Review

Prevention and Management of Gestational Diabetes Using Vitamin D Supplementation: An Overview and Appraisal of Clinical Trials

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Abstract: A number of studies have examined the role of vitamin D in reproductive processes and disorders of pregnancy such as gestational diabetes mellitus (GDM). Although observational studies have linked maternal vitamin D deficiency with a plethora of adverse pregnancy outcomes including GDM, intervention trials generally do not support the use of vitamin D supplementation for GDM prevention or management. This narrative review provides an up-to-date overview and critical appraisal of randomised controlled trials (RCTs) to describe the current state of knowledge regarding the efficacy of vitamin D supplementation for preventing and/or managing GDM. Overall, although RCT data indicates a potential benefit of vitamin D in maternal glycaemic control, results are highly disparate and the data published to date have not conclusively established the efficacy of vitamin D in GDM prevention. There are, however, several limitations within the existing literature, including some considerable challenges that are unique to vitamin D trials, which should be carefully considered in the interpretation of the evidence and design of future studies. For now, many unanswered questions remain, and there is still a need for adequately powered and well-designed trials before routine supplementation can be recommended in the context of GDM.

Keywords: vitamin D; 25(OH)D; gestational diabetes mellitus; GDM; pregnancy; glycaemic control; randomized controlled trials; critical appraisal

1. Introduction

Vitamin D has a well-recognised role in calcium-phosphate homeostasis and bone mineralisation [1]. Accumulating evidence has implicated vitamin D in a number of disease states not related to bone health, including autoimmune [2,3], infectious [4,5], metabolic and cardiovascular diseases [6–9], as well as some cancers [10,11] and neurological or mental health conditions [12–14], including schizophrenia [15]. Vitamin D has also been associated with reproductive processes and disorders of pregnancy [16,17]. Discovery of the nuclear vitamin D receptor (VDR) and metabolizing enzyme, 1 α -hydroxylase, in reproductive organs including the decidua, placenta, ovary, and endometrium, has seen an upsurge of studies examining potential links between vitamin D and adverse pregnancy outcomes [18]. In particular, there has been growing interest in the potential role of vitamin D in gestational diabetes mellitus (GDM), a common disorder of glucose intolerance with onset or first recognition occurring during pregnancy. However, the evidence is largely disparate and classifications for vitamin D deficiency or optimal concentrations during pregnancy remain contentious [19,20].

Several lines of evidence support the potential involvement of vitamin D in the pathogenesis of GDM. In vitro studies have shown that active vitamin D has potent anti-inflammatory properties [21], and directly activates transcription of the insulin receptor gene [22], as well as regulating transcription of genes associated with placental invasion, normal implantation, and angiogenesis [23]. These studies

posit that vitamin D deficiency may lead to increased inflammation and decreased insulin action, which could contribute to an increased risk of GDM. Observational studies have reported mixed results, with some finding inverse associations between maternal 25(OH)D concentrations and GDM risk factors including obesity [24], insulin resistance [25], dysglycaemia [26], dyslipidaemia [27], hypertension [28], and inflammation [29,30]; while others report no associations [31,32]. Similarly, some prospective studies link maternal vitamin D deficiency with an increased risk of incident GDM [33], while others do not [34–36].

Higher levels of evidence, such as that from randomised controlled trials (RCTs) have also produced mixed results, with some [37–40], but not all [41–43], supporting the use of vitamin D supplementation for preventing or managing GDM. These RCTs have been limited by small sample sizes and low statistical power, as well as low doses of vitamin D and variability in the baseline risk profiles of participants. Newly published trials [44–46] have sought to address these limitations, but the overall evidence from RCTs varies in scope, design, results and conclusions, making it difficult for decision-makers to reach definitive conclusions and recommendations for the use of vitamin D in clinical practice.

The aim of this paper is to provide an up-to-date overview of all published RCTs to describe the current state of knowledge regarding the efficacy of vitamin D supplementation for preventing and/or managing GDM. This review is not systematic and is not intended to introduce new data or conclusions, nor does it address all studies of vitamin D in pregnancy. Rather, the aim is to provide a comprehensive overview and critical appraisal of high-level evidence related to vitamin D and GDM and to present this in an objective, accessible and compact manner to aid in decision-making and future research endeavours.

2. Methods

A non-systematic search was conducted on electronic databases including PubMed, Google Scholar and Scopus using relevant search terms including: ‘vitamin D’; ‘supplements’; ‘supplementation’; ‘gestational diabetes’; ‘GDM’; ‘diabetes in pregnancy’; ‘pregnancy’; ‘reproductive’; ‘randomized controlled trials’; ‘RCTs’, and combinations of these terms. Studies in English with an RCT design were collated for narrative synthesis if they reported on GDM incidence or glycaemic control during pregnancy (e.g., oral glucose tolerance test results or glycated haemoglobin A1c [HbA1c]) after vitamin D supplementation. Observational or non-randomized studies as well as studies reporting postpartum or other obstetric outcomes or not reporting at least one glycaemic parameter during pregnancy were excluded.

3. Overview of Vitamin D

3.1. Vitamin D Physiology and Metabolism

Vitamin D is a fat-soluble vitamin, which can be derived from dietary sources such as oily fish or fortified dairy products or from supplements in the form of ergocalciferol (vitamin D₂) or cholecalciferol (vitamin D₃). However, the primary source of vitamin D in humans is through skin exposure to ultraviolet-B (UVB) radiation [47]. The length of time needed to produce adequate vitamin D from skin exposure to sunlight depends on the strength of UVB rays (i.e., place of residence) and skin pigmentation [48].

Upon sun exposure, vitamin D is photobiosynthesised in the epidermis by 7-dehydrocholesterol, a normal byproduct of cholesterol synthesis [7]. Bound to a vitamin D binding protein (DBP), and to a lesser extent to albumin, this biologically inert form of vitamin D is transported to the liver where it undergoes hydroxylation to form 25-hydroxyvitamin D₃ (25(OH)D; the main measure of vitamin D status). A second hydroxylation occurs in the kidneys by the enzyme 1 α -hydroxylase (CYP27B1) to form 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the biologically active form [49]. This active hormonal form, 1,25(OH)₂D₃, exerts pleiotropic biological functions by binding to a nuclear VDR, which is present

in most human cells and tissues, including in the liver, kidney, ovary, pituitary, and endometrium, as well as in pancreatic β -cells [49]. The affinity of $1,25(\text{OH})_2\text{D}_3$ to bind to the VDR is 1000 times that of $25(\text{OH})\text{D}$. However, the half-life of $25(\text{OH})\text{D}$ is approximately 3 weeks, whereas $1,25(\text{OH})_2\text{D}_3$ has a shorter half-life of 4–6 h [49]. Vitamin D controls calcium absorption in the small intestine and works with parathyroid hormone (PTH) to mediate skeletal mineralization and maintain calcium homeostasis in the bloodstream [50].

3.2. Measurement, Definition and Prevalence of Vitamin D Deficiency

Measuring $1,25(\text{OH})_2\text{D}_3$ in serum is impractical due to a number of factors including its short half-life (hours), its highly lipophilic and unstable properties, and because it is present at picomolar concentrations within the circulation (a thousand-fold less than $25(\text{OH})\text{D}$) [51]. The formation and concentration of $1,25(\text{OH})_2\text{D}_3$ is also not directly regulated by vitamin D intake. Rather, it is regulated by other factors (such as serum PTH) and, even in the presence of severe vitamin D deficiency, $1,25(\text{OH})_2\text{D}_3$ levels may be normal or elevated due to an upregulation of the 1α -hydroxylase enzyme. Serum $25(\text{OH})\text{D}$ is thus currently considered the best indicator of vitamin D status [51].

Several commercially available methods are used for measuring vitamin D status, including competitive protein binding assays (CPBA), radioimmunoassays (RIA), enzyme-linked immunoassays (EIA or ELISA), random access automated assays using chemiluminescence technology (RAAA or chemiluminescent immunoassay [CLIA]), high-performance liquid chromatography (HPLC), and what is now considered to be the gold standard: liquid chromatography–tandem mass spectrometry (LC/MS) [7,51].

The blood concentration of $25(\text{OH})\text{D}$ used to define deficiency remains an area of debate. The Institute of Medicine (IOM) [52] and National Institutes of Health (NIH) [53] suggest that circulating $25(\text{OH})\text{D}$ concentrations <50 and <25 nmol/L are considered deficient and severely deficient, respectively. These definitions would translate to 40–98% of pregnant women globally being deficient ($25(\text{OH})\text{D} <50$ nmol/L), with 15–84% being severely deficient ($25(\text{OH})\text{D} <25$ nmol/L) [54]. However, there is currently no consensus regarding optimal vitamin D levels during pregnancy, and the amount of supplementation required remains relatively unknown [19]. Epidemiological studies have postulated a U-shaped exposure–risk relationship between serum $25(\text{OH})\text{D}$ concentrations and several disease outcomes (i.e., increased risk at both low [<30 nmol/L] and high [>75 or >125 nmol/L] concentrations) [55]. These U-shaped curves may reflect biologically meaningful differences such as genetic variants, or may stem from confounding factors such as sun exposure or the use of vitamin D supplements by participants prior to study enrolment. Although both explanations are plausible, further investigations are needed to confirm these observations and how they may influence biological responses to vitamin D.

Low vitamin D status is prevalent across all age-groups, geographic regions, and seasons [56]. Vitamin D concentration in blood varies cyclically over the course of the year in relation to genetic (gender, ethnicity, skin pigmentation, VDR polymorphisms) and environmental factors (sun exposure and sun safety practices, diet, food-fortification, use of vitamin D supplements). Data from the National Health and Nutrition Examination Surveys (NHANES) demonstrate a three-fold increase in the prevalence of severe vitamin D deficiency over the past 10–13 years in North America, with nearly 36% of the US population being deficient [57]. Similarly, an estimated 31% of adults in Australia have $25(\text{OH})\text{D}$ concentrations <50 nmol/L, increasing to more than 50% during winter–spring and in people residing in Southern states [58].

The rising prevalence of vitamin D deficiency is due to several lifestyle changes including the global obesity epidemic and increase in sedentary indoor lifestyles, more common use of sunscreen and protective clothing due to fears of skin cancer, limited sources of vitamin D-rich foods or fortified food products, as well as insufficient amounts of vitamin D in dietary or prenatal supplements. Of concern is that populations with the greatest physiological needs for vitamin D, such as pregnant women, neonates, and children tend to be at highest risk for vitamin D deficiency [59].

3.3. Vitamin D in Pregnancy

Discovery of the nuclear VDR and 1α -hydroxylase enzyme in pregnancy-specific tissues such as decidua and placenta has generated substantial interest into the role of vitamin D in pregnancy. In normal pregnancy, maternal $1,25(\text{OH})_2\text{D}_3$ levels are twice as high by 12 weeks of gestation compared to the non-pregnant state, and peak at levels 2–3 fold higher ($>700 \text{ pmol/L}^{-1}$) by the third trimester [60]. These concentrations would be toxic due to hypercalcaemia in a non-pregnant individual, yet, they appear to be essential in pregnancy with an apparent decoupling with calcium homeostasis. It is thought that the increase in $1,25(\text{OH})_2\text{D}_3$ may have an immunomodulatory rather than calcium-regulatory function (although it maintains the latter), and is highly dependent on bioavailability of the $25(\text{OH})\text{D}$ substrate for conversion [60]. Thus, vitamin D deficiency in pregnancy is of particular importance because at no other time in life is $25(\text{OH})\text{D}$ so closely linked with $1,25(\text{OH})_2\text{D}_3$ production.

Low vitamin D status in pregnancy predisposes to hypocalcaemia in the immediate postpartum period, and then to rickets in the following months. Infants breastfed by vitamin D-deficient mothers remain at high risk as the amount of vitamin D in breast milk correlates with maternal vitamin D status [61]. Conversely, vitamin D supplementation during pregnancy improves maternal vitamin D status and may positively affect the availability of vitamin D to the fetus and neonate [62]. This has important clinical implications and as such, the IOM recommends daily intakes of 600 IU and 400 IU of vitamin D for pregnant [52] and lactating women [61], respectively. However, these doses are likely inadequate to sufficiently raise blood $25(\text{OH})\text{D}$ concentrations, particularly in the case of existing deficiency and limited sun exposure. In 2011, guidelines released by the Endocrine Society [63] suggested that all pregnant and lactating women with vitamin D deficiency receive 1000–2000 IU of vitamin D daily in addition to the 400 IU provided in prenatal vitamins. These levels are consistent with current recommendations in Australia, where daily supplements containing 1000 or 2000 IU of vitamin D_3 are recommended for pregnant women with $25(\text{OH})\text{D}$ levels <50 and $<30 \text{ nmol/L}$, respectively, while women with levels $>50 \text{ nmol/L}$ should consume 400 IU daily as part of a pregnancy multivitamin [64].

In addition to bone health and infant transfer of vitamin D, maternal vitamin D deficiency has been associated with an increased risk of several other adverse pregnancy outcomes including GDM [59,65], pre-eclampsia [66], and preterm birth [67]. Although observational data supports a strong and consistent association between low vitamin D status and GDM, cause-effect relationships and the potential benefits of maternal vitamin D replenishment for the prevention and management of GDM remain unclear.

4. Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a condition of glucose intolerance with onset or first recognition during pregnancy, and is characterised by insulin resistance and impaired insulin secretion [19]. In most women, GDM is asymptomatic and, depending on severity, it may be adequately controlled without medication (diet-controlled GDM), otherwise insulin and/or oral hypoglycaemic agents may be needed to achieve euglycaemia [68]. A diagnosis of GDM is generally made following an oral glucose tolerance test (OGTT) at 26–28 weeks gestation; however, the individuals targeted for screening as well as the timing and criteria used can vary. For instance, the American College of Obstetricians and Gynaecologists (ACOG) criteria requires two or more threshold values and use a 100 g glucose solution, while the Canadian Diabetes Association (CDA), the International Association of Diabetes and Pregnancy Study Groups Consensus Panel (IADPSG), and the World Health Organization (WHO) require only one threshold value and use a 75 g glucose solution [68–71]. Similarly, diagnostic cut-offs also differ, with a fasting glucose $\geq 5.3 \text{ mmol/L}$ for CDA and ACOG and 5.1 mmol/L for IADPSG and WHO. A 1-h plasma glucose cut-off $\geq 10.0 \text{ mmol/L}$ is adopted by all except CDA, which uses 10.6 mmol/L ; and a 2-h glucose cut-off $\geq 8.5 \text{ mmol/L}$ is adopted by IADPSG and WHO, whereas the CDA cut-off is ≥ 9 and ACOG is $\geq 8.6 \text{ mmol/L}$. Only ACOG includes a 3-h plasma glucose, with a cut-off of 7.8 mmol/L [68–71].

An estimated 7% of pregnancies in the US were complicated by diabetes in 2009 and roughly 86% of these cases were women with GDM [72]. In Australia, GDM affects approximately 10–13% of pregnancies depending on the diagnostic criteria used [73]. GDM increases the risk of macrosomia, birth trauma, pre-eclampsia and neonatal hypoglycaemia [68]. The prevalence of GDM varies by ethnicity and is directly proportional to the prevalence of type 2 diabetes in the population [68]. Vitamin D has been implicated in both GDM and type 2 diabetes via its reported effects on insulin resistance and inflammation, and given the similar pathophysiology of these conditions [74,75]. However, the potential links between vitamin D status and GDM remain contentious, with several clinical trials attempting to clarify whether supplementation may reduce the risk of GDM or ameliorate its clinical presentation.

5. Vitamin D and GDM: An Overview of Randomised Controlled Trials

5.1. Characteristics of Randomised Controlled Trials

A total of 18 trials (20 articles with 18 unique samples) were identified and synthesized for the purpose of this review. The characteristics of these studies are summarised in Table 1. Most of the trials were conducted in Iran ($n = 11$), with two in China [39,76], and the remaining single studies conducted in India [41], Pakistan [77], Australia [42], and the United States (US) [43]. One study was a multi-centre RCT across seven European countries (United Kingdom, Ireland, Austria, Poland, Italy, Spain, and Belgium) [44]. Sample sizes ranged from 54 [37,38,78] to 500 [40] participants, but approximately half the studies ($n = 9$ RCTs) included <100 participants and nearly all ($n = 16/18$ RCTs) included ≤ 200 participants. Four trials had open-label designs [40,41,77,79], one was a single-blind RCT [44], while the rest were double-blind RCTs (Table 1).

In 13 of the 18 trials, the mean baseline 25(OH)D level of participants was <50 nmol/L, whereas three trials [43,44,78] noted sufficient mean vitamin D levels at baseline (25(OH)D <75 nmol/L) and two [76,80] did not report mean baseline vitamin D status. Twelve trials [37,38,40–44,77,79–83] recruited women without GDM (healthy women or women at risk of GDM or preeclampsia) and aimed to examine whether vitamin D supplementation can prevent or reduce the risk of incident GDM or improve glycaemic measures. The remaining six trials [39,45,46,76,78,84,85] included women diagnosed with GDM to examine the effects of vitamin D supplementation on glycaemic control or insulin resistance in these women (Table 1).

Table 1. Characteristics of randomised controlled trials examining the efficacy of vitamin D supplementation on reducing incident GDM or improving maternal glycaemic control.

No	Author, Year, Country	Study Design	Population	n	Baseline 25(OH)D (nmol/L) *	GDM Criteria	Intervention	Average Daily Dose (IU)	Control	Micronutrient Co-Interventions	Summary of Results	25(OH)D Method
1	Asemi, 2013a [37,38], Iran	Double-blind parallel RCT	Healthy	54	36.2	NA	Oral VD ₃ 400 IU daily From 25 GW for 9 weeks	400	Placebo	Folic acid, iron	↓ FPG and insulin; ↓ HOMA-IR and HOMA-β; ↑ QUICKI score; GDM outcome NR	ELISA
2	Asemi, 2013b [78], Iran	Double-blind parallel RCT	GDM	54	50.9	ADA	Oral VD ₃ 50,000 IU twice From 24–28 and 27–31 GW	NA	Placebo	Folic acid, iron	↓ FPG, insulin, HOMA-IR and ↑ QUICKI; no effect on HOMA-β	ELISA
3	Asemi, 2015 [84], Iran	Double-blind parallel RCT	GDM	56	49.1	ADA	Oral VD ₃ 50,000 IU VD ₃ twice + 1000 mg calcium/day At mean 25 GW and on day 21 of intervention	NA	Placebo	Calcium	↓ FPG, insulin, HOMA-IR and ↑ QUICKI; no effect on HOMA-β	ELISA
4	Corcoy, 2020 [44], Europe (x7 countries)	Multi-centre RCT	Healthy with pre-pregnancy BMI ≥ 29 kg/m ²	154	69.6	IADPSG	Oral VD ₃ 1600 IU daily From ~15 weeks until delivery	1600	Placebo	-	Small ↓ in FPG; no effect on insulin, HOMA-IR, 1h or 2h glucose or insulin or GDM risk	LC/MS
5	Hossain, 2014 [77], Pakistan	Open label RCT	Healthy	200	13.7	O'Sullivan and Mahan	Oral VD ₃ 4000 IU daily From 20 GW until delivery	4000	200 mg ferrous sulfate + 600 mg calcium daily	Iron, calcium	No effect on GDM incidence (determined by abnormal glucose challenge test)	CLIA
6	Karamali, 2015 [81], Iran	Double-blind parallel RCT	Healthy women at risk of pre-eclampsia	60	42.7	NA	Oral VD ₃ 50,000 IU biweekly From 20 GW for 12 weeks	3571	Placebo (+folic acid, iron and MV)	Folic acid, iron, MV containing 400 IU VD ₃	↓ insulin, HOMA-IR and HOMA-β, ↑ QUICKI; no effect on FPG; GDM outcome NR	ELISA

Table 1. Cont.

No	Author, Year, Country	Study Design	Population	n	Baseline 25(OH)D (nmol/L) *	GDM Criteria	Intervention	Average Daily Dose (IU)	Control	Micronutrient Co-Interventions	Summary of Results	25(OH)D Method
7	Karamali, 2018 [46] and Jamilian, 2019 [45], Iran	Double-blind parallel RCT	GDM	60	33.8	ADA	Oral VD (type NR) 200 IU VD + 400 mg calcium + 4 mg zinc + 100 mg magnesium daily (+usual prenatal supplements) From 24–28 GW for 6 weeks	200	Placebo (all women received usual prenatal supplements: 1000 IU VD ₃ + 400 ug vitamin B9 + 60 mg iron daily)	Magnesium, zinc, calcium, B9, iron	↓ FPG, insulin, HOMA-IR and ↑ QUICKI	ELISA
8	Li, 2016 [39], China	Double-blind parallel RCT	GDM	103	40.5	ADA	Oral VD ₃ (fortified yoghurt) x2 servings daily of 100 g yoghurt with 500 IU VD ₃ From 14 GW for 16 weeks	1000	Plain yoghurt (not fortified)	-	↓ FPG, insulin, HOMA-IR, and HOMA-β	NR
9	Mojibian, 2015 [40], Iran	Open label RCT	Healthy	500	38.2	ADA	Oral VD ₃ 50,000 IU biweekly From 12–16 GW until delivery	3571	400 IU VD ₃ daily	-	↓ GDM risk	ELISA
10	Sablok, 2015 [41], India	Open-label RCT	Heathy	180	24	NR	Oral VD ₃ BL VD >50 nmol/L: 60,000 IU once at 20 GW BL VD 25–50 nmol/L: 120,000 IU at 20 and 24 GW <BL VD <25 nmol/L: 120,000 IU at 20, 24, 28, and 32 GW	NA	No supplementation	-	No effect on GDM incidence	ELISA
11	Samimi, 2016 [82], Iran	Double-blind parallel RCT	Healthy women at risk of pre-eclampsia	60	40.5	NR	Oral VD ₃ 50,000 IU vitamin D ₃ biweekly + 1000 mg day ⁻¹ calcium From 20 to 32 GW	3571	Placebo	Calcium	↓ FPG, insulin, HOMA-IR, HOMA-β and improved QUICKI; GDM outcome NR	ELISA
12	Shahgheibi, 2016 [83], Iran	Double-blind parallel RCT	Healthy women at risk of GDM	90	17.4	Specified levels	Oral VD (type NR) 5000 IU weekly From 12 GW until 26 GW	714	Placebo	-	↓ GDM risk (determined by ↓ proportion of abnormal glucose tolerance/challenge tests at 26 GW)	Liebermann–Burchard method

Table 1. Cont.

No	Author, Year, Country	Study Design	Population	n	Baseline 25(OH)D (nmol/L) *	GDM Criteria	Intervention	Average Daily Dose (IU)	Control	Micronutrient Co-Interventions	Summary of Results	25(OH)D Method
13	Soheilykhah, 2013 [79], Iran	Open-label RCT	Healthy	120	20.7	NA	Oral VD (type NR) I ₁ = 50,000 IU monthly I ₂ = 50,000 IU biweekly From 12 GW until delivery	I ₁ = 1667 I ₂ = 3571	200 IU VD daily	-	↓ HOMA-IR and insulin in high-dose (biweekly) group vs controls; no effect on FPG; GDM outcome NR	CLIA
14	Tehrani, 2017 [80], Iran	Double-blind parallel RCT	Healthy	210	NR	Specified levels	Oral VD ₃ 50,000 IU biweekly for 10 weeks From 14–16 GW for 10 weeks	3571	C ₁ = Placebo (omega-3 pearls); C ₂ = pregnant women with VD > 25 nmol/L	-	No effect on GDM incidence	NR
15	Yap, 2014 [42], Australia	Double-blind parallel RCT	Healthy	179	44.9	ADIPS	Oral VD ₃ 5000 IU daily From <20 GW until delivery	5000	400 IU Oral VD ₃ daily (low dose)	-	No effect on GDM incidence or glucose levels	CLIA
16	Yazdchi, 2016 [85], Iran	Double-blind parallel RCT	GDM	76	25.8	IADPSG	Oral VD ₃ 50,000 IU biweekly From 24–28 GW for 2 months	3571	Placebo (paraffin oil)	Folic acid, iron	↓ FPG; no effect on insulin or HOMA-IR; ↑ HbA1c in placebo group	CLIA
17	Zerofsky, 2014 [43], USA	Double-blind parallel RCT	Healthy	57	69.6	NR	Oral VD ₃ 2000 IU daily From 20 GW until delivery	2000	400 IU VD ₃ daily	-	No effect on FPG or measures of glucose tolerance (1 h or 2 h glucose) or HbA1c; GDM outcome NR	LC/MS
18	Zhang, 2016 [76], China	Double-blind multi-arm RCT	GDM	133	NR	NR	Oral VD ₃ I ₁ = 200 IU daily I ₂ = 50,000 IU monthly I ₃ = 50,000 IU biweekly From 24–28 weeks until delivery (I ₁), for 25 days (I ₂) or 12.5 days (I ₃)	I ₁ = 200 I ₂ = 1667 I ₃ = 3571	Placebo (sucrose)	-	↓ insulin and HOMA-IR with high-and medium dose but not with low doses; no effect on FPG	ELISA

* Reflects mean baseline 25(OH)D in the placebo/control group. ADA, American Diabetes Association; ADIPS, Australasian Diabetes in Pregnancy Society; BL, baseline; BMI, body mass index; C, control group; CLIA, chemiluminescent immunoassay; ELISA, enzyme linked immunosorbent assay; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; GW, gestational week/s; HbA1c, glycated haemoglobin A1c; HOMA-IR/β; homeostatic model assessment of insulin resistance/ beta-cell function; I, Intervention group; IADPSG, International Association for Diabetes and Pregnancy Study Group; IU, international units; LC/MS, liquid chromatography/mass spectrometry; NR, not reported; NA, not applicable; QUICKI, qualitative RCT, randomised controlled trial; VD, vitamin D.

Dosage regimens varied substantially between studies, whereby seven trials used daily or weekly doses ranging between 200 IU to 5000 IU [37,38,42–46,77,83], and the remaining studies used biweekly or monthly doses of 50,000 IU [40,79–82,85], or bolus doses of 50,000 [37,78], 60,000 or 120,000 IU [41] at different frequencies and time points during the course of the pregnancy. Across the 15 studies using daily, biweekly or monthly doses, the average daily dose equates to approximately 2414 IU per day (Table 1). Only one study by Zhang et al. [76] compared daily doses with weekly and monthly bolus doses in a multi-arm trial, while Li et al. [39] used fortified yoghurt to administer vitamin D. All studies used oral cholecalciferol, with the exception of three studies which did not specify the type of vitamin D used [45,46,79,83]. Co-interventions were reported in approximately half the trials ($n = 8$ RCTs), most of which included calcium, iron and/or folic acid (Table 1), and it is likely that women across all trials were receiving multi-supplements as part of their routine antenatal care.

For the control groups, placebo was the comparator in 11 trials (Table 1), whereas four trials used low dose vitamin D (200 to 400 IU daily) [40,42,43,79] and the rest used either no supplementation [41], plain yoghurt [39], or calcium and iron [77] as the comparison/control group. Blood concentrations of 25(OH)D were assessed by ELISA in nine trials [37,38,40,41,45,46,76,78,81,82,84], four used CLIA [42,77,79,85] and only two employed the gold-standard LC/MS [43,44]. Of the remaining three studies, two did not report the method for determining circulating 25(OH)D [39,80] and one reported using the Liebermann–Burchard method [84].

5.2. Efficacy of Vitamin D Supplementation for Reducing Incident GDM

Regarding the effects of vitamin D supplementation on risk of incident GDM, results from the included RCTs were highly disparate. Of the 12 RCTs conducted in healthy women without GDM, only seven reported on incident GDM as an outcome [40–42,44,77,80,83]. Among these seven trials, five reported no change in the risk of GDM after supplementation with 1600 [44], 4000 [77], or 5000 IU [42] of cholecalciferol daily from ≤ 20 weeks gestation or after receiving bolus doses of 50,000 IU biweekly from 14–16 weeks gestation [80] or 60,000 to 120,000 IU once, twice or four times from 20 weeks gestation [41]. Of note, two of these studies were open-label trials [41,77], one was single-blind [44] and all five were conducted in different geographical regions (Iran, India, Australia, Pakistan, Europe). All the trials also had different comparators including placebo, no supplements, iron and calcium, or low dose vitamin D. Hence, directly comparing these results or drawing conclusions as to the reasons for the lack of findings is challenging given the substantial heterogeneity between the study settings, designs and participants.

In the two remaining trials [40,83], both of which were conducted in Iran, a reduced risk of GDM following vitamin D supplementation was reported. One of these trials was a double-blind RCT of 90 women which supplemented 5000 IU of vitamin D daily (type not reported) from 12 to 26 weeks gestation [83]. Here, Shahgheibi et al. [83] found a reduction in the proportion of abnormal glucose tolerance tests at 26 weeks gestation in the supplemented group compared with placebo. Participating women were at risk of GDM and given the small sample size, the generalisability of these results is questionable. The second trial was an open-label RCT and the largest trial to date as it included 500 women [40]. This trial found that women receiving biweekly supplementation with 50,000 IU of cholecalciferol from 12 weeks gestation had a significantly reduced risk of GDM, compared with a control group receiving the standard prenatal daily dose of 400 IU [40]. These findings suggest that perhaps commencing supplementation at higher doses earlier in pregnancy may be more effective in preventing GDM. However, given the overall divergent results and null findings in most of the studies, there is a clear need for further well-designed trials using larger cohorts and a range of time points to clarify the benefits of vitamin D supplementation in reducing the risk of incident GDM, should any benefit exist.

5.3. Efficacy of Vitamin D Supplementation for Improving Glycaemic Control in Pregnant Women with and without GDM

Twelve studies reported the effects of vitamin D supplementation on glycaemic parameters, of which six were in women with GDM [39,45,46,76,78,84,85] and six were in healthy (or at risk) populations [37,38,43,79,81,82].

The six studies in women with GDM were conducted in Iran [45,46,78,84,85] and China [39,76]. All studies reported a reduction in fasting glucose, with the exception of one study by Zhang et al. [76] which reported no effect in 133 women receiving cholecalciferol at doses of 200 IU daily, 50,000 IU monthly or 50,000 IU biweekly compared with placebo. Similarly, all studies reported a reduction in insulin levels and insulin resistance measured by the homeostatic model assessment of insulin resistance (HOMA-IR), except for one study by Yazdchi et al. [85] which found no effect on these measures in 76 women receiving 50,000 IU biweekly from 24–28 weeks gestation for two months. However, Yazdchi et al. [85] did note a significant increase in HbA1c in the placebo group (compared with HbA1c remaining stable in the vitamin D group), whereas HbA1c was not assessed in the remaining studies. Insulin sensitivity using the quantitative insulin sensitivity check index (QUICKI) was assessed in three studies from Iran, all of which found improvements after vitamin D supplementation, despite the different doses used (50,000 IU twice or 200 IU daily from ~24 weeks gestation) and different regimens including vitamin D alone [78] or co-supplementing with calcium [84], or with magnesium, zinc and calcium [45,46]. Conversely, findings for HOMA of beta-cell function (HOMA- β) were inconsistent. One study from China [39] reported a reduction in HOMA- β in 103 women receiving 1000 IU daily cholecalciferol in fortified yoghurt from 14 weeks gestation for 16 weeks, while two smaller studies ($n = 54$ and $n = 56$) from Iran [78,84] found no significant effect after two doses of 50,000 IU cholecalciferol from ~24 weeks gestation. Hence, in women with GDM, vitamin D appears to have some favourable effects on fasting glucose and insulin resistance, but results tend to vary for most outcomes, likely a reflection of the varied study designs, populations and supplementation regimens.

Of the six studies in healthy women, four were conducted in Iran, with one study in the US [43] and one across Europe [44]. The US study reported that 2000 IU of cholecalciferol daily from 20 weeks gestation had no effect on insulin, HbA1c or fasting, 1-h or 2-h glucose post-OGTT [43]. Similarly, the multi-centre European trial [44] found a small reduction in fasting glucose but no effect on insulin, HOMA-IR, or 1-h or 2-h glucose post-OGTT after supplementation with 1600 IU of cholecalciferol daily from 15 weeks gestation until delivery. It should be noted that in both studies, participants were not vitamin D-deficient at baseline (mean 25(OH)D > 50 nmol/L). In contrast, all four studies from Iran [37,38,79,81,82] reported that vitamin D supplementation reduced insulin levels and HOMA-IR. Fasting glucose was reduced in two studies [37,38,82] while two reported no effect [79,81]; and three of the four studies [37,38,81,82] showed a reduction in HOMA- β and an increase in QUICKI (the fourth [79] did not measure these outcomes). Overall, the studies from Iran suggest an improvement in insulin resistance and glycaemic control with vitamin D supplementation, despite having quite different study regimens. For example, Asemi et al. [37,38] provided only 400 IU of cholecalciferol daily from 25 weeks gestation, while the remaining three studies provided 50,000 IU biweekly from 12 [79] or 20 weeks gestation [81,82]. The doses in the latter studies average to approximately 3571 IU daily, a dose nine times higher than that provided in the studies by Asemi and colleagues [37,38]. Yet, with the exception of fasting glucose, these studies seem to be in agreement that vitamin D supplementation can improve measures of glycaemic control and insulin resistance in pregnant women without GDM, irrespective of the dose and duration of supplementation. This contrasts with the study by Soheilykhah et al. [79] which included three comparison groups (50,000 IU biweekly; 50,000 IU monthly, and 200 IU daily [controls]), and reported that differences in glycaemic measures were only significant when comparing the high dose (biweekly) group with the controls, but not when comparing the lower dose (monthly) group with controls. Whether the effect of vitamin D on these outcomes is influenced by dosage regimens or other factors such as ethnicity, baseline risk profiles including

glycaemic control or vitamin D status, or other study or population characteristics, remains unclear from the available evidence.

6. Critical Appraisal and Considerations for Future Trials

The literature summarised in this review represents the highest level of evidence of the efficacy of vitamin D supplementation for preventing or managing GDM. Despite a number of trials examining this topic over the last decade, results remain largely divergent and, in general, there is no strong evidence to support the use of vitamin D supplementation for GDM prevention or management. However, like observational studies that are limited by confounding and the inability to determine causation, RCTs are also subject to a number of limitations that likely contribute to these inconsistent results and threaten their external (e.g., low response rates) and internal (e.g., poor adherence, withdrawal, unblinding) validity. These limitations are summarised below and should be considered in the interpretation of results and in the design and execution of future trials.

First, RCTs often have low response rates and small sample sizes leading to poor external validity. This is demonstrated in the trials identified in this review, whereby half had less than 100 participants and most had less than 200 participants. The most recent trial by Corcoy et al. [44] indicated that 220 participants would be required to achieve statistical power to detect differences in insulin sensitivity and fasting glucose, with even more required to detect differences in a binary outcome such as GDM. Hence, most existing trials were underpowered which may explain some of the null results or inconsistencies identified in this review. Moreover, self-selection bias is problematic in RCT study designs since participants who volunteer to take part are more likely to be healthy or health conscious and less likely to be vitamin D-deficient [86]. While this may be less problematic in trials of pregnant women (since many women are typically more health conscious during pregnancy), there will always be some uncertainty as to whether results can be extrapolated to the general population. Together, these factors can reduce external validity and introduce bias, and are important to consider when interpreting the overall evidence.

A second key issue in vitamin D RCTs pertains to 25(OH)D levels and the common use of doses which are insufficient to adequately increase circulating 25(OH)D to target ranges. Indeed, a non-linear association between vitamin D status and disease risk has been demonstrated for all-cause mortality and cardiovascular disease, and may also exist for other conditions such as GDM [86]. This strongly suggests that vitamin D trials should include only participants with 25(OH)D concentrations below the inflection point where disease risk starts to rise, since supplementation would not be expected to reduce disease risk in people with 25(OH)D levels above this inflection point. This inflection point varies depending on the disease in question, with a previous review of the literature [87] concluding that the thresholds for beneficial vitamin D status could range from a 25(OH)D of 25 nmol/L for bone diseases to up to 100 nmol/L for cancer. Importantly, inflection points or thresholds for observing benefits in the context of pregnancy or GDM have not been established. Nevertheless, the presence of vitamin D-sufficient individuals in a trial is sure to weaken its statistical power because their risk of disease remains unchanged irrespective of whether they receive vitamin D or placebo [86]. This, in turn, can mask the potential benefits of vitamin D, leading to erroneous results and conclusions. The trials reviewed herein demonstrate this concept to some extent, since the two trials [43,44] which found no effect on most or any of the measured glycaemic outcomes (including GDM risk) both reported a non-deficient mean baseline 25(OH)D level (69 nmol/L). Much like we would not include slim individuals in a trial examining obesity reduction, if we are to optimize the detection of potential benefits from vitamin D supplements, participants with sufficient vitamin D levels should not be included in trials examining the efficacy of vitamin D supplementation. This is even more pertinent in vitamin D trials as baseline 25(OH)D can influence the half-life of ingested vitamin D, since higher concentrations can lead to faster catabolism of vitamin D to maintain equilibrium [88]. Therefore, despite a lack of consensus regarding the inflection points or optimal concentrations required to achieve health gains

in pregnancy, at the very least, RCTs should aim to recruit participants with vitamin D deficiency (<50 nmol/L) and provide supplementation regimens adequate for achieving sufficiency (>75 nmol/L).

A detailed discussion of this issue is provided by Heaney [89], where, in addition to recruiting participants who are vitamin D-deficient, it is recommended that trials provide a sufficiently large dose of vitamin D to shift their status into the range where disease risk decreases (or benefit increases), and assess temporal changes in vitamin D status to verify the adequacy of the dose and adherence. As noted above, the exact 'point' at which disease risk shifts can vary depending on the disease in question and is generally not known, especially in pregnancy. Moreover, the exact dose and regimen required to achieve sufficiency also varies and can heavily influence results. For instance, bolus doses may lead to short-lived peaks in intracellular vitamin D, followed by longer periods of insufficient vitamin D levels, resulting in an overall absence of physiological effects [90]. Similarly, a daily dose of 400 IU for several months will achieve little or no detectable effect on circulating vitamin D, whereas extended periods of 2000–6000 IU daily can maintain stable concentrations in the range of 25–100 nmol/L in a linear fashion [91,92]. These factors have clear implications in vitamin D trials but remain to be fully understood within the pregnancy context, emphasising the need for further research to clarify the optimal 25(OH)D concentrations for pregnancy-related benefits and the doses needed to achieve these.

An important caveat to note with regard to 25(OH)D levels is the ethical dilemma introduced by providing placebo in the presence of deficiency. Ethics review committees may require that participants in the control arm also be given vitamin D, particularly in the case of pregnancy where deficiency (especially severe deficiency) can pose undue risk to the mother or fetus. While the vitamin D doses in prenatal vitamins are usually low (e.g., 200–400 IU daily), they are still high enough to shift control participants out of severe vitamin D deficiency, hence impacting the power to detect differences as noted above. An alternative would be to store blood samples at baseline for measurement of 25(OH)D later once the trial is completed. However, while this can be justified on the basis of equipoise about the effect of vitamin D supplementation on most outcomes, this is not possible during pregnancy as treatment of severe deficiency is critical for preventing infantile complications such as rickets. The use of multivitamins or low dose vitamin D supplements in the control group is therefore inevitable and will always be an issue for vitamin D trials in pregnancy, one which requires appropriate planning and consideration in the design phase of these trials.

Closely linked to the point above regarding baseline 25(OH)D status and target levels, the third issue with vitamin D trials, both in and outside of pregnancy, is that they do not follow the traditional model for conducting clinical trials, where the evaluation of interventions is entirely under the control of clinicians and researchers. Unlike drug trials for example, participants in vitamin D trials have easy access to vitamin D supplements, widely available from pharmacies, supermarkets and online. This contributes to a high risk of contamination among control participants, should they take supplements outside the trial protocol [86]. Contamination can in turn influence 25(OH)D levels and, as described above, alterations in 25(OH)D levels can lead to a number of problems with analysing and interpreting RCT results. It can be argued that self-purchased vitamin D is likely to be similar in both intervention and control arms of a double-blind trial, thereby cancelling out any differences in effects between groups. In reality however, this is not the case since taking supplements will reduce the proportion of vitamin D-deficient participants in the control arm (without really impacting the intervention arm) and hence reduce the chances of detecting a beneficial effect, particularly if the association between vitamin D status and disease risk is non-linear as has been demonstrated [86]. Participants can also have their vitamin D levels tested relatively easily, which is especially problematic among pregnant women who often have their vitamin D levels checked as part of routine antenatal care. This can risk unblinding and further exacerbate contamination should they decide to take supplements or make lifestyle changes such as increasing their sun exposure or intake of vitamin D-fortified foods to self-treat their deficiency. These limitations can reduce the internal validity of RCTs and influence their findings, and may in part explain some of the discrepancies demonstrated in the literature. To counter these issues, trials need to incorporate close monitoring of 25(OH)D status, with frequent sampling and

testing over the course of a trial to assess potential protocol violations, but this comes with substantial costs and time commitments. Of note, none of the included trials measured vitamin D status at different time points across the course of pregnancy, hence the above problems could have been encountered and remained undetected.

Fourth, the clinical trials reviewed herein demonstrate substantial heterogeneity and variations in methodological rigour that should be considered in the interpretation of their findings. For instance, there are considerable differences in study designs, populations and settings, including varying cut-off points used to define vitamin D sufficiency and/or deficiency, different diagnostic criteria for GDM (ADA, IADPSG, etc.), different assays used to determine 25(OH)D levels (with only two studies using the gold-standard LC/MS), and different geographies and population groups studied, with the majority of trials ($n = 11/18$) being conducted in Iran, limiting generalisability (Table 1). Participants also had different risk profiles at baseline, with some having GDM or being classified as high-risk and others being relatively healthy, hence, direct comparisons become challenging. Some studies [78,84] noted significant differences in baseline glycaemic parameters between intervention and control groups (e.g., the placebo group was less insulin-resistant at baseline), making their results difficult to interpret. It is also important to consider the selection of factors to be controlled in an experimental setting, since some aspects such as body mass index, ethnicity, and increased maternal age can increase the risk of both GDM and vitamin D deficiency [93]. Other factors strictly related to vitamin D, such as endogenous biosynthesis (sun exposure) and amounts of dietary intake, should be taken into account when interpreting results from vitamin D trials. Yet, lifestyle factors such as these have seldom been incorporated in the existing trials and may explain some of the variations observed in the evidence.

Finally, there remain uncertainties regarding the functions of vitamin D at different stages of pregnancy, how these functions may be related to different clinical outcomes, and the appropriate 'critical windows' for measuring and treating deficiency or insufficiency in line with relevant pregnancy outcomes. For instance, gestational age is a crucial consideration for intervention studies since repletion needs to be restored prior to observing changes in the outcome of interest. Although blood 25(OH)D levels may promptly respond to supplementation (assuming sufficient doses), peripheral cellular deficiency may require more time. Intervention studies designed during late pregnancy may therefore not have sufficient time to restore the physiological conditions required for the prevention of dysfunction, or indeed, GDM [94]. The short durations of many of the existing trials (i.e., weeks or months) may also be ineffective in altering the pathogenic pathways of disorders such as GDM, which is thought to have initial manifestations from the very early stages of pregnancy [94]. This may explain some of the null or inconsistent findings from existing studies since, of the 18 included trials, only three [40,79,83] started supplementation in the first trimester (i.e., from ~12 weeks), while the majority ($n = 12/18$ RCTs) commenced at or after 20 weeks gestation, with many lasting less than three months.

When considering the above limitations, coupled with small sample sizes, lack of statistical power, open-label designs, and the use of low dose vitamin D supplements in the control groups (which is largely unavoidable in pregnancy), the equivocal findings presented in this review are not surprising. This is further compounded by a lack of defined knowledge around the appropriate doses or optimal concentrations of vitamin D, and the duration of supplementation necessary to appreciate any benefits, particularly during pregnancy when the vitamin D system undergoes substantial adaptations to support increased maternal and fetal requirements. Co-supplementation was also often used, making it impossible to isolate the effects of vitamin D alone. This is particularly important when considering co-supplementation with magnesium since magnesium is an integral cofactor in the enzymatic conversion of vitamin D to 25(OH)D and 1,25(OH)D, and is present in most pregnancy multivitamins [95]. Studies also varied in their supplementation regimens, ranging between daily, weekly, biweekly, monthly or intermittent supplementation of varying doses. As mentioned earlier, these regimens may influence the measured outcomes since bolus doses can be absorbed rapidly and become undetectable, while a steady state can be achieved by supplying constant doses of vitamin D for

3–4 months [96]. In addition, as outlined in this review, there are many uncertainties regarding whether vitamin D is only effective in certain subgroups or whether a certain threshold exists at which vitamin D ceases to incur additional benefits. It is possible that vitamin D may have differential effects in women of different ethnicities, age groups or baseline risk profiles, but this is yet to be fully delineated. Finally, there is a need to ascertain the long-term effects of vitamin D supplementation during pregnancy to determine whether improvements in glycaemic control following supplementation may translate into a reduced risk of type 2 diabetes or recurrent GDM in subsequent pregnancies. Taken together, while the existing research indicates potential benefits of vitamin D for glycaemic control in pregnancy but not for GDM prevention per se, more conclusive evidence is needed to confirm these findings and to determine the mechanistic pathways underlying the protective features of vitamin D, if any.

7. Conclusions

In summary, RCT data published to date have not conclusively established the efficacy of vitamin D in the prevention or management of GDM. Importantly, there are several challenges and limitations within the existing literature that should be carefully considered in the interpretation of the evidence. In light of these limitations, there is currently insufficient evidence to support the use of vitamin D for preventing or managing GDM, despite some clinical trials indicating that vitamin D may improve glycaemic parameters in pregnancy. For now, there remain unanswered questions regarding the potential use of vitamin D supplementation as a GDM prevention or management strategy, and there is a need for large-scale, adequately powered, and well-designed RCTs addressing the above limitations before routine supplementation can be recommended for this purpose.

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