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Artikelen

Vitamin D supplementation according to guidelines may be insufficient to correct preexisting deficiency in pregnancy

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Background: In current international guidelines, the daily dosage of vitamin D supplementation in pregnant women varies between 200 IU and 1200 IU. No advice is given to tailor supplementation to the initial status of the patient or to measure risk patients, hence, current guidelines might be interpreted as an "one size fits all" approach.
Objective: We investigated the efficacy of 400 IU and 800 IU daily doses of vitamin D in a group of initially deficient pregnant women (serum calcidiol <50 nmol/L) and measured the fraction still deficient after the supplementation period.
Design: Retrospectively we selected 372 initially vitamin D deficient pregnant women from the laboratory database, being treated either at the hospital, or at two primary midwifery practices in Amersfoort, the Netherlands. Prescription for vitamin D was either 400 IU per day or 800 IU per day. Calcidiol (25-hydroxyvitamin D3) levels were measured at baseline and after on average three months of suppletion.
Results: Women taking 400 IU per day (n= 253) showed a mean increase in calcidiol of 15 nmol/L, and after supplementation 57% was still deficient. When using 800 IU per day (n=119), we observed a mean increase of 23 nmol/L, and 35% of the women remained deficient.
Conclusions: Supplementation with either 400 or 800 IU vitamin D per day seems to be insufficient to correct preexisting vitamin D deficiency for a large part of our pregnant women population. Current international guidelines for vitamin D supplementation in pregnancy may need a revision.

An adequate vitamin D status during pregnancy is important for both mother and child (1-4). Vitamin D deficiency is widespread throughout the world with prevalence's at 50 nmol/L ranging from about one quarter to nearly the full population (1). Malaise, fatigue, loss of energy, muscle pain, and weakness as manifestations of vitamin D deficiency during pregnancy are often unrecognized. In addition, vitamin D deficiency during pregnancy is associated with a broad range of events including an increased risk of preeclampsia, cesarean section, gestational diabetes, intrauterine infection, preterm birth and dysmaturity (5-12). The vitamin D status of the fetus and neonate is dependent on that of the mother (9, 10, 13) and therefor on her sun exposure and cultural habits, her skin pigmentation and her dietary pattern, amongst others (1, 4). Vitamin D and calcium are essential for adequate mineralization of the fetal skeleton and normal development of the brain, lungs, and immunosystem (10, 12-15). Severe intrauterine vitamin D deficiency is associated with neonatal hypocalcaemia with seizures (16), craniotabes, hypotonia, occasionally neonatal rickets (10, 17), and with respiratory syncytial virus (RSV) infection in the first year of life (18). For these reasons, it is very important to attain sufficient vitamin D levels during pregnancy. From the data of our previous study (13) we estimated the prevalence of deficiency (based on < 50 nmol/L) in our region for West-European pregnant women to be 41%, while non-Western pregnant women had a prevalence of 88%.

There is no consensus with regard to the optimal dose for vitamin D supplementation in pregnancy (2, 19). This may explain why the supplementation recommendations vary largely between countries. In Australia and New Zealand (20), a daily use of 200 IU is advised in accordance to the WHO 2012 recommendation. In Great Britain (21), the Scandinavian countries (22) and in the Netherlands (23), 400 IU is recommended. Higher doses are considered appropriate in the USA (24) (600 IU), Belgium (25), Germany, Switzerland and Austria (26) (800 IU) and the highest dose is used in Turkey (27) (1200 IU). In our experience, medical subscribers generally consider supplementation doses of 400 or 800 IU per day to be adequate for pregnant women, without checking the initial serum calcidiol level.

The target level of calcidiol for pregnant women in the Netherlands is set at 30 nmol/L by the Dutch Health Council (23). However most Dutch physicians regard 50 nmol/L as the lower limit, being defined by both the Institute of Medicine in the USA (24) and the German Nutritional Society (26) as sufficient for 97.5% of the population.

In the region of Amersfoort, the Netherlands, serum levels of vitamin D are measured in pregnant women

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at their first visit to the clinic at approximately ten weeks of gestation and often repeated on average three months later. The objective of our study was to investigate the effect of daily administration of 400 or 800 IU on the serum calcidiol level in vitamin D deficient pregnant women.

Methods

Study population

In a retrospective study we searched the hospital laboratory database and the clinical database from January 2007 until July 2010. Consequently, we included 269 pregnant women from the Department of Gynaecology of Meander Medical Centre and 103 pregnant women from two primary midwifery practices in Amersfoort, the Netherlands who met the following inclusion criteria (see flowchart):

1) Pregnant and a baseline calcidiol serum below 50 nmol/L, followed by another calcidiol measurement within five months;
2) Treated with oral vitamin D supplementation of either 400 or 800 IU per day;
3) At least two months of supplementation before the second calcidiol measurement;
4) Pregnant throughout the course of supplementation and when blood was drawn the second time;

The prescribed amount of vitamin D (cholecalciferol) in our region was subject to a change in prescription practice. Between 2007 and 2010 a dose of 400 IU per day was given to all women with a serum calcidiol level <50 nmol/L, irrespective of the actual baseline calcidiol level, season or characteristics of the individual woman. In 2010 the protocol was changed and the daily dosage was raised to 800 IU per day. This change in policy was based on new publications and the clinical observation that a significant number of patients did not reach the target level of 50 nmol/L while using 400 IU of daily vitamin D. As of 2009 vitamin D was also prescribed to pregnant women seen by local midwifes.

The time period of at least two months in between two succeeding measurements has been chosen since a steady state level is not achieved over a shorter period of time for such daily doses (28).

The median dietary vitamin D intake for women of fertile age in the Netherlands is 2.6-2.8 ug/day, so approximately 100 IU/day.

Data collection

Patients: Consecutive calcidiol requests from the Department of Gynaecology and Obstetrics of Meander Medical Centre and from two participating midwifery practices were collected from the laboratory database from January 2007 until July 2010. When suitable according to the first inclusion criterion, the corresponding clinical data were retrieved from the obstetrical databases or from the records of the midwifery practices to find out if the three other inclusion criteria were met. We recorded the following data from their files: age, ethnicity (Caucasian/ European origin versus non-Caucasian), Body Mass Index (BMI) at intake, calcidiol levels and the date of measurement, dosage of vitamin D supplementation, and the starting month of supplementation to distinguish between the summer (April until October) and winter period. Initial vitamin D status was defined "deficient" at a calcidiol concentration below 50 nmol/L and "severely deficient" below 25 nmol/L.

Laboratory analysis: calcidiol-concentrations were measured in serum samples with a electrochemiluminescence method and a polyclonal antibody against calcidiol using a CobasE601 analyzer (Roche). This method measures only 25-hydroxyvitamin D3, however no 25-hydroxyvitamin D2 is used in prescriptions or available in the

Flowchart 25OHvit D3 requests from 2007 - half 2010

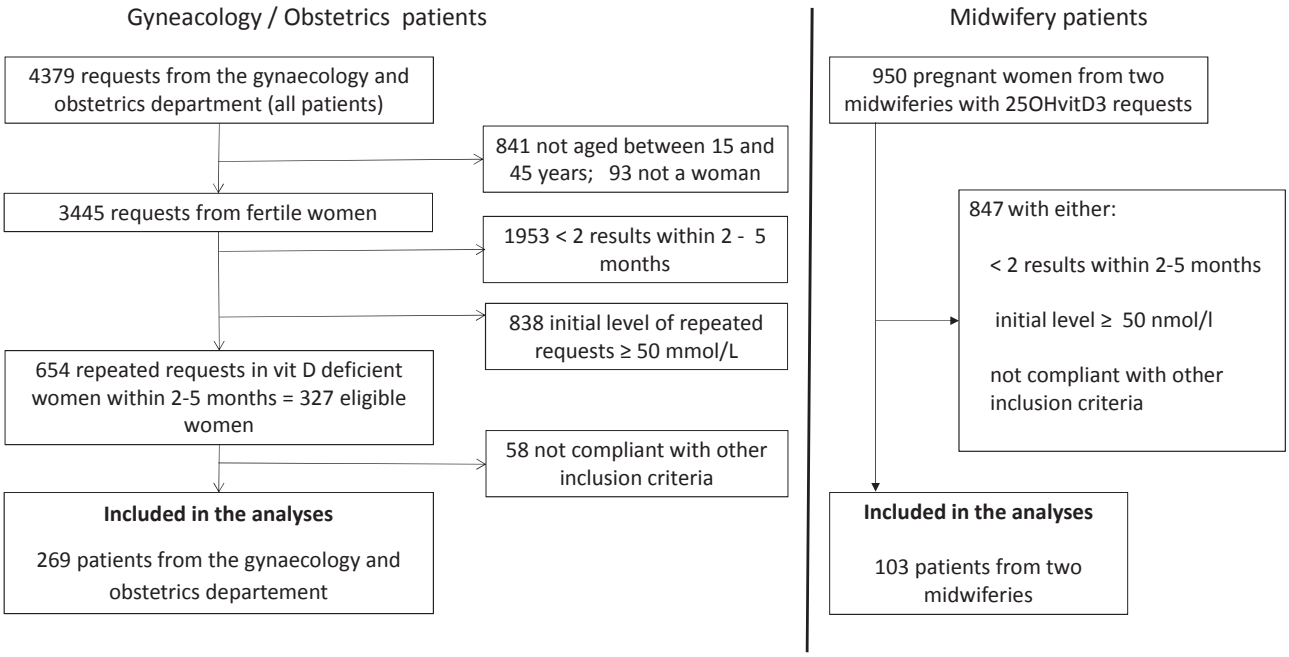

Table 1. Characteristics of the study population sorted by dosage of vitamin D

	Dose vitamin D supplementation	
	400 IU (n=253)	800 IU (n=119)
Treatment center Outpatient gynaecologist Meander MC	91%	32%*
Ethnicity: Non-Caucasian	52%	26%*
Age (years)	34.1 (SD 5.7)	32.0 (SD 5.1)*
Body Mass Index (kg/m^2)	26.4 (SD 6.0)	24.3 (SD 4.6)*
Start supplementation in winter	67%	65%
End supplementation in winter	46%	30%*
Baseline calcidiol status (nmol/L)	31.9 (SD 12)	32.9 (SD 11)
Severely deficient at baseline (calcidiol <25 nmol/L)	28%	18%*
Duration of supplementation (months)	3.3 (SD 1.0)	2.7 (SD 0.8)*

* p <0.05 for difference between the group advised to supplement with 400 IU and the group advised to supplement with 800 IU

Table 2. Average increase in calcidiol and the difference in increase for dose of supplementation, treatment centre, ethnicity, age, BMI, start of supplementation, severity of deficiency, and duration of supplementation

		Increase in calcidiol levels (nmol/L)	Difference in increase in calcidiol levels (nmol/L)		
			Model 1	Model 2	Model 3
Pregnant women	All	17 (SD 15)			
Dose of supplementation	400 IU	15 (SD 14)			
	800 IU	23 (SD 15)	8 (5-11)		11 (7-15)
Treatment center	Midwifery practice	18 (SD 14)			
	Outpatient gynaecologist	17 (SD 15)	-1 (-5-2)	7 (2-11)	7 (2-11)
Ethnicity	Caucasian	18 (SD 15)			
	Non-Caucasian	16 (SD 15)	-2 (-5-1)	0 (-3-3)	-3 (-6-0)
Age	< 35 years	17 (SD 15)			
	≥ 35 years	17 (SD 15)	0 (-4-3)	1 (-2-4)	0 (-2-3)
BMI	< 30 kg/m^2	18 (SD 15)			
	≥ 30 kg/m^2	15 (SD 15)	-2 (-6-1)	-1 (-5-2)	-3 (-7-0)
Start of supplementation	Winter	16 (SD 14)			
	Summer	19 (SD 17)	3 (0-7)	3 (0-6)	2 (-1-6)
End of supplementation	Winter	12 (SD 13)			
	Summer	21 (SD 15)	8 (5-11)	7 (5-10)	7 (4-10)
Severity of deficiency at baseline	Moderately (25-50 nmol/L)	16 (SD 15)			
	Severely (<25 nmol/L)	21 (SD 14)	5 (1-8)	6 (3-9)	6 (3-10)
Duration of supplementation	2 months	17 (SD 13)			
	≥ 3 months	18 (SD 16)	1 (-2-4)	3 (0-7)	2 (-1-5)

Model 1: crude analysis, model 2: adjusted for dose of supplementation, model 3: as model 2 + mutually adjusted for the other factors

Netherlands. The interassay CVs for pooled serum analyses (frozen serum aliquots) were 6.9% at 25.5 nmol/L 25(OH)-vitamin D3 and 3.2% at 72.5 nmol/L.

Statistical analysis

Statistical analyses were performed using SPSS version 18.0 (SPSS Inc, Chicago, IL). Results were tested at a significance of $\alpha < 0.05$. We examined the differences in patient characteristics between the two study groups (supplementation dose of 400 or 800 IU per day) using the unpaired Student's T-test for continuous variables and using the χ^2 - test for ordinal or dichotomous variables. The relationship between average increase in calcidiol levels and supplementation dose (400 / 800 IU), treatment center (midwifery practice / outpatient care), ethnicity (Caucasian / non-Caucasian), age (<35 years / ≥35 years), BMI (< 30 kg/m2 / ≥ 30 kg/m2), start/end of supplementation period (winter/summer), severity of deficiency at baseline (25-50 nmol/L / <25 nmol/L) and duration of supplementation period (2 months / ≥ 3 months) was tested using linear regression with and without adjustment for the other factors. To investigate the difference between women attaining the target calcidiol values of 50 nmol/L after supplementation and supplementation dose, treatment center, ethnicity, age, BMI, start/end of supplementation period, severity of deficiency at baseline and duration of supplementation period, we used logistic regression analysis with and without adjustment for the other factors.

Results

We retrieved 372 vitamin D deficient pregnant women from 4380 laboratory files who received vitamin D supplementation during a period of at least two months and were measured before and after. About two third of all pregnant women received a daily dose of 400 IU, the others daily doses of 800 IU. At the

Table 3. Percentage of initially deficient pregnant women (< 50 nmol/L) not attaining the target of 50 nmol/L after supplementation and Odds Ratios

		After supplementation	Odds Ratio (95%CI)		
		< 50 nmol/L	model 1	model 2	model 3
Pregnant women	All	50%			
Dose of supplementation	400 IU	57%			
	800 IU	35%	0.4 (0.3- 0.6)		0.3 (0.2- 0.7)
Treatment centre	Midwifery practice	41%			
	Outpatient gynaecologist	53%	1.6 (1.0- 2.6)	0.9 (0.5-1.6)	0.5 (0.2- 0.9)
Ethnicity	Caucasian	39%			
	Non-Caucasian	64%	2.9 (1.9- 4.4)	2.5 (1.6- 3.8)	2.2 (1.3- 3.7)
Age	< 35 years	50%			
	≥ 35 years	50%	1.0 (0.7-1.5)	0.9 (0.6- 1.3)	0.9 (0.6-1.5)
BMI	< 30 kg/m^2	48%			
	≥ 30 kg/m^2	55%	1.3 (0.8-2.2)	1.2 (0.7 -2.0)	1.4 (0.8-2.6)
Start of supplementation	Winter	52%			
Period	Summer	45%	0.8 (0.5-1.2)	0.8 (0.5- 1.2)	0.7 (0.4- 1.2)
End of supplementation	Winter	62%			
period	Summer	41%	0.4 (0.3- 0.6)	0.5 (0.3- 0.7)	0.5 (0.3- 0.7)
Severity of deficiency	Moderately				
at baseline	(25-50 nmol/L)	41%			
	Severely				
	(<25 nmol/L)	76%	4.6 (2.7- 7.8)	4.3 (2.5- 7.4)	4.2 (2.3- 7.7)
Duration of	2 months	49%			
supplementation period	≥ 3 months	50%	1.0 (0.7-1.6)	0.8 (0.5-1.3)	0.9 (0.5-1.4)

Model 1: crude analysis, model 2: adjusted for dose of supplementation, model 3: as model 2 + mutually adjusted for the other factors

outpatient clinic of the Department of Gynaecology and Obstetrics, 86% of the pregnant women of our study population were prescribed 400 IU vitamin D daily, while at the midwifery practices 79% were prescribed 800 IU. The origin of this difference is that gynaecologists started to prescribe vitamin D as of 2007, when a daily dosage of 400 IU was given, whereas midwifes started in 2009 prescribing 400 IU, which was changed to 800 IU pending 2010. The average baseline calcidiol level in the total study group was 32 nmol/L (SD 11 nmol/L), and 92 of the 372 deficient pregnant women (25%) were severely vitamin D deficient (< 25 nmol/L). The supplementation period observed over the whole group ranged from two to five months, and averaged 3.1 months (SD 1.0 months). Table 1 shows the characteristics of our study population sorted out by dose of vitamin D prescribed. Compared to women supplemented with 800 IU of vitamin D, the women supplemented with 400 IU were more often treated by a gynaecologist, non-Caucasian, initially severely deficient, older, had a slightly higher BMI, a longer supplementation period, and the second measurement was more often in the winter. There was no significant difference between pregnant women advised to supplement with 400 or 800 IU daily in mean calcidiol levels at baseline and the period in which supplementation started (winter or summer). Table 2 shows the average change in calcidiol serum concentration and the difference in change for dose of supplementation, treatment center, ethnicity, age, BMI, start and end of supplementation, severity of deficiency, and duration of supplementation, being split up for the two dosage groups. Calculations were based on three models: model 1 was the crude model, model 2 adjusted for dose of supplementation and model 3: as model 2 + mutually adjusted for the other factors. The increase in calcidiol over on average 3.1 months of supplementation for the total study population was 17 nmol/L (SD 15 nmol/L). For women receiving 400 IU per day, the average increase in calcidiol was 15 nmol/L compared to 23 nmol/L for those receiving 800 IU per day ($p < 0.001$) as visualized in figure 1 by box and whisker plots. At mean baseline calcidiol levels of 32 nmol/L, we observed an average increase of 15 nmol/L for women advised to supplement with 400 IU per day, compared with 23 nmol/L at 800 IU per day. This is an effective mean response of 1.5 nmol/L per microgram given daily for the 400 IU group and 1.2 nmol/L per microgram given daily for the 800 IU group. After adjustment for dose of supplementation, women treated by a gynaecologist had a 7 nmol/L higher increase in calcidiol compared to those who received care in a midwifery practice. Women having their second measurement in the summer period had a 7 nmol/L higher increase in calcidiol compared to women having a second measurement in the winter. This difference persisted after adjustment for ethnicity, age, BMI, baseline vitamin D status, and duration of supplementation. Severely deficient pregnant women (baseline < 25 nmol/L) showed an 6 nmol/L higher increase in calcidiol compared to those with moderate deficiency (baseline between 25 and 50 nmol/L), independent of the supplementation dose. The increase in calcidiol was not dependent on ethnicity, age, BMI, start of supplementation, and duration of the supplementation period. Table 3 shows the percentage of initially deficient pregnant women (< 50 nmol/L) who do not reach the target calcidiol level of 50 nmol/L. This target was not met by 57% of the initially deficient pregnant women

(<50 nmol/L) when advised to supplement with 400 IU per day. In the group of pregnant women who were advised to use 800 IU daily, 35% did not attain the target calcidiol level ($p < 0.001$). Those with a supplementation dose of 800 IU had a 70% lower risk of vitamin D deficiency after adjustment for treatment center, ethnicity, age, BMI, severity of deficiency, start and end of supplementation, and duration of supplementation. In the fully adjusted model, those treated by a gynaecologist had a 50% lower odds of remaining deficient, and starting or ending the supplementation in the summer period resulted in respectively a 30% and 50% lower odds of remaining deficient. Non-Caucasians had a 220% higher odds of remaining deficient than Caucasians. Those with a severe deficiency at baseline had an even 420% higher odds of remaining deficient than those who were moderately deficient at baseline. For the other determinants no significant association with vitamin D deficiency in the fully adjusted model was observed. The formal Dutch target of at least 30 nmol/L was not met by 38% of the initially severely deficient pregnant women (<25 nmol/L) advised to supplement with 400 IU per day and not met by 10% of those advised to supplement with 800 IU per day ($p = 0.014$).

Discussion

Main Findings

We investigated the increase of serum calcidiol levels after supplementation in pregnancy and whether the advice of many international guidelines to supplement with 400 IU or 800 IU of vitamin D per day was sufficient to attain calcidiol levels of at least 50 nmol/L for initially vitamin D deficient pregnant women. When advised to supplement with 400 IU per day (n= 253), we observed a mean increase of 15 nmol/L, and after supplementation 57% was still deficient. When advised to supplement with 800 IU per day (n=119) we observed a mean increase of 23 nmol/L, and 35% remained deficient. This implies that these doses do not appear to be effective in achieving a sufficient vitamin D status (≥ 50 nmol/L) for a large moiety of initially deficient pregnant women.

Strengths and limitations

The supplementation regimen was not randomized. The prescribed dose of vitamin D was subject to local protocols and individual subscribers. In the beginning of the study period, most prescribers followed the Dutch advice of 400 IU per day for pregnant women. In 2010, local subscription advice was increased to 800 IU per day based on new international studies. The choice for 400 or 800 IU per day for the individual patient was not based on baseline calcidiol levels, season, or characteristics of the individual women. The fact that baseline characteristics may differ amongst those advised to supplement with 400 IU and 800 IU per day is taken into account by adjustment in the tables for the respective differing characteristics. Unfortunately, some factors that may influence the response to vitamin D supplementation, such as individual sun exposure, dietary habits, and adherence to supplementation were not documented, which may have led to residual confounding. A major strength of our "real-life study" is that the results may better reflect the true effect of advising vitamin D supplementation, including possible individual variation in compliance compared to a randomized controlled trial with strict guidance of the included patients. However, based on the efficacy observed we believe that overall compliance was comparable to other studies as discussed below in 'Interpretation'.

Interpretation

In the Netherlands, a target of 30 nmol/L is used for people below the age of 70, including pregnant women, which is based solely on the prevention of bone pathology (23). However, avoiding bone pathology is not the primary objective for pregnant women. Medical care should aim at achieving and maintaining optimal health for the mother and at optimal development of her child (2,3,10). In accordance with literature and international guidelines (24,26), we therefore defined a target for sufficiency of 50 nmol/L although there is still debate about this level and the use of terms like sufficiency and deficiency. Pregnancy it selves has no significant effect on the vitamin D status (29).

In the present study, 62% of the severely deficient pregnant women did not reach the target of 50 nmol/L when advised to supplement with 800 IU per day, which is comparable to the results of other trials about supplementation in pregnancy. In a Turkish study (27) using a dose of 600 IU over three months at mean calcidiol baseline of 24 nmol/L, the target of 50 nmol/L was not reached by 58% of the group. In an English trial, 63% of the severely deficient pregnant women (median baseline 25 nmol/L) remained below the target of 50 nmol/L after supplementation with 800 IU per day in the third trimester of pregnancy (30).

In our study, the efficacy of supplementation and the increase in calcidiol levels depended on the dose of supplementation, baseline vitamin D status, supplementation period in winter or summer, and treatment center. We observed a large variability in the

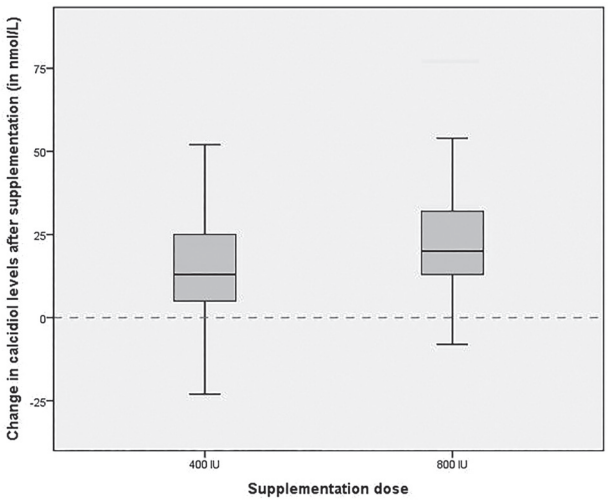

Figure 1. Efficacy of the supplementation for the 400 iU/d and the 800 iU/d group. The box shows the 1st and 3rd quartile and the median of the calcidiol increase.

change of the calcidiol status over the supplementation period for the patients included as demonstrated by figure 1. This is partly attributed to the confounders mentioned above. Theoretically the variability could also be caused by variation in compliance of our patients. To check whether the average compliance in our retrospective study was good or at least similar to published studies we calculated the efficacy and compared it to other (prospective) studies. At a mean baseline calcidiol levels of 32 nmol/L, we observed an average efficacy of 1.5 nmol/L per microgram given daily for the 400 IU group, and 1.2 nmol/L per microgram given daily for the 800 IU group. These results are compliant with the published efficacy bandwidth of 0.7 to 2.0 nmol/L per microgram given daily depending on the baseline calcidiol levels (31). Moreover, from a prospective Turkish study in pregnant women with baseline around 26 nmol/L and supplementation over three months with 600 IU per day, an efficacy of 1.2 nmol per microgram daily could be calculated. This suggests that compliance in our study group is similar to the published dose response studies. Severely deficient pregnant women showed a greater increase in calcidiol levels than pregnant women with moderate deficiency, in accordance with the previously published inverse correlation between baseline calcidiol levels and response efficacy (32).

The additional increase in vitamin D levels in the summer months compared to supplementation in winter is most likely attributable to the effect of sun exposure. The degree of increase in calcidiol was not significantly associated with the length of the supplementation period, which confirms that the steady state of calcidiol concentration is generally reached after approximately two months of supplementation (28).

Pregnant women treated by a gynaecologist showed higher increases in calcidiol levels than pregnant women treated by a midwife. This may be attributable to higher adherence to supplementation in pregnant women treated by gynaecologists. In the fully adjusted model, treatment by a midwife, starting or stopping supplementation in the winter period, being Non-Caucasian and having a severe vitamin D deficiency at baseline all resulted in a higher odds ratio of remaining vitamin D deficient. Though these results need to be confirmed in other studies, this may imply that supplementation doses need to be higher in the winter period, in Non-Caucasians and in those with severe vitamin D deficiency.

We found that for vitamin D deficient pregnant women, a daily dose of 400 or 800 IU over a period of two months is too low to achieve a calcidiol status of 50 nmol/L for the whole group. A similar conclusion was obtained from the prospective Turkish study for doses of 600 and 1200 IU (27). In prospective, randomized trials in both the US and Abu Dahbi, 2000 or 4000 IU per day of vitamin D was observed to be safe and effective for the correction of maternal vitamin D deficiency (33, 34). A daily dose of 4000 IU is the upper level for pregnant women defined by the American Institute of Medicine (24). We do not intend to promote 4000 IU per day for pregnant women. The difference between the IOM upper level and the present advices for supplementation in pregnancy seems to be large enough to allow further optimization of the dosage in future studies.

Conclusions

An advice according to present guidelines to supplement with 400 IU of vitamin D per day was inadequate for about half of initially deficient pregnant women in our population to attain sufficiency (calcidiol ≥ 50 nmol/L). When advised 800 IU per day, still about one third did not attain sufficiency. Since vitamin D deficiency is often unrecognized but highly prevalent among pregnant women (1, 10-13), revision of the current vitamin D guidelines for pregnant women seems necessary. Additional studies are needed concerning the supplementation dose in pregnancy guidelines allowing deficient women to reach a sufficient state of 50 nmol/L.

Especially those who need it most (the severely deficient) have the highest odds for remaining deficient when supplemented with 400 or 800 IU per day. We suggest measuring the status of those belonging to a risk group, or with complaints compatible to vitamin D deficiency. In case of proven deficiency the supplement prescription should be adjusted. One size of vitamin D supplementation "does not fit all" to promote the health of pregnant women and their baby's.

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