM development. Through inhibiting production and action of inflammatory cytokines, $1,25(OH)_2D$ can lower systemic inflammation and promote beta-cell survival (67,68).

A major strength of the present study is the use of longitudinal measurements of plasma levels of 25(OH)D, VDBP, and albumin before GDM diagnosis and throughout pregnancy, which provided the opportunity to examine the temporal relationship between longitudinal vitamin D status during pregnancy and risk of GDM. In particular, I was able to examine a panel of vitamin D biomarkers and compare them for the strength of their associations with GDM risk. The multiethnic diversity of the study cohort increased the generalizability of my results. Furthermore, findings from this observational research will complement and extend findings from existing and future clinical research of the effects of vitamin D supplements on GDM. Clinical trials are ideal to define a causal relationship between vitamin D and GDM; however, some of logistical limitations may restrict their ability to address some unanswered questions about vitamin D as follows: (1) only certain fixed dose levels of vitamin D can be tested; (2) a relatively narrow range of vitamin D levels in the trial participants cannot allow for assessment of the full spectrum

of vitamin D levels; (3) inability to assess a panel of novel vitamin D biomarkers relative to physiological levels of total 25(OH)D, including VDBP, free or bioavailable 25(OH)D, may hinder our further understandings of the physiological role of vitamin D in relation to GDM.

However, some limitations need to be acknowledged. First, the relatively small sample size limited my ability to fully address ethnic disparities in the relationship between vitamin D and GDM risk, and I was only able to explore possible effect modification of these associations by race/ethnicity. Second, a monoclonal ELISA assay used for VDBP measurements has been criticized for lack of sensitivity to genetically determined isoforms, yielding an underestimation of VDBP levels in Blacks due to a high frequency of Gc-1F alleles in this population, compared with polyclonal or LC-MS/MS-based VDBP measurements (45-48,69,70). Thus, my main findings have focused primarily on total 25(OH)D levels assessed by the LC-MS/MS, which is the most widely used and clinically accepted biomarker of vitamin D status, and its associations with GDM risk across race/ethnicity. It is worth noting that a recent study of 368 healthy white pregnant women found that directly measured free 25(OH)D had stronger correlations with gestational age and markers of bone metabolism, lipid metabolism, and kidney function than total 25(OH)D (71). Their findings implicate the importance of free 25(OH)D in monitoring of maternal vitamin D status, although further research is needed to clarify the validity and utility of free 25(OH)D in reflecting tissue-specific activities or overall status of vitamin D. Third, parathyroid hormone (PTH), known for its synergistic role with vitamin D endocrine system (56), was not measured. Emerging evidence has also suggested the potential effects of an interaction between vitamin D and PTH on

glucose metabolism (72,73). Lastly, due to lack of information on determinants of vitamin D levels such as sun exposure or outdoor activities, residual confounding from them cannot be completely ruled out.

In conclusion, my results suggest that early-pregnancy vitamin D deficiency may increase the risk of developing GDM in pregnant women. These findings suggest that the assessment of vitamin D in the first trimester of gestation may contribute to the identification of women at risk for developing GDM. For those identified as high-risk, clinical vitamin D supplementation and dietary recommendations might be considered in clinical-care strategies to aid in the prevention of GDM associated with vitamin D deficiency. Further longitudinal studies with larger sample size and accurate assessment of vitamin D-related biomarkers measured by well-validated and standardized assays are required to confirm my findings. If confirmed, future randomized controlled trials are warranted to clarify the preventive dosage and therapeutic time windows of vitamin D supplementation to prevent GDM and address potential racial/ethnic disparities.

Chapter 3

Independent and Joint Associations of 25(OH)D and PTH with Cardiometabolic Biomarkers in U.S. White and Black Postmenopausal Women

3.1 Introduction

Evidence from mechanistic studies indicates that the vitamin D and PTH endocrine system regulates diverse physiological functions, including insulin/glucose metabolism, the renin-angiotensin-aldosterone system (RAAS), vascular and cardiac cell function, inflammatory pathways, cell proliferation and differentiation, and immune response modulation (6-10). The hypothesized mechanisms underlying the relationship between vitamin D and cardiometabolic health may operate through binding to the nuclear vitamin D receptor in a variety of tissues. Epidemiological studies suggest that vitamin D deficiency or PTH excess may be associated with intermediate cardiometabolic biomarkers, including homeostatic model assessment of insulin resistance and beta-cell function (HOMA-IR and HOMA-B), high sensitivity C-reactive protein (hs-CRP), and estimated glomerular filtration rate (eGFR) (74-76), although the available evidence remains inconclusive (77-79). It has been consistently reported that, compared to whites, blacks have a higher prevalence of vitamin D deficiency, PTH excess, and the aforementioned cardiovascular risk factors (24,80-82). There is also evidence for racial disparities in the associations of 25(OH)D or PTH with cardiometabolic biomarkers (83-85).

However, most previous studies have focused on independent associations of total 25(OH)D and PTH with cardiovascular risk factors (74-79). Given the well-established interrelations between 25(OH)D and PTH, further investigation of their joint associations

with cardiovascular risk is needed to better understand their comparative impact on CVD and racial/ethnic disparities in cardiometabolic health.

This study specifically evaluated the independent and joint associations of plasma total 25(OH)D and PTH with four core cardiometabolic biomarkers (HOMA-IR, HOMA-B, hs-CRP, and eGFR) among a random subcohort of American white and black postmenopausal women without CVD from the WHI-OS. My aims were 1) to examine both linear and nonlinear independent associations of total 25(OH)D and PTH with each cardiometabolic biomarker and 2) to explore the joint association of 25(OH)D and PTH with each cardiometabolic biomarker, separately, for U.S. white and black postmenopausal women.

3.2 Methods

3.2.1 Study population

I leveraged data from a case-cohort ancillary study conducted within the WHI-OS (86). The WHI-OS consisted of 93,676 multiracial women aged 50-79 years recruited at 40 clinical centers across U.S. between 1994 and 1998. With a 20% minority enrollment rate, the WHI-OS cohort roughly parallels the racial/ethnic diversity of the U.S. population (87).

The ancillary case-cohort study included 2,050 CVD cases and 2,800 controls after excluding women with a history of stroke or myocardial infarction, or of receiving dialysis at baseline from the original WHI-OS cohort (86). All non-CVD controls, being representative samples of the entire WHI-OS cohort, were included in this study, yielding a final sample of 1,500 white women and 1,300 black women without baseline prevalent

or incident CVD. All participants provided written informed consent at study entry, and the study was approved by the institutional review boards of each participating center.

3.2.2 Biomarker assessment

Blood samples were collected from all WHI-OS participants at baseline after at least 12 hours of fasting and stored at -80°C before laboratory assays. All assays were performed in the laboratory of Dr. Nader Rifai (CERLab) at Boston Children's Hospital. Total 25(OH)D was measured by an enzyme immunoassay from Immunodiagnostic Systems Inc (Fountain Hills, AZ). PTH was determined by electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN) with a lower limit of detection of 1.2 pg/mL. Plasma hs-CRP was measured using an immunoturbidimetric assay, creatinine by an enzymatic method, fasting glucose enzymatically, and fasting insulin by an electrochemiluminescence immunoassay; all assays were performed on the Roche E Modular system using Roche Diagnostic reagents (Roche Diagnostics, Indianapolis, IN). The averaged intra-assay coefficients of variation for each assay were: total 25(OH)D, 6.95%; PTH, 3.46%; hs-CRP, 3.34%; creatinine, 1.82%; fasting glucose, 3.26%; and fasting insulin, 2.49%. HOMA-IR was calculated by multiplying FPI μIU/mL by FPG mmol/L, then dividing by the constant 22.5, i.e. HOMA-IR = (FPI × FPG)/22.5 (52). HOMA-B was computed using the following formula: HOMA-B = 20 × FPI (μIU/mL)/FPG (mmol/L) − 3.5 (52). I calculated eGFR using the well-validated Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which has been shown to provide more accurate eGFR estimates than the Modification of Diet in Renal Disease (MDRD) equation (88).

Estimated GFR (in ml/min/1.73 m^2) = 141 x min(creatinine/κ, 1)$^{\alpha}$ x max(creatinine/κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018 (if female) x 1.159 (if Black)

Where creatinine = standardized serum creatinine measures in mg/dL

κ = 0.7 for females or 0.9 for males

α = -0.329 for females or -0.411 for males

min = indicates the minimum of creatinine/κ or 1

max = indicates the maximum of creatinine/κ or 1

Age = years

3.2.3 Covariates

Information on demographics, lifestyle behaviors, and medication history was collected from each woman at study entry (i.e., baseline) via self-administered questionnaires, including age (year), race/ethnicity (white vs. black), clinical center (Southern: <35°N, Middle: 35-40°N, and Northern: >40°N), education (≤ high school graduate/GED, post high school, and college graduate or higher), season of blood draw (Spring, Summer, Autumn, and Winter), cigarette smoking status (never, past, and current), alcohol consumption (never, past, and current), postmenopausal hormone therapy (never, past, and current), and physical activity levels (MET-hour/week). A physical examination was also performed, including height, weight, and other anthropometric measurements of each participant (87). BMI (kg/m^2) was calculated.

3.2.4 Statistical analysis

I compared white and black women in terms of total 25(OH)D, PTH, and other baseline characteristics using Student t-tests for continuous variables and Chi-square tests

for categorical variables. Age-adjusted Spearman's partial correlation coefficients were computed to examine the correlations of vitamin D biomarkers with each of the following cardiometabolic biomarkers: eGFR, hs-CRP, HOMA-IR, HOMA-B, fasting glucose, and fasting insulin.

As a random subsample of the WHI-OS cohort, the present study population represents the entire cohort. Therefore, I performed a weighted linear regression analysis to assess the independent associations between vitamin D biomarkers and each cardiometabolic biomarker at baseline. To reflect the WHI-OS population characteristics, I used an inverse probability weighting method based on the Barlow's approach (89). Each vitamin D biomarker was parameterized as a continuous variable by assuming that it has a linear relationship with the cardiometabolic biomarkers. Results for direct comparison of effect sizes between 25(OH)D and PTH were reported per one standard deviation (SD) increment in biomarker levels.

Plasma levels of HOMA-IR, HOMA-B, and hs-CRP were log-transformed due to their skewed distributions. For ease of interpretation, regression coefficients (β) obtained from these models were back-transformed to a relative difference, which can be interpreted in terms of percent change. Because of the potential nonlinear associations between vitamin D biomarkers and cardiometabolic biomarkers, I also divided all participants according to quartiles of 25(OH)D and PTH levels. For separate analyses with 25(OH)D and PTH as a continuous variable and as a categorical variable by quartiles, covariates adjusted in the main models included age, race/ethnicity, clinical center, education, season of blood draw, cigarette smoking status, alcohol consumption, postmenopausal hormone therapy, physical activity levels, and BMI. I tested for linear

trends across quartiles of vitamin D biomarkers by using the median values of each category as a continuous variable in the models. In addition, I used quadratic and cubic terms of each vitamin D biomarker as a continuous variable to capture potential nonlinear trends. To investigate black-white differences in the associations between vitamin D biomarkers and cardiometabolic biomarkers, the multivariable analyses were repeated, stratifying by race/ethnicity. I also tested for interaction between race/ethnicity and vitamin D biomarkers by including an interaction term in the main models. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC), unless otherwise specified.

I applied a novel penalized spline-based semiparametric regression model, developed by Tu et al., to explore the joint associations of total 25(OH)D and PTH with each cardiometabolic biomarker (90,91). A nonlinear bivariate surface function was used to depict the simultaneous influences of 25(OH)D and PTH on the cardiometabolic biomarkers, including HOMA-IR, HOMA-B, hs-CRP, and eGFR in blacks and whites. The estimated surface functions were presented in the form of colored contour plots, where the height of the surface function at each combination of 25(OH)D and PTH represented the mean levels of each cardiometabolic biomarker. By contrasting the shapes of the contour surfaces between blacks and white participants, one could make inferences about the potentially differential influences of 25(OH)D and PTH on CVD in the two racial/ethnic groups. I implemented the analysis using the *mgcv* package in R software (Version 3.4.2; R Foundation for Statistical Computing, Vienna, Austria).

3.3 Results

3.3.1 Population characteristics

The baseline characteristics of the present study population by ethnicity are presented in **Table 3**. Briefly, compared to white women, black women had significantly higher BMI, lower levels of physical activity, education, and current alcohol consumption, and less hormone therapy use, and were more likely to be current smokers and have a history of diabetes or hypertension, but less likely to have a family history of CVD. Plasma levels of total 25(OH)D were significantly lower, but PTH, HOMA-IR, HOMA-B, hs-CRP, and eGFR were significantly higher in black women than in white women (all P-values<0.0001).

Table 3. Baseline characteristics of postmenopausal women without prevalent or incident CVD from the WHI-OS by ethnicity.

Variables		Black women (n=1300)	White women (n=1500)	P-value*
Age, years	mean ± SD	62 ± 7.1	63 ± 7.1	< 0.0001
BMI, kg/m^2	mean ± SD	30.6 ± 6.5	26.7 ± 5.4	< 0.0001
Family history of CVD	n (%)	495 (42.2)	739 (52.2)	< 0.0001
History of diabetes	n (%)	115 (18.4)	81 (7.5)	< 0.0001
History of hypertension	n (%)	676 (52.8)	413 (28.2)	< 0.0001
History of high cholesterol	n (%)	193 (15.2)	206 (14.1)	0.437
Physical activity, MET-hour/week	median (IQR)	6.5 (1.3, 16)	10.5 (3.8, 21)	< 0.0001
Cigarette smoking status	n (%)			
Never		628 (49.3)	715 (48.5)	
Past		513 (40.2)	681 (46.2)	< 0.0001
Current		134 (10.5)	77 (5.2)	
Alcohol consumption status	n (%)			
Never		246 (19.2)	128 (8.7)	
Past		400 (31.2)	249 (16.9)	< 0.0001
Current		636 (49.6)	1099 (74.5)	
Hormone therapy use	n (%)			
Never		758 (58.6)	533 (35.8)	< 0.0001
Past		169 (13.1)	226 (15.2)	

Current		366 (28.3)	731 (49.1)	
Statin use	n (%)	186 (14.4)	212 (14.2)	0.914
Educational Levels	n (%)			
≤ High school graduate/GED		342 (26.4)	301 (20.2)	
Post high school		484 (37.4)	512 (34.3)	< 0.0001
College graduate or higher		469 (36.2)	678 (45.5)	
Geographical latitude (Clinical center)	n (%)			
Southern: <35°N		442 (32.6)	444 (29.8)	
Middle: 35-40°N		428 (33.1)	434 (29.1)	0.001
Northern: >40°N		445 (34.4)	613 (41.1)	
Season of blood draw	n (%)			
Spring		381 (29.8)	437 (29.4)	
Summer		348 (27.2)	431 (29.0)	0.74
Autumn		279 (21.8)	318 (21.4)	
Winter		271 (21.2)	299 (20.1)	
Vitamin D biomarkers				
Total 25(OH)D, nmol/l	median (IQR)	42.5 (33.4, 54.7)	63.3 (51.1, 76.7)	< 0.0001
PTH, pg/mL	median (IQR)	40.2 (31.4, 51.7)	35.6 (28.4, 44.2)	< 0.0001
Cardiometabolic biomarkers				
HOMA-IR	median (IQR)	2.2 (1.3, 3.6)	1.5 (1.0, 2.4)	< 0.0001
HOMA-B	median (IQR)	98.1 (62.9, 145.9)	81.4 (57.3, 116.4)	< 0.0001
hs-CRP, mg/L	median (IQR)	3.3 (1.4, 7.2)	2.2 (0.9, 4.9)	< 0.0001
eGFR, mL/min/1.73m2	mean ± SD	94.1 ± 18	86.2 ± 13	< 0.0001

*P-value for differences between black and white women were obtained by Student t-test for continuous variables and Chi-square test for categorical variables.
SD, standard deviation; IQR, interquartile range; BMI, body mass index; MET, metabolic equivalent of task.

3.3.2 Correlations between vitamin D biomarkers and cardiometabolic biomarkers

I examined the correlations of vitamin D biomarkers with each cardiometabolic biomarker (**Table 4**). Among all participants, total 25(OH)D was inversely correlated with PTH and all cardiometabolic biomarkers, whereas PTH was positively correlated with HOMA-IR, HOMA-B, hs-CRP, fasting insulin, and fasting glucose. While total 25(OH)D was significantly and inversely correlated with hs-CRP (r=-0.08; P=0.001) and HOMA-B (r=-0.19; P=<0.0001) in white women only, PTH showed a significant inverse correlation with eGFR only in black women (r=-0.07; P=0.014). Although the

correlations of 25(OH)D with eGFR were similar between white (r=-0.05) and black women (r=-0.06), white women had a stronger correlation between 25(OH)D and HOMA-IR (r=-0.23) than black women (r=-0.13). In contrast, the correlations of PTH with HOMA-IR and HOMA-B appeared to be stronger in black women (r=0.13 for HOMA-IR; r=0.15 for HOMA-B) than in whites (r=0.11 for HOMA-IR; r=0.08 for HOMA-B).

Table 4. Age-adjusted Spearman correlation coefficients for vitamin D biomarkers and cardiometabolic biomarkers among postmenopausal women, stratified by race/ethnicity.

Biomarkers	Total 25(OH)D	Fasting glucose	Fasting insulin	PTH	eGFR	hs-CRP	HOMA-IR	HOMA-B
All participants (n=2800)								
Total 25(OH)D	1	-0.13†	-0.26†	-0.37†	-0.16†	-0.14†	-0.26†	-0.17†
Fasting glucose		1	0.49†	0.07†	0.06†	0.18†	0.62†	-0.14†
Fasting insulin			1	0.17†	0.03	0.36†	0.98†	0.73†
PTH				1	0.01	0.08†	0.16†	0.14†
eGFR					1	0.04	0.04*	-0.04*
hs-CRP						1	0.36†	0.25†
HOMA-IR							1	0.61†
HOMA-B								1
American white women (n=1500)								
Total 25(OH)D	1	-0.12†	-0.24†	-0.29†	-0.05*	-0.08†	-0.23†	-0.19†
Fasting glucose		1	0.47†	0.06*	0.04	0.1†	0.58†	-0.08†
Fasting insulin			1	0.11†	-0.01	0.31†	0.99†	0.81†
PTH				1	0.003	0.05	0.11†	0.08†
eGFR					1	0.02	-0.002	-0.04
hs-CRP						1	0.3†	0.28†
HOMA-IR							1	0.71†
HOMA-B								1
American blacks (n=1300)								
Total 25(OH)D	1	-0.07†	-0.12†	-0.36†	-0.06*	-0.05	-0.13†	-0.05
Fasting glucose		1	0.5†	0.05	0.06*	0.24†	0.67†	-0.24†
Fasting insulin			1	0.16	-0.03	0.35†	0.97†	0.64†
PTH				1	-0.07*	0.04	0.13†	0.15†
eGFR					1	-0.01	0.004	-0.11†
hs-CRP						1	0.37†	0.18†
HOMA-IR							1	0.46†
HOMA-B								1

* P<0.05; † P<0.01.

3.3.3 Independent associations between vitamin D biomarkers and cardiometabolic biomarkers

I assessed the independent associations between total 25(OH)D levels and each cardiometabolic biomarker at baseline (**Table 5a**). Higher total 25(OH)D levels were independently associated with lower levels of HOMA-IR, HOMA-B, and eGFR in a dose-response manner among all participants, adjusting for age, race/ethnicity, clinical center, education, season of blood draw, cigarette smoking status, alcohol consumption, postmenopausal hormone therapy, physical activity, and BMI. The statistically significant association between 25(OH)D and hs-CRP attenuated to non-significance after additional adjustment for BMI. When stratified by race/ethnicity, the observed associations of 25(OH)D with HOMA-IR (6.59% decrease per 1 SD increase in 25(OH)D; P for linear trend=0.0001) and HOMA-B (3.21% decrease per 1 SD increase in 25(OH)D; P for linear trend=0.03) persisted in white women only. In contrast, an inverse association between 25(OH)D and eGFR persisted in black women only (β=-0.99; P=0.028). However, the interaction term between race/ethnicity and 25(OH)D was significant for HOMA-B only (P for interaction=0.029).

Table 5a. Multivariable weighted linear regression analysis between total 25(OH)D and cardiometabolic biomarkers among postmenopausal women.

Model		Least-Squares Mean (95% CI or SE)§				P for linear trend	P for non-linearity	β (SE)	RD (%)‖
		Quartile1	Quartile2	Quartile3	Quartile4				
HOMA-IR									
All participants	Model 1*	2.23 (2.11, 2.35)	1.99 (1.89, 2.10)**	1.69 (1.60, 1.79)**	1.45 (1.37, 1.54)**	<0.0001	0.098	-0.27 (0.02)**	-23.93
	Model 2†	2.17 (2.03, 2.31)	1.96 (1.84, 2.09)#	1.71 (1.60, 1.83)**	1.51 (1.40, 1.62)**	<0.0001	0.192	-0.24 (0.02)	-21.12
	Model 3‡	2.01 (1.90, 2.13)	1.94 (1.83, 2.05)	1.82 (1.71, 1.94)**	1.72 (1.61, 1.83)**	<0.0001	0.221	-0.11 (0.02)**	-10.65
American white women	Model 1*	2.00 (1.87, 2.13)	1.72 (1.61, 1.84)**	1.54 (1.44, 1.64)**	1.31 (1.22, 1.40)**	<0.0001	0.521	-0.16 (0.02)**	-14.77
	Model 2†	1.97 (1.80, 2.14)	1.71 (1.57, 1.86)**	1.58 (1.45, 1.72)**	1.36 (1.24, 1.49)**	<0.0001	0.643	-0.14 (0.02)**	-13.29
	Model 3‡	1.82 (1.69, 1.96)	1.73 (1.61, 1.86)	1.65 (1.53, 1.78)#	1.55 (1.43, 1.68)**	0.0001	0.583	-0.07 (0.02)**	-6.59
American black women	Model 1*	2.43 (2.22, 2.66)	2.29 (2.09, 2.50)	2.23 (2.04, 2.44)	1.85 (1.69, 2.02)**	<0.0001	0.783	-0.12 (0.02)**	-10.91
	Model 2†	2.32 (2.10, 2.57)	2.21 (1.99, 2.44)	2.23 (2.01, 2.47)	1.88 (1.70, 2.09)**	0.002	0.826	-0.08 (0.02)**	-8.14
	Model 3‡	2.22 (2.02, 2.44)	2.13 (1.94, 2.35)	2.26 (2.06, 2.49)	2.03 (1.84, 2.23)	0.196	0.626	-0.04 (0.02)	-3.45
HOMA-B									
All participants	Model 1*	93.44 (89.67, 97.37)	93.44 (89.58, 97.55)	83.27 (79.62, 87.09)**	76.62 (73.14, 80.26)**	<0.0001	0.018	-0.15 (0.02)**	-13.58
	Model 2†	92.26 (87.71, 97.03)	93.08 (88.35, 98.05)	84.48 (80.04, 89.17)**	78.95 (74.52, 83.64)**	<0.0001	0.0497	-0.12 (0.02)**	-11.52
	Model 3‡	88.18 (84.07, 92.49)	92.28 (87.83, 96.94)	87.41 (83.05, 92.01)	85.41 (80.82, 90.26)	0.103	0.037	-0.05 (0.02)#	-4.64

American white women	Model 1*	92.66 (87.95, 97.63)	86.30 (81.94, 90.88)	79.60 (75.59, 83.83)**	72.78 (69.13, 76.64)**	<0.0001	0.231	-0.09 (0.01)**	-8.85
	Model 2†	91.87 (85.81, 98.36)	86.22 (80.66, 92.17)	81.33 (75.92, 87.12)**	75.16 (69.95, 80.76)**	<0.0001	0.317	-0.08 (0.01)**	-7.66
	Model 3‡	88.02 (82.68, 93.70)	87.06 (81.90, 92.55)	83.94 (78.81, 89.40)	81.93 (76.68, 87.54)#	0.03	0.249	-0.03 (0.01)#	-3.21
American black women	Model 1*	95.97 (89.46, 103.0)	92.32 (86.07, 99.03)	95.21 (88.76, 102.1)	90.65 (84.44, 97.31)	0.353	0.859	-0.03 (0.02)	-3.28
	Model 2†	95.77 (88.14, 104.1)	91.44 (84.15, 99.35)	95.08 (87.38, 103.5)	92. 20 (84.67, 100.4)	0.642	0.893	-0.02 (0.02)	-2.42
	Model 3‡	93.32 (86.11, 101.1)	89.88 (82.94, 97.39)	96.13 (88.58, 104.3)	96.32 (88.69, 104.6)	0.337	0.75	0.004 (0.02)	0.35
hs-CRP									
All participants	Model 1*	2.83 (2.59, 3.09)	2.81 (2.57, 3.08)	2.18 (1.98, 2.40)**	2.33 (2.11, 2.57)**	0.0002	0.457	-0.17 (0.04)**	-15.97
	Model 2†	2.81 (2.53, 3.12)	2.92 (2.62, 3.26)	2.36 (2.11, 2.64)**	2.42 (2.15, 2.73)#	0.004	0.84	-0.16 (0.04)**	-14.64
	Model 3‡	2.58 (2.33, 2.85)	2.89 (2.61, 3.20)	2.55 (2.29, 2.83)	2.95 (2.63, 3.31)#	0.17	0.921	0.02 (0.04)	1.85
American white women	Model 1*	2.59 (2.30, 2.92)	2.09 (1.86, 2.35)#	1.92 (1.71, 2.17)**	2.03 (1.80, 2.29)**	0.004	0.357	-0.10 (0.03)**	-9.83
	Model 2†	2.42 (2.08, 2.82)	2.09 (1.80, 2.43)	1.92 (1.65, 2.24)**	1.93 (1.64, 2.27)**	0.007	0.592	-0.10 (0.03)**	-9.81
	Model 3‡	2.21 (1.91, 2.54)	2.11 (1.83, 2.42)	2.05 (1.77, 2.37)	2.30 (1.97, 2.67)	0.65	0.98	0.001 (0.03)	0.12
American black women	Model 1*	3.33 (2.93, 3.79)	3.10 (2.73, 3.53)	3.01 (2.65, 3.43)	2.91 (2.56, 3.32)	0.163	0.492	-0.06 (0.03)	-5.82
	Model 2†	3.48 (3.00, 4.04)	3.18 (2.74, 3.69)	3.42 (2.94, 3.98)	3.37 (2.89, 3.92)	0.958	0.282	-0.02 (0.03)	-2.05
	Model 3‡	3.28 (2.86, 3.76)	2.99 (2.61, 3.43)	3.50 (3.05, 4.02)	3.74 (3.26, 4.30)	0.042	0.303	0.05 (0.03)	5.46
eGFR									

All participants	Model 1*	91.30 (0.51)	89.54 (0.52)#	90.10 (0.55)	88.98 (0.57)**	0.01	0.131	-1.25 (0.46)**	0.006
	Model 2†	92.04 (0.63)	90.28 (0.65)#	90.76 (0.67)	89.71 (0.72)**	0.016	0.139	-1.25 (0.48)**	0.010
	Model 3‡	92.07 (0.64)	90.28 (0.66)#	90.93 (0.69)	89.80 (0.74)**	0.018	0.384	-1.21 (0.51)#	0.017
American white women	Model 1*	87.38 (0.62)	86.50 (0.61)	85.51 (0.61)#	85.80 (0.61)	0.045	0.246	-0.63 (0.31)#	0.040
	Model 2†	88.16 (0.83)	87.16 (0.81)	86.24 (0.83)#	86.86 (0.87)	0.125	0.227	-0.5 (0.33)	0.125
	Model 3‡	88.20 (0.83)	87.34 (0.81)	86.24 (0.84)#	86.96 (0.88)	0.137	0.505	-0.47 (0.34)	0.172
American black women	Model 1*	95.36 (0.92)	93.97 (0.92)	94.07 (0.91)	93.06 (0.92)	0.102	0.436	-0.70 (0.46)	0.130
	Model 2†	96.14 (1.08)	94.58 (1.08)	95.00 (1.10)	93.01 (1.10)#	0.032	0.543	-0.97 (0.48)#	0.045
	Model 3‡	96.09 (1.09)	94.63 (1.09)	94.96 (1.11)	92.88 (1.11)#	0.028	0.507	-0.99 (0.50)#	0.046

* Model 1 adjusted for age, clinical center, and race/ethnicity.
† Model 2 further adjusted for education, season of blood draw, cigarette smoking status, alcohol intake, postmenopausal hormone therapy use, and physical activity levels.
‡ Model 3 additionally adjusted for BMI.
§ Least-Square Mean with 95% CI was presented for log-transformed HOMA-IR, HOMA-B, and hs-CRP; Least-Square Mean with SE (standard error) was shown for eGFR.
‖ RD, relative difference, is interpreted as percent lower (negative value) or higher (positive value) of levels of HOMA-IR, HOMA-B, or hs-CRP on the original scale for every SD increase in total 25(OH)D.
# P<0.05; ** P<0.01.

I also assessed the independent associations between PTH levels and each cardiometabolic biomarker at baseline (**Table 5b**). After adjusting for the same covariates as above, higher PTH levels were independently associated with higher levels of HOMA-IR and HOMA-B, and lower eGFR among all participants. In a race/ethnicity-stratified analysis, higher PTH levels were significantly associated with higher HOMA-IR levels in white women only (3.29% increase per 1 SD increase in PTH) and higher HOMA-B levels in black women only (4.55% increase per 1 SD increase in PTH; P for nonlinearity=0.003). While there was a linear trend towards higher PTH associated with lower hs-CRP in black women (P for linear trend=0.01), I found a nonlinear relationship between PTH and hs-CRP among white women (P for nonlinearity=0.039). There was also a significant interaction between race/ethnicity and PTH on hs-CRP (P for interaction=0.035). PTH was nonlinearly associated with eGFR across racial/ethnic groups (P for nonlinearity=0.005 for white women and 0.036 for black women).

Table 5b. Multivariable weighted linear regression analysis between PTH and cardiometabolic biomarkers among postmenopausal women.

Model		Least-Squares Mean (95% CI or SE)§				*P* for linear trend	*P* for non-linearity	β (SE)	RD (%)‖
		Quartile1	Quartile2	Quartile3	Quartile4				
HOMA-IR									
All participants	Model 1*	1.72 (1.63, 1.82)	1.78 (1.68, 1.88)	1.88 (1.78, 1.99)#	2.12 (2.01, 2.24)**	<0.0001	0.042	0.15 (0.02)**	15.6
	Model 2†	1.77 (1.66, 1.89)	1.78 (1.67, 1.91)	1.91 (1.79, 2.04)#	2.09 (1.96, 2.22)**	<0.0001	0.693	0.13 (0.02)**	14
	Model 3‡	1.88 (1.77, 2.00)	1.83 (1.72, 1.94)	1.93 (1.82, 2.05)	1.95 (1.84, 2.07)	0.14	0.761	0.05 (0.02)**	5.41
American white women	Model 1*	1.49 (1.39. 1.59)	1.59 (1.48, 1.70)	1.60 (1.50, 1.72)	1.81 (1.69, 1.94)**	<0.0001	0.621	0.08 (0.02)**	8.24
	Model 2†	1.57 (1.44, 1.72)	1.63 (1.49, 1.78)	1.66 (1.52, 1.81)	1.85 (1.69, 2.02)**	0.001	0.453	0.08 (0.02)**	7.9
	Model 3‡	1.70 (1.58, 1.83)	1.68 (1.55, 1.81)	1.68 (1.56, 1.81)	1.76 (1.63, 1.89)	0.352	0.37	0.03 (0.01)#	3.29
American black women	Model 1*	1.89 (1.73, 2.07)	2.07 (1.90, 2.27)	2.33 (2.13, 2.55)**	2.52 (2.30, 2.75)**	<0.0001	0.183	0.10 (0.02)**	10.74
	Model 2†	1.93 (1.74, 2.14)	2.06 (1.86, 2.29)	2.28 (2.05, 2.52)#	2.36 (2.13, 2.61)**	0.001	0.644	0.08 (0.02)**	8.16
	Model 3‡	2.08 (1.89, 2.29)	2.09 (1.90, 2.30)	2.31 (2.10, 2.54)	2.16 (1.97, 2.37)	0.372	0.948	0.03 (0.02)	2.65
HOMA-B									
All participants	Model 1*	81.48 (78.00, 85.11)	85.38 (81.77, 89.15)	88.36 (84.67, 92.21)**	94.73 (90.89, 98.73)**	<0.0001	0.012	0.09 (0.02)**	9.51
	Model 2†	83.74 (79.41, 88.30)	86.00 (81.57, 90.68)	88.89 (84.42, 93.60)#	94.18 (89.51, 99.09)**	<0.0001	0.606	0.08 (0.02)**	8.43
	Model 3‡	86.72 (82.48, 91.18)	86.92 (82.69, 91.37)	89.06 (84.83, 93.50)	90.97 (86.68, 95.46)	0.075	0.988	0.04 (0.02)#	3.83

American white women	Model 1*	77.77 (73.79, 81.96)	82.31 (78.12, 86.72)	82.41 (78.20, 86.84)	87.32 (82.86, 92.02)**	0.0003	0.323	0.04 (0.01)**	4.37
	Model 2†	80.90 (75.51, 86.68)	83.93 (78.32, 89.95)	83.99 (78.45, 89.92)	88.56 (82.67, 94.87)#	0.019	0.219	0.04 (0.01)**	4.14
	Model 3‡	85.12 (79.93, 90.66)	85.45 (80.23, 91.02)	84.99 (79.88, 90.43)	86.50 (81.23, 92.10)	0.659	0.188	0.02 (0.01)	1.51
American black women	Model 1*	80.24 (74.81, 86.06)	91.43 (85.29, 98.01)**	99.50 (92.84, 106.6)**	105 (97.94, 112.6)**	<0.0001	<0.0001	0.08 (0.02)**	8.33
	Model 2†	81.91 (75.32, 89.09)	90.72 (83.48, 98.58)#	98.80 (90.99, 107.3)	103.62 (95.50, 112.4)	<0.0001	<0.0001	0.07 (0.02)**	7.34
	Model 3‡	85.30 (78.60, 92.57)	91.32 (84.27, 98.97)	100.1 (92.32, 108.5)**	98.92 (91.36, 107.1)**	0.003	0.003	0.04 (0.02)#	4.55
hs-CRP									
All participants	Model 1*	2.49 (2.27, 2.73)	2.34 (2.14, 2.57)	2.61 (2.39, 2.86)	2.83 (2.59, 3.09)#	0.01	0.999	0.12 (0.04)**	13.24
	Model 2†	2.69 (2.41, 3.00)	2.47 (2.21, 2.76)	2.64 (2.37, 2.94)	2.85 (2.56, 3.17)	0.162	0.97	0.09 (0.04)#	9.83
	Model 3‡	2.95 (2.66, 3.27)	2.57 (2.32, 2.85)#	2.72 (2.46, 3.01)	2.61 (2.36, 2.88)#	0.12	0.002	-0.01 (0.03)	-1.24
American white women	Model 1*	2.08 (1.84, 2.34)	2.00 (1.78, 2.25)	2.19 (1.94, 2.47)	2.34 (2.08, 2.64)	0.089	0.111	0.09 (0.03)**	9.48
	Model 2†	2.08 (1.78, 2.43)	2.02 (1.74, 2.36)	2.07 (1.78, 2.41)	2.26 (1.94, 2.64)	0.249	0.2	0.07 (0.03)#	7.31
	Model 3‡	2.30 (1.99, 2.65)	2.09 (1.81, 2.41)	2.12 (1.84, 2.44)	2.13 (1.84, 2.45)	0.427	0.039	0.02 (0.03)	1.6
American black women	Model 1*	3.14 (2.77, 3.58)	2.58 (2.27, 2.94)#	2.53 (3.10, 4.01)	3.18 (2.80, 3.62)	0.271	0.482	0.03 (0.03)	3.46
	Model 2†	3.61 (3.10, 4.20)	2.83 (2.43, 3.28)**	3.69 (3.18, 4.29)	3.39 (2.93, 3.93)	0.773	0.796	0.01 (0.03)	0.64
	Model 3‡	4.05 (3.53, 4.65)	2.90 (2.53, 3.32)**	3.75 (3.27, 4.30)	2.95 (2.58, 3.38)**	0.01	0.943	-0.08 (0.03)#	-7.56
eGFR									

All participants	Model 1*	90.37 (0.53)	90.42 (0.53)	90.86 (0.52)	89.11 (0.51)	0.077	0.034	-2.20 (0.42)**	<0.0001
	Model 2†	91.20 (0.66)	91.22 (0.66)	91.66 (0.64)	89.88 (0.63)	0.066	0.032	-2.18 (0.43)**	<0.0001
	Model 3‡	91.23 (0.67)	91.24 (0.67)	91.84 (0.65)	89.91 (0.65)	0.04	0.043	-2.24 (0.43)**	<0.0001
American white women	Model 1*	85.88 (0.62)	86.84 (0.61)	86.90 (0.61)	85.47 (0.62)	0.504	0.002	-0.97 (0.31)**	0.002
	Model 2†	86.80 (0.83)	87.77 (0.83)	87.74 (0.82)	86.30 (0.83)	0.425	0.003	-1.03 (0.31)**	0.001
	Model 3‡	86.96 (0.83)	87.90 (0.83)	87.84 (0.82)	86.27 (0.83)	0.31	0.005	-1.09 (0.31)**	0.001
American black women	Model 1*	95.80 (0.92)	94.69 (0.91)	94.50 (0.91)	91.41 (0.91)**	0.001	0.006	-2.14 (0.46)**	<0.0001
	Model 2†	96.36 (1.10)	95.18 (1.08)	95.41 (1.08)	92.13 (1.06)**	0.001	0.025	-1.98 (0.47)**	<0.0001
	Model 3‡	96.31 (1.11)	95.15 (1.09)#	95.22 (1.10)	92.20 (1.08)**	0.002	0.036	-1.93 (0.48)**	<0.0001

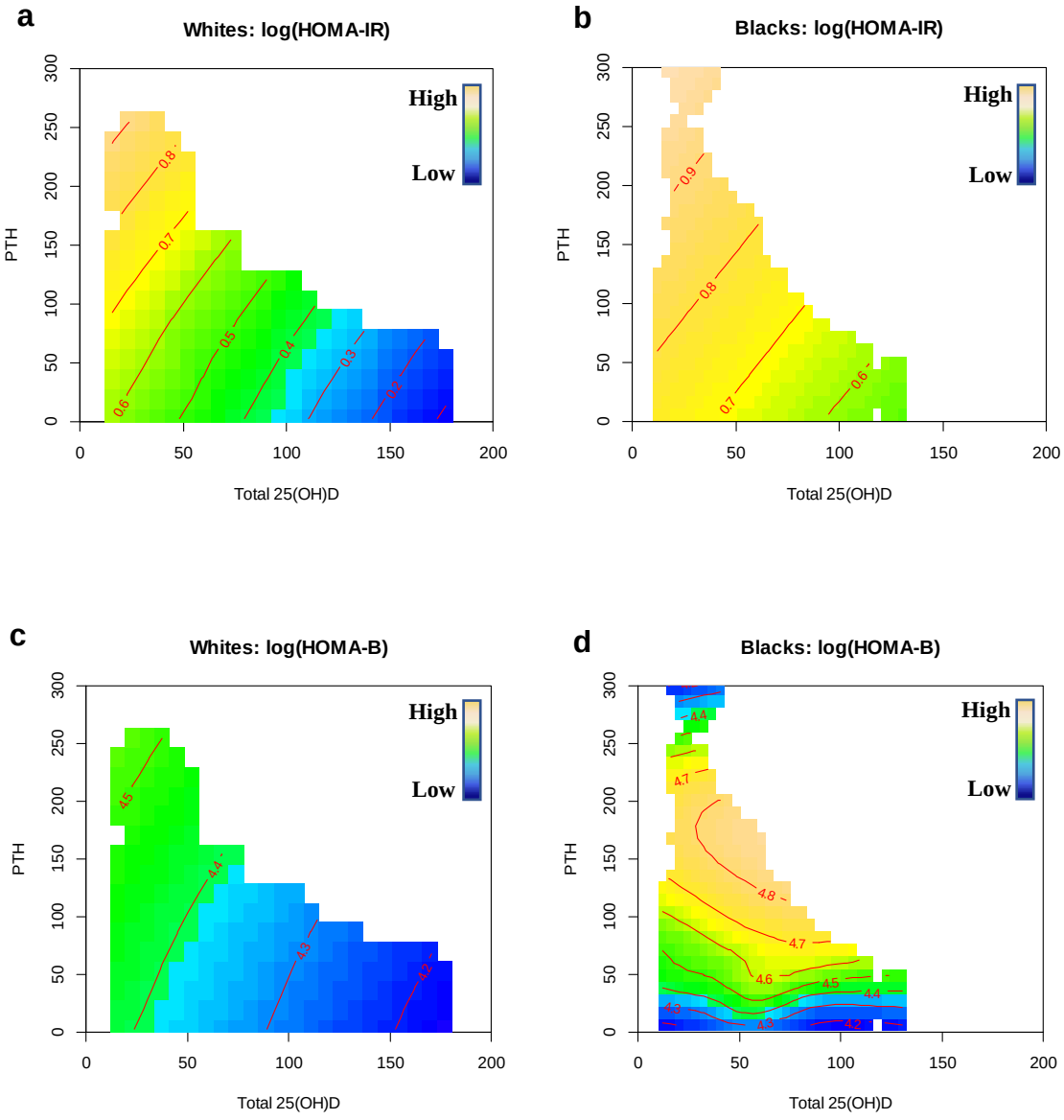
* Model 1 adjusted for age, clinical center, and race/ethnicity.
† Model 2 further adjusted for education, season of blood draw, cigarette smoking status, alcohol intake, postmenopausal hormone therapy use, and physical activity levels.
‡ Model 3 additionally adjusted for BMI.
§ Least-Square Mean with 95% CI was presented for log-transformed HOMA-IR, HOMA-B, and hs-CRP; Least-Square Mean with SE (standard error) was shown for eGFR.
‖ RD, relative difference, is interpreted as percent lower (negative value) or higher (positive value) of levels of HOMA-IR, HOMA-B, or hs-CRP on the original scale for every SD increase in PTH.
# P<0.05; ** P<0.01.

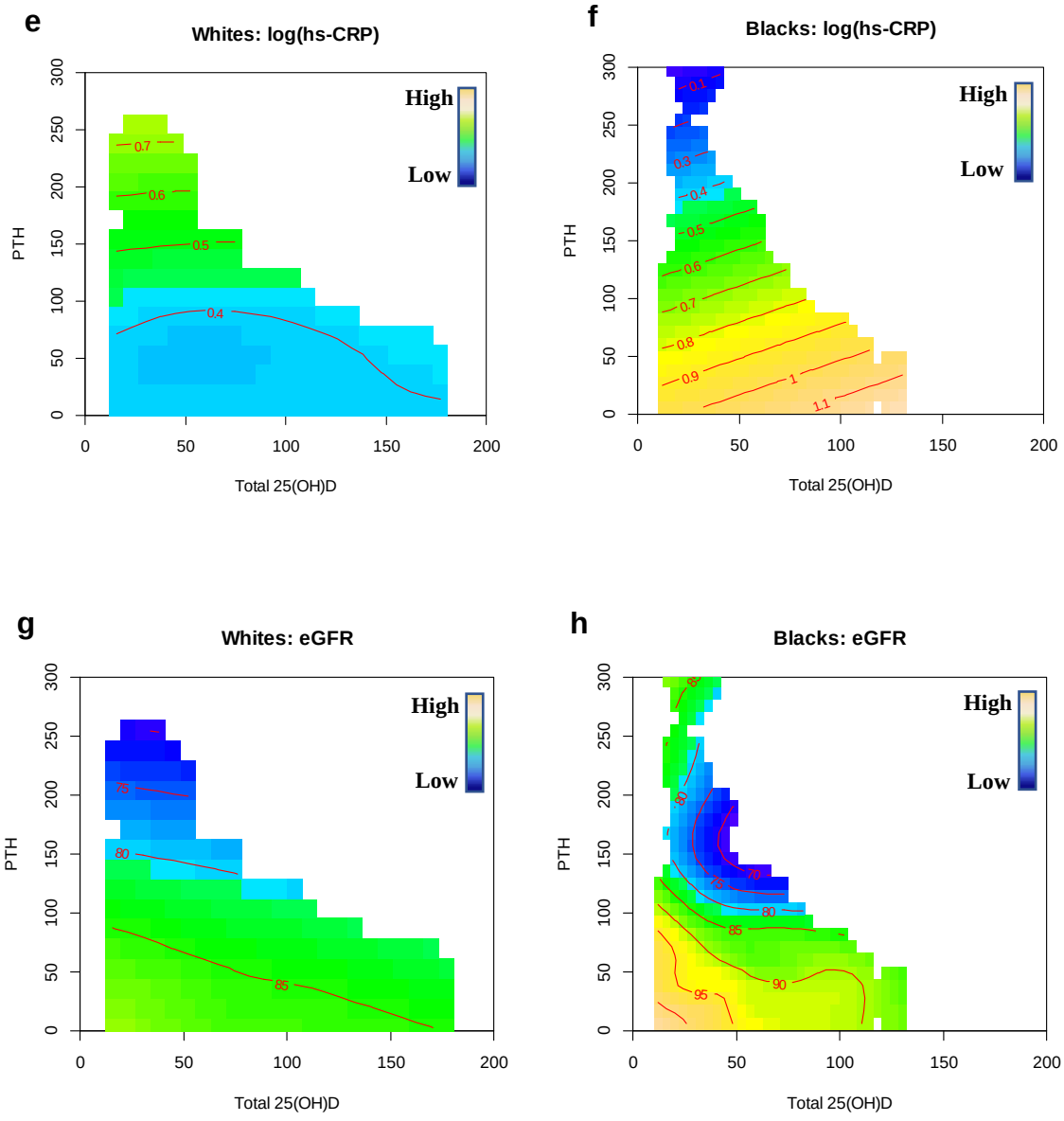
3.3.4 Race-specific joint associations of 25(OH)D and PTH with cardiometabolic biomarkers

I further explored the joint associations of total 25(OH)D and PTH with each cardiometabolic biomarker (**Figure 4, a-h**). In the contour plots, the mean response levels of each cardiometabolic biomarker at all combinations of 25(OH)D and PTH levels were indicated by colors and numbers on the contour lines with adjustment of confounding factors. On average, white women had lower levels of HOMA-IR, HOMA-B, hs-CRP, and eGFR than black women. The combination of lower total 25(OH)D and higher PTH was associated with higher HOMA-IR in both white and black women with similar linear trends, suggesting a lack of racial/ethnic differences (**Figure 4a and 4b**). Conversely, black-white differences in the joint associations of 25(OH)D and PTH with HOMA-B, hs-CRP, and eGFR were evident (**Figure 4c-h**). Overall, the combination of lower 25(OH)D levels and higher PTH levels was jointly and linearly associated with higher levels of HOMA-B in white women, but there was a nonlinear association between PTH and HOMA-B in black women (**Figure 4c and 4d**). While PTH alone was positively associated with hs-CRP in white women, there was a joint association of higher 25(OH)D and lower PTH with higher hs-CRP in black women (**Figure 4e and 4f**). Although 25(OH)D and PTH were simultaneously associated with eGFR among both black and white women, there were notable racial/ethnic differences in the shape of the associations (**Figure 4g and 4h**). Specifically, PTH appeared to be inversely and linearly associated with eGFR in white women, mainly confined to low 25(OH)D (**Figure 4g**); however, their joint association with eGFR took on a nonlinear and complex shape in black women and appeared to be predominantly driven by PTH levels (**Figure 4h**).

Figure 4. Estimated concurrent associations of 25(OH)D and PTH on cardiometabolic biomarkers by race/ethnicity (blacks vs. whites).

The estimated mean response levels of each cardiometabolic biomarker for all 25(OH)D-PTH combinations in blacks and whites is indicated by the numbers on the contour lines, adjusting for age, clinical center, education, season of blood draw, BMI, cigarette smoking status, alcohol consumption, postmenopausal hormone therapy, and physical activity levels.





3.4 Discussion

In this large cohort of U.S. postmenopausal women without CVD, total 25(OH)D was inversely correlated with PTH and all cardiometabolic biomarkers in both white and black participants but the joint association of 25(OH)D and PTH with beta-cell function, systemic inflammation, and kidney function differed by race. Specifically, High PTH and low 25(OH)D were jointly associated with high HOMA-B in white women and low hs-

CRP in black women. In contrast, PTH was independently associated with HOMA-B in black women and hs-CRP in white women. Although 25(OH)D and PTH were jointly associated with eGFR among black and white women, racial/ethnic differences in the shape of the associations were complex and mainly due to PTH levels. However, higher PTH and lower total 25(OH)D were independently and jointly associated with higher HOMA-IR in both white and black women, with similar linear patterns. These findings suggest that the vitamin D-PTH endocrine system may play a role in explaining racial disparities in cardiometabolic health.

My findings are consistent with most previous studies of the individual associations of 25(OH)D and PTH with cardiometabolic biomarkers of interest among ethnically diverse populations (92-99). Data from two national surveys suggested that 25(OH)D was inversely associated with HOMA-IR and HOMA-B (92,93). Similar to the present study, evidence from recent observational studies showed that PTH was positively correlated with HOMA-IR and HOMA-B across different populations (94,95). No association between 25(OH)D and hs-CRP was observed in a cohort from the Framingham Offspring Study (n=1,381) (96). Several studies have demonstrated an inverse association of PTH and 25(OH)D with eGFR in patients with chronic kidney disease and in general populations, respectively (97-99).

Whereas the independent associations of either 25(OH)D or PTH with cardiometabolic biomarkers have been repeatedly shown in a handful of studies, some findings have been inconsistent. For instance, cross-sectional data from a largely obese ethnic-minority cohort of non-diabetic Canadian adults (n=510) indicated that 25(OH)D was not associated with either HOMA-IR or HOMA-B, potentially due to distinct genetic

and behavioral traits of the Canadian Cree population (100). A cross-sectional study of 374 African and 350 Asian Indian healthy adults reported a negative association between PTH and HOMA-IR, the opposite of my findings (101). Data from NHANES 2003-2006 (n=8,948) showed a positive relationship between PTH and hs-CRP (102). Also, a population study of 1,042 white and black Americans reported that the inverse association of 25(OH)D with hs-CRP was significant in unadjusted analysis, but attenuated to non-significance after adjusting for age, race, sex, and geographic region of residence (75). Contrary to my results, data from the 5th Korean National Health and Nutritional Examination Survey (KNHANES) 2011-2012 indicated a positive relationship between 25(OH)D and eGFR in moderate and severe CKD (99). Residual confounding due to different population characteristics, especially determinants of vitamin D status, may explain these null or contradictory findings. In addition, small sample size and different biomarker categorizations and model specifications may also explain these inconsistent results.

The reciprocal relation between 25(OH)D and PTH is dynamic, complex, and very sensitive to racial/ethnical background (24). Contrary to studies of independent associations of 25(OH)D and PTH with cardiometabolic biomarkers, there are limited data on their joint associations. The strategy in previous studies has generally been to use the ratio, subgroup analyses by broad categorizations of 25(OH)D and PTH, or adjusted parameter estimates. A small case-control study consisting of 15 obese and 15 matched normal-weight adolescent girls from the Partners HealthCare network found that the ratio of PTH/25(OH)D was positively associated with hs-CRP (103). Their findings suggested a joint association of high PTH and low 25(OH)D with high hs-CRP, which is similar to

my results for black women, but in the opposite direction. To explore the inconsistent results, I stratified the analysis by BMI (<25 vs. ≥25 kg/m^2) in black women. Unexpectedly, the association of 25(OH)D with hs-CRP was positive in those with normal weight, synergistically with PTH (**Supplemental Figure B-1a and B-1b in Appendix B**). This could be, at least partly, due to limited variability of hs-CRP among black women with normal weight (6.29+7.23 mg/L for overweight/obesity vs. 2.91+4.62 mg/L for normal weight). Also, the results could be sensitive to extreme values (**Supplemental Figure B-2 in Appendix B**). In contrast, the correlations of 25(OH)D and PTH with hs-CRP are more likely stable for overweight/obese individuals who have a wider range of hs-CRP values. In line with my findings, the KNHANES data from 2009-2011 showed an inverse PTH-eGFR relationship when 25(OH)D<20 ng/mL without covariate adjustment and a nonlinear trend for PTH levels across eGFR tertiles, after accounting for 25(OH)D and other covariates in Korean women (99). A recent hospital-based case-control study among 225 elderly Greek patients found that participants with vitamin D deficiency and high PTH levels (third tertile) had the highest HOMA-IR levels but no changes in HOMA-B than all other groups with either vitamin D sufficiency or low PTH levels (first to second tertile) (104). However, the small sample size of that study limited its ability to identify joint associations of vitamin D and PTH with HOMA-B, especially with grouped data on vitamin D and PTH.

To account for the nonlinear race-specific relations between 25(OH)D and PTH and their possible interactions with CVD biomarkers in the present study, I used a novel model-based color contour plots to delineate those joint associations with each biomarker. I found consistent linear associations of higher PTH and lower total 25(OH)D

with higher HOMA-IR, independently and jointly, in both white and black women. The results also showed black-white differences in the associations of 25(OH)D and PTH with HOMA-B, hs-CRP, and eGFR. Although the nonlinear association between PTH and HOMA-B I observed in black women is in agreement with findings from vitro studies, indicating the calcium-dependent effects of PTH on insulin release by pancreatic islets in a dose-dependent manner (105), my observed linear relationship of higher PTH and lower 25(OH)D with higher HOMA-B is contrary to the existing biological evidence linking vitamin D/PTH system to pancreatic beta-cell function. It is worth mentioning that HOMA-B is not a reliable or sensitive surrogate of beta-cell function, as evidenced by findings from the same cohort and other population studies (52,106,107). HOMA-B was strongly correlated with fasting glucose or insulin levels, which reflect insulin resistance to varying extents depending on population characteristics. Contrary to the joint associations of 25(OH)D and PTH with hs-CRP in black women, the association between PTH and hs-CRP in white women was independent of 25(OH)D. The anti-inflammatory property of PTH may be more active in white women than in black women.

Racial differences in associations of 25(OH)D and PTH may explain racial disparities in inflammation-related cardiovascular health. The shape of the joint association of 25(OH)D and PTH with eGFR was linear in white women and nonlinear in black women. The existing evidence mainly focuses on the independent associations of either 25(OH)D or PTH with eGFR and suggests that their relationships may be complex, synergistic, and vary by race. A cross-sectional study of 203 CKD patients from a U.K. hospital found no association between 25(OH)D and eGFR, but PTH increased with worsening eGFR, especially in those not taking vitamin D supplements (108). A Korean

nationally representative survey reported that 25(OH)D started to decrease when eGFR<55.4 mL/min/1.73m^2 (109). Another cross-sectional analysis of laboratory data collected on 2,028 CKD patients (including 505 African Americans) from 170 U.S. practices found that the slope of the inverse relationship between PTH and eGFR was steeper in blacks than in whites (110). CKD has been described as a state of stagnant vitamin D metabolism with decreased vitamin D catabolism; the racial/ethnic heterogeneity in the joint association of 25(OH)D and PTH with eGFR that I observed, therefore, may suggest biological differences between whites and blacks in altered vitamin D catabolism related to impaired kidney function. Overall, these findings may contribute to a better understanding of racial/ethnic differences in the complex associations of vitamin D and PTH with cardiovascular risk and thus inform the design of future clinical interventions to reduce racial/ethnic disparities related to CVD.

My findings may be explained by the pleiotropic effects of the vitamin D-PTH endocrine system on the cardiovascular system. Both vitamin D and PTH receptors are expressed in vascular smooth muscle and endothelium. Vitamin D stimulates insulin secretion and action, regulates the RAAS, and inhibits pro-inflammatory cytokine production, which can induce insulin resistance and inflammation-linked vascular endothelial dysfunction (6-8). PTH plays an important role in calcium metabolism, the RAAS, endothelial function, and systemic inflammation and may therefore influence cardiovascular risk independently or jointly with vitamin D (98,111-113). PTH increases endothelial expression of factors implicated in endothelial dysfunction and atherosclerosis, including IL-6, endothelin-1, and the receptor of advanced glycation end products (113). Elevated PTH levels also stimulate the release of cytokine IL-6 from liver

and osteoblasts and may in turn increase hepatic production of CRP (114-116). PTH independently affects glucose/insulin metabolism via either direct action on beta-cells or indirectly through elevating intracellular calcium in major target tissues for insulin (117,118).

The present study has several strengths. The well-characterized cohort of white and black postmenopausal women allowed us to thoroughly examine and address racial/ethnic disparities in the associations of total 25(OH)D and PTH with a panel of core cardiometabolic biomarkers. Many potential confounders were accounted for in my analysis, and participants with rigorously adjudicated CVD were excluded. Further, I used a novel analytic approach with 2-D contour plots to visually illustrate and elucidate possible differences in the complex concurrent associations of total 25(OH)D and PTH with cardiometabolic biomarkers between whites and blacks, by simultaneously taking account of possible nonlinear relations and interactions, as well as controlling confounding from conventional risk factors.

This study also has some limitations. First, I used the HOMA indexes as surrogate measures for assessing insulin resistance and beta-cell function, because they have been widely used and validated in epidemiological and clinical studies (52,106,107). However, there is evidence that the HOMA model may underestimate insulin sensitivity and overestimate beta-cell function, without incorporating proinsulin secretion (52,107). C-peptide, as a proinsulin cleavage product, has been recognized as a robust marker of insulin secretion; nonetheless, the c-peptide assay requires additional cost and critical calibration compared to insulin data (52,119). Further research with a more reliable and feasible marker of insulin secretion and activity is needed to confirm my results. Second,

this study lacked directly measured free and bioavailable 25(OH)D or 1,25(OH)D, which may be a better measure of vitamin D activity than total 25(OH)D. However, their measures using different assays have not been rigorously validated or standardized for large population studies. Third, the present study was cross-sectional in design that cannot be for inferring any cause-to-effect relation. Fourth, there still may be the potential for residual or unmeasured confounding, such as sun exposure and dietary or supplemental vitamin D intake. Finally, the lack of data on U.S. minority groups other than blacks limits the generalizability of the findings.

In conclusion, I found a similar pattern of joint associations of total 25(OH)D and PTH with insulin resistance between U.S. postmenopausal white and black women, but black-white differences in the associations of total 25(OH)D and PTH with biomarkers of beta-cell function, systemic inflammation, and kidney function. Future longitudinal studies are warranted to determine race/ethnicity-specific thresholds of both 25(OH)D and PTH levels and their trajectories in relation to future risk of cardiometabolic diseases. Although the mechanisms underlying racial disparities in cardiometabolic biomarkers attributable to the vitamin D-PTH endocrine system still remain to be elucidated, the present study with assessment of the full spectrum of vitamin D and PTH levels provides empirical data to augment and complement our mechanistic understanding of the overall null findings from clinical trials on the effect of vitamin D supplementation on several health outcomes (120,121). Further studies are needed to determine the optimal dose of vitamin D supplements.

Chapter 4

Combined Associations of 25(OH)D and PTH with Diabetes Risk and Related Cardiometabolic Comorbidities in U.S. White and Black Postmenopausal Women

4.1 Introduction

There is consistent evidence that African Americans tend to have lower total 25(OH)D levels than white Americans do, potentially due to reduced cutaneous biosynthesis of vitamin D (19,122). Disparities also exist in cardiometabolic risk between blacks and whites (80). Vitamin D and PTH endocrine system, through binding to the nuclear vitamin D receptor in a variety of tissues, regulates insulin/glucose metabolism, the RAAS, endothelial function, immune response modulation, cell differentiation and growth, and vascular and cardiac cell function (6-10). Despite inconclusive results from randomized clinical trials regarding the effect of vitamin D supplementation on risk of diabetes and its metabolic risk factors (121,123), observational studies have consistently associated vitamin D deficiency with diabetes-related cardiometabolic disorders, such as obesity, hypertension, and chronic kidney disease (124-126).

Emerging evidence has also suggested the potential synergistic effects of vitamin D and PTH on cardiometabolic outcomes (18). However, few studies have specifically examined joint associations of circulating 25(OH)D and PTH levels with diabetes development, or with risk of related comorbid cardiometabolic conditions in a multiethnic setting. Thus, the combined assessment of 25(OH)D and PTH may provide an optimal evaluation of the influence of vitamin D and PTH endocrine system on diabetes and its associated cardiometabolic comorbidities. As previously reported, in the WHI-OS cohort, PTH excess was associated with incident CVD in white women only

after adjusting for diabetes status at baseline, regardless of 25(OH)D levels (86). Considering the potential modifying effect of diabetes and growing evidence of long-term risk for diabetic CVD (127), further investigation is needed to evaluate the combined associations of 25(OH)D and PTH with CVD risk among diabetic women compared to non-diabetic women.

In the present study, using data from the same WHI-OS cohort, I specifically examined 1) the joint associations of 25(OH)D and PTH with risk for developing diabetes among U.S. white and black postmenopausal women; 2) the joint associations of 25(OH)D and PTH with prevalent risk of cardiometabolic comorbidities, including obesity, hypertension, and chronic kidney disease, among diabetic women compared to non-diabetic women; and 3) whether plasma levels of 25(OH)D and PTH are jointly associated with incident CVD among diabetic women compared to non-diabetic women, and whether these associations vary between white and black women.

4.2 Methods

4.2.1 Study population

This study used data from an existing case-cohort study that is an ancillary study of the WHI-OS that enrolled 93,676 postmenopausal women aged 50 to 79 years from 40 clinical centers throughout the United States from 1994 to 1998 (86). In this prospective case-cohort study, 4,850 women were selected after excluding those with history of stroke or myocardial infarction (MI) or who were receiving dialysis at baseline, yielding a randomly chosen subcohort of 2800 participants at baseline and a total of 550 incident cases of CVD in black women and 1500 incident cases of CVD in non-Hispanic white

women. CVD events were ascertained by self-report and medical records, including incident nonfatal MI, nonfatal stroke, or CVD mortality, as of the September 2013 database. Details of the WHI-OS cohort and the ancillary study design have been reported elsewhere (86).

Of the 4,850 women, 622 diabetic women and 4,228 non-diabetic women were identified at baseline. After removing 37 women who were missing information on newly diagnosed diabetes, data on 4,191 women were analyzed to examine the prospective associations between vitamin D biomarkers and incident diabetes among high-risk women. I further prospectively assessed the associations between vitamin D biomarkers and CVD development among diabetic women compared to non-diabetic women. Of 2,050 cases of CVD, 355 cases were diabetic women and 1,695 cases were non-diabetic women at baseline. All participants provided written informed consent and the study was approved by institutional review boards at each participating institution.

4.2.2 Outcomes

Prevalent and Incident Diabetes. Prevalent diabetes was defined as a self-report of ever having received a physician diagnosis of diabetes when not pregnant or fasting plasma glucose levels of 126 mg/dL or higher at baseline. Incident treated diabetes was defined as a self-report of a new physician diagnosis of diabetes treated with oral drugs or insulin shots during study follow-up (128).

Diabetes-related cardiometabolic comorbidities. Obesity was defined as BMI $\geq$ 30 kg/m^2, calculated based on clinical measurements of height and weight at baseline. Hypertension was defined as self-report of current treatment for hypertension, and/or self-report of being told by a doctor they had high blood pressure, and/or baseline

measurement of systolic blood pressure ≥ 140mm Hg or diastolic blood pressure ≥ 90mm Hg (129). CKD was defined as an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 at study baseline. A composite endpoint of obesity, hypertension, or CKD was classified as no comorbidity or at least one comorbidity. In the ancillary study, CVD outcomes were ascertained by self-report using annual questionnaires and documented by medical records, including nonfatal MI, nonfatal stroke (hemorrhagic/ischemic), and CVD mortality. Nonfatal MI was defined as acute MI that required overnight hospitalization and was confirmed by electrocardiography or cardiac enzyme elevations. Nonfatal stroke was diagnosed based on the rapid onset of a neurologic deficit lasting more than 24 hours, requiring hospitalization and supported by imaging studies when available (86). CVD mortality was defined as a death due to definite or possible diagnoses of coronary heart disease, pulmonary embolism, cerebrovascular disease, or other or unknown cardiovascular conditions.

4.2.3 Biomarker measurement

A 12-hour fasting blood sample was collected at baseline from each participant and stored at -80°C until assay. All biochemical assays were completed by Dr. Nader Rifai's Clinical & Epidemiologic Research Laboratory. Plasma total 25(OH)D was assayed using an enzyme immunoassay with a competitive binding technique from Immunodiagnostic Systems Inc. (Fountain Hills, AZ). PTH, creatinine, and fasting glucose were measured by an electrochemiluminescence immunoassay on the Roche E Modular system using Roche Diagnostic reagents (Roche Diagnostics, Indianapolis, IN). Mean intra-assay coefficients of variation for each analyte were 6.95% for 25(OH)D, 3.46% for PTH, 1.82% for creatinine, and 3.26% for fasting glucose.

eGFR was calculated using the CKD-EPI equation, which provides more-accurate prognostic information in the higher eGFR range than the MDRD equation (88). eGFR (in ml/min/1.73 m^2) = 141 x min(creatinine/κ, 1)$^{\alpha}$ x max(creatinine/κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018 (if female) x 1.159 (if Black); where, creatinine = standardized serum creatinine measures in mg/dL; κ = 0.7 for females or 0.9 for males; α = -0.329 for females or -0.411 for males; min = indicates the minimum of creatinine/κ or 1; max = indicates the maximum of creatinine/κ or 1; Age = years.

4.2.4 Covariates

Information on age (year), race/ethnicity (white or black), clinical center (Southern: <35°N, Middle: 35-40°N, and Northern: >40°N), education (≤ high school graduate/GED, post high school, and college graduate or higher), season of blood draw (Spring, Summer, Autumn, and Winter), smoking status (never, past, and current), alcohol consumption (never, past, and current), physical activity levels (MET-hour/week), family history of diabetes, family history of CVD (yes or no), history of high cholesterol (yes or no), postmenopausal hormone therapy use (never, past, and current), and statin use (yes or no) were obtained by self-report questionnaires from each participant at baseline. Physical measurements (including height, weight, and blood pressure) were assessed by clinical interviews (87). BMI (kg/m^2) was calculated based on clinical measurements of height and weight.

4.2.5 Statistical analysis

I compared the baseline characteristics of white and black women using the Wilcoxon rank sums test or Chi-square test. Age-adjusted Spearman correlated

coefficients were calculated to explore associations between vitamin D biomarkers and cardiometabolic biomarkers among non-diabetic women, stratified by race/ethnicity.

To evaluate the prospective associations between vitamin D biomarkers and risk for incident diabetes, I fitted inverse-probability weighted Cox proportional-hazards models in women without diabetes at baseline. Follow-up time was calculated from enrollment to self-reported endpoint at annual visit, to death, or to last follow-up as of September 2013. In the analysis for independent associations, baseline total 25(OH)D and PTH levels were modeled as both continuous variables and quartiles. To assess their joint associations with diabetes, I also divided the sample into four subgroups by 25(OH)D (<50 nmol/L vs. ≥50 nmol/L) and PTH (≤65 pg/mL vs. >65 pg/mL), with women having high 25(OH)D levels (>50 nmol/L) and low PTH levels (≤65 pg/mL) as the reference group. I further conducted a race/ethnicity-stratified analysis to examine the possible black-white differences in the associations between vitamin D biomarkers and incident diabetes. My main models were adjusted for age, race/ethnicity, clinical center, BMI, education, season of blood draw, smoking status, alcohol consumption, physical activity levels, family history of diabetes, and postmenopausal hormone therapy use.

To take into account the sampling strategies used in the case-cohort study, I compared the weighted distributions of each vitamin D biomarker stratified by status of diabetes at baseline between white and black women, as well as between women with and without any related cardiometabolic comorbidity. To cross-sectionally examine the independent and joint associations of vitamin D biomarkers with risk of diabetes-related cardiometabolic comorbidities, weighted logistic regression models of women with and without prevalent or incident diabetes among all participants and each racial/ethnic group

were constructed. In the main models for prevalent obesity and a composite cardiometabolic endpoint, I controlled for age, race/ethnicity, clinical center, education, season of blood draw, smoking status, alcohol consumption, physical activity, and postmenopausal hormone therapy use. BMI was additionally adjusted in the analyses of prevalent CKD and hypertension. I also added the interaction term between race/ethnicity and each vitamin D biomarker into the main models to test for black-white differences. Multivariable weighted logistic regression models were also constructed to assess the joint associations of 25(OH)D and PTH with risk of the composite endpoint in both diabetic and non-diabetic women, separately.

To further prospectively examine the associations of vitamin D biomarkers with incident CVD in women with and without baseline diabetes, weighted Cox proportional-hazards models were constructed. I defined follow-up time as the interval between enrollment and the first occurrence of: adjudication date of CVD onset, last follow-up date, or date of reported death. Covariates adjusted in the main model included age, race/ethnicity, clinical center, BMI, education, season of blood draw, smoking status, alcohol consumption, physical activity, postmenopausal hormone therapy use, family history of CVD, and eGFR. A stratified analysis was performed for each CVD event among white and black women, separately. I also tested the interaction between each vitamin D biomarker and diabetes status at baseline by including an interaction term in each model and performed the analysis for their joint association with incident CVD using the four aforementioned predefined subgroups of vitamin D biomarkers among diabetic women compared to non-diabetic women, not only among all participants, but in each racial/ethnic group.

To test the robustness of my results and explore potential effect modifiers, I also performed several sensitivity analyses by further adjusting for eGFR (for incident diabetes, and prevalence of obesity and hypertension only), history of high cholesterol, and statin use and defining prevalent diabetes as a self-report of ever having received a physician diagnosis of diabetes, without fasting glucose ≥126 mg/dL at baseline. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

4.3 Results

4.3.1 Population characteristics

Table 6 depicts the characteristics of white and black women at baseline. Compared to white women, black women were younger and had higher BMI, lower physical activity and education, were more likely to be current smokers and have a family history of diabetes, and were less likely to have a family history of CVD, current hormone therapy and statin use, or current alcohol consumption. White women had significantly higher total 25(OH)D levels and lower PTH levels than black women. While black women had higher levels of creatinine, hs-CRP, fasting glucose and insulin, and eGFR than white women, the prevalence of diabetes, obesity and hypertension was also higher in black women. With an average follow-up of 12.1 years, I identified 453 incident cases of treated diabetes among 4,191 women without diabetes at baseline, 3,322 prevalent cases of obesity, hypertension, or CKD, and 2,050 incident cases of CVD. The incidence rates of diabetes and CVD were 9.5 and 40.7 per 1000 person-years, respectively.

Table 6. Baseline characteristics of postmenopausal women without CVD at baseline from the WHI-OS by ethnicity.

Variables		Blacks (n=1850)	Whites (n=3000)	p-value
Age (years)	mean ± SD	62.7 ± 7.5	65.7 ± 7.3	< 0.0001
BMI (kg/m^2)	median (IQR)	29.7 (26.2, 34.5)	26.2 (23.3, 30)	< 0.0001
Family history of CVD	n (%)	740 (44.3%)	1594 (56.2%)	< 0.0001
Family history of diabetes	n (%)	850 (46.3%)	882 (29.6%)	< 0.0001
History of high cholesterol	n (%)	297 (16.4%)	483 (16.4%)	0.999
Physical activity (MET-hour/week)	median (IQR)	6.3 (1, 15.1)	9.6 (3.4, 19)	< 0.0001
Smoking status	n (%)			
Never		887 (48.7%)	1425 (48.3%)	
Past		718 (39.4%)	1338 (45.4%)	< 0.0001
Current		216 (11.9%)	187 (6.3%)	
Alcohol consumption	n (%)			
Never		350 (19.1%)	295 (10%)	
Past		610 (33.3%)	581 (19.5%)	< 0.0001
Current		873 (47.6%)	2098 (70.5%)	
Hormone therapy use	n (%)			
Never use		1110 (60.2%)	1194 (39.8%)	
Past use		247 (13.4%)	506 (16.9%)	< 0.0001
Current use		488 (26.4%)	1298 (43.3%)	
Statin use	n (%)	285 (15.4%)	532 (17.7%)	0.035
Educational Levels	n (%)			
≤ High school graduate/GED		502 (27.1%)	663 (22.1%)	
Post high school		694 (37.5%)	1111 (37%)	< 0.0001
College graduate or higher		654 (35.4%)	1226 (40.9%)	
Geographical latitude (Clinical center)	n (%)			
Southern: < 35 degrees N		617 (33.4%)	862 (28.7%)	
Middle: 35-40 degrees N		624 (33.7%)	865 (28.8%)	< 0.0001
Northern: > 40 degrees N		609 (32.9%)	1273 (42.4%)	
Season of blood draw	n (%)			
Spring		532 (29%)	870 (29.1%)	
Summer		491 (26.8%)	854 (28.5%)	0.36
Autumn		403 (22%)	661 (22.1%)	
Winter		406 (22.2%)	606 (20.3%)	
Vitamin D biomarkers				
Total 25(OH)D, nmol/L	median (IQR)	42.4 (33.4, 54.6)	61.6 (49.7, 75.1)	< 0.0001
PTH, pg/mL	median (IQR)	40.9 (31.7, 52.6)	37 (29.3, 46.4)	< 0.0001
Cardiometabolic biomarkers				
Creatinine, mg/dL	median (IQR)	0.78 (0.69, 0.89)	0.72 (0.64, 0.81)	< 0.0001
hs-CRP, mg/L	median (IQR)	3.6 (1.5, 7.8)	2.5 (1.1, 5.8)	< 0.0001
Glucose, mg/dL	median (IQR)	95 (87, 107)	94 (88, 101)	0.001
Insulin, uIU/mL	median (IQR)	9.2 (5.8, 13.8)	7.2 (4.9, 11.2)	< 0.0001

eGFR, mL/min/1.73 m^2	mean ± SD	91.9 ± 19.4	83.7 ± 14.1	< 0.0001
Prevalent Diabetes	n (%)	370 (20.0%)	252 (8.4%)	< 0.0001
Diabetes-related comorbidities				
Obesity	n (%)	881 (48.5%)	748 (25.2%)	< 0.0001
Hypertension	n (%)	1243 (67.6%)	1516 (50.8%)	< 0.0001
Chronic kidney disease	n (%)	121 (6.6%)	197 (6.6%)	0.926

4.3.2 Correlations between vitamin D biomarkers and cardiometabolic biomarkers among non-diabetic women

As expected, total 25(OH)D and PTH were significantly and inversely correlated across racial/ethnic groups among all non-diabetic women (**Table 7).** Also, creatinine, hs-CRP, fasting glucose and insulin were inversely correlated with 25(OH)D and positively correlated with PTH. After stratifying by race/ethnicity, the correlation between 25(OH)D and creatinine turned from inverse to positive, while all others persisted among white women. In black women, only the inverse correlation between 25(OH)D and fasting insulin remained significant.

Table 7. Age-adjusted Spearman's correlation coefficients between vitamin D biomarkers and cardiometabolic biomarkers among non-diabetic women stratified by race/ethnicity.

Biomarkers	Total 25(OH)D	PTH	Creatinine	hs-CRP	Glucose	Insulin
All non-diabetic women (n=4228)						
Total 25(OH)D	1	-0.36**	-0.07**	-0.09**	-0.08**	-0.2**
PTH		1	0.09**	0.08**	0.09**	0.15**
Creatinine			1	0.02	-0.01	0.09**
hs-CRP				1	0.11**	0.32**
Glucose					1	0.43**
Insulin						1
American white non-diabetic women (n=2748)						
Total 25(OH)D	1	-0.3**	0.06**	-0.06**	-0.14**	-0.2**

PTH		1	0.05*	0.06**	0.1**	0.12**
Creatinine			1	-0.01	-0.02	0.05**
hs-CRP				1	0.08**	0.29**
Glucose					1	0.44**
Insulin						1
American black non-diabetic women (n=1480)						
Total 25(OH)D	1	-0.36**	0.02	-0.03	-0.04	-0.1**
PTH		1	0.07**	0.07**	0.1**	0.16**
Creatinine			1	0.01	0.02	0.07**
hs-CRP				1	0.16**	0.34**
Glucose					1	0.41**
Insulin						1

*p<0.05; **p<0.01.

4.3.3 Associations between vitamin D biomarkers and incident diabetes

In the multivariate-adjusted analysis, as expected, I found that higher 25(OH)D levels were significantly and independently associated with decreased risk for developing diabetes in a dose-response manner among all participants and among white women [hazard ratio (HR)=0.78, 95% CI: 0.68-0.88; P for linear trend<0.0001]. However, I observed no significant association between PTH and diabetes (**Supplemental Table C-1 in Appendix C**). When assessed jointly, compared to those with both non-deficient levels of 25(OH)D (≥50 nmol/L) and normal PTH levels (≤65 pg/mL), women with deficient 25(OH)D levels (<50 nmol/L) had significantly higher risk for developing diabetes, whether they had excess PTH levels (HR=1.92; 95% CI: 1.42-2.58) or normal PTH levels (HR=1.32; 95% CI: 1.12-1.57). The observed joint association of vitamin D deficiency and PTH excess with incident diabetes remained significant and became even stronger in white women (**Figure 5).** Compared to the same reference group, white women with deficient 25(OH)D levels (<50 nmol/L) had higher risk of incident diabetes, regardless of their PTH levels (HR=1.46; 95% CI: 1.20-1.78 for those with normal PTH levels;

HR=2.30; 95% CI: 1.51-3.49 for those with excess PTH levels). Based on the HR being 1.46 for the group with vitamin D deficiency alone and 0.88 for the group with PTH excess only, the excess relative risk (ERR) related to deficient 25(OH)D levels was calculated as1.46-1=0.46 and the ERR related to PTH excess was -0.12, separately. In this sense, the joint influence of deficient 25(OH)D and excess PTH levels on incident diabetes (HR=2.30) showed a trend towards synergism on an additive scale [relative excess risk due to interaction (RERI)=1.30-0.46-(-0.02)=0.96] in white women, but was not statistically significant. However, there was no evidence of such an association among black women.

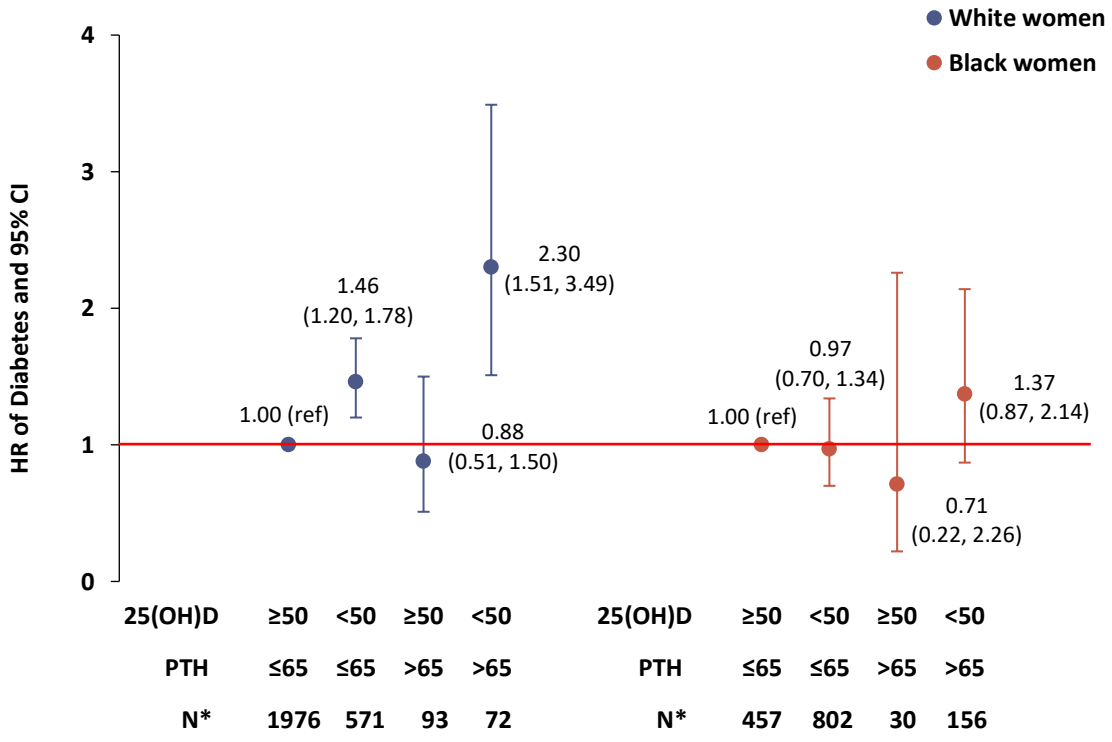
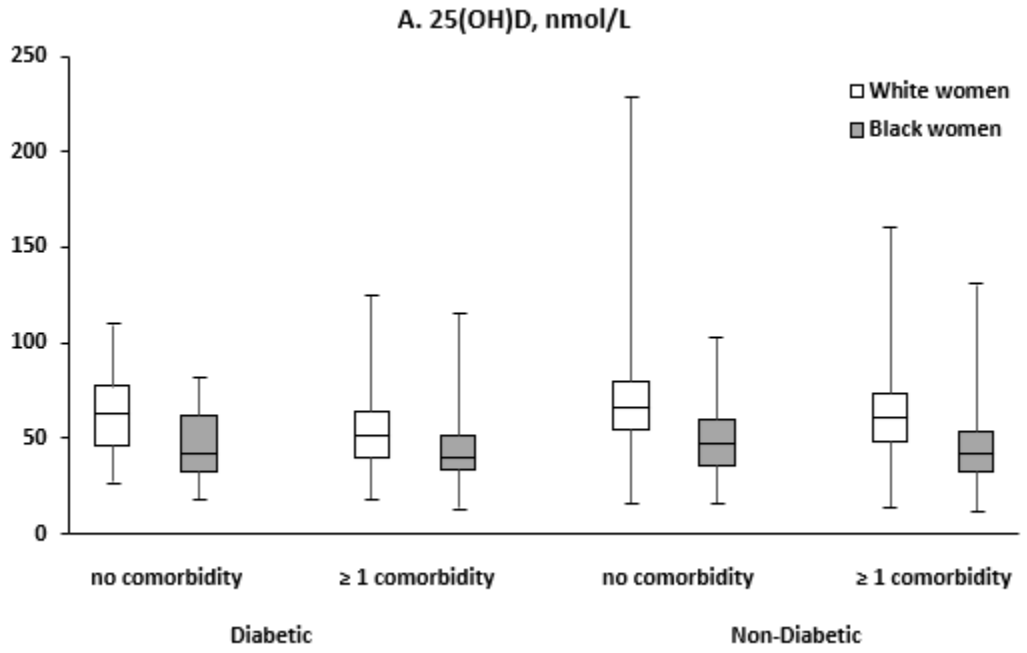


Figure 5. Joint associations of 25(OH)D and PTH with incident diabetes among U.S. White and Black women. Models were adjusted for age, clinical center, race/ethnicity, BMI, family history of diabetes, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw. *N represents number of participants in each group.

4.3.4 Associations between vitamin D biomarkers and prevalent risk of obesity, hypertension, and CKD

Weighted distributions of 25(OH)D and PTH differed significantly between white and black women when stratified by diabetes status and cardiometabolic comorbidities (all P-values<0.0001; **Figure 6**). In addition, there were consistent differences in their distributions between white and black women within the group with at least one comorbidity and the other without any comorbidity, separately (all P-values<0.0001).



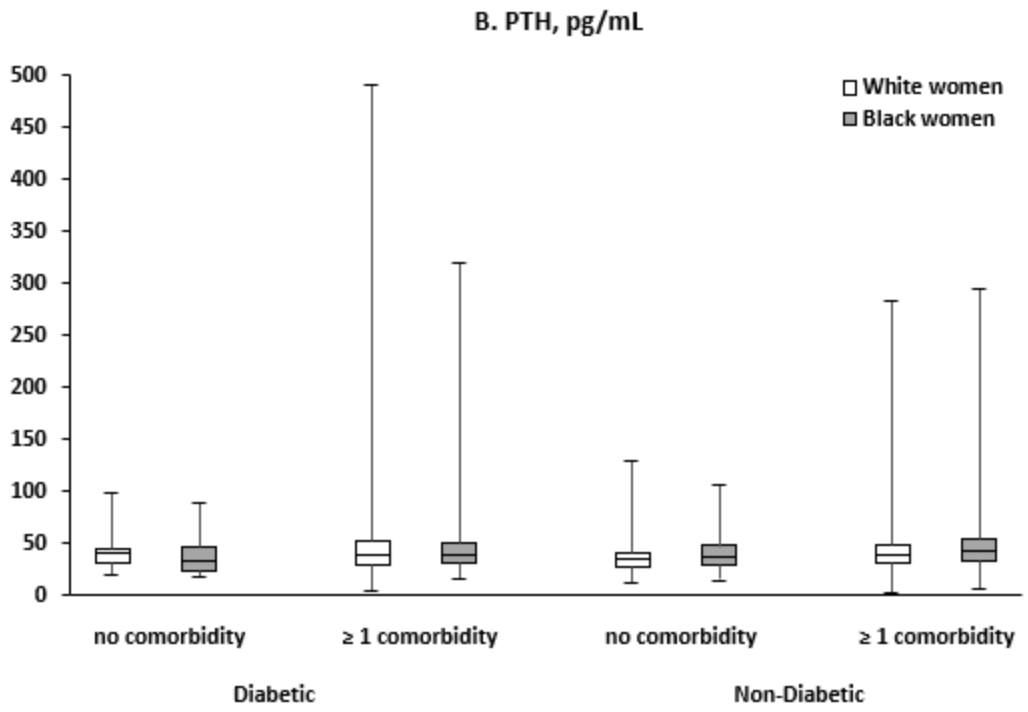


Figure 6. Distributions of 25(OH)D (A) and PTH (B) by diabetes status at baseline and cardiometabolic comorbidities among U.S. White and Black women.

Individually, lower 25(OH)D or higher PTH levels were associated with greater risks of obesity and the composite endpoint across racial/ethnic groups, whereas their independent associations with hypertension were statistically significant in white women only (**Supplemental Table C-2 and Supplemental Table C-3 in Appendix C**). In addition, I found a positive relationship between PTH and CKD among both white and black women (**Supplemental Table C-3 in Appendix C**). These results were not modified by additional adjustment for history of high cholesterol, statin use, and eGFR (for obesity and hypertension only). When 25(OH)D and PTH were assessed jointly (**Figure 7**), women with either deficient 25(OH)D (<50 nmol/L) or excess PTH levels (>65 pg/mL) had a higher risk of obesity, hypertension, or CKD, compared to those with higher 25(OH)D (≥50 nmol/L) and normal PTH levels (≤65 pg/mL), regardless of

diabetes status. However, the combination of deficient 25(OH)D levels and PTH excess was associated with elevated risk for at least one cardiometabolic outcome among women without prevalent or incident diabetes (OR=4.23, 95% CI: 2.90-6.18), but not for those who had diabetes at baseline or who developed diabetes later (OR=1.89, 95% CI: 0.96-3.71).

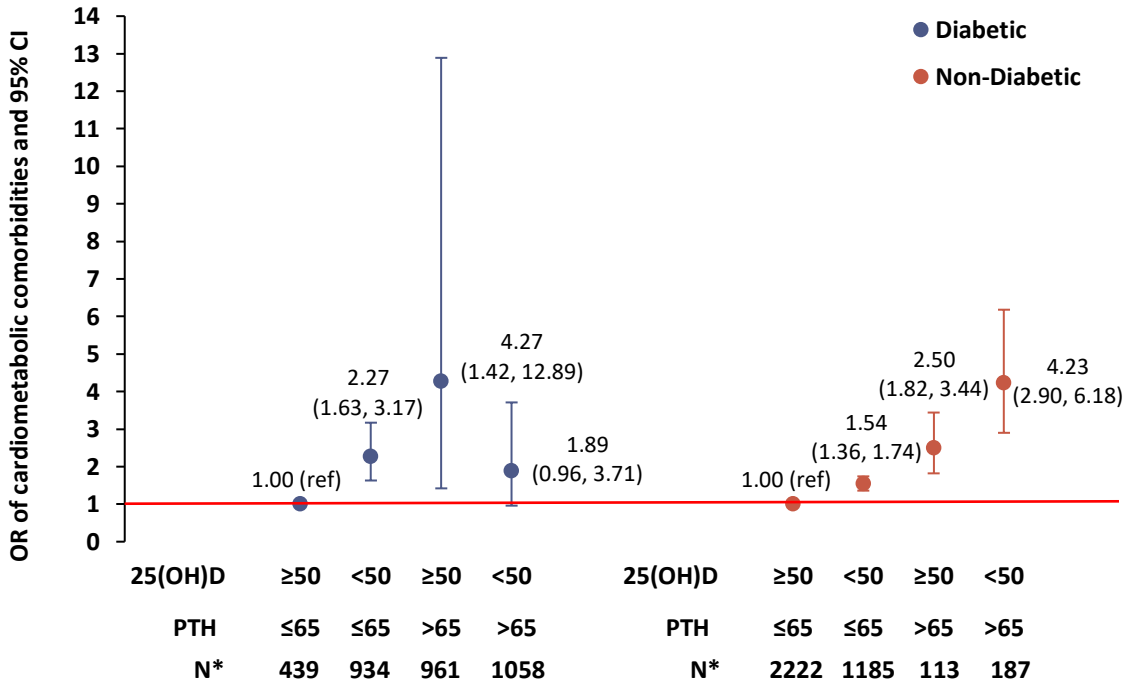


Figure 7. Joint associations of 25(OH)D and PTH with prevalence of cardiometabolic comorbidities between women with and without prevalent or incident diabetes. Models were adjusted for age, clinical center, race/ethnicity, family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw. *N represents number of participants in each group.

4.3.5 Associations between vitamin D biomarkers and incident CVD

After adjusting for potential confounding factors, PTH was positively associated with risk for developing CVD among non-diabetic women only (**Supplemental Table C-4 in Appendix C**). The observed association between PTH and CVD persisted in white

women without diabetes at baseline, but not for black women (P for interaction by diabetes status=0.041). In contrast, 25(OH)D was inversely associated with risk of CVD among white women without diabetes, after adjusting for age, race/ethnicity, and clinical center. But the association became attenuated and non-significant after additional adjustment for other covariates (**Supplemental Table C-5 in Appendix C**). The difference in joint influence of 25(OH)D and PTH on the risk for developing CVD between women with and without diabetes at baseline is presented in **Figure 8**. Compared to the reference group with non-deficient vitamin D (≥50 nmol/L) and normal PTH levels (≤65 pg/mL), non-diabetic women with either deficient 25(OH)D (<50 nmol/L) (HR=1.25; 95% CI: 1.02-1.52) or excess PTH (>65 pg/mL) (HR=1.61; 95% CI: 1.02-2.56) or both (HR=1.76; 95% CI: 1.18-2.61) were at higher risk for developing CVD. However, these associations were not observed in women with diabetes at baseline. When further stratified by race/ethnicity (**Figure 9A-B**), the combination of deficient 25(OH)D (<50 nmol/L) and excess PTH levels (>65 pg/mL) was significantly associated with increased risk of developing CVD in white women without diabetes at baseline (**Figure 9B**; HR=2.96; 95% CI: 1.61-5.45). In addition, the adjusted HR of 2.96 was higher than the expected association based on their independent association effect sizes on an additive scale (i.e. RERI=1.96-0.22-0.65=1.09) with statistical significance, which suggested a possible synergistic effect of vitamin D deficiency and PTH excess on CVD risk in white women without diabetes.

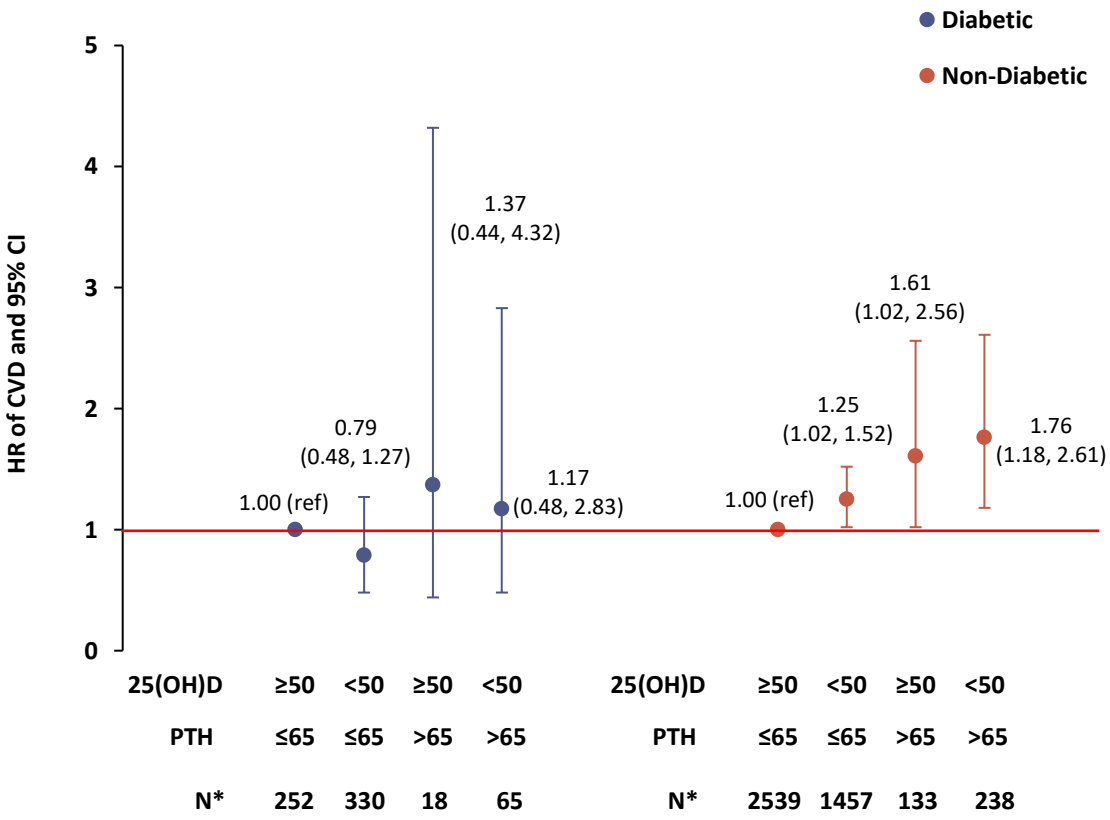


Figure 8. Joint associations of 25(OH)D and PTH with incident CVD by diabetes status at baseline. Models were adjusted for age, clinical center, race/ethnicity, eGFR, BMI, family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw. *N represents number of participants in each group.

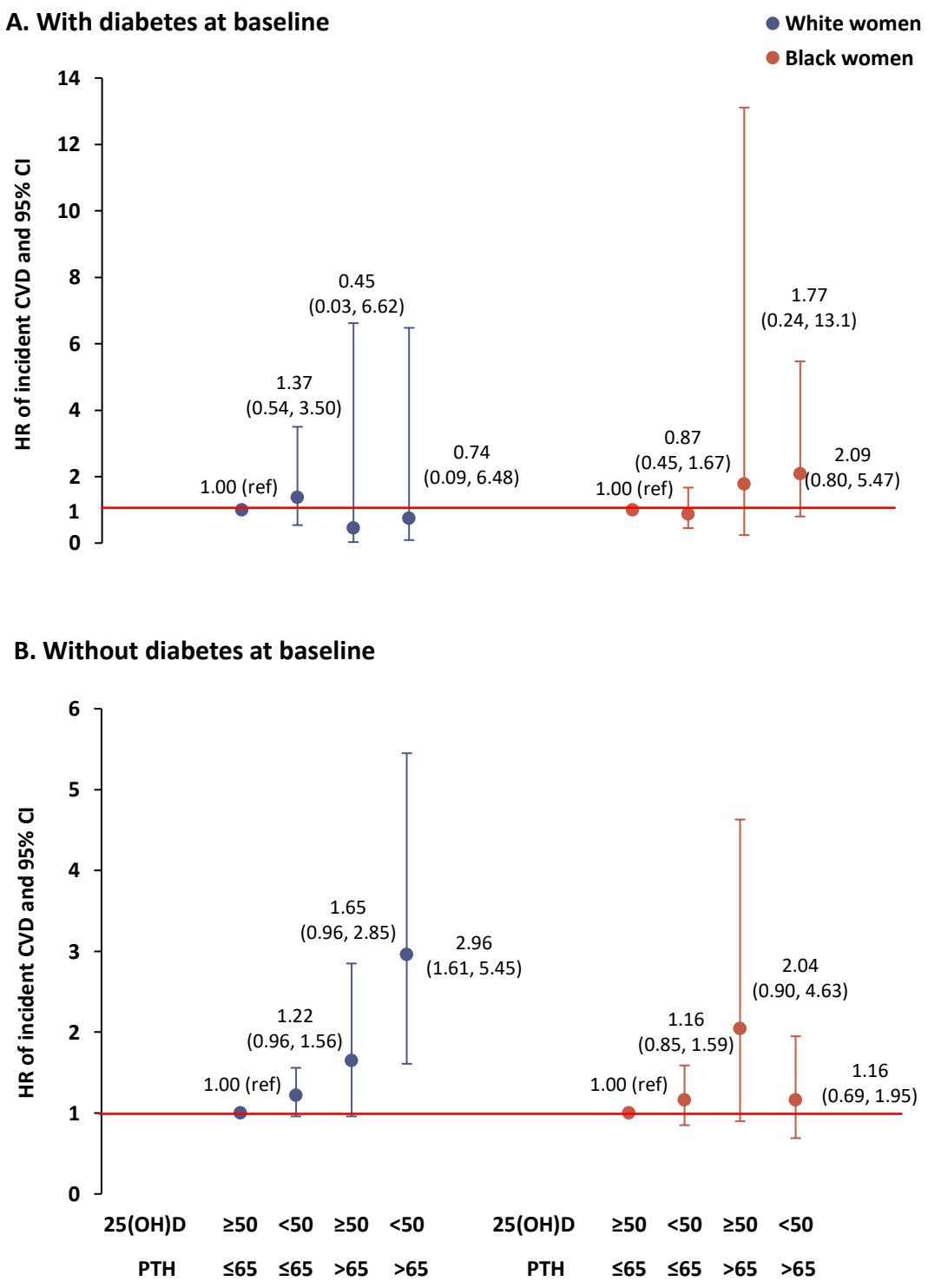


Figure 9. Joint associations of 25(OH)D and PTH with incident CVD stratified by race/ethnicity and diabetes status (A with and B without any prevalent or incident diabetes). Models were adjusted for age, clinical center, eGFR, BMI, family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw.

Even after changing my definition of prevalent diabetes to self-report of diagnosed diabetes alone, the results remained robust (data not shown).

4.4 Discussion

In this large, ethnically diverse cohort of U.S. postmenopausal women, deficient 25(OH)D levels (<50 nmol/L) were significantly associated with increased risk for developing diabetes in white women only, regardless of PTH levels. Compared to those with non-deficient levels of 25(OH)D (≥50 nmol/L) and normal PTH levels (≤65 pg/mL), women with either deficient 25(OH)D or excess PTH levels had a greater risk of obesity, hypertension, or CKD, irrespective of their diabetes status; whereas the combination of vitamin D deficiency and PTH excess was associated with increased risk of at least one of the aforementioned cardiometabolic comorbidities among non-diabetic women only. Similarly, deficient 25(OH)D and excess PTH levels were jointly associated with increased risk for incident CVD among non-diabetic women, especially white women. My findings suggest that vitamin D and PTH endocrine system may be involved in diabetes development, especially among white women; and that their combined associations with cardiometabolic outcomes such as obesity, hypertension, CKD, and CVD appeared to be more pronounced among non-diabetic women and largely attenuated by diabetes status.

4.4.1 Vitamin D and PTH with incident diabetes

My findings agree with the results of two recent prospective population-based cohort studies and a meta-analysis of observational studies assessing independent

association of vitamin D with diabetes development (130-132). Data from a U.S. cohort of 903 Caucasian adults aged 74 years old on average and free of diabetes or pre-diabetes at study entry through a 12.5-year follow-up period suggested that 25(OH)D was inversely associated with incidence of diabetes in a dose-response manner, after adjusting for age, BMI, waist circumference, calcium supplement intake, triglycerides, and HDL-cholesterol (130). Another German population study of 7,791 initially diabetes-free subjects aged 50-74 years reported a non-linear inverse relationship between 25(OH)D and risk of diabetes with a threshold of below 40 nmol/L in women only after a 8-year follow-up, accounting for age, sex, season of blood draw, multi-vitamin supplement intake, frequent fish consumption, BMI, HbA1C, family history of diabetes, education, physical activity, smoking, hypertension, renal dysfunction, C-reactive protein, and fasting triglycerides (131). A recent randomized clinical trial in Canada among 96 individuals with vitamin D insufficiency [mean 25(OH)D=51.1 nmol/L at baseline] and at high risk of diabetes or with newly diagnosed type 2 diabetes also found that vitamin D3 supplementation (5,000 IU daily) for 6 months significantly increased peripheral insulin sensitivity and beta-cell function (123).

Data on joint associations between vitamin D and PTH with diabetes and related risk factors are limited. In line with my findings, a prospective study of 494 postpartum women in Canada suggested that vitamin D deficiency/insufficiency with PTH in the highest tertile at three months postpartum were associated with worsening beta-cell function and insulin sensitivity and increased fasting and 2-hour glucose nine months later, after controlling for age, ethnicity, family history of type 2 diabetes, previous gestational diabetes, BMI, glucose, duration of breast-feeding, physical activity, and

season of blood draw (73). Another case-control study in a Greek cohort of 144 patients aged ≥65 years with prediabetes and 81 healthy age-matched controls reported that individuals with 25(OH)D <50 nmol/L and PTH ≥40 pg/mL had significantly higher fasting plasma glucose levels than those with either 25(OH)D ≥50 nmol/L or PTH <40 pg/mL or both, after adjustment for age, sex, BMI, and season of sampling (104).

However, conflicting findings have also been reported. An Italian observational study in a cohort of 2,227 elderly Caucasians of 76.1 years average age found no association between baseline 25(OH)D and incidence of diabetes over 4.4 years follow-up, potentially due to a competing risk of death (22.4% lost to follow-up due to death) and an underestimation of newly diagnosed diabetes by using fasting glucose only (133). Recently, a meta-analysis of 47 randomized controlled trials involving 44,161 non-diabetic individuals found no effect of vitamin D supplementation on incidence of type 2 diabetes, but the majority of the trials included were very small and included different populations with non-deficient levels of 25(OH)D at baseline (mean levels of 41 nmol/L) (134). Their overall dose-response analyses suggested that vitamin D supplementation with a dose > 4000 IU/d is sufficient to achieve optimal 25(OH)D levels (>90 nmol/L) and improve glucose and insulin homeostasis (134). Similarly, a recent large randomized clinical trial (n=2,423) indicated that intake of 4,000 IU daily of vitamin D3 by prediabetic participants did not significantly lower risk of diabetes onset through a median follow-up of 2.5 years (121). Their null findings may be due mainly to adequate vitamin D status at baseline of the trial participants with mean 25(OH)D levels of 70.5 nmol/L at baseline, which may limit the ability of those researchers to detect a significant effect. It is generally recommended that a daily dose of vitamin D3 ranging from 2000

IU/day to 4000 IU/day may have beneficial effects on cardiometabolic health without potential toxic effects. However, further interventional studies are needed to determine the minimum effective dose of vitamin D to prevent or treat diabetes in at-risk populations, incorporating accurate assessment of vitamin D/PTH responses to intervention.

4.4.2 Vitamin D and PTH with cardiometabolic comorbidities

Similar to my findings for hypertension in white women, data from the third National Health and Nutrition Examination Survey indicated an inverse relationship between 25(OH)D and systolic blood pressure among white Americans (135). Conversely, a cross-sectional study in 1205 adults aged 65 years or older in the Netherlands (predominantly Caucasians) reported that higher PTH levels, unlike 25(OH)D, were associated with risk of hypertension with adjustments for age, sex, region, season, waist circumference, physical activity, smoking, and alcohol intake (136). Supporting my findings of an independent, positive association of CKD with PTH but not with 25(OH)D, the results from another recent cross-sectional study in a community population of 4,080 adults in Taiwan suggested that either elevated PTH levels or hyperparathyroidism was associated with higher risk of CKD after adjusting for age, sex, 25(OH)D, calcium, and phosphate (76). In contrast, an inverse association between 25(OH)D and risk of incident CKD was observed in a Chinese elderly cohort (n=1,037) accounting for potential confounders (137). While a large number of studies suggest that obesity is inversely associated with 25(OH)D and positively associated with PTH (138,139), there are also inconsistent results (140,141). For example, a retrospective

study of 316 patients in a Spanish hospital found that neither 25(OH)D nor PTH was associated with obesity after adjusting for potential confounding factors (140).

The association of vitamin D and PTH with CVD risk also remains controversial. As previously reported, higher circulating vitamin D levels were not associated with lower risk of incident CVD, whereas PTH excess may be significantly associated with CVD development in white women (86). My current estimates, using the same data, further confirm a null association between 25(OH)D and incident CVD but a positive association between PTH and CVD risk in white women, and extend these findings by showing the combined impacts of vitamin D deficiency and PTH excess on risk of CVD among nondiabetic white women. After comparing the characteristics of four groups, i.e., race/ethnicity (white or black) by diabetes status at baseline (yes or no), I found that differences in their levels of 25(OH)D, PTH, and hs-CRP were statistically significant (all P-values<0.0001). Compared to white women with diabetes (and all other groups, for that matter), white women without diabetes had higher mean 25(OH)D levels (64.3 nmol/L) and lower mean levels of PTH (39.7 pg/mL) and hs-CRP (4.65 mg/L). Both non-human experimental and epidemiological studies have suggested that an inflammatory state may reduce vitamin D concentrations and lead to dysregulation of immune and inflammatory responses (142,143). I speculate that thresholds for the cardiovascular effects of vitamin D and PTH may vary by race/ethnicity and inflammatory state. Further, I found that diabetic women had more extreme outliers in PTH levels compared to non-diabetic women (**Supplemental Figure C-1 in Appendix C**), potentially due to status of glycemic control (144). However, the extent to which glycemic control, inflammatory state, or other metabolic abnormalities among diabetic patients may disrupt or nullify the

impact of VD/PTH on cardiovascular health remains unknown. In addition, the relatively small number of diabetic women at baseline in the present study may also lead to the null associations observed in this group.

The present research is a large biracial study designed to comprehensively examine the combined associations of 25(OH)D and PTH with multiple established diabetes-related cardiometabolic comorbidities. The combined associations I observed between vitamin D deficiency and PTH excess with at least one cardiometabolic condition (i.e., obesity, hypertension, or CKD) and their joint influence on CVD development in non-diabetic women imply that the combined assessment of vitamin D and PTH may more accurately reflect the functional status of vitamin D relevant to cardiometabolic health compared with assessment of one factor alone. These results also indicate that the combined associations of deficient vitamin D and elevated PTH levels with the aforementioned cardiometabolic conditions may vary depending on diabetes status. My estimated partial Spearman's correlations show that creatinine, hs-CRP, as well as fasting glucose and insulin, were inversely correlated with 25(OH)D and positively correlated with PTH among nondiabetic individuals. Among diabetic women, I found that 25(OH)D was still inversely and significantly associated with fasting glucose but not with fasting insulin, while PTH was not associated with either fasting glucose or fasting insulin in white diabetic women. Overall, my findings may help explain inconsistent results regarding the associations of vitamin D and PTH with cardiometabolic conditions, and thus inform the design of future observational and interventional studies assessing the impact of vitamin D and PTH endocrine system on cardiometabolic health.

4.4.3 Potential mechanisms

It is well documented that excess activation of the RAAS is associated with hypertension, CKD, diabetes, and CVD through its crucial role in the regulation of blood pressure, fluid volume, and electrolyte homeostasis (145-147). Mechanistic studies in both animals and humans have shown vitamin D to be a negative endocrine regulator of the RAAS (6,148). Increasing evidence also indicates a bi-directional relationship between PTH and the RAAS. While expression of both the angiotensin type I receptor and the mineralocorticoid receptor in parathyroid tissue may suggest a plausible mechanism of the RAAS action on PTH levels (149), PTH excess may stimulate adrenal aldosterone synthesis both directly via binding to PTH receptors and indirectly by stimulating renin release and thus increasing angiotensin II (150). In addition, vitamin D deficiency may directly promote adiposity accumulation, which may lead to greater production of PTH. PTH excess can increase calcium influx into adipocytes and thus enhances lipogenesis and inhibits lipolysis (151). Adipose tissue, on the other hand, may regulate vitamin D via vitamin D receptor in adipocytes. Vitamin D stored in excess fat tissue may become sequestered, reducing calcium absorption due to decreased bioavailability (152). Adiposity, in turn, induces the further secretion of PTH to maintain calcium levels. Other possible mechanisms underlying the complex interplay between vitamin D, PTH, and cardiometabolic disorders include stimulation of insulin secretion, inhibition of pro-inflammatory cytokine production, and modulation of endothelial functions, through the expression of their receptors in vascular smooth muscle and endothelial cells (7,8,113,153).

4.4.4 Strengths and Limitations

The present study has several strengths. The large and well-characterized cohort of white and black postmenopausal women with a long follow-up allowed us to address racial/ethnic disparities in the associations of total 25(OH)D and PTH with cardiometabolic risk. I was also able to examine multiple established diabetes-related cardiometabolic comorbidities, accounting for many potential confounders. However, the data source did not allow me to examine other diabetes-related cardiometabolic comorbid conditions. Although potential biases due to the inclusion of misclassified diabetes deserve attention, my sensitivity analyses yielded similar results with and without defining prevalent diabetes using a fasting glucose ≥ 126 mg/dl at baseline. Also, random measurement error from single measurement of biomarkers might have biased the results towards the null. However, this seems unlikely, since all the coefficients of variation were below 10% and were similar to those reported in the majority of previous population studies. In addition, residual or unmeasured confounding cannot be ruled out, such as that derived from sun exposure and dietary or supplemental vitamin D intake. Lastly, I am unable to generalize my results from postmenopausal women to men or other populations.

4.4.5 Conclusions

This prospective population-based cohort study suggested a joint influence of vitamin D deficiency and excess PTH on the risk of diabetes among U.S. white postmenopausal women. While either vitamin D deficiency or excess PTH was significantly associated with higher prevalence of obesity, hypertension, or CKD, irrespective of diabetes status, their combination may increase the risk of CVD in non-

diabetic women. Larger longitudinal studies are needed to confirm my results and fully address race/ethnicity-specific associations of 25(OH)D and PTH with these cardiometabolic conditions. Assuming such confirmation, future clinical trials are warranted to determine the effective dosage of vitamin D supplementation to prevent diabetes and other cardiometabolic conditions based on assessment of both 25(OH)D and PTH as integrated indicators of intervention response.

Chapter 5

Summary

In this research, I found that 1) maternal vitamin D deficiency as early as the first trimester of pregnancy was associated with an elevated risk for developing GDM, with a stronger association for women who were persistently deficient through the second trimester; 2) the joint associations of total 25(OH)D and PTH with biomarkers of beta-cell function, systemic inflammation, and kidney function apparently differed between U.S. white and black postmenopausal women; and 3) vitamin D deficiency and PTH excess were jointly associated with development of diabetes and non-diabetic CVD in white women only, whereas their combined associations with obesity, hypertension, and CKD might be non-diabetes-dependent. These findings implied that assessment of vitamin D status in combination with PTH may help us identify individuals at risk for cardiometabolic diseases. The present study provides evidence of the temporal association between vitamin D status and cardiometabolic risk among pregnant and postmenopausal women from racially/ethnically diverse groups, and contributes to elucidate possible black-white differences in these associations. These findings also enhance our understanding of the contribution of vitamin D-PTH endocrine system to racial disparities in cardiometabolic health. Further research is warranted to determine the race/ethnicity-specific thresholds of 25(OH)D and PTH for cardiometabolic risk and clarify their clinical utilities in evaluating effective dosage of vitamin D supplementation for the prevention and treatment of cardiometabolic diseases.

Appendices

Appendix A

Supplemental Table A-1. Partial Spearman correlation coefficients for vitamin D biomarkers and glucose homeostasis biomarkers adjusted for maternal age, the NICHD Fetal Growth Studies-Singleton cohort.

Biomarkers		Total 25(OH)D		Free 25(OH)D		Bioavailable 25(OH)D		VDBP		Fasting Glucose
		GWs 10-14	GWs 15-26	GWs 10-14	GWs 15-26	GWs 10-14	GWs 15-26	GWs 10-14	GWs 15-26	GWs 15-26
Total 25(OH)D	GWs 10-14	1	0.80**	0.30**	0.16**	0.31**	0.15**	0.25**	0.27**	-0.08
	GWs 15-26		1	0.22**	0.31**	0.24**	0.31**	0.22**	0.23**	-0.12*
Free 25(OH)D	GWs 10-14			1	0.76**	0.99**	0.75**	-0.80**	-0.66**	-0.10
	GWs 15-26				1	0.75**	0.99**	-0.68**	-0.82**	-0.14*
Bioavailable 25(OH)D	GWs 10-14					1	0.75**	-0.78**	-0.64**	-0.10
	GWs 15-26						1	-0.67**	0.81**	-0.15**
VDBP	GWs 10-14							1	0.85**	0.06
	GWs 15-26								1	0.09
Fasting Glucose	GWs 15-26									1

P values were adjusted for age.
GWs, gestational weeks.
* P<0.05; ** P<0.01.

Supplemental Table A-2. ORs for risk of GDM by season-specific quartiles of plasma vitamin D biomarkers at gestational weeks 10-14 and 15-26, the NICHD Fetal Growth Studies-Singleton cohort.

	OR (95%CI)				P-trend
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Gestational weeks 10-14					
Total 25(OH)D					
Model 1†	1	0.49 (0.24, 1.01)	0.46 (0.21, 1.03)	0.41 (0.19, 0.91)	0.04
Model 2‡	1	0.44 (0.20, 0.98)	0.42 (0.17, 1.03)	0.48 (0.20, 1.13)	0.12
Model 3§	1	0.48 (0.21, 1.09)	0.44 (0.17, 1.13)	0.45 (0.18, 1.08)	0.09
25(OH)D3					
Model 1†	1	0.52 (0.24, 1.09)	0.47 (0.21, 1.04)	0.38 (0.17, 0.87)	0.03
Model 2‡	1	0.47 (0.21, 1.08)	0.45 (0.18, 1.12)	0.45 (0.19, 1.09)	0.11
Model 3§	1	0.51 (0.22, 1.20)	0.45 (0.18, 1.18)	0.42 (0.17, 1.04)	0.08
25(OH)D2					
Model 1†	1	0.80 (0.38, 1.68)	0.66 (0.30, 1.43)	0.88 (0.42, 1.81)	0.74
Model 2‡	1	0.63 (0.27, 1.47)	0.48 (0.20, 1.19)	0.74 (0.33, 1.68)	0.45
Model 3§	1	0.61 (0.26, 1.44)	0.49 (0.20, 1.23)	0.71 (0.31, 1.62)	0.41
Free 25(OH)D					
Model 1†	1	1.04 (0.51, 2.14)	0.78 (0.36, 1.68)	0.69 (0.33, 1.48)	0.31
Model 2‡	1	1.30 (0.59, 2.85)	0.72 (0.30, 1.73)	0.72 (0.31, 1.66)	0.23
Model 3§	1	1.26 (0.57, 2.77)	0.70 (0.29, 1.72)	0.70 (0.31, 1.63)	0.22
bioavailable 25(OH)D					
Model 1†	1	0.62 (0.29, 1.29)	0.60 (0.29, 1.26)	0.56 (0.27, 1.17)	0.19
Model 2‡	1	0.71 (0.32, 1.57)	0.56 (0.24, 1.33)	0.54 (0.24, 1.24)	0.13
Model 3§	1	0.73 (0.33, 1.63)	0.57 (0.24, 1.36)	0.54 (0.24, 1.25)	0.13
VDBP					
Model 1†	1	0.77 (0.36, 1.68)	1.34 (0.64, 2.78)	0.93 (0.42, 2.07)	0.79
Model 2‡	1	0.93 (0.39, 2.22)	1.48 (0.65, 3.36)	0.99 (0.41, 2.43)	0.65
Model 3§	1	0.96 (0.40, 2.32)	1.57 (0.68, 3.66)	0.93 (0.38, 2.32)	0.76
Gestational weeks 15-26					
Total 25(OH)D					
Model 1†	1	0.47 (0.22, 0.999)	0.64 (0.30, 1.35)	0.59 (0.29, 1.22)	0.24
Model 2‡	1	0.47 (0.20, 1.13)	0.84 (0.36, 1.98)	0.72 (0.32, 1.61)	0.71
Model 3§	1	0.50 (0.20, 1.23)	0.94 (0.39, 2.27)	0.86 (0.37, 2.01)	0.71
25(OH)D3					
Model 1†	1	0.52 (0.25, 1.10)	0.60 (0.29, 1.26)	0.48 (0.23, 1.03)	0.09
Model 2‡	1	0.58 (0.25, 1.35)	0.89 (0.38, 2.06)	0.62 (0.27, 1.42)	0.43
Model 3§	1	0.72 (0.30, 1.72)	1.07 (0.44, 2.55)	0.75 (0.32, 1.78)	0.72
25(OH)D2					
Model 1†	1	0.76 (0.36, 1.62)	0.94 (0.43, 2.06)	1.27 (0.61, 2.63)	0.42
Model 2‡	1	0.72 (0.30, 1.73)	0.86 (0.36, 2.08)	1.17 (0.51, 2.65)	0.59
Model 3§	1	0.71 (0.29, 1.76)	0.95 (0.39, 2.35)	1.26 (0.54, 2.93)	0.46

Free 25(OH)D					
Model 1†	1	0.41 (0.19, 0.89)	0.51 (0.25, 1.06)	0.53 (0.25, 1.14)	0.15
Model 2‡	1	0.71 (0.30, 1.69)	0.68 (0.30, 1.55)	0.62 (0.26, 1.44)	0.26
Model 3§	1	0.71 (0.29, 1.75)	0.67 (0.29, 1.58)	0.57 (0.23, 1.39)	0.22
bioavailable 25(OH)D					
Model 1†	1	0.43 (0.20, 0.92)	0.54 (0.26, 1.13)	0.51 (0.24, 1.08)	0.13
Model 2‡	1	0.84 (0.35, 2.02)	0.73 (0.31, 1.71)	0.68 (0.29, 1.57)	0.34
Model 3§	1	0.83 (0.33, 2.08)	0.72 (0.30, 1.71)	0.64 (0.27, 1.54)	0.29
VDBP					
Model 1†	1	0.85 (0.41, 1.79)	1.05 (0.50, 2.22)	1.17 (0.54, 2.53)	0.71
Model 2‡	1	0.91 (0.39, 2.14)	0.98 (0.41, 2.34)	1.22 (0.50, 2.95)	0.62
Model 3§	1	0.90 (0.38, 2.16)	1.00 (0.40, 2.49)	1.26 (0.51, 3.12)	0.54

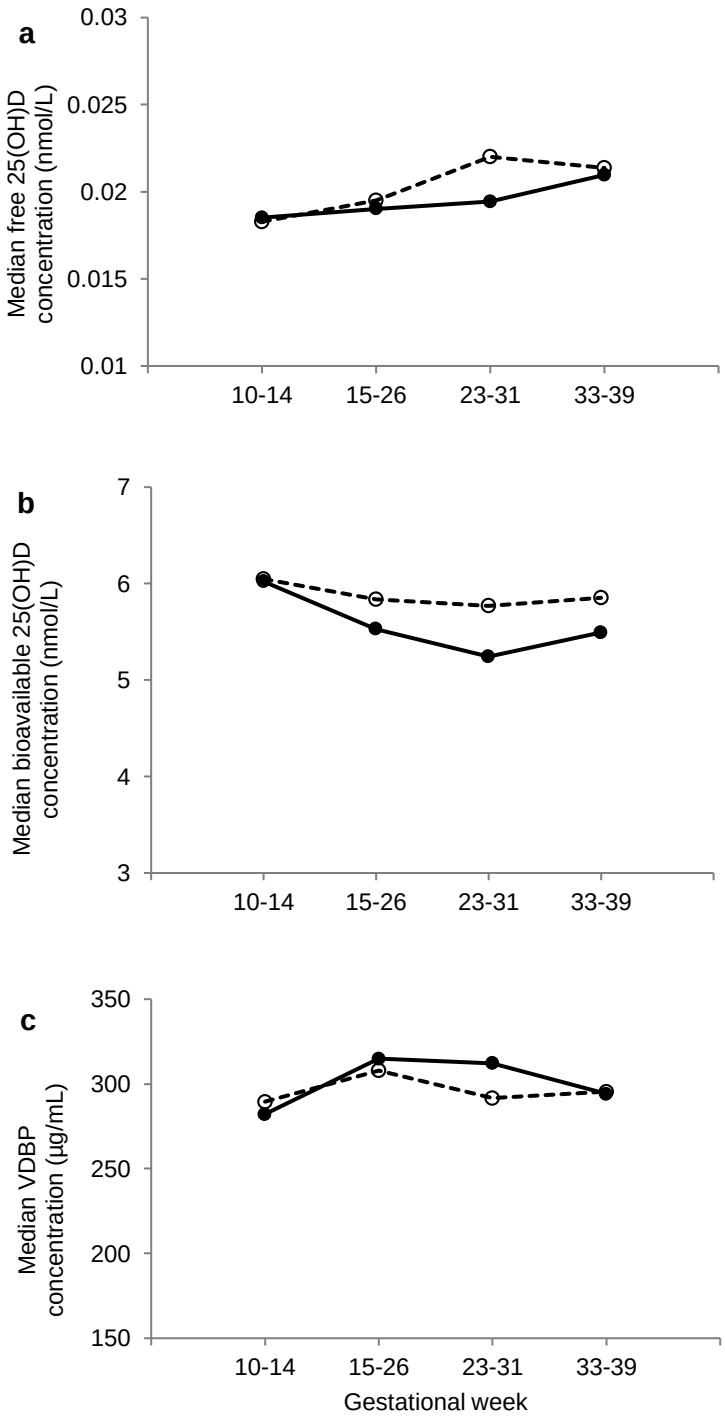
†Model 1 adjusted for race/ethnicity, maternal age, gestational age at blood collection, and geographical latitude (clinical center).
‡Model 2 (main model) further adjusted for pre-pregnancy BMI, parity, and family history of diabetes.
§Model 3 additionally adjusted for physical activity.

Supplemental Table A-3. Change of free and bioavailable 25(OH)D, and VDBP from the 1st to 2nd trimester for individuals with GDM and normal glucose levels in multivariate mixed-effects models.

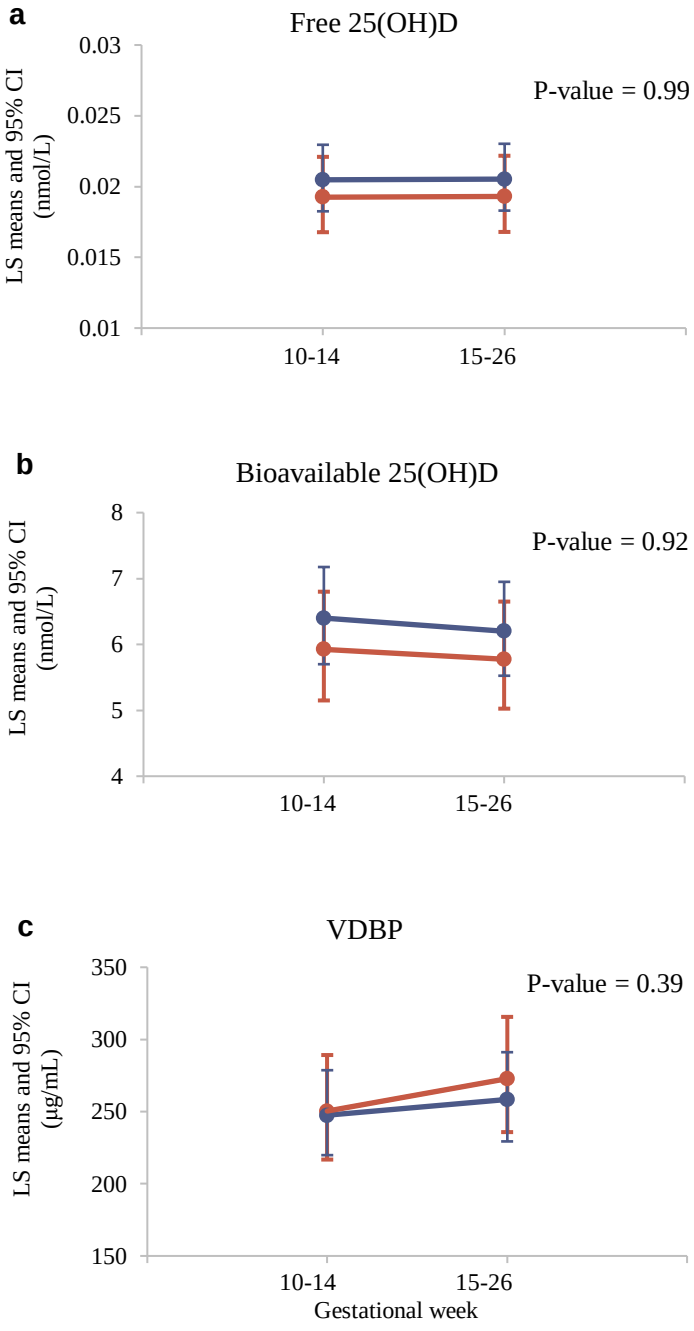
	Model 2†			Model 3‡		
	β	95% CI	P-value	β	95% CI	P-value
Free 25(OH)D						
GDM	-0.06	(-0.20, 0.09)	0.42	-0.06	(-0.21, 0.09)	0.42
Visit	0.05	(-0.01, 0.11)	0.13	0.05	(-0.01, 0.11)	0.13
GDM*Visit	-0.001	(-0.11, 0.11)	0.99	-0.001	(-0.11, 0.11)	0.99
Bioavailable 25(OH)D						
GDM	-0.07	(-0.22, 0.07)	0.32	-0.07	(-0.22, 0.07)	0.32
Visit	-0.06	(-0.13, -0.001)	0.048	-0.06	(-0.13, -0.001)	0.047
GDM*Visit	0.01	(-0.10, 0.11)	0.92	0.01	(-0.10, 0.12)	0.92
VDBP						
GDM	0.01	(-0.14, 0.17)	0.88	0.01	(-0.14, 0.17)	0.83
Visit	0.06	(0.01, 0.12)	0.03	0.06	(0.01, 0.12)	0.03
GDM*Visit	0.04	(-0.05, 0.14)	0.39	0.04	(-0.05, 0.14)	0.39

†Model 2 (main model) adjusted for race/ethnicity, maternal age, gestational age at blood collection, geographical latitude (clinical center), pre-pregnancy BMI, parity, season of blood draw, and family history of diabetes.
‡Model 3 further adjusted for physical activity.
β coefficient for "GDM" represents the difference of vitamin D biomarker levels between GDM cases and non-GDM controls when their gestational age was 10-14 weeks;
β coefficient for "Visit" represents the longitudinal change of vitamin D levels from 10-14 to 15-26 weeks of gestation among non-GDM controls;
β coefficient for "GDM*Visit" represents the difference in longitudinal change of vitamin D biomarker levels from 10-14 to 15-26 weeks of gestation between GDM cases and non-GDM controls.

Supplemental Figure A-1. Median levels of free 25(OH)D (**a**), bioavailable 25(OH)D (**b**), and VDBP (**c**) according to gestational age at blood collection among women with GDM (solid line) and their matched control subjects (dashed line).

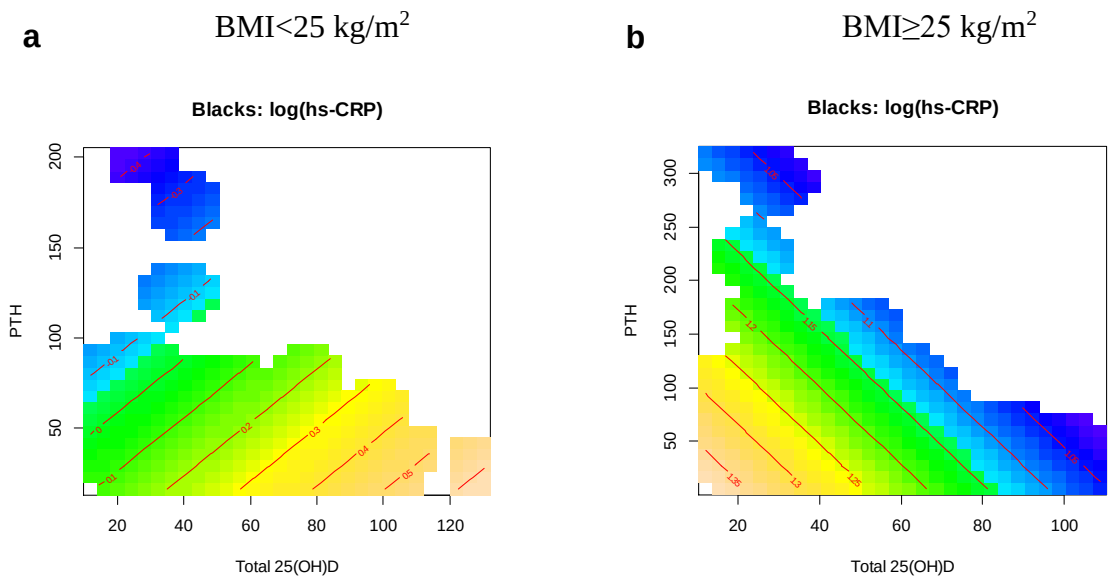


Supplemental Figure A-2. Longitudinal change of vitamin D biomarkers, including free 25(OH)D (a), bioavailable 25(OH)D (**b**), and VDBP (**c**), for individuals with GDM (case, orange line) and normal glucose levels (control, blue line).
Back-transformed LS means and the corresponding 95% CIs of vitamin D biomarkers at gestational weeks of 10-14 and 15-26 from linear mixed-effects models, adjusting for race/ethnicity, maternal age, gestational age at blood collection, geographical latitude (clinical center), pre-pregnancy BMI, parity, season of blood draw, and family history of diabetes (Model 2).

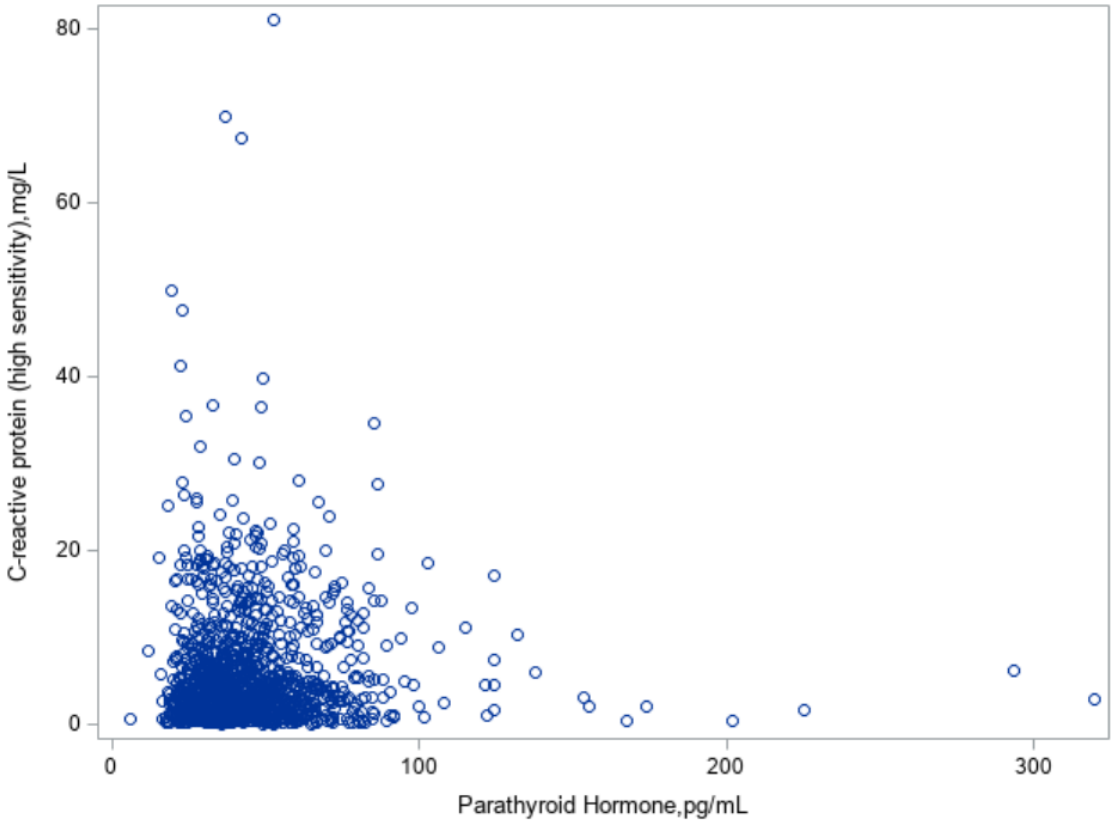


Appendix B

Supplemental Figure B-1. Estimated concurrent associations of 25(OH)D and PTH on hs-CRP by BMI (<25 vs. ≥25 kg/m^2) among black women.
The estimated mean response levels of each cardiometabolic biomarker for all 25(OH)D-PTH combinations in blacks and whites is indicated by the numbers on the contour lines, adjusting for age, clinical center, education, season of blood draw, cigarette smoking status, alcohol consumption, postmenopausal hormone therapy, and physical activity levels.



Supplemental Figure B-2. Scatterplot of parathyroid hormone to high sensitivity C-reactive protein among black women.



Appendix C

Supplemental Table C-1. Independent associations of 25(OH)D and PTH with risk of diabetes.

Model		HR (95%CI)				P for linear trend	$HR_{per\text{-}SD}$ (95%CI)‡
		Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Total 25(OH)D							
All participants	Model 1*	1	0.63 (0.53, 0.76)	0.53 (0.44, 0.64)	0.42 (0.34, 0.52)	<0.0001	0.63 (0.56, 0.71)
	Model 2†	1	0.76 (0.63, 0.92)	0.69 (0.56, 0.85)	0.57 (0.45, 0.72)	<0.0001	0.78 (0.68, 0.88)
	Model 3¶	1	0.76 (0.63, 0.92)	0.67 (0.54, 0.83)	0.58 (0.45, 0.73)	<0.0001	0.78 (0.68, 0.89)
American white women	Model 1*	1	0.60 (0.48, 0.74)	0.6 (0.49, 0.74)	0.44 (0.35, 0.55)	<0.0001	0.60 (0.52, 0.69)
	Model 2†	1	0.66 (0.53, 0.82)	0.75 (0.60, 0.94)	0.55 (0.42, 0.71)	<0.0001	0.73 (0.62, 0.86)
	Model 3¶	1	0.64 (0.51, 0.80)	0.70 (0.56, 0.89)	0.57 (0.44, 0.73)	<0.0001	0.73 (0.62, 0.86)
American black women	Model 1*	1	0.86 (0.62, 1.21)	0.74 (0.52, 1.05)	0.48 (0.32, 0.71)	0.0001	0.80 (0.69, 0.94)
	Model 2†	1	0.96 (0.67, 1.38)	0.98 (0.67, 1.43)	0.65 (0.42, 0.997)	0.052	0.93 (0.79, 1.10)
	Model 3¶	1	0.96 (0.67, 1.39)	1.01 (0.69, 1.47)	0.64 (0.42, 0.99)	0.051	0.94 (0.79, 1.11)
PTH							
All participants	Model 1*	1	1.03 (0.84, 1.25)	0.88 (0.72, 1.07)	1.43 (1.19, 1.72)	<0.0001	1.17 (1.08, 1.27)
	Model 2†	1	0.93 (0.76, 1.13)	0.78 (0.63, 0.96)	1.11 (0.92, 1.35)	0.181	1.05 (0.96, 1.16)
	Model 3¶	1	0.93 (0.76, 1.14)	0.78 (0.63, 0.97)	1.10 (0.90, 1.34)	0.249	1.04 (0.94, 1.15)
American white women	Model 1*	1	1.25 (0.99, 1.57)	0.93 (0.73, 1.19)	1.39 (1.11, 1.74)	0.021	1.16 (1.04, 1.29)
	Model 2†	1	1.10 (0.87, 1.40)	0.83 (0.65, 1.07)	1.15 (0.91, 1.46)	0.385	1.05 (0.93, 1.18)
	Model 3¶	1	1.08 (0.85, 1.37)	0.8 (0.62, 1.04)	1.11 (0.87, 1.40)	0.594	1.02 (0.90, 1.15)
	Model 1*	1	0.72 (0.50, 1.06)	0.85 (0.58, 1.23)	1.35 (0.97, 1.88)	0.02	1.17 (1.03, 1.34)

American black women	Model 2†	1	0.63 (0.43, 0.94)	0.62 (0.41, 0.92)	0.96 (0.67, 1.38)	0.648	1.04 (0.89, 1.23)
	Model 3¶	1	0.64 (0.43, 0.96)	0.64 (0.42, 0.96)	0.96 (0.66, 1.38)	0.673	1.05 (0.89, 1.24)

* Model 1 adjusted for age, clinical center, and race/ethnicity.
† Model 2 further adjusted for BMI, family history of diabetes, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw.
¶ Model 3 additionally adjusted for eGFR, history of high cholesterol and statin use.
‡ HRs represent per-standard deviation increases in biomarker measures.

Supplemental Table C-2. Independent associations of plasma 25(OH)D levels with prevalent risk of comorbidities (obesity, hypertension, and CKD) among postmenopausal women.

Model		OR (95%CI)				P for linear trend	$OR_{per\text{-}SD}$‡ (95%CI)
		Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Obesity							
All participants	Model 1*	1	0.71 (0.63, 0.80)	0.45 (0.39, 0.51)	0.23 (0.20, 0.27)	< 0.0001	0.41 (0.38, 0.45)
	Model 2†	1	0.77 (0.68, 0.88)	0.51 (0.44, 0.59)	0.28 (0.23, 0.33)	< 0.0001	0.45 (0.41, 0.49)
	Model 3¶	1	0.77 (0.68, 0.88)	0.51 (0.44, 0.59)	0.28 (0.23, 0.33)	< 0.0001	0.45 (0.41, 0.49)
American white women	Model 1*	1	0.60 (0.52, 0.68)	0.40 (0.35, 0.46)	0.21 (0.18, 0.25)	< 0.0001	0.35 (0.32, 0.39)
	Model 2†	1	0.66 (0.57, 0.77)	0.48 (0.41, 0.56)	0.24 (0.20, 0.30)	< 0.0001	0.39 (0.35, 0.44)
	Model 3¶	1	0.66 (0.56, 0.76)	0.48 (0.41, 0.56)	0.25 (0.20, 0.30)	< 0.0001	0.39 (0.35, 0.44)
American black women	Model 1*	1	0.89 (0.69, 1.14)	0.65 (0.50, 0.83)	0.45 (0.35, 0.58)	< 0.0001	0.68 (0.62, 0.75)
	Model 2†	1	0.89 (0.67, 1.17)	0.76 (0.57, 1.00)	0.51 (0.38, 0.69)	< 0.0001	0.73 (0.65, 0.81)
	Model 3¶	1	0.88 (0.66, 1.16)	0.74 (0.56, 0.99)	0.49 (0.37, 0.66)	< 0.0001	0.72 (0.64, 0.80)
Hypertension							
All participants	Model 1*	1	0.82 (0.72, 0.92)	0.69 (0.61, 0.78)	0.56 (0.50, 0.64)	< 0.0001	0.74 (0.69, 0.78)
	Model 2†	1	0.84 (0.74, 0.96)	0.79 (0.69, 0.90)	0.66 (0.58, 0.77)	< 0.0001	0.82 (0.76, 0.89)
	Model 3¶	1	0.83 (0.73, 0.95)	0.78 (0.68, 0.90)	0.68 (0.59, 0.78)	< 0.0001	0.83 (0.77, 0.90)
American white women	Model 1*	1	0.71 (0.63, 0.81)	0.58 (0.51, 0.65)	0.51 (0.45, 0.58)	< 0.0001	0.70 (0.64, 0.75)
	Model 2†	1	0.75 (0.65, 0.86)	0.66 (0.57, 0.76)	0.61 (0.52, 0.70)	< 0.0001	0.79 (0.72, 0.87)
	Model 3¶	1	0.71 (0.62, 0.82)	0.65 (0.56, 0.75)	0.61 (0.52, 0.71)	< 0.0001	0.79 (0.73, 0.87)
American black women	Model 1*	1	1.20 (0.92, 1.56)	0.93 (0.72, 1.20)	0.80 (0.62, 1.04)	0.022	0.91 (0.82, 1.00)
	Model 2†	1	1.23 (0.92, 1.66)	0.96 (0.71, 1.28)	0.96 (0.71, 1.29)	0.428	0.97 (0.87, 1.09)

	Model 3¶	1	1.25 (0.92, 1.69)	1.00 (0.74, 1.35)	1.00 (0.74, 1.36)	0.663	0.99 (0.88, 1.11)
CKD							
All participants	Model 1*	1	0.94 (0.73, 1.22)	0.87 (0.67, 1.14)	1.03 (0.80, 1.34)	< 0.0001	0.95 (0.83, 1.10)
	Model 2†	1	0.99 (0.75, 1.31)	0.96 (0.72, 1.29)	1.30 (0.96, 1.74)	< 0.0001	1.08 (0.92, 1.27)
	Model 3¶	1	1.00 (0.76, 1.32)	0.94 (0.70, 1.27)	1.26 (0.94, 1.71)	< 0.0001	1.06 (0.90, 1.24)
American white women	Model 1*	1	1.23 (0.95, 1.60)	1.07 (0.81, 1.41)	1.22 (0.93, 1.61)	0.277	0.96 (0.82, 1.14)
	Model 2†	1	1.39 (1.04, 1.86)	1.31 (0.96, 1.78)	1.52 (1.11, 2.09)	0.018	1.08 (0.89, 1.30)
	Model 3¶	1	1.38 (1.03, 1.84)	1.27 (0.93, 1.74)	1.45 (1.05, 2.00)	0.043	1.04 (0.86, 1.26)
American black women	Model 1*	1	1.64 (0.86, 3.13)	1.15 (0.58, 2.28)	0.74 (0.36, 1.54)	0.141	0.92 (0.73, 1.18)
	Model 2†	1	1.53 (0.74, 3.18)	1.00 (0.44, 2.23)	1.01 (0.45, 2.27)	0.689	1.07 (0.81, 1.41)
	Model 3¶	1	1.55 (0.74, 3.23)	1.17 (0.51, 2.66)	1.04 (0.46, 2.37)	0.783	1.08 (0.82, 1.42)
Composite outcome (at least one)							
All participants	Model 1*	1	0.66 (0.58, 0.75)	0.53 (0.46, 0.60)	0.39 (0.35, 0.45)	< 0.0001	0.62 (0.58, 0.66)
	Model 2†	1	0.68 (0.60, 0.78)	0.57 (0.49, 0.65)	0.41 (0.36, 0.48)	< 0.0001	0.63 (0.59, 0.68)
	Model 3¶	1	0.67 (0.59, 0.77)	0.56 (0.49, 0.65)	0.42 (0.36, 0.48)	< 0.0001	0.63 (0.59, 0.68)
American white women	Model 1*	1	0.65 (0.57, 0.74)	0.49 (0.43, 0.56)	0.40 (0.35, 0.46)	< 0.0001	0.59 (0.54, 0.64)
	Model 2†	1	0.66 (0.57, 0.76)	0.53 (0.46, 0.61)	0.41 (0.35, 0.47)	< 0.0001	0.59 (0.54, 0.65)
	Model 3¶	1	0.63 (0.55, 0.73)	0.52 (0.45, 0.61)	0.41 (0.35, 0.47)	< 0.0001	0.60 (0.55, 0.65)
American black women	Model 1*	1	0.98 (0.71, 1.36)	0.73 (0.53, 1.00)	0.52 (0.39, 0.71)	< 0.0001	0.78 (0.69, 0.87)
	Model 2†	1	0.91 (0.64, 1.30)	0.74 (0.52, 1.05)	0.57 (0.41, 0.81)	0.001	0.82 (0.72, 0.93)
	Model 3¶	1	0.91 (0.63, 1.30)	0.75 (0.53, 1.06)	0.58 (0.41, 0.82)	0.001	0.82 (0.73, 0.94)

* Model 1 adjusted for age, clinical center, and race/ethnicity.

† Model 2 further adjusted for BMI (for prevalent CKD and hypertension only), family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw.

¶ Model 3 additionally adjusted for eGFR (for prevalent obesity and hypertension only), history of high cholesterol and statin use.
‡ ORs represent per-standard deviation increases in biomarker measures.

Supplemental Table C-3. Independent associations of plasma PTH levels with prevalent risk of comorbidities (obesity, hypertension, and CKD) among postmenopausal women.

Model		OR (95%CI)				P for linear trend	$OR_{per\text{-}SD}$‡ (95%CI)
		Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Obesity							
All participants	Model 1*	1	1.30 (1.14, 1.49)	1.45 91.27, 1.66)	2.49 (2.19, 2.83)	< 0.0001	1.75 (1.61, 1.89)
	Model 2†	1	1.25 (1.08, 1.44)	1.40 (1.21, 1.62)	2.10 (1.82, 2.42)	< 0.0001	1.58 (1.45, 1.72)
	Model 3¶	1	1.25 (1.08, 1.44)	1.38 (1.19, 1.60)	2.10 (1.82, 2.42)	< 0.0001	1.58 (1.45, 1.72)
American white women	Model 1*	1	1.52 (1.30, 1.78)	1.40 (1.19, 1.64)	2.51 (2.16, 2.92)	< 0.0001	1.86 (1.68, 2.06)
	Model 2†	1	1.45 (1.22, 1.71)	1.30 (1.09, 1.54)	2.18 (1.85, 2.57)	< 0.0001	1.64 (1.47, 1.83)
	Model 3¶	1	1.45 (1.23, 1.72)	1.27 (1.07, 1.51)	2.18 (1.85, 2.57)	< 0.0001	1.63 (1.46, 1.82)
American black women	Model 1*	1	1.42 (1.10, 1.84)	1.81 (1.40, 2.34)	2.83 (2.19, 3.67)	< 0.0001	1.53 (1.35, 1.72)
	Model 2†	1	1.47 (1.10, 1.96)	1.50 (1.12, 2.00)	2.38 (1.79, 3.17)	< 0.0001	1.46 (1.28, 1.67)
	Model 3¶	1	1.47 (1.10, 1.96)	1.50 (1.12, 1.99)	2.40 (1.80, 3.20)	< 0.0001	1.47 (1.28, 1.68)
Hypertension							
All participants	Model 1*	1	1.06 (0.94, 1.18)	1.43 (1.28, 1.60)	1.84 (1.64, 2.07)	< 0.0001	1.54 (1.43, 1.67)
	Model 2†	1	0.98 (0.87, 1.11)	1.38 (1.22, 1.57)	1.56 (1.37, 1.77)	< 0.0001	1.40 (1.29, 1.53)
	Model 3¶	1	0.98 (0.87, 1.11)	1.37 (1.21, 1.55)	1.59 (1.39, 1.81)	< 0.0001	1.40 (1.29, 1.53)
American white women	Model 1*	1	1.07 (0.94, 1.22)	1.45 (1.28, 1.65)	1.86 (1.64, 2.12)	< 0.0001	1.73 (1.57, 1.92)
	Model 2†	1	0.95 (0.83, 1.09)	1.40 (1.22, 1.61)	1.57 (1.36, 1.81)	< 0.0001	1.55 (1.39, 1.73)
	Model 3¶	1	0.96 (0.83, 1.10)	1.38 (1.20, 1.59)	1.60 (1.39, 1.85)	< 0.0001	1.56 (1.40, 1.74)
American black women	Model 1*	1	1.20 (0.93, 1.55)	1.37 (1.06, 1.77)	1.63 (1.26, 2.12)	0.0002	1.21 (1.08, 1.36)
	Model 2†	1	1.21 (0.91, 1.61)	1.32 (0.99, 1.76)	1.41 (1.05, 1.89)	0.028	1.15 (0.998, 1.31)

	Model 3¶	1	1.16 (0.86, 1.55)	1.31 (0.98, 1.75)	1.37 (1.02, 1.85)	0.037	1.13 (0.98, 1.30)
CKD							
All participants	Model 1*	1	0.92 (0.68, 1.25)	1.29 (0.97, 1.70)	2.57 (1.99, 3.32)	< 0.0001	1.76 (1.59, 1.95)
	Model 2†	1	0.75 (0.54, 1.06)	1.08 (0.79, 1.46)	2.30 (1.75, 3.03)	< 0.0001	1.84 (1.63, 2.09)
	Model 3¶	1	0.76 (0.54, 1.06)	1.03 (0.76, 1.40)	2.23 (1.69, 2.93)	< 0.0001	1.84 (1.63, 2.08)
American white women	Model 1*	1	0.86 (0.62, 1.19)	1.09 (0.80, 1.49)	2.29 (1.75, 3.01)	< 0.0001	1.84 (1.61, 2.09)
	Model 2†	1	0.71 (0.50, 1.02)	0.90 (0.64, 1.25)	2.01 (1.50, 2.68)	< 0.0001	1.87 (1.62, 2.17)
	Model 3¶	1	0.72 (0.51, 1.03)	0.85 (0.60, 1.18)	1.94 (1.45, 2.59)	< 0.0001	1.88 (1.62, 2.17)
American black women	Model 1*	1	1.46 (0.59, 3.64)	2.45 (1.06, 5.67)	4.70 (2.13, 10.39)	< 0.0001	1.62 (1.40, 1.89)
	Model 2†	1	1.60 (0.57, 4.45)	2.16 (0.83, 5.63)	4.23 (1.70, 10.56)	0.0002	1.87 (1.51, 2.32)
	Model 3¶	1	1.58 (0.56, 4.40)	2.12 (0.81, 5.55)	3.96 (1.58, 9.91)	0.001	1.89 (1.51, 2.36)
Composite outcome (at least one)							
All participants	Model 1*	1	1.15 (1.03, 1.29)	1.55 (1.38, 1.74)	2.56 (2.26, 2.90)	< 0.0001	2.13 (1.94, 2.34)
	Model 2†	1	1.08 (0.96, 1.22)	1.55 (1.37, 1.76)	2.31 (2.02, 2.63)	< 0.0001	2.01 (1.82, 2.22)
	Model 3¶	1	1.08 (0.96, 1.23)	1.54 (1.36, 1.75)	2.34 (2.05, 2.67)	< 0.0001	2.01 (1.82, 2.23)
American white women	Model 1*	1	1.24 (1.10, 1.41)	1.52 (1.34, 1.73)	2.51 (2.20, 2.87)	< 0.0001	2.32 (2.07, 2.60)
	Model 2†	1	1.16 (1.01, 1.32)	1.51 (1.32, 1.73)	2.33 (2.02, 2.68)	< 0.0001	2.22 (1.97, 2.50)
	Model 3¶	1	1.17 (1.02, 1.34)	1.49 91.30, 1.71)	2.37 (2.05, 2.73)	< 0.0001	2.23 (1.98, 2.52)
American black women	Model 1*	1	1.25 (0.94, 1.66)	1.97 (1.45, 2.68)	2.28 (1.67, 3.12)	< 0.0001	1.74 (1.46, 2.07)
	Model 2†	1	1.26 (0.92, 1.73)	1.65 (1.18, 2.30)	2.00 (1.43, 2.81)	< 0.0001	1.59 (1.33, 1.91)
	Model 3¶	1	1.22 (0.89, 1.68)	1.62 (1.16, 2.27)	1.95 (1.39, 2.75)	< 0.0001	1.58 (1.31, 1.90)

* Model 1 adjusted for age, clinical center, and race/ethnicity.

† Model 2 further adjusted for BMI (for prevalent CKD and hypertension only), family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw.

¶ Model 3 additionally adjusted for eGFR (for prevalent obesity and hypertension only), history of high cholesterol and statin use.
‡ ORs represent per-standard deviation increases in biomarker measures.

Supplemental Table C-4. Independent associations of total 25(OH)D and PTH with risk of incident CVD between postmenopausal women with and without diabetes at baseline.

Model		HR (95%CI)				P for linear trend	$HR_{per\text{-}SD}$‡ (95%CI)
		Quartile 1	Quartile 2	Quartile 3	Quartile 4		
Total 25(OH)D							
All Participants							
With diabetes	Model 1*	1	1.22 (0.77, 1.92)	1.14 (0.66, 1.97)	0.93 (0.49, 1.74)	0.954	0.95 (0.69, 1.29)
	Model 2†	1	1.12 (0.65, 1.94)	1.26 (0.63, 2.51)	1.12 (0.53, 2.38)	0.607	1.02 (0.70, 1.49)
	Model 3¶	1	1.26 (0.72, 2.22)	1.75, 0.82, 3.71)	1.42 (0.63, 3.22)	0.233	1.13 (0.75, 1.71)
Without diabetes	Model 1*	1	0.85 (0.69, 1.05)	0.66 (0.53, 0.82)	0.74 (0.59, 0.92)	0.002	0.82 (0.73, 0.93)
	Model 2†	1	0.88 (0.70, 1.11)	0.70 (0.55, 0.90)	0.87 (0.68, 1.12)	0.172	0.92 (0.80, 1.05)
	Model 3¶	1	0.87 (0.69, 1.09)	0.69 (0.54, 0.88)	0.87 (0.67, 1.12)	0.177	0.92 (0.80, 1.05)
PTH							
All participants							
With diabetes	Model 1*	1	1.16 (0.65, 2.04)	1.25 (0.74, 2.12)	1.42 (0.87, 2.30)	0.152	1.08 (0.97, 1.21)
	Model 2†	1	1.25 (0.63, 2.45)	1.29 (0.68, 2.45)	1.45 (0.80, 2.61)	0.241	1.13 (0.98, 1.31)
	Model 3¶	1	1.58 (0.77, 3.24)	1.38 (0.69, 2.72)	1.48 (0.79, 2.77)	0.339	1.14 (0.97, 1.33)
Without diabetes	Model 1*	1	1.10 (0.89, 1.35)	1.27 (1.03, 1.56)	1.52 (1.23, 1.86)	< 0.0001	1.30 (1.18, 1.43)
	Model 2†	1	1.07 (0.85, 1.35)	1.19 (0.95, 1.50)	1.37 (1.10, 1.72)	0.003	1.24 (1.10, 1.39)
	Model 3¶	1	1.08 (0.86, 1.36)	1.21 (0.96, 1.52)	1.39 (1.11, 1.75)	0.003	1.24 (1.10, 1.39)

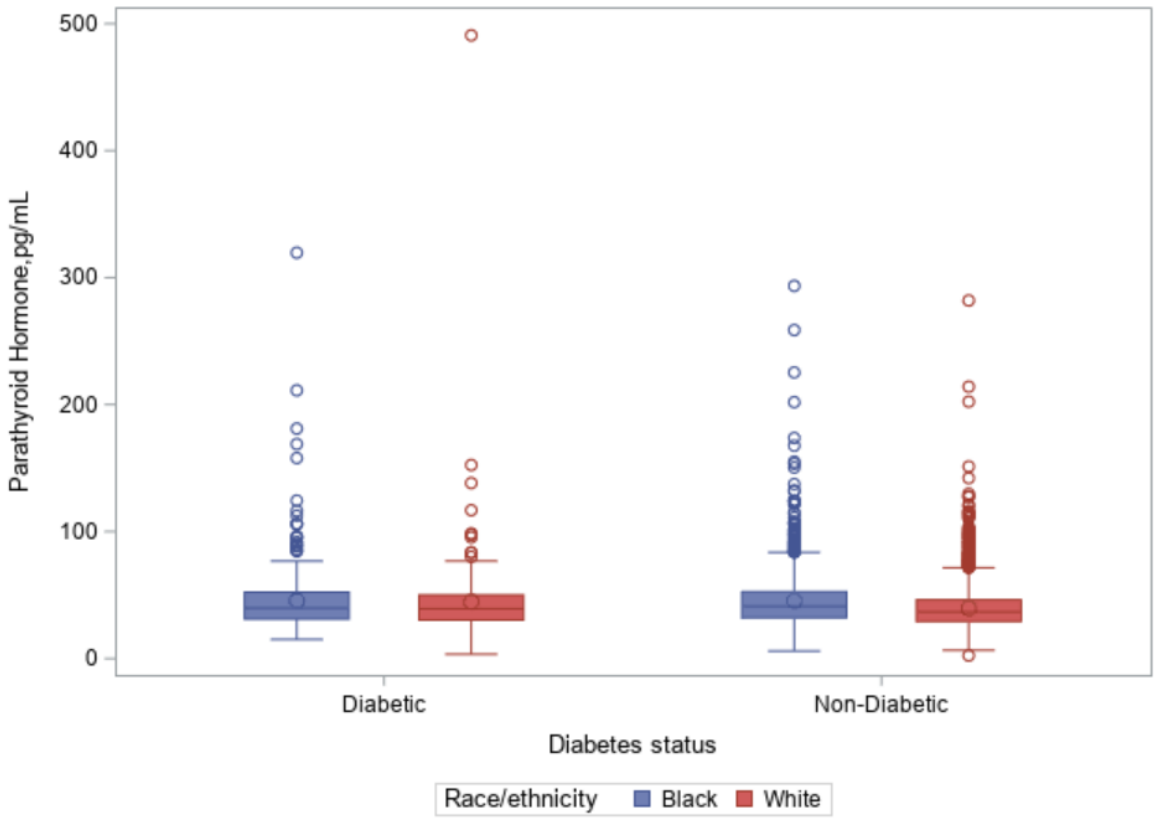
* Model 1 adjusted for age, clinical center, and race/ethnicity.
† Model 2 further adjusted for eGFR, BMI, family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw.
¶ Model 3 additionally adjusted for history of high cholesterol and statin use.
‡ HRs represent per-standard deviation increases in biomarker measures.

Supplemental Table C-5. Independent associations of total 25(OH)D and PTH with risk of incident CVD stratified by race/ethnicity and diabetes status.

Model		White women		Black women	
		$HR_{per\text{-}SD}$‡ (95%CI)	P for interaction	$HR_{per\text{-}SD}$‡ (95%CI)	P for interaction
Total 25(OH)D					
With diabetes	Model 1*	0.998 (0.63, 1.58)	0.229	0.995 (0.77, 1.29)	0.37
	Model 2†	0.68 (0.31, 1.48)	0.31	0.95 (0.68, 1.32)	0.933
	Model 3¶	0.78 (0.32, 1.87)	0.24	0.98 (0.69, 1.40)	0.87
Without diabetes	Model 1*	0.83 (0.71, 0.97)	—	0.90 (0.79, 1.02)	—
	Model 2†	0.90 (0.76, 1.08)	—	0.996 (0.85, 1.16)	—
	Model 3¶	0.90 (0.76, 1.08)	—	0.98 (0.84, 1.15)	—
PTH					
With diabetes	Model 1*	1.03 (0.85, 1.26)	0.0005	1.08 (0.93, 1.25)	0.225
	Model 2†	1.24 (0.90, 1.71)	0.041	1.26 (0.93, 1.70)	0.662
	Model 3¶	1.07 (0.49, 2.35)	0.035	1.25 (0.92, 1.69)	0.593
Without diabetes	Model 1*	1.37 (1.20, 1.57)	—	1.17 (1.03, 1.33)	—
	Model 2†	1.28 (1.10, 1.49)	—	1.17 (0.98 1.39)	—
	Model 3¶	1.29 (1.10, 1.50)	—	1.16 (0.97, 1.39)	—

* Model 1 adjusted for age, clinical center, and race/ethnicity.
† Model 2 further adjusted for eGFR, BMI, family history of CVD, educational levels, alcohol intake, physical activity levels, cigarette smoking status, postmenopausal hormone therapy use, and season of blood draw.
¶ Model 3 additionally adjusted for history of high cholesterol and statin use.
‡ HRs represent per-standard deviation increases in biomarker measures.

Supplemental Figure C-1. Boxplots of PTH by diabetes status at baseline and race/ethnicity.



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Curriculum Vitae

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Education

- Ph.D. in Epidemiology – Indiana University (February 2020)
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Professional Experience

- Research Assistant, Indiana University-Purdue University Indianapolis (2013 Aug-Dec, 2015-present)
- Sr. Statistician-Computation, Eli Lilly and Company (2012-2013)
- Biostatistician, Johns Hopkins University Center on Aging and Health (2008-2012)
- Graduate Assistant, Florida International University (2004-2007)

Publications

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Presentations

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- Xia J, Song Y, Wu J, Hinkle S, Li M, Tsai MY, Zhang C. Biomarkers of vitamin D3 C-3 epimers during pregnancy and the risk of gestational diabetes mellitus: a longitudinal study in a multiracial cohort. Poster presentation at the American

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- Xia J, Song Y, Rawal S, Wu J, Hinkle S, Tsai M, Zhang C. Vitamin D status during pregnancy and the risk of gestational diabetes mellitus: a longitudinal study in a multiracial cohort. Poster presentation at the American Diabetes Association's 78th Scientific Sessions, Orlando, Florida, June 24, 2018.
- Xia J, Song Y. Joint associations of diabetes and sarcopenia on all-cause mortality among U.S. older adults. Poster presentation at the American Diabetes Association's 78th Scientific Sessions, Orlando, Florida, June 23, 2018.