# Obstetric and Neonatal Outcomes of Maternal Vitamin D Supplementation: Results of an Open-Label, Randomized Controlled Trial of Antenatal Vitamin D Supplementation in Pakistani Women

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**Objective:** The objective of the study was to determine whether vitamin D (vitD) supplementation during pregnancy affects obstetric and neonatal outcomes.

**Setting:** The study was conducted at a university hospital in Karachi, Pakistan.

**Methods:** The study was a single-center, open-label, randomized, controlled trial of routine care (group A, 200 mg ferrous sulfate and 600 mg calcium daily) vs vitD supplementation (group B, 4000 IU vitamin D3 daily), started at 20 weeks and continued till delivery. Maternal serum samples of 25-hydroxyvitamin D (25OHD) were collected at baseline and delivery. Neonatal vitD status was assessed in cord blood or in neonatal serum samples within 48 hours of birth. Obstetric outcomes included gestational hypertension, gestational diabetes, and preterm labor, and neonatal well-being included small for gestational age, birth weight, length, head circumference, and 1- and 5-minute Apgar scores.

**Results:** Of 207 gravidae enrolled, 193 completed the trial. Maternal age, vitD status, and gestational age at enrollment were comparable between the two groups. At delivery, maternal 25OHD was increased in group B (18.3 $\pm$ 11 ng/dL vs 8.82 $\pm$ 11.84 ng/dL ($P = .001$) compared with group A (6.9 $\pm$ 7.0 ng/dL vs 6.32 $\pm$ 3.97 ng/dL, $P = .06$). The obstetric outcomes were comparable between the two groups ($P > .05$). Neonatal 25OHD levels were significantly higher in group B compared with group A (19.22 $\pm$ 12.19 ng/dL vs 6.27 $\pm$ 5.2 ng/dL). There was positive correlation between maternal and neonatal 25OHD levels ($r = 0.83$, $P = .001$). One- and 5-minute Apgar scores were significantly higher in group B (7.10 $\pm$ 0.66 vs 6.90 $\pm$ 0.50, $P = .026$, and 8.53 $\pm$ 0.68 vs 8.33 $\pm$ 0.81, $P = .051$, respectively). Neonatal anthropometric parameters were comparable between the two groups ($P > .05$).

**Conclusion:** Maternal vitD supplementation improved maternal and neonatal vitD status. **(*J Clin Endocrinol Metab* 99: 2448–2455, 2014)**

Vitamin D (vitD) plays important roles in cell functioning, bone mineral metabolism, and calcium homeostasis. Low levels of vitamin D have been identified in chronic conditions like multiple sclerosis, depression, tuberculosis, and human immune deficiency virus infection (1–3). vitD deficiency during pregnancy is emerging as a

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Abbreviations: BP, blood pressure; CI, confidence interval; GDM, gestational diabetes mellitus; IQR, interquartile range; 25OHD, 25-hydroxyvitamin D; RCT, randomized controlled trial; SGA, small for gestational age; vitD, vitamin D.

public health issue globally; approximately 36% of the population in the United States has been shown to be vitD deficient (4). All age groups are vulnerable to vitD deficiency, including newborns, children, adolescents, pregnant women, and the elderly of either gender (5).

Much debate has recently centered on the serum threshold level of 25-hydroxyvitamin D (25OHD), a metabolite that reliably reflects vitD status, that reflects evidence of vitD deficiency. The Endocrine Society of North America acknowledges serum 25OHD less than 30 ng/mL to reflect vitD deficiency (6). The Institute of Medicine, however, proposes 20 ng/mL as a threshold above which an individual is deemed as vitD sufficient (7). Although Institute of Medicine and some other organizations adhere to the proposed guidelines, regardless of pregnancy status, the importance of ensuring serum 25OHD greater than 30 ng/mL in pregnancy is underscored by The Endocrine Society and prioritized by other organizations.

A role of vitD is identified in all stages of pregnancy, from implantation to delivery (8, 9). Maternal vitD deficiency has been related to adversely affect skeletal, cardiovascular, respiratory, and neuronal functions of the newborn (10, 11). In a prospective study of more than 400 pregnant women, decreased maternal 25OHD levels were associated with an increased splaying index of fetal femora, evident as early as at 19 weeks of pregnancy (10). Although decreased birth weight and neonatal length, delay in fontanelle closure, and an increased risk for neonatal infections are described in association with maternal vitD insufficiency, positive correlations are reported between maternal vitD levels and birth weight, neonatal length, and Apgar scores, albeit inconsistently (12). Maternal vitD status is additionally recognized to hold long-term implications for the progeny. In a longitudinal study from the United Kingdom, lower maternal vitD levels were associated with lower whole-body and lumbar spine bone mineral content of children at 9 years of age (13).

Maternal vitD deficiency has been associated with a risk of common maternal morbidities including preeclampsia, gestational diabetes mellitus, preterm labor (14), and operative delivery; Merewood et al (8) reported that the likelihood for delivering by primary cesarean section was 4-fold higher in pregnant women with serum 25OHD levels of 37.5 nmol/L or greater, after controlling for confounding factors like age, ethnicity, education, and insurance status.

We and others have previously reported on the concerning magnitude and severity of hypovitaminosis D in Pakistani parturients (15, 16). Almost 90% of Pakistani women delivering at a tertiary care institution were observed to be deficient in vitD (25OHD $<$ 30 ng/dL) (15). We herein report results of the first randomized controlled trial (RCT) of maternal vitD supplementation undertaken in Pakistani gravidae with a focus on effects of maternal vitD supplementation on maternal and neonatal vitD status and on specified maternal and neonatal outcomes.

## Materials and Methods

The study was approved by the Ethical Review Board of Dow University of Health Sciences and was registered at clinical trials.gov (identifier number NCT01418664). In the period between September 2010 and May 2012, pregnant women attending the outpatient obstetric clinic at the Civil Hospital Karachi (Karachi, Pakistan) were deemed eligible for participation if they were at 20 weeks or less of gestation with a singleton pregnancy and deemed to be normoglycemic and normotensive at the time of antenatal booking; accessibility via a valid telephone contact number was further considered as an inclusion criterion. History of gestational diabetes, hypertension, thyroid disorder, chronic liver disease, and evidence of fetal anomaly in current pregnancy were predetermined exclusion criteria. Eligible gravidae provided verbal informed consent.

At baseline, details regarding demographics (age; marital status; parity; educational and socioeconomic status), diet (intake frequency of dairy products including milk, cheese, and yogurt assessed by verbal recall), and sun exposure (assessed by questions about dress code, ie, extent of coverage of arms and face, and by weekly estimate of time spent in the sun) were captured using predesigned questionnaires previously reported (15). Sequential randomization schema was used and eligible gravidae were randomized into two groups: group A was assigned to routine antenatal care, which included ferrous sulfate 200 mg, twice daily, and 600 mg of calcium lactate daily. Group B, in addition to the routine care regimen, received oral liquid formulation (400 IU/drop) of vitamin D3 at a daily dose of 4000 IU (10 drops daily) starting at completed 20 weeks of gestation (Figure 1). Frequency of antenatal visits was per routine clinical care, ie, every 4 weeks until 28 weeks, every 2 weeks until 36 weeks, and then weekly until delivery. At each antenatal visit, participants in either group were provided with study medications; those assigned to group B were requested to bring back used vitD bottles to allow the assessment of compliance; those demonstrating 80% adherence with dispensed supplements were deemed as compliant. Participants were reminded about their scheduled antenatal checkups by telephone calls.

Figure 2 outlines the details of antenatal screening and testing. At each antenatal visit, participants (either group) underwent routine clinical evaluation [examination in-

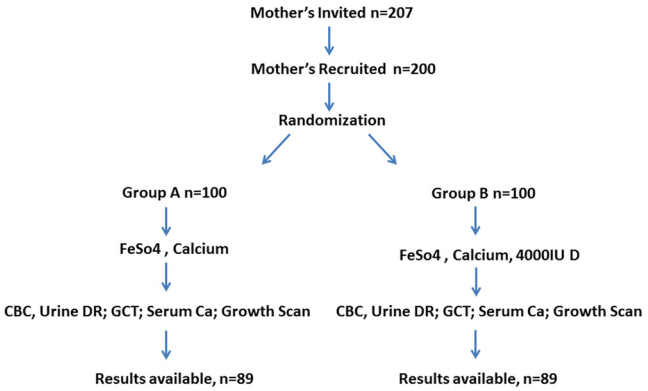


**Figure 1.** Flow chart showing enrollment of study participants. calcium, 600 mg; CBC, complete blood count; FeSo4, ferrous sulfate (200 mg); GCT, glucose challenge test; serum Ca, serum calcium; urine DR, urine detailed report.

cluding monitoring of blood pressure (BP)], and laboratory assessment (urinalysis for proteinuria and blood sampling for estimation of complete blood count), serum calcium, and screening for gestational diabetes mellitus (GDM) was undertaken at 24 weeks' gestation by an oral glucose challenge test using a 50-g glucose load (17) with plans for proceeding with an oral glucose tolerance test with 75 g glucose for those failing the oral glucose challenge test (18). Gestational hypertension was defined as a BP of 140/90 mm Hg or greater, without evidence of proteinuria; preeclampsia was defined as a BP of 140/90 mm Hg with proteinuria of 1+ or greater (on urine dipstick) in a previously normotensive woman, after 20 weeks of gestation (19). Preterm labor was defined as the onset of labor before 37 completed weeks of gestation (20); small for gestational age (SGA) was defined as birth weight of less than 2500 g (14). Fetal death was defined as the death of the fetus before complete expulsion or extraction from the mother, irrespective of duration of pregnancy.

Study participants were excluded from the clinical trial if three consecutive antenatal follow-up visits were missed. Pregnancy data including complications (eg, preeclampsia, intrauterine fetal demise, stillbirth, gestation at delivery, and mode of delivery) were collected from patient records.

Assessment of newborns was undertaken by trained staff at the time of delivery, using the World Health Organization's standardized protocol (21). This included assessment of neonatal Apgar scores at minutes 1 and 5 after birth, weight (in kilograms), length (in centimeters), head circumference (in centimeters), and the longest diameter of the anterior fontanelle (in centimeters).

## Biochemical data

Maternal, nonfasting venous samples (5 cc) were collected at recruitment and at delivery. Newborn's serum samples included either cord blood or venous samples collected within 48 hours of birth. Serum samples were centrifuged and stored at −80°C for subsequent assessment of 25OHD (nanograms per milliliter) by chemiluminescence immunoassay based on a direct competitive immunoassay technique. Two levels of commercial controls were run with each lot of the samples with the coefficient of variation in the range between 4% and 6%. Maternal serum calcium levels were assessed by the spectrophotometery method as a safety measure to assess for hypercalcemia, a manifestation of vitamin D intoxication, on a monthly basis (22). As per the guidelines of The Endocrine Society of North America (6), a serum 25OHD level less than 30 ng/mL was deemed as evidence of vitD deficiency and a level of 10 ng/mL or less defined severe vitD deficiency.

## Statistical analysis

Data were imputed into Excel (Microsoft Inc), and individual variables were examined to ensure against inadvertent errors. Continuous variables were examined for distribution, and skewed data were log transformed and reexamined for the attainment of normal distribution. Parametric analyses (Pearson's correlation, Student *t* test, and linear regression) compared normally distributed data across treatment groups (A vs B), whereas nonparametric analyses (Spearman correlation, Mann-Whitney *U*) compared skewed data between the two groups. Multivariable logistic regression analyses examined the effect of intervention (group B vs reference group A) on dichotomized outcomes (preeclampsia, GDM, preterm delivery, neonatal death, intrauterine fetal demise, SGA, delivery by cesarean section).

The goodness-of-model fit was assessed (23). Multivariable linear regression analyses assessed for the impact of vitD supplementation on nominal outcomes (maternal BP, gestational age at delivery, neonatal birth weight, head circumference, length, and Apgar scores). Variation inflation factor analyses were

| | 18-20 weeks | 24 weeks | 28 weeks | 32 weeks | 36 weeks | Delivery | Postnatal |
|---|---|---|---|---|---|---|---|
| Screening, randomization | √ | | | | | | |
| Consent | √ | | | | | | |
| Anomaly scan | √ | | | | | | |
| Demographic details | √ | | | | | | |
| Maternal height, weight, BP | √ | | | | | | |
| Maternal blood for 25(OH)D | √ | | | | | | |
| CBC, GCT, Ca, urine DR, BP | | √ | | | | | |
| CBC, Urine, Ca, US, BP | | | √ | | | | |
| CBC, Urine, Ca, US, BP | | | | √ | | | |
| CBC, Urine, Ca, US, BP | | | | | √ | | |
| Maternal 25(OH)D, BP | | | | | | √ | |
| Neonatal 25(OH)D, length, weight, head circumference, Apgar scores at 1 and 5 minutes | | | | | | | √ |

**Figure 2.** Summary of trial events. Ca, serum calcium; CBC, complete blood count; GCT, glucose challenge test; US, ultrasound.

**Table 1.** Baseline Characteristics

| Baseline Data | Group A (Routine Care) | Group B (vitD) | *P* Value |
|---|---|---|---|
| Age, y[a] | 25.19 ± 4.36 | 25.96 ± 3.13 | .14 |
| BMI, kg/m$^{2}$[a] | 23.26 ± 4.09 | 24.04 ± 3.82 | .06 |
| Parity[b] | 1 (0–3) | 2 (1–3) | .40 |
| Antenatal visits, n[a] | 4.71 ± 0.62 | 4.88 ± 1.13 | .33 |
| Serum calcium, mg/dL[a] | 8.69 ± 0.66 | 9.22 ± 0.72 | .18 |
| Serum 25OHD at 20 wk, ng/dL[b] | 5.31 (3–8.2) | 4.74 ( 3–9) | .80 |

Abbreviation: BMI, body mass index.

[a] Mean (SD).

[b] Median (IQR).

conducted to assess for multicollinearity in multivariable linear regression analyses (24). Continuous data (normal in distribution) are presented as mean ± SD or median and interquartile range (skewed distribution), and categorical data are presented as percentage. Regression (r) and correlation coefficients [$\beta$-coefficients reflect strength of associations between continuous variables; generalized linear model with Poisson regression estimated relative risk and 95% confidence intervals (CI) for dichotomized outcomes]. Hypothesizing a 30% reduction in cumulative maternal-infant morbidity with vitD supplementation, a priori power analyses identified the study to be powered at 80% for a fixed $\alpha$ of .05 with a sample size of 100 in each arm. Two-tailed $P < .05$ was considered to reflect statistical significance, and STATA 12.0 was used for analyses.

## Results

Figure 1 outlines enrollment details. Of 200 gravid women recruited, 193 (87.5%) were followed up until delivery. Results were available for 175 women (n = 89) subjects in group A and (n = 86) subjects in group B).

### Baseline data (Table 1)

The two groups were comparable in demographic and baseline characteristics including vitD status.

### Improved maternal vitD status with supplementation

At delivery, the parturients assigned to group B (ie, daily supplementation with 4000 IU vitamin D3) demonstrated significantly higher serum levels of 25OHD compared with baseline, whereas the levels were essentially unchanged in the women assigned to routine care ($P$ = .001, Figure 2). Notably, despite adequate compliance, daily supplementation with 4000 IU vitamin D3 starting at 20 weeks' gestation failed to achieve normal serum 25OHD levels in almost four fifths of the women; only 15% of those in group B attained serum levels of 30 ng/mL or greater. In contrast, 99% of the population in group A was deficient in vitD at delivery [relative risk for vitD deficiency at the time of delivery for group A was 1.16 (95% CI 1.06–1.28), $P$ = .001].

### Effect of maternal vitD supplementation on pregnancy outcomes (Table 2)

As demonstrated in Table 2, specified outcomes of interest were comparable between the two groups; maternal vitD supplementation failed to impact on risks for preterm delivery, gestational hypertension, preeclampsia, GDM, or the incidence of delivery by cesarean section ($P > .05$).

### Effect of maternal vitamin D supplementation on the neonate (Table 3)

Babies of mothers assigned to group B (vitD supplementation) demonstrated significantly higher 1-minute Apgar scores ($P$ = .03); 5-minute Apgar scores were similarly higher in group B compared with group A newborns ($P$ = .05). Maternal vitD supplementation failed to impact on neonatal anthropometric parameters. Newborns of

**Table 2.** Pregnancy Outcome Data: Results of RCT of Routine Care (Group A) vs Daily Supplementation With 4000 IU Vitamin D3 (Group B) Starting at 20 Weeks' Gestation and Continued Until Delivery

| Pregnancy Outcome | Group A (n = 89) | Group B (n = 86) | *P* Value |
|---|---|---|---|
| Duration of gestation, wk | 37.66 ± 2.0 | 37.56 ± 1.9 | .29 |
| Preterm birth, %[a] | 10 (10.75%) | 12 (12.63%) | .67 |
| Gestational hypertension, %[b] | 7 (7.5%) | 11 (11.57%) | .32 |
| Preeclampsia[c] | 6 (6.45%) | 10 (10.52%) | .36 |
| Abnormal GCT[d] | 6 (6.45%) | 10 (10.52%) | .36 |
| SGA[e] | 18 (19.35%) | 19 (20%) | .84 |
| Intrauterine fetal demise | 0 | 1 | .05 |
| Cesarean section | 46 (49.46%) | 49 (51.57%) | .97 |

Abbreviation: GCT, glucose challenge test. Data are presented in n (percentage) or as mean ± SD.

[a] Less than 37 completed weeks of gestation.

[b] BP of 140/90 mm Hg or greater without proteinuria.

[c] BP of 140/90 mm Hg or greater with +1 or greater proteinuria.

[d] An abnormal glucose level greater than 140 mg/dL at 1 hour, after a glucose load of 50 g.

[e] Birth weight less than 2500 g.

**Table 3.** Neonatal Data: Results of RCT of Routine Antenatal Care (Group A) vs Daily Maternal Supplementation With 4000 IU Vitamin D3 (Group B) Starting at 20 Weeks' Gestation and Continued Until Delivery

| Variable | Group A | Group B | *P* Value |
|---|---|---|---|
| Neonatal serum 25OHD (ng/ml) | | | |
| Less than 30 ng/mL, % | 18% | 69% | .01 |
| Less than 10 ng/mL, % | 82% | 31% | .01 |
| Gender | | | |
| Female | 51 | 48 | |
| Birth weight, kg | 2.75 ± 0.44 | 2.81 ± 0.52 | .40 |
| Head circumference, cm | 34 ± 1.57 | 34 ± 1.51 | .33 |
| Length, cm | 48.8 ± 2.37 | 48.9 ± 2.76 | .44 |
| Apgar score at 1 min | 6.90 ± 0.50 | 7.10 ± 0.66 | .03 |
| Apgar score at 5 min | 8.33 ± 0.81 | 8.53 ± 0.68 | .05 |

mothers assigned to vitD supplementation demonstrated significantly higher serum 25OHD levels compared with those whose mothers received routine care (Table 3); maternal and neonatal serum 25OHD levels were highly correlated ($P = .001$).

### Tolerance and safety of vitD dosing regimen

A total of 87.5% of the subjects were compliant. There were no adverse events relating to use of vitD supplementation (22). Baseline maternal serum calcium levels were nonsignificantly higher in group B compared with group A [median 8.8, interquartile range (IQR) 8.4–9.1 mg/dL in group B vs median 8.6, IQR 8.3–9 mg/dL in group A, $P = .17$]; the difference in the maternal serum calcium levels between the two groups persisted and was of statistical significance at 36 weeks' gestation (median 9.2 mg/dL, IQR 8.7–9.6 in group B vs median 8.5, IQR 8.25–8.8 mg/dL in group A, $P < .001$). The proportion of women demonstrating hypercalcemia at 36 weeks' gestation (serum calcium > 10.2 mg/dL) was, however, comparable in the two groups (three cases in group A vs nine cases in group B, $P = .10$). All those with biochemical hypercalcemia at 36 weeks were normocalcemic at baseline and were without symptoms. Furthermore, each of the three patients in group A with hypercalcemia was deficient in vitD (serum 25OHD < 20 ng/mL), and the same was true for eight of nine women in group B with evidence of hypercalcemia; information on the serum 25OHD level at delivery was not available for one of nine women.

## Discussion

We report on the results of the first RCT of antenatal vitD supplementation undertaken in Pakistani gravidae. The magnitude of maternal and neonatal vitD deficiency in pregnant Pakistani women and the dependency of neonate on maternal vitD stores as observed are comparable with our earlier observations (15). Whereas on the one hand, our data demonstrate tolerability of vitD in a daily dose of 4000 IU starting at 20 weeks' gestation and continued until delivery, on the other hand, this study establishes inefficacy of the used regimen in impacting on maternal well-being. Other than improved Apgar scores observed in babies born to mothers who received vitD supplement, the remainder of the neonatal parameters were unaffected by maternal supplementation.

Wagner et al (25) identify 4000 IU daily as a recommended dose in pregnancy; our data, however, highlight inadequacy of this regimen in achieving normalization of maternal and neonatal vitD status in the indigent Pakistani population. With daily vitD supplementation with 4000 IU starting at 20 weeks and continued until delivery, normalization of vitD status (≥30 ng/mL) was achieved in only 15% of the population, with almost 23% demonstrating persisting severe vitD deficiency (<10 ng/mL). Based on estimates previously detailed by Vieth (26), the cumulative vitD dosage received by women delivering at term (at or beyond 37 completed wk of gestation) who were assigned to group B is anticipated to range between 476 000 IU (17 wk × 7 days × 4000 IU) and 560 000 IU (20 wk × 7 days × 4000 IU) (26). Disregarding the severity of baseline vitD deficiency, as shown previously, the rise of 25OHD levels from baseline is usually not above 30 ng/dL after 500 000 IU. The chosen dose in relation to the severity of vitD deficiency in our population thus may underlie our inability to attain normalization of vitD status.

Issues of compliance are an unlikely mechanism, given that compliance with vitD was assessed at each antenatal visit and significant improvement in both maternal and neonatal serum 25OHD levels was noted with maternal supplementation. Oral as well as transcutaneous strategies represent physiological routes for vitD supplementation; im megadose regimens are also commonly used strategies for managing vitD deficiency. A prospective pharmacokinetic study undertaken in nonpregnant healthy adults compared a bolus dose of 600 000 IU of vitD administered by oral and im routes; bioavailability was found to be earlier with oral supplementation,

whereas im administration resulted in a more sustained response (27). Thus, when aiming for a rapid response, and in the absence of malabsorption-related concerns, oral vitD formulations should be preferentially considered. Although poor gastrointestinal absorption of the ingested supplement is a plausible mechanism to explain our failure to achieve normalization of vitD status, this aspect was not a consideration in our study design.

Preeclampsia and eclampsia are common contributors to maternal and perinatal morbidity and mortality (28). In recent years, maternal vitD deficiency has been suggested as a modifiable risk for hypertensive disorders of pregnancy. In a cohort of more than 23 000 Norwegian women, supplementation with vitD was found to be associated with a 27% reduction in the risk of preeclampsia [odds ratio 0.73 (95% CI 0.58–0.92)] (29). Bodnar et al (30), in a nested case-control study, identified decreased maternal levels of serum 25(OH)D at 16 weeks' gestation as a risk for the subsequent development of gestational hypertension and preeclampsia. In a recent case-control study from our neighboring country of Bangladesh, Ullah et al (31) assessed the relationship between pregnancy-related hypertensive disorders and vitD status in a population of similar demographics as ours. The authors observed a higher likelihood of eclampsia and preeclampsia in vitD-deficient women (<30 ng/dL). These findings are consistent with published literature, as discussed. Although observational data suggest a causative role for vitD deficiency in the pathophysiology of pregnancy-related hypertensive disorders, our RCT failed to impact on incident preeclampsia through vitD supplementation. Whereas these null findings negate a relevance of vitD in the pathophysiology of preeclampsia, the null effect could be secondary to a failure of the dosing regimen to normalize vitD levels in most of the supplemented population. Future studies should include dose-finding strategies to comprehensively explore whether the incidence of preeclampsia may be reduced through the normalization of the vitD status.

An accruing body of observational data relates hypertensive disorders of pregnancy to maternal vitD deficiency (32). The observed lack of efficacy of vitD against hypertensive disorders of pregnancy in our clinical trial places under scrutiny prior interpretations primarily based on observational data; alternatively, the observed lack of effect may be a reflection of power constraints in our study sample, given that our study was powered for cumulative morbidity rather than incident hypertensive disease of pregnancy. Yet another explanation may lie in an inadequacy of our dosing and failure to attain normalization of vitD status. In keeping with our observation, however, others have failed to substantiate a relationship between maternal vitD status with the subsequent development of hypertensive disorders of pregnancy (33).

A relationship between vitD deficiency and type 2 diabetes mellitus is well described in the nonpregnant population (34). Relevance of maternal vitD deficiency for GDM is also suggested. Zhang et al (17), in a nested case-control study, related an increased risk for GDM with low levels of vitD in early pregnancy. Others have related low maternal vitD levels with subsequent abnormal response to a glucose challenge test (35). In a nested case-control study, Baker et al (36), however, failed to identify vitD deficiency as a risk for GDM, and our data corroborate this impression. As acknowledged earlier, our observed lack of efficacy of vitD against GDM questions prior interpretations that were predominantly centered on observational data; alternatively, a lack of effect of vitD supplementation on the incidence of GDM may be a reflection of power constraint and/or our inability to successfully address the magnitude of vitD insufficiency.

Premature birth is a leading cause of perinatal mortality. In a RCT of vitD supplementation vs placebo, Wagner and colleagues (20) observed a dose-titrated effect of vitD supplementation on the prevention of preterm birth. The risk of preterm delivery was observed to be attenuated in gravidae who received 4000 IU of vitD daily, compared with women who were given smaller doses of vitD (20). Although plausible mechanism/s that may explain the observed phenomenon are unclear, improved maternal vitD status may act through reducing the risk of infections, including bacterial vaginosis that has been implicated in the causation of preterm labor (37). In contrast to Wagner et al (25), we failed to observe any effect of maternal vitD supplementation on the risk of preterm birth. Herein, again, we acknowledge that our study may not be adequately powered, and/or our inadequacy of dosing regimen could theoretically have resulted in the null findings. An alternative explanation that merits consideration, however, is that the previously reported associations between vitD status and clinical end points such as GDM may in reality be spurious, with vitD status being a surrogate for an unidentified variable of clinical relevance.

Maternal vitD deficiency has been related to decreased birth weight and neonatal length. In a Caucasian population, Bodnar et al (14) demonstrated a U-shaped association between maternal vitD levels and fetal growth. Sabour et al (38) reported an association of vitD adequacy with improved fetal length but not weight in an Iranian population. Weiler et al (39), reporting on mother-infant pairs from Canada, observed that vitD-deficient newborns were larger at birth, as well as a later stage, although they were found to have decreased bone mass. In contrast, studies of Spanish and Indian cohorts failed to substantiate a relationship between maternal vitD status with fetal growth and neonatal anthropometrics (12, 40). Although our findings are consistent with the latter observations, our observations contrast with an earlier study undertaken in Asian women living in London wherein

supplementation with 1000 IU of vitD during pregnancy was associated with an increased birth weight of newborns (41). Differences in population characteristics and in the magnitude of vitD deficiency at baseline can be theorized to explain why our study failed to recapitulate the latter findings.

Secondary hyperparathyroidism can be seen in 20%–40% of cases of moderate to severe vitD deficiency, and vitD deficiency is a recognized contributor to skeletal compromise in individuals of all ages (42). Information on biomarkers relevant to skeletal health would offer highly relevant information regarding therapeutic implications of maternal vitD supplementation on maternal and neonatal skeletal well-being; financial constraints limited our ability to include a study on the skeletal benefits of maternal vitD supplementation.

Our study affirmed the tolerance and overall safety of maternal vitD supplementation at a dose of 4000 IU daily from 20 weeks' gestation until delivery. Not a single patient reported intolerance of the supplementation regimen, and serial urinalyses failed to identify evidence of calciuria. Although the incidence of asymptomatic hypercalcemia at 36 weeks' gestation was higher in the population randomized to vitD supplementation compared with the population receiving routine care (nine vs three), the difference was not of statistical significance. All 12 participants were normocalcemic at baseline, and hypercalcemia occurred despite persistent vitD deficiency in 11 of 12 (25OHD level at delivery assessment was not available for one patient in group B). The observed phenomenon is thus likely secondary to exogenous calcium supplementation (because all women received calcium supplementation as a part of routine clinical care) rather than a reflection of vitD toxicity. Wagner et al (25) have previously demonstrated safety of an identical regimen (daily 4000 IU vitD) in pregnancy, whereas Garrett-Mayer et al (43) affirmed safety and tolerance of a similar dose over 12 months in adult males.

A rigorous study design and excellent follow-through (87% of the population completed the clinical trial) add credence to our data. Our findings reflect the inefficacy of maternal vitD supplementation on the incidence of common obstetric morbidities. Our inability to successfully address the magnitude of vitD insufficiency despite a daily supplementation with 4000 IU vitD can be hypothesized to underlie our essentially null observations. Our study did not consider variability in vitD signaling as a potential determinant of the magnitude of treatment effect.

In summary, our observed lack of efficacy of vitD against common obstetric morbidities places under scrutiny prior interpretations that were predominantly centered on observational data (44). The only parameter to suggest a neonatal benefit from maternal supplementation was higher Apgar scores of babies born to mothers who had received antenatal vitD supplementation. In a large data set from the American population, decreased neonatal and postneonatal mortality rates were noted in both singleton and twin term gestations (45). Conversely, higher neonatal mortality rates were reported in term neonates with low 5-minute Apgar scores (1–3); at 5 minutes, a higher neonatal mortality rate was found until term of 37 weeks or longer. The observed differences in Apgar scores, although of statistical significance, are unlikely to be of clinical relevance. Although neonatal mortality rates were comparable in the two groups, information on newborns' well-being prior to discharge from the hospital was not collected due to lack of financial and logistic resources and is a limitation of this study. Implications of subtle differences in Apgar scores remain unclear, and long-term follow-up is required to determine whether higher neonatal Apgar scores with maternal vitD supplementation hold any lasting benefit for infantile well-being.

Given that the relevance of maternal vitD status for skeletal health of the mother and child is well established, the observed prevalence and the magnitude of vitD deficiency in the Pakistani women and the newborns and our failure to achieve normalization of maternal and neonatal vitD status with antenatal supplementation with daily 4000 IU vitD underscore a need for longitudinal studies to determine the implications of maternal vitD status on the infantile and pediatric skeleton and dose-finding studies to adequately address vitD requirements of the Pakistani population.

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