Vitamin D during pregnancy : why observational studies suggest deficiency and interventional studies show no improvement in clinical outcomes ? A narrative review

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Vitamin D during pregnancy: Why observational studies suggest deficiency and interventional studies show no improvement in clinical outcomes?

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Abstract

A considerable number of studies have examined vitamin D status during pregnancy. Although data from observational studies denote vitamin D hypovitaminosis (deficiency or insufficiency) during pregnancy, data from interventional (supplementation) trials fail to reveal a significant impact on maternal and offspring health. The aim of this review was to critically appraise the methodology of the published trials in an attempt to explain the difference between observational and supplementation data. We found that this difference could be attributed to a variety of factors, namely: i) study design (lack of a specific outcome, enrolment of participants with low vitamin D concentrations only), ii) vitamin D expenditure (lack of determination of plasma half-life), iii) supplementation regimen (administration of a wide range of regimens, in terms of dose, bolus and form), iv) geographical characteristics (vitamin D needs could vary significantly within a country, particularly in areas with a wide range of latitude gradient), v) altered metabolism during pregnancy (vitamin D and calcium equilibrium are changed during pregnancy compared with the non-pregnant state) and vi) assay methodology (most studies report on a minority of vitamin D metabolites). All these parameters should be taken into consideration in the design of future vitamin D supplementation trials.

Key words: hypovitaminosis D, pregnancy, complications, neonate, maternal, supplement.

1. Introduction

Pregnancy is a unique state in a woman's life, characterized by a continuum of biologic events that enables tissue maturation, aiming for fetal adaptation to future environmental and nutritional influences (1, 2). On this critical time frame, several exogenous stimuli could affect maternal - neonatal syncytium and have an impact on pregnancy outcome, maternal health and future offspring development (1-3). A wide range of nutritional deficiencies have been recognized as a preventable cause of adverse health events, rendering scientific communities and health organizations worldwide to establish specific nutritional recommendations aiming for optimal fetal development (4). This suggested diet model, although very similar to a balanced healthy one suggested for most adults, incorporates recommendations, which apply to consumption of micronutrients and vitamins, which have been recognized to be of critical importance in protecting the developing fetus (4, 5).

On that basis, data from observational studies during the last decade have suggested a potential adverse effect of maternal hypovitaminosis D during pregnancy on maternal and offspring health outcomes (6, 7). Gradually, interventional studies have been conducted, focusing on the potential beneficial effects of vitamin D supplementation on these outcomes. However, the majority of these studies failed to show any benefit from vitamin D supplementation during pregnancy. The reasons for absence of an agreement between data from observational and supplementation studies remain obscure. The aim of this review was to critically appraise the methodology of the published trials, in an attempt to explain why data from supplementation trials fail to reveal a significant impact on maternal and offspring health outcomes as date from observational studies are suggesting.

2. Maternal outcomes

Results for pre-eclampsia and gestational diabetes mellitus (GDM) fail to converge to a meaningful outcome, revealing the potential for systematic failures within the field. At that basis, this review included most representative studies on the field according to study sample and methodology (Table 1).

2.1. Pre-eclampsia

A series of supplementation studies have examined potential adverse maternal and pregnancy outcomes, mainly pre-eclampsia and GDM. Studies with pre-eclampsia risk reduction as their primary outcome can be divided into observational and randomized. A large, prospective observational trial from Norway (8) studied 23.423 nulliparous women, categorized into two groups, according to the use of vitamin D supplements before or during pregnancy. Vitamin D supplementation during pregnancy with 400 - 600 IU daily resulted in 27% risk reduction of pre-eclampsia [odds ratio (OR) 0.73, 95% confidence interval (CI) 0.58 - 0.92]. However, the beneficial effect of vitamin D intake should not be attributed to supplementation *per se*, due to the high intake of vitamin D-rich long n-3 fatty acids in the local diet. In another large, Hungarian study (9), routine vitamin D supplementation, either as a mono-therapy or contained in a multi-vitamin regimen, with a dose of 3,000 IU/week (average 400 IU/day) starting from the 20th gestational week, resulted in a reduced risk of pre-eclampsia in a dose-response manner.

In the field of randomized trials, Marya et al. (10) in a placebo-controlled, non-blinded trial studied two groups of 200 pregnant women (supplementation group: 375 mg calcium and 1200 IU of vitamin D daily from the 20 - 24th gestational week; non-supplementation group: normal diet) and found no statistical difference in the incidence of pre-eclampsia between them. The only blinded, supplementation study available so far (11) administered 400

(control group), 2,000 or 4,000 IU of vitamin D_3 daily during pregnancy. Cord blood 25-hydroxy-vitamin D [25(OH)D] concentrations were 45.5 ± 25.3 nmol/l in the 2000 IU group and 66.3 ± 25.8 nmol/l in the 4000 IU group. Higher 25(OH)D concentrations did not alter cord blood calcium or phosphorus. The study suffered from a high dropout rate, while powered only for a biochemical endpoint. However, by combining all available randomized data so far, a reduced OR of pre-eclampsia is evident in supplemented women with a pooled OR of 0.66 (95% CI 0.52 - 0.83, p = 0.001) (12).

2.2. Gestational diabetes mellitus

Although some observational studies support an association between maternal hypovitaminosis D during pregnancy and the development of GDM (13-15), data from supplementation studies are limited. A double-blinded, randomized controlled trial (RCT) in 54 women diagnosed with GDM reported an improvement in fasting glucose and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index after two doses of oral 50,000 IU vitamin D, given 21 days apart, compared with placebo (16). However, significant differences in these parameters were noted at baseline, making the results difficult to interpret. In an open label RCT of vitamin D supplementation in two groups of Pakistani women, 4,000 IU of vitamin D were administered compared to calcium and ferrous sulphate supplementation (17). The obstetrical outcomes were identical in both groups. This effect was attributed to the inefficacy of the given vitamin D regimen to achieve normalization of maternal vitamin D status (only 15 % of pregnant women achieved concentrations above 30 ng/ml), leaving the majority in the deficiency range.

In an Australian double-blind controlled supplementation study (18), a cohort of 209 pregnant women before 20^{th} gestational week were randomized in either 5,000 IU (n = 89) or

400 IU (n = 90) of oral vitamin D_3 daily, until delivery. Endpoints were maternal glucose concentrations in Oral Glucose Tolerance Test (OGTT, 26^{th} - 28^{th} gestational week), neonatal 25(OH)D concentrations, obstetric and neonatal outcomes and assessment of maternal insulin resistance. Although an inverse association between baseline 25(OH)D and fasting and 2-h glucose concentrations was found in *post hoc* analysis, the 5,000 IU group failed to demonstrate significant differences in mean fasting, 2-h blood glucose concentrations and HOMA-IR, compared to the 400 IU group. Overall, all women in the 5,000 IU group which developed GDM (n = 7) manifested adequate vitamin D status at the time of diagnosis. However, this study was not adequately powered to detect a difference between groups in the incidence of GDM.

In summary, as far as maternal outcomes are concerned, although supplementation studies homogeneously indicate a beneficial effect on the reduction of pre-eclampsia risk, the absence of pre-conception vitamin D values, the limited power and the heterogeneity in thresholds used do not allow for definitive conclusions to be drawn. In the case of GDM, risk reduction data do not indicate a beneficial effect, so far.

3. Fetal outcomes

Anthropometry at birth or during early life is the most studied extra-skeletal clinical outcome of maternal vitamin D supplementation. So far, only two (11,19) out of six RCTs (11,19-24) defined 25(OH)D concentrations as their primary outcome and had adequate sample size. The only study, which demonstrated effects on neonatal clinical parameters (20), was conducted in India, an area with profound vitamin D deficiency. Administration of two doses of 600,000 IU vitamin D in the third trimester of pregnancy resulted in a significant increase in birth weight of the offspring, compared to the non-supplemented group. This study did not define a

primary outcome parameter, used no placebo and reported no data on 25(OH)D concentrations.

3.1. Anthropometry

In an effort to assess post-partum beneficial effects of optimization of maternal vitamin D status in offspring linear growth, a randomized, double-blind, supplementation trial (24) (35,000 IU/week vs. placebo) during the first trimester of pregnancy evaluated longitudinally neonatal anthropometry from birth to 12 months of age. The primary analysis included evaluation of mean length-for-age Z-score based on international standards. At birth, no differences among the two groups were evident, whereas at one year of age, mean length-for-age was higher in the supplemented group, in conjunction with an increase in longitudinal growth during early infancy, interpreted in an increase of 1.1 cm throughout the first year of life, after controlling for sex. A large-scale, supplementation study (11) treated 257 pregnant women with 400, 2,000 or 4,000 IU of vitamin D_3 daily during pregnancy. Achieved cord blood 25(OH)D concentrations were 45.5 ± 25.3 nmol/l in the low-dose group and 66.3 ± 25.8 nmol/l in the high-dose group. A positive association between vitamin D dose and neonatal weight percentile, as well as a negative association between 25(OH)D concentrations and premature labour and infection was evident. These results might have been biased by the small study sample (n = 162, supplemented until term) and the high percentage of maternal vitamin D deficiency (35%) in the group supplemented with 4,000 IU daily.

3.2. Immune system

The effect of maternal vitamin D supplementation on offspring immune profile remains controversial. In a large cohort of more than 5,000 young adults (25), supplementation with

more than 2,000 IU vitamin D daily in the first year of life was found to be associated with an increased prevalence of allergic rhinitis. Although this trial centred on supplementation during infancy and not during pregnancy, it could comprise a spectrum of long-term effects of vitamin D equilibrium on immune regulation. This research question was the main objective in a RCT where 180 pregnant women were allocated randomly from 27th gestational week to either, single bolus of 200.000 IU *per os* or 800 IU d of ergocalciferol daily (26). No significant effect of maternal supplementation was evident on the risk of offspring wheeze, atopy (assessed by skin test) or lung function at 3 years of age. It has to be noted, however, that the supplemented groups demonstrated inadequate vitamin D status at both dosing regimens (daily dose: 26 nmol/l, bolus dose: 25 nmol/l), an effect that might influence potential beneficial effects of vitamin D supplementation.

In summary, as far as fetal outcomes are concerned, supplementation studies indicate a potential beneficial effect on offspring anthropometry. However, several parameters regarding timing, duration and dose of supplementation regimen remain to be elucidated before incorporating this evidence into daily clinical practice.

4. Why data from supplementation studies fail to reveal favourable clinical outcomes?

Based on the data by the supplementation studies discussed above, a series of reasons can be implicated. These reasons are discussed in the following paragraphs and illustrated in Table 2.

4.1. Study design

It could be argued that the link between maternal hypovitaminosis D and adverse outcomes is not causative and that decreased vitamin D concentrations are a consequence or a confounder,

rather than a disease *per se*. However, recent data on cardiovascular disease (CVD) risk (27), suggest a causal association between serum 25(OH)D concentrations and CVD risk, as do most well-designed RCTs. The term "well-designed RCT" is of utmost importance in understanding the potential beneficial effects of vitamin D supplementation during pregnancy. As described recently by Heaney (28), a vitamin D RCT should focus on a specific outcome, enrol only participants with low 25(OH)D concentrations and supplement with appropriate doses and regimens of vitamin D_3.

4.2. Vitamin D expenditure

Theoretically, serum vitamin D concentrations are the result of endogenous production and dietary intake (29,30). As a consequence, the potential beneficial effects of vitamin D supplementation should be interpreted in the context of attained concentrations of serum 25(OH)D and not just the dose administered. Vitamin D supplementation markedly differs from other interventions, where a pharmaceutical compound is given. In the former case, serum 25(OH)D concentrations are under the confounding contribution of newly synthesized 25(OH)D, whereas in the latter, serum / plasma concentrations depend on the administered dose (29). This phenomenon is of particular importance in defining the vitamin D dose-response relationship in supplementation trials. Thus, incorporation of vitamin D expenditure parameters, such as 25(OH)D plasma half-life, could provide an additional insight to supplementation studies, by identifying vitamin D kinetics (e.g. storage and release from fat and muscle) (31).

4.3. Supplementation regimen

An ideal vitamin D supplementation trial in pregnancy would use a reference population, with different baseline vitamin D status, aiming at attaining sufficient serum 25(OH)D

concentrations, in order to establish a "supplementation and result" relationship (28). Nevertheless, this outcome could be significantly affected by the regimen and dose of vitamin D used in each study. Since even a large bolus of 50,000 or 100,000 IU of vitamin D would rapidly (in a few days) be absorbed and undetectable from the serum (32). It has to be noted that several supplementation trials used this type of bolus administration, with a potential effect on their outcomes (19,23). In this context, the duration of supplementation could also play a role in maintaining adequate vitamin D concentrations. Although the optimal dosing and duration for specific outcomes remains to be defined, by supplying constant doses of vitamin D for 3 - 4 months, a steady state will be attained (33, 34). This is not the case in bolus regimens with monthly or weekly patterns of supplementation. The short duration of vitamin D supplementation during pregnancy (i.e. weeks or months) may also not be adequate to alter the pathogenetic pathways in which vitamin D is speculated to be involved, such as in pre-eclampsia and GDM states. Thus, supplementation before pregnancy and achievement of vitamin D sufficiency may be more effective than supplementation during pregnancy for both maternal and fetal outcomes.

4.4. Geographical characteristics

As vitamin D is generated by an environmental factor (sunshine exposure), it can be affected by geographical factors (35,36). Reports from Europe, USA and Africa indicate that populations from different countries share more common vitamin D-related characteristics than cohorts from the same country (37,38). This concept is further supported by Kimlin et al. (39), who assessed vitamin D data from seven USA locations. During eight months (March to October), no latitude gradient (from 18° to 44°N) of vitamin D was observed. In contrast, during cooler months (November to February) vitamin D was strongly determined by latitude. These observations indicate that vitamin D could vary significantly within a country,

particularly in areas with a wide range of latitude gradient. Moreover, vitamin D status of immigrant populations in Europe was poor compared with that of the indigenous European populations (40), indicating that social and cultural habits are different as well. Indeed, the approach to vitamin D status taking into account specific geographical characteristics, such as latitude, ultra-violet B (UVB) radiation and microclimate, as well as the specific social and dietary habits, could minimize heterogeneity among studies.

4.5. Metabolism during pregnancy

Vitamin D and calcium equilibrium are altered during pregnancy compared with the non-pregnant state (7). Pregnant women manifest extremely high concentrations of $1,25(OH)_2D$ without evidence of hypercalcemia, whereas vitamin D-binding protein (VDBP) concentrations increase in response to high estrogen concentrations (41). VDBP is increasingly recognized as a vital parameter in the interpretation of vitamin D status (42). Recently, results from a large study in community-dwelling black Americans, demonstrated low concentrations of total 25(OH)D and VDBP compared to white population, resulting in similar concentrations of estimated bioavailable 25(OH)D (43). Although the assay methodology used in this was prone to overestimation of bio-available 25-hydroxyvitamin D by 2 to 2.5 times owing to underestimation of vitamin D–binding protein in black people, racial differences could theoretically explain these findings. Conversely, the increase in VDBP during pregnancy could decrease bioavailable vitamin D, albeit concentrations considered as normal according to current vitamin D criteria.

These adaptive changes of vitamin D metabolism during pregnancy could interpret attained vitamin D concentrations, as optimal, although not. Interpreting bioavailable vitamin D concentrations in future trials in conjunction with VDBP concentrations could offer a new

insight in the field. In addition, maternal vitamin D receptor (VDR) polymorphisms have been associated with an increase risk of GDM in Iranian population, as well as increased birth weight (44).

4.6. Assay methodology

Although the existence of various vitamin D forms, such as epimers, has been established, their clinical significance remains obscure. Most studies report on a minority of vitamin D metabolites, which are usually the circulating ones. The latter are convenient to be measured, but they are essentially inactive. Furthermore, recent data show that at least one epimer form has activity *in vitro* (45). In studies where both the active and circulating forms have been measured, there was no significant correlation between them. Indeed, a recent study has revealed that higher concentrations of the active form exist in diseases, such rheumatoid arthritis, and diabetes mellitus type 1 (46).

In recent years, there have been considerable advances in techniques for vitamin D measurement (47,48). High quality assays for multiple vitamin D forms include liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (49,50). With the development of more advanced assays, a thorough understanding of the interplay among the various vitamin D forms can be achieved. The accurate assay highlights a considerable proportion of vitamin D exists as epimers and there is a lack of correlation between the circulating and active forms. These results underscore the need for accurate measurements to appraise vitamin D status. The results, based on specific and accurate measurement, revealed that maternal characteristics and active forms of vitamin D, along with their epimers explain 56% of neonatal vitamin D concentration (45).

5. Conclusions

Data from supplementation trials with vitamin D during pregnancy fail to reveal a significant impact on maternal and offspring health, at least in consistent way. Possible reasons for this fact may include:

1. Study design: methodology flaws, such lack of a specific outcome and enrolment of participants with low 25(OH)D concentrations only.
2. Vitamin D expenditure: lack of parameters that describe vitamin D expenditure, such as plasma half-life.
3. Supplementation regimen: administration of a wide range of regimens, in terms of dose, bolus and form that prevent safe interpretation of study results.
4. Geographical characteristics: vitamin D needs could vary significantly within a country, particularly in areas with a wide range of latitude gradient.
5. Metabolism during pregnancy: vitamin D and calcium equilibrium are altered during pregnancy compared with the non-pregnant state.
6. Assay methodology: most studies report on a minority of vitamin D metabolites.

Supplementation with a biomolecule that also derives from endogenous production and undergoes significant transformation in order to accomplish its skeletal and extra-skeletal actions is a challenging task. By taking into account the whole spectrum of parameters that could affect vitamin D homeostasis during pregnancy, it can be concluded that supplementation regimens should be specifically tailored on each population, taking into account parameters such the ones suggested by this study.

ETHICAL APPROVAL (RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS): not required due to type of manuscript (review)

Informed consent : not required due to type of manuscript (review)

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