Prospective Associations of Vitamin D Status with 
Beta-cell function, Insulin Sensitivity and Glycemia: 
The Impact of Parathyroid Hormone Status

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ABSTRACT

Previous studies have yielded conflicting findings on the relationship between low vitamin D (25-OH-D) and impaired glucose homeostasis. In this context, we hypothesized that combined assessment of 25-OH-D with its regulator parathyroid hormone (PTH) may be required for optimal evaluation of the impact of vitamin D status on glucose metabolism. Thus, we evaluated the prospective associations of 25-OH-D and PTH at 3-months postpartum with beta-cell function (Insulin Secretion-Sensitivity Index-2 (ISSI-2)), insulin sensitivity (Matsuda index) and glycemia at 12-months postpartum in 494 women undergoing serial metabolic characterization. Notably, 32% of those with pre-diabetes/diabetes at 12-months postpartum had both vitamin D deficiency and PTH in the highest tertile at 3-months postpartum. On multiple-adjusted linear regression analyses, vitamin D deficiency/insufficiency with PTH in the highest tertile at 3-months independently predicted poorer beta-cell function (P=0.03) and insulin sensitivity (P=0.01), and increased fasting (P=0.03) and 2-hour glucose (P=0.002) at 12-months postpartum. In contrast, vitamin D deficiency/insufficiency with lower PTH did not predict these outcomes. In conclusion, only vitamin D deficiency/insufficiency with increased PTH is an independent predictor of beta-cell dysfunction, insulin resistance and glycemia, highlighting the need for consideration of the PTH/25-OH-D axis when studying the impact of vitamin D status on glucose homeostasis.
INTRODUCTION

In the recent years, a growing body of evidence has demonstrated extra-skeletal associations of both vitamin D (25-OH-D)(1-3) and parathyroid hormone (PTH)(4-6), with particular focus on their metabolic implications. Several studies have suggested that low levels of vitamin D may play a role in the development of type 2 diabetes (T2DM)(7-9). Indeed, in a meta-analysis of 21 studies, the circulating level of 25-OH-D was inversely associated with the risk of future T2DM (7). Furthermore, deterioration in beta-cell function has been suggested as a pathophysiologic mechanism through which lower vitamin D may increase the risk of T2DM (10-11). Previous studies have also reported that increased PTH is associated with insulin resistance (12) and metabolic syndrome (4;13), and deterioration of insulin sensitivity and beta-cell function has been described in hyperparathyroid states (14). Conversely, however, many investigators have questioned the association of vitamin D and PTH with glucose metabolism, particularly in light of several observational studies (13;15-18) and interventional trials (1;19-21) that have been either negative or showed only modest beneficial effects of vitamin D supplementation on glucose homeostasis.

A possible explanation for this conflicting evidence is that previous studies have generally evaluated the respective metabolic implications of vitamin D and PTH in isolation, rather than considering both hormones together as a reflection of the status of the PTH-vitamin D axis. Indeed, for the comprehensive assessment of many endocrine axes (e.g. thyroid), it is often necessary to consider both upstream regulators (e.g. thyroid-stimulating hormone) and downstream effector hormones (e.g. thyroxine) in conjunction with one another. Moreover, in the case of vitamin D, the 25-OH-D concentration that provides maximal PTH suppression is widely variable, suggesting that there is an individual threshold for the serum 25-OH-D concentration below which PTH rises (22). In this context, we hypothesized that combined...
assessment of PTH and 25-OH-D together may be needed for optimal evaluation of the impact of vitamin D status on glucose metabolism. Specifically, it may be that glucose metabolism is only adversely affected when circulating 25-OH-D falls to a level that causes PTH to rise, reflecting true functional vitamin D inadequacy. Thus, our objective in this study was to collectively evaluate vitamin D and PTH in relation to changes over time in beta-cell function, insulin sensitivity and glycemia in a cohort of subjects reflecting a broad range of diabetic risk.

METHODS

Participants

This study was performed in the setting of a prospective observational cohort consisting of women representing the full spectrum of glucose tolerance in a recent pregnancy (from normal to gestational diabetes mellitus (GDM)), who thereby have a broad range of risk for the future development of pre-diabetes and T2DM in the years after delivery (23-25). This cohort provided a model for studying the longitudinal relationship between vitamin D/PTH and glucose metabolism in the first year postpartum for two reasons: (i) lactating women in the first year postpartum in Toronto, Canada (latitude 43°42′N) are a population at risk for vitamin D deficiency/insufficiency and (ii) the range of future diabetic risk within this cohort has been shown to manifest in changes in beta-cell function, insulin sensitivity, and glycemia between 3- and 12-months postpartum (23;26).

As previously described (23-25), the women comprising this cohort are recruited at the time of antepartum screening for GDM in late 2nd trimester and undergo metabolic characterization at recruitment and at both 3-months and 12-months postpartum. At our institution, women are screened for GDM by 50g glucose challenge test (GCT) in late 2nd trimester, followed by referral for diagnostic oral glucose tolerance test (OGTT) if the GCT
is abnormal. In this cohort study, women are recruited either before or after the GCT, and all participants undergo a 3-hour 100g OGTT for determination of GDM status (regardless of the GCT result)(27). The resultant cohort thus reflects the full spectrum of glucose tolerance in pregnancy from normal to GDM, which translates to a gradient of future risk for postpartum progression to pre-diabetes and T2DM. For this cohort study, participants return to the clinical investigation unit at both 3- and 12-months postpartum to undergo repeat metabolic characterization, including evaluation of glucose tolerance by 2-hour 75g OGTT. The protocol has been approved by the Mount Sinai Hospital Research Ethics Board and all women have provided written informed consent for their participation. The current study was performed in 494 women who have completed their 12-month postpartum visit and had vitamin D and PTH measured at 3-months postpartum, thereby enabling assessment of the longitudinal relationships between vitamin D/PTH status and metabolic outcomes (beta-cell function, insulin sensitivity, glycemia) 9 months later.

**Study Visits at 3- and 12-months Postpartum**

At the study visits at 3- and 12-months postpartum, interviewer-administered questionnaires were completed including assessment of current medications/supplements, duration of breastfeeding, and physical activity. As previously described (26), physical activity was assessed by the validated Baecke questionnaire. This instrument measures total physical activity and its component domains of sport-related physical activity (sport index), non-sport leisure-time activity (leisure-time index), and occupation-associated activity (work index). Work index is not measured at 3-months postpartum, as most women would not yet have returned to their usual occupation at that time. At each study visit, physical examination was performed including measurement of weight and waist circumference.
Laboratory Measurements and Physiologic Indices

Vitamin D status was assessed with measurement of serum 25-OH-D by competitive electrochemiluminescent immunoassay on the Roche Modular E170 (Catalogue number 05894913190; Laval, Canada). This assay has a lower reporting limit of 8 nmol/l. Serum PTH was measured using an electrochemiluminescence immunoassay on the Roche Modular E170 Analyzer (Catalogue number 11972103122; Laval, Canada), which has a detection range from 0.6 to 530 pmol/L.

All OGTTs at 3- and 12-months postpartum were performed in the morning after overnight fast. Venous blood samples were drawn for the measurement of glucose and specific insulin at fasting and at 30-, 60-, and 120-minutes following the ingestion of the glucose load, as previously described (23-24).

At each OGTT, current glucose tolerance status was determined according to Canadian Diabetes Association guidelines (28). Dysglycemia refers to pre-diabetes (impaired glucose tolerance, impaired fasting glucose or both) or T2DM. Area-under-the-insulin-curve (AUC_{ins}) and area-under-the-glucose-curve (AUC_{gluc}) during the OGTT were calculated using the trapezoidal rule. Insulin sensitivity was measured using the Matsuda index, an established measure of whole-body insulin sensitivity that has been validated against the euglycemic-hyperinsulinemic clamp (29). Beta-cell function was assessed on each OGTT with the Insulin Secretion-Sensitivity Index-2 (ISSI-2). ISSI-2 is a validated measure of beta-cell function that is analogous to the disposition index obtained from the intravenous glucose tolerance test (ivGTT) (30-31). ISSI-2 has been directly validated against the disposition index from the ivGTT, with which it exhibits stronger correlation than do other OGTT-derived measures of beta-cell function (31), and has been used to measure beta-cell function.
in several previous studies, including both clinical trials and observational studies (10;23;32-35). ISSI-2 is defined as the product of (i) insulin secretion measured by the ratio of the area-under-the-insulin-curve to the area-under-the-glucose-curve and (ii) insulin sensitivity measured by Matsuda Index (30-31).

**Statistical Analyses**

All analyses were conducted using SAS 9.1 (SAS Institute, Cary,NC). Continuous variables were tested for normality of distribution, and natural log transformations of skewed variables were used, where necessary, in subsequent analyses.

Participants were initially stratified into groups according to (i) vitamin D status at 3-months postpartum and (ii) tertiles of PTH at 3-months postpartum, respectively. Vitamin D status was classified as per Endocrine Society guidelines as vitamin D deficient (25-OH-D <50nmol/L)(n=161), vitamin D insufficient (25-OH-D ≥50nmol/L and <75nmol/L)(n=178), or vitamin D sufficient (25-OH-D ≥75nmol/L) (n=155) (2;36). The tertiles of PTH were defined as 1st tertile (PTH ≤2.6pmol/l)(n=167), 2nd tertile (PTH >2.6pmol/l and ≤3.8pmol/l)(n=174), and 3rd tertile (PTH >3.8pmol/l)(n=153). Univariate differences across both the vitamin D groups and the tertiles of PTH were assessed by ANOVA and Wilcoxon rank sum test for continuous variables and the $\chi^2$ test for categorical variables (Table 1).

To evaluate whether these vitamin D and PTH groups at 3-months postpartum predict insulin sensitivity, beta-cell function and glycemia at 12-months postpartum, multiple linear regression models were constructed with metabolic outcomes of insulin sensitivity (Matsuda index), beta-cell function (ISSI-2) and glycemia (fasting glucose and 2-hr glucose) at 12-months postpartum as dependent variables (Table 2). In each case, Model 1 was adjusted for risk factors for diabetes (age, ethnicity, family history of diabetes, previous GDM, body mass...
index (BMI) at 3-months) and baseline levels of the outcome. The subsequent models were further adjusted for possible confounders that could impact the association of vitamin D/PTH and the metabolic outcomes: (Model 2) duration of breastfeeding; and (Model 3) total physical activity and season of blood collection. The models were tested for collinearity of covariates using the variance inflation factor, which confirmed no significant collinearity. Sensitivity analyses were performed with adjustment for use of calcium and vitamin D supplements, smoking status, and change in BMI between 3- and 12-months postpartum. In addition, all analyses were repeated after excluding the 19 participants with PTH above the upper limit of the laboratory normal range (6.5 pmol/l).

To evaluate the combined impact of vitamin D status and PTH tertile at 3-months on future glucose tolerance, we evaluated the prevalence of dysglycemia (pre-diabetes or diabetes) at 12-months postpartum in groups defined by both vitamin D status and PTH using \( \chi^2 \) test (Figure 1). To further evaluate the combined impact of vitamin D and PTH status on metabolic outcomes, participants were stratified into four groups as follows: (i) vitamin D sufficient and PTH in the 1\textsuperscript{st}/2\textsuperscript{nd} tertile (reference group); (ii) vitamin D sufficient and PTH in the 3\textsuperscript{rd} tertile; (iii) vitamin D deficient/insufficient and PTH in the 1\textsuperscript{st}/2\textsuperscript{nd} tertile, and (iv) vitamin D deficient/insufficient and PTH in the 3\textsuperscript{rd} tertile. We then tested for a biological interaction between vitamin D deficiency/insufficiency and PTH in the 3\textsuperscript{rd} tertile. Using the equation described by Rothman (37) and others (38-40), we evaluated whether the combined impact of vitamin D deficiency/insufficiency with PTH in the 3\textsuperscript{rd} tertile on the outcomes of dysglycemia, lowest tertile of Matsuda index, and lowest tertile of ISSI-2 at 12-months postpartum exceeded the sum of the individual effects of these conditions alone (Figure 2). With this approach (38-40), the following three measures of biological interaction are calculated to quantify the amount of interaction on a multiplicative scale: the relative excess
risk due to interaction (RERI), the attributable proportion due to interaction (AP), and the synergy index (S). RERI can be interpreted as the risk that is additional to that which is to be expected on the basis of addition of the odds ratios (OR) under exposure, calculated as the difference between the expected risk and the observed risk (RERI = OR_{12} – OR_1 – OR_2 + 1). AP can be interpreted as the proportion of disease that is due to interaction amongst persons with both exposures (AP = RERI/OR_{12}). S can be interpreted as the excess risk from both exposures in the setting of interaction, relative to the risk from exposure without interaction (S = [OR_{12} - 1]/[(OR_1 - 1) + (OR_2 – 1)]. As previously described (37-38), RERI=0, AP=0 and S=1 indicate the absence of biological interaction.

Using ANCOVA, we compared the percentage change from 3- to 12-months postpartum for insulin sensitivity (Matsuda index), beta-cell function (ISSI-2) and glycemia (fasting glucose and 2-hour glucose) between these four vitamin D/PTH groups, adjusted for age, ethnicity, family history of diabetes, previous GDM, and BMI at 3-months (Figure 4). Finally, sequentially-adjusted multiple linear regression models (Table 3) were constructed with Matsuda index, ISSI-2, fasting glucose and 2-hour glucose at 12-months postpartum as the outcomes using the same approach to covariate adjustment as described earlier for Table 2.

RESULTS

The study population consisted of 494 women aged 34.8±4.3 years. There were no women with renal disease or other serious medical co-morbidities. We first evaluated the respective metabolic implications of their vitamin D status (Section I) and their PTH status (Section II), in turn, before considering both vitamin D and PTH together (Section III).
(I) Vitamin D Status: cross-sectional associations

Table 1 shows baseline (3-months postpartum) characteristics of the participants stratified into the following 3 groups based on their vitamin D status: vitamin D deficient (n=161; 33% of study population), insufficient (n=178; 36%), and sufficient (n=155; 31%). As anticipated, the groups differed in ethnicity (P<0.001) with the vitamin D deficient group having the greatest proportion of non-white ethnicity (39.8%). In addition, the vitamin D deficient group had higher prevalence rates of family history of diabetes (P=0.04) and current smoking (P<0.008), and lower levels of physical activity at 3-months postpartum (all P<0.03).

Metabolically, BMI (P<0.001) and waist circumference (P=0.03) differed across the groups, with both being highest in the deficient group. Consistent with these differences in adiposity, the vitamin D groups also differed with respect to insulin sensitivity, beta-cell function and glycemia at 3-months postpartum. Specifically, there was a stepwise decrease in Matsuda index (P<0.001) and ISSI-2 (P=0.04) from the sufficient to insufficient to deficient group, coupled with an analogous progressive increase in fasting glucose (P<0.001) and 2-hour glucose (P=0.002) across these groups.

Vitamin D Status: longitudinal covariate-adjusted analyses

On multiple linear regression analyses (Table 2), vitamin D deficiency at 3-months postpartum independently predicted increased fasting glucose (P=0.008) and 2-hour glucose (P=0.01) at 12-months postpartum, as compared to the vitamin D sufficient (reference) group. However, by itself, vitamin D deficiency did not independently predict poorer insulin sensitivity (Matsuda index) or beta-cell function (ISSI-2). Vitamin D insufficiency at 3-
months predicted decreased beta-cell function (P=0.006) and higher 2-hour glucose (P=0.04) at 12-months postpartum as compared to the reference group.

(II) **PTH Status: cross-sectional associations**

As shown in Table 1, the study population was also stratified into tertiles based on serum PTH at 3-months postpartum. In contrast to the vitamin D groups, these PTH groups did not differ in ethnicity, family history of diabetes, and current smoking. Leisure-time index was the only physical activity measure that differed between the PTH tertiles and was lowest in the 3rd tertile (P=0.03). As expected, 25-OH-D differed across the groups (P<0.001).

In the same way as observed across the vitamin D groups, BMI and waist differed across the PTH tertiles, being highest in the 3rd tertile (both P<0.001). In addition, insulin sensitivity progressively decreased (P<0.001) and fasting glucose increased (P=0.004) from the 1st to 2nd to 3rd tertile. ISSI-2 was lowest in the 3rd tertile but the overall comparison across the groups did not reach significance (P=0.06).

**PTH Status: longitudinal covariate-adjusted analyses**

On multiple linear regression analyses (Table 2), the 2nd tertile of PTH at 3-months postpartum did not independently predict insulin sensitivity, beta-cell function, fasting glucose or 2-hour glucose at 12-months, as compared to the (reference) 1st tertile. However, the 3rd tertile of PTH at 3-months independently predicted lower insulin sensitivity (P=0.008) and higher 2-hour glucose (P=0.007) nine months later.
(III) Vitamin D and PTH Status Combined

Recognizing that evaluation of vitamin D in conjunction with PTH might better reflect the status of the vitamin D-PTH axis than would consideration of either hormone in isolation, we next assessed the prevalence of dysglycemia (prediabetes or diabetes) at 12-months in relation to vitamin D status and PTH tertile combined. Indeed, as shown in Figure 1, there was a striking difference in the prevalence of dysglycemia at 12-months postpartum across the nine groups defined by vitamin D status (sufficient, insufficient, deficient) and PTH tertile at 3-months postpartum (P=0.003), with the highest prevalence in the group with vitamin D deficiency and PTH in the 3rd tertile (30%). Overall, of the 79 participants with pre-diabetes/diabetes at 12-months postpartum, 32% were in the group with vitamin D deficiency and PTH in the 3rd tertile at 3-months.

To further evaluate the combined impact of vitamin D and PTH on metabolic parameters, we stratified participants into the following 4 groups based on vitamin D and PTH status at 3-months postpartum: (i) vitamin D sufficient and PTH in the 1st/2nd tertile (reference group)(n=130); (ii) vitamin D sufficient and PTH in the 3rd tertile (n=25); (iii) vitamin D deficient/insufficient and PTH in the 1st/2nd tertile (n=212), and (iv) vitamin D deficient/insufficient and PTH in the 3rd tertile (n=127). To test for a biological interaction, we investigated whether the combined impact of vitamin D deficiency/insufficiency and PTH in the 3rd tertile on the outcomes of (i) dysglycemia, (ii) lowest tertile of Matsuda index, and (iii) lowest tertile of ISSI-2 at 12-months postpartum, exceeded the sum of the individual effects of these conditions.

With respect to dysglycemia at 12-months postpartum, the presence of vitamin D deficiency/insufficiency and PTH in the 3rd tertile alone conferred increments in the risk for dysglycemia of 48.4% and 71.4%, respectively, as compared to the reference group (vitamin
D sufficiency and PTH in 1st/2nd tertile). Interestingly, when both conditions were present, there was a 203.2% increased risk for dysglycemia which resulted in RERI=83.4%, AP=27.5% and S=1.69 (Figure 2A). In other words, the combined effect of vitamin D deficiency/insufficiency and PTH in the 3rd tertile conferred an excess risk of 83.4% beyond the sum of the individual effects. Furthermore, as indicated by the AP parameter, the effect of the interaction accounts for 27.5% of the dysglycemia amongst individuals with both vitamin D deficiency/insufficiency and PTH in the 3rd tertile.

Similarly, for the outcome of being in the lowest tertile of Matsuda index at 12-months postpartum, the presence of vitamin D deficiency/insufficiency and PTH in the 3rd tertile alone conferred excess risks of 109.8% and 21.3%, respectively, while the combined effect conferred an increased risk of 360.9% (RERI=229.8%, AP=49.8%, and S=2.75)(Figure 2B). Lastly, for the lowest tertile of ISSI-2, the presence of vitamin D deficiency/insufficiency and PTH in the 3rd tertile alone conferred risk increments of 90.8% and -5%, respectively, while the combined effect conferred an increased risk of 152% (RERI=66.2%, AP=26.3% and S=1.77)(Figure 2C).

**Vitamin D and PTH Status Combined: longitudinal covariate-adjusted analyses**

Figure 3 shows the adjusted estimates at 3- and 12-months postpartum for insulin sensitivity, beta-cell function, fasting glucose and 2-hour glucose in the four vitamin D/PTH groups, after adjustment for the diabetes risk factors age, ethnicity, family history of diabetes, previous GDM, and BMI. Notably, vitamin D deficiency/insufficiency with PTH in the 3rd tertile was associated with lower Matsuda index (Panel A) and ISSI-2 (Panel B), and increased fasting glucose (Panel C) and 2-hour glucose (Panel D). In addition, this group
exhibited a pronounced deterioration in ISSIE2 and 2-hour glucose between 3- and 12-months postpartum that was not observed in the other 3 groups.

To further explore the differential changes in metabolic outcomes between the four groups defined by vitamin D and PTH together, we evaluated the adjusted percentage change in insulin sensitivity, beta-cell function and glycemia in each of these groups between 3- and 12-months postpartum. As shown in Figure 4, only the group with vitamin D deficiency/insufficiency and PTH in the 3rd tertile had a significant decline in ISSI-2 (-4.7 ±3.4% vs. +7.6 ±4.4%, P=0.04) and 2-hour glucose (+6.6 ±2.6% vs. -0.4 ±2.8%, P=0.05) as compared to the reference group. Of note, none of the other vitamin D/PTH groups showed changes in these metabolic outcomes compared to the reference group (all P>0.15).

Finally, we performed multiple linear regression analyses to evaluate the longitudinal associations between combined vitamin D/PTH status at 3-months postpartum and metabolic outcomes, after full covariate adjustment. As shown in Table 3, vitamin D deficiency/insufficiency accompanied by PTH in the 3rd tertile was the only group that independently predicted lower insulin sensitivity (P=0.01) and beta-cell function (P=0.008), and increased fasting glucose (P=0.03) and 2-hour glucose (P=0.002) at 12-months postpartum, as compared to the reference group (Table 3). Of note, vitamin D deficiency/insufficiency with PTH in the 1st/2nd tertile did not independently predict any of these metabolic outcomes. In sensitivity analyses, these results were unchanged with further adjustment for use of vitamin D/calcium supplements, smoking status, and change in BMI between 3- and 12-months postpartum (data not shown). In addition, none of the results changed after excluding the 19 participants with PTH above the laboratory normal range, with the exception of fasting glucose with which vitamin D deficiency/insufficiency and PTH in the 3rd tertile was now associated at borderline significance (P=0.06) (data not shown).
DISCUSSION

In this study, we show that the combination of vitamin D deficiency/insufficiency and increased PTH is an independent predictor of deterioration in insulin sensitivity, beta-cell function and glycemia in a cohort of women in the 1st year postpartum. Notably, vitamin D deficiency/insufficiency with higher PTH conferred an excess risk for future dysglycemia, decreased insulin sensitivity and poorer beta-cell function that exceeded the sum of the individual risks associated with these conditions. While both vitamin D and PTH alone were independently associated with some of the outcomes, only the coupling of vitamin D deficiency/insufficiency with higher PTH was consistently associated with declining insulin sensitivity and beta-cell function, and rising glycemia over time.

Previous studies have suggested an association of integrated assessment of vitamin D/PTH with glucose metabolism (41-42). Specifically, in a cross-sectional analysis evaluating 15 obese girls and 15 matched controls, Stanley et al demonstrated that the ratio of PTH/vitamin D was associated with insulin sensitivity and high-sensitivity C-reactive protein (41). These results were confirmed by another study of 133 obese adolescents that showed an association of the PTH/vitamin D ratio with presence of metabolic syndrome (42). Our study further extends this concept by demonstrating an interaction between vitamin D deficiency/insufficiency and PTH on longitudinal changes in glucose metabolism in a prospective cohort. This novel analysis has two key implications. First, it supports an effect of vitamin D status on glucose homeostasis by demonstrating an independent association of the PTH-vitamin D axis with the development of hyperglycemia through the worsening of insulin sensitivity and beta-cell function. Second and most importantly, these data highlight the need for assessment of the entire PTH-vitamin D axis when studying the effect of vitamin D on glucose metabolism. Specifically, by showing that vitamin D deficiency/insufficiency
has a differential association with glucose metabolism and its underlying physiology depending on the concurrent level of PTH, our study suggests a new perspective for future clinical trials of vitamin D supplementation.

Previous studies evaluating the association of vitamin D and glucose metabolism have yielded inconsistent results. Although lower 25-OH-D has been associated with incident T2DM (7-9;43) and decline in beta-cell function over time (10-11) in several observational studies, other investigators have noted conflicting results (13;15-16;18). In addition, interventional studies aiming to evaluate the effect of vitamin D supplementation on glucose metabolism have yielded inconsistent conclusions (19-21;44-45). Physiologic studies have found either no impact of vitamin D supplementation on clamp-derived insulin secretion (21) or an effect restricted to first-phase insulin secretion (44). In the same way, the results of clinical trials have not been consistent or robust in supporting a role for vitamin D supplementation in the prevention of T2DM (46). In a randomised trial of 71 obese men, vitamin D supplementation improved postprandial insulin sensitivity but had no effect on insulin secretion or hepatic insulin resistance (20). Moreover, in a study of 92 adults at risk for diabetes, supplementation with vitamin D yielded a slight improvement in beta-cell function and a marginal effect on glycemic control (45). There are several possible reasons for these inconsistencies such as differences in study populations, diverse methodologies in the assessment of beta-cell function and insulin sensitivity, and the potential impact of confounders (such as obesity and outdoor physical activity). However, the results of the current study suggest that the lack of careful documentation of the PTH-vitamin D axis may in part explain the contradictory findings of these previous reports.

Our analyses demonstrate that vitamin D deficiency/insufficiency of sufficient biologic impact to result in an increase in PTH was independently associated with
dysglycemia, declining insulin sensitivity and beta-cell dysfunction. These data raise the possibility that previous randomised controlled trials failed to detect a robust effect of vitamin D supplementation on glucose metabolism because they were not performed in the specific patient population that would most benefit from this intervention.

Our findings are supported by biological factors suggesting a link between vitamin D status and glucose homeostasis. First, vitamin D receptors are expressed in pancreatic beta-cells and target tissues for insulin action such as skeletal muscle and adipose tissue (9). Second, vitamin D receptor polymorphisms impact insulin secretion and sensitivity in humans (47-48). Finally, the active form of vitamin D, calcitriol, has an effect in modulating calcium influx and gene expression in beta-cells (49). Most notably, it has been reported that calcitriol does not impact insulin release when pancreatic islets are under normal conditions, instead requiring a stressed environment such as exposure to pathologic cytokines or vitamin D deficiency for the detection of its effect (49). Thus, it would appear that low 25-OH-D coupled with increased PTH may be a better indicator reflecting the state of vitamin D deficiency/insufficiency that leads to dysregulation of glucose homeostasis.

Our study is robust as it was performed in a well-characterized cohort undergoing serial metabolic evaluation at a time (1st year postpartum when women may be breastfeeding and often indoors to limit infant sun exposure) and geographic location (northern latitude) that may contribute to an increased likelihood of vitamin D deficiency/insufficiency. Accordingly, this cohort provides a unique opportunity to study the impact of PTH-vitamin D status on glucose metabolism. Furthermore, to our knowledge, this is the first study to prospectively evaluate both vitamin D and PTH together in relation to glucose homeostasis and its underlying physiology. A possible limitation of this study is that we did not measure 1,25-dihydroxyvitamin D and vitamin D–binding protein, which could provide a more
comprehensive evaluation of this pathway. However, 25-OH-D and PTH are the standard clinical measures for assessment of vitamin D status in practice. In addition, because vitamin D and PTH are associated with adiposity, it is reasonable to consider the possibility that the impact of the vitamin D/PTH axis on glucose metabolism is due to its association with obesity. However, it should be noted that all models were adjusted for BMI and that change in BMI was further evaluated in sensitivity analyses which supported an independent association between the vitamin D/PTH axis and glucose homeostasis. Another consideration is that the study population consisted of young women in the postpartum period, such that the results should be confirmed in large population-based studies outside this setting. Lastly, beta-cell function and insulin sensitivity were assessed with surrogate indices, rather than more time-consuming and cumbersome clamp studies which would have been difficult to implement in 494 new mothers on two occasions in the first year after delivery. Moreover, ISSI-2 and Matsuda index are validated measures that have been widely used in previous studies (10-11;23;32-35).

In conclusion, we demonstrate that PTH status needs to be considered when evaluating the associations of vitamin D status with glucose homeostasis and its underlying determinants. Specifically, vitamin D deficiency/insufficiency with increased PTH is independently associated with deterioration in insulin sensitivity, beta-cell function and glycemia over time, which was not observed in women with vitamin D deficiency/insufficiency in conjunction with lower PTH. This concept provides novel pathophysiologic insight relevant to the impact of vitamin D status on T2DM and warrants further evaluation in future clinical trials of vitamin D supplementation.
AUTHOR CONTRIBUTIONS

CKK wrote the first draft of the manuscript. CKK, BS and RR contributed to statistical analysis. AJH, PWC, MS, BZ, and RR contributed to study conception and design. All authors contributed to analysis and interpretation of the data, and revision of the manuscript for intellectual content. All authors gave approval for submission.

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DUALITY OF INTEREST

There are no conflicts of interest to declare in relation to this manuscript.
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Table 1: Baseline comparisons (i) between groups of vitamin D status (deficient/insufficient/sufficient) at 3-months postpartum and (ii) between tertiles of PTH at 3-months postpartum

<table>
<thead>
<tr>
<th>Vitamin D Status</th>
<th>Deficient (25-OH-D &lt;50 nmol/l)</th>
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<td>178</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>Months postpartum (months)</td>
<td>3 (3-4)</td>
<td>3 (3-4)</td>
<td>3 (3-4)</td>
<td>0.10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.5 ± 4.2</td>
<td>34.9 ± 4.5</td>
<td>35.0 ± 4.1</td>
<td>0.47</td>
</tr>
<tr>
<td>Ethnicity:</td>
<td>White (%)</td>
<td>60.0</td>
<td>73.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asian (%)</td>
<td>14.3</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (%)</td>
<td>25.5</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>Family history of T2DM (%)</td>
<td>57.0</td>
<td>54.0</td>
<td>43.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>8.1</td>
<td>3.3</td>
<td>1.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Season of blood sample collection:</td>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>Winter (%)</td>
<td>27.9</td>
<td>20.2</td>
<td>20.7</td>
<td></td>
</tr>
<tr>
<td>Spring (%)</td>
<td>27.9</td>
<td>28.0</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>Summer (%)</td>
<td>17.4</td>
<td>21.3</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td>Fall (%)</td>
<td>26.7</td>
<td>30.3</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>Total physical activity</td>
<td>4.7 ± 1.0</td>
<td>5.0 ± 1.0</td>
<td>5.1 ± 1.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Sport index</td>
<td>1.8 (1.5-2.3)</td>
<td>2.0 (1.5-2.5)</td>
<td>2.0 (1.8-2.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Leisure time index</td>
<td>2.8 (2.5-3.3)</td>
<td>3.0 (2.5-3.3)</td>
<td>3.0 (2.5-3.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Duration of breastfeeding (months)</td>
<td>3.0 (2.0-4.0)</td>
<td>3.0 (2.5-3.0)</td>
<td>3.0 (3.0-3.5)</td>
<td>0.30</td>
</tr>
<tr>
<td>25-OH-D (nmol/l)</td>
<td>35.7 ± 10.2</td>
<td>64.4 ± 7.4</td>
<td>91.9 ± 12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>4.0 (3.2-4.8)</td>
<td>2.9 (2.2-3.7)</td>
<td>2.7 (2.1-3.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 (23.9-32.3)</td>
<td>25.3 (23.4-28.9)</td>
<td>24.5 (22.1-27.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.0 (83-100)</td>
<td>88.0 (82-96)</td>
<td>86.0 (81-93)</td>
<td>0.03</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>7.7 (5.0-11.6)</td>
<td>11.6 (7.8-15.5)</td>
<td>14.0 (9.0-17.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ISSI-2</td>
<td>706 (543-933)</td>
<td>733 (625-994)</td>
<td>797 (602-1045)</td>
<td>0.04</td>
</tr>
<tr>
<td>Fasting glucose on OGTT (mmol/l)</td>
<td>4.7 ± 0.6</td>
<td>4.6 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-hour glucose on OGTT (mmol/l)</td>
<td>6.3 (5.3-7.5)</td>
<td>6.0 (5.1-7.3)</td>
<td>5.7 (4.8-6.7)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Continuous data presented as mean ± SD (if normally distributed) or median followed by (25th-75th) if skewed
Table 2: Multiple linear regression models showing adjusted estimates for vitamin D status (deficiency, insufficiency) at 3-months postpartum and PTH tertile (second, third) at 3-months postpartum in predicting the following glucose/metabolic outcomes at 12-months postpartum: (i) Matsuda index, (ii) ISSI-2 (iii) fasting glucose and (iv) 2-hour glucose (adjusted for the indicated covariates in each model). For the vitamin D groups, the reference group is vitamin D sufficiency at 3-months postpartum. For PTH tertiles, the reference group is the first tertile at 3-months postpartum.

<table>
<thead>
<tr>
<th>Outcomes at 12-months</th>
<th>Vitamin D Status at 3-months</th>
<th>Tertiles of PTH at 3-months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deficient (25-OH-D &lt;50 nmol/l)</td>
<td>Insufficient (50 ≤ 25-OH-D &lt;75 nmol/l)</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
<td>P</td>
</tr>
<tr>
<td>(i) Matsuda index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, Matsuda at 3-months</td>
<td>-0.105</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>-0.107</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>-0.099</td>
<td>0.08</td>
</tr>
<tr>
<td>(ii) ISSI-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, ISSI-2 at 3-months</td>
<td>-0.051</td>
<td>0.23</td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>-0.051</td>
<td>0.23</td>
</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>-0.062</td>
<td>0.16</td>
</tr>
<tr>
<td>(iii) Fasting glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, fasting glucose at 3-months</td>
<td>0.023</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>0.023</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>0.026</td>
<td>0.008</td>
</tr>
<tr>
<td>(iv) 2-hour glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, 2-hour glucose at 3-months</td>
<td>0.069</td>
<td>0.009</td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>0.069</td>
<td>0.009</td>
</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>0.070</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Bold indicates p<0.05
Outcome variables are log-transformed
Table 3. Multiple linear regression models showing adjusted estimates for vitamin D/PTH groups at 3-months postpartum in predicting the following glucose/metabolic outcomes at 12-months postpartum: (i) Matsuda index, (ii) ISSI-2 (iii) fasting glucose and (iv) 2-hour glucose (adjusted for the indicated covariates in each model). The reference group is vitamin D sufficient with PTH in 1st/2nd tertile at 3-months postpartum.

<table>
<thead>
<tr>
<th>Outcomes at 12-months</th>
<th>Vitamin D / PTH groups</th>
<th>Vitamin D sufficient</th>
<th>PTH 1st/2nd tertile (25-OH-D ≥75 nmol/l and PTH ≤ 3.8 pmol/l)</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
<th>Estimate</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Matsuda index</td>
<td></td>
<td></td>
<td>VITAMIN D SUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, Matsuda at 3-months</td>
<td>REF.</td>
<td>---</td>
<td>-0.105</td>
<td>0.29</td>
<td>-0.0289</td>
<td>0.58</td>
<td>-0.151</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>REF.</td>
<td>---</td>
<td>-0.104</td>
<td>0.30</td>
<td>-0.0280</td>
<td>0.59</td>
<td>-0.154</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>REF.</td>
<td>---</td>
<td>-0.089</td>
<td>0.38</td>
<td>-0.0188</td>
<td>0.72</td>
<td>-0.156</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ii) ISSI-2</td>
<td></td>
<td></td>
<td>VITAMIN D SUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, ISSI-2 at 3-months</td>
<td>REF.</td>
<td>---</td>
<td>0.0030</td>
<td>0.86</td>
<td>0.015</td>
<td>0.10</td>
<td>0.020</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>REF.</td>
<td>---</td>
<td>0.0031</td>
<td>0.85</td>
<td>0.015</td>
<td>0.10</td>
<td>0.020</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>REF.</td>
<td>---</td>
<td>0.0034</td>
<td>0.84</td>
<td>0.015</td>
<td>0.09</td>
<td>0.023</td>
<td>0.03</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(iii) Fasting glucose</td>
<td></td>
<td></td>
<td>VITAMIN D SUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
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<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, fasting glucose at 3-months</td>
<td>REF.</td>
<td>---</td>
<td>0.0030</td>
<td>0.86</td>
<td>0.015</td>
<td>0.10</td>
<td>0.020</td>
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<td>---</td>
<td>0.0031</td>
<td>0.85</td>
<td>0.015</td>
<td>0.10</td>
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<td>Model 3: model 2 + physical activity + season</td>
<td>REF.</td>
<td>---</td>
<td>0.0034</td>
<td>0.84</td>
<td>0.015</td>
<td>0.09</td>
<td>0.023</td>
<td>0.03</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(iv) 2-hour glucose</td>
<td></td>
<td></td>
<td>VITAMIN D SUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
<td>VITAMIN D DEFICIENT/INSUFFICIENT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: age, ethnicity, family history of T2DM, previous GDM, BMI, 2-hour glucose at 3-months</td>
<td>REF.</td>
<td>---</td>
<td>-0.026</td>
<td>0.58</td>
<td>0.038</td>
<td>0.12</td>
<td>0.091</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2: model 1 + duration of breastfeeding</td>
<td>REF.</td>
<td>---</td>
<td>-0.026</td>
<td>0.58</td>
<td>0.038</td>
<td>0.13</td>
<td>0.091</td>
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</tr>
<tr>
<td>Model 3: model 2 + physical activity + season</td>
<td>REF.</td>
<td>---</td>
<td>-0.027</td>
<td>0.58</td>
<td>0.035</td>
<td>0.17</td>
<td>0.094</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bold indicates p<0.05
Outcome variables are log-transformed
Figure 1: Prevalence of dysglycemia (pre-diabetes or diabetes) at 12-months postpartum within each strata of vitamin D status and PTH tertile at 3-months postpartum. P value refers to overall comparison across the groups.
Figure 2: Excess risk for (Panel A) dysglycemia, (Panel B) lowest tertile of Matsuda index, and (Panel C) lowest tertile of ISSI-2 at 12-months postpartum attributed to vitamin D insufficiency/deficiency, PTH in 3rd tertile and their combined effect, as compared to (reference group) vitamin D sufficiency with PTH in 1st/2nd tertile.
Figure 3: Adjusted mean levels for (Panel A) Matsuda index, (Panel B) ISSI-2, (Panel C) fasting glucose, and (Panel D) 2-hour glucose in each of the 4 vitamin D/PTH groups at 3- and 12-months postpartum, adjusted for age, ethnicity, family history of diabetes, previous gestational diabetes, and BMI at 3-months.
Figure 4: Percentage change in (Panel A) Matsuda index, (Panel B) ISSI-2, (Panel C) fasting glucose, and (Panel D) 2-hour glucose in each of the 4 vitamin D/PTH groups between 3- and 12-months postpartum, adjusted for age, ethnicity, family history of diabetes, previous gestational diabetes, and BMI at 3-months.

**P ≤ 0.05 for comparison with reference group (vitamin D sufficient with PTH in 1st/2nd tertile)