Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i4.5602

A CRITICAL ANALYSIS OF CALCIUM AND VITAMIN D3 IN YOUNG PREGNANT WOMEN AND THEIR ASSOCIATION WITH MATERNAL AND FETAL OUTCOME

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Abstract

Background: These interventions are available to policy makers interested in improving calcium and Vitamin D_3 intake.

Objectives: The objectives of the study were to find out the frequency of calcium and vitamin D in young pregnant women and their effects and association on fetus as well as mothers.

Study Design: Interventional study design.

Place and Duration of Study: Gynecology ward and outpatient department of PMC Hospital Nawabshah, Sindh, Pakistan from 1st January 2023 to 31st December 2023.

Methodology: Two hundred participants were enrolled with proper evidence of pregnancy and divided in two equal groups "study group" and "comparison group". All participants from age 18 years to age 26 years with no any malabsorption syndrome or chronic disease were included and those women who have some other diseases or age outliers were excluded. Nonprobability Convenience Sampling was used. 09 participants were missing due to no follow up from study group. Data was collected from 91 Study group and 100 comparison group fulfilling the inclusion criteria.

Results: The frequency of Hypocalcemia before intervention in study group was 50.55% which was significantly decreased to 17.58% after intervention for 03 months as compared to comparison group having 41% hypocalcemia. The frequency of Vitamin D_3 deficiency/ insufficiency was 87.91% before intervention and was reduced to 58.24% after intervention in study group while in comparison group it was 84%. There is a significance difference in the vitamin D and calcium during the period of pregnancy. In the study group the significance association was observed between maternal calcium level and health outcome of the mother (P=0.001) while the vitamin D3

level was (P=0.021). In study group there was significant association between Serum calcium level of the mother and outcome of baby (p= 0.042) while there was no significant association between Serum Vitamin D3 level of mother and baby outcome (p= 0.053).

Conclusion: It was statistically found that the Calcium and Vitamin D_3 values show significances changes during pregnancy. To help improve calcium and Vitamin D_3 intake, there are various interventions that can be executed that include promoting the consumption of foods naturally high in calcium and Vitamin D_3. These interventions are available to policy makers interested in improving calcium and Vitamin D_3 intake.

Key words: Calcium, Vitamin D, Young Pregnant women

Introduction

Every year, more than 20 million babies are born with low birth weight around the world, and roughly 3.6 million of those babies pass away shortly after delivery as a result. Pregnant women frequently experience deficiencies in micronutrients such iron, folate, Calcium, Vitamin D_3, B12, C, and E, as well as riboflavin. Multiple micronutrient supplements may be a helpful strategy for lowering pregnancy-related outcomes during pregnancy. Inadequate consumption of meat, fruits, and vegetables can lead to micronutrient deficiencies, as can infections. Multiple micronutrient supplementations during pregnancy may be a viable tactic for lowering negative pregnancy outcomes by enhancing the nutritional and immunological condition of the mother.[1] In the growth and development of the fetus inside the placenta, maternal nourishment is crucial.[2] Micronutrients, including vitamins and minerals that can be obtained through food, are crucial for biological function. The status of micronutrients varies greatly between communities and during pregnancy. The rigors of gestation can exacerbate micronutrient deficiencies, which can have a negative impact on the health of the fetus in low-income nations where pregnancies are frequently started by malnourished women.[3] One of the most important factors in ensuring the health of the mother and the unborn child is prenatal care (ANC). The best integral component of ANC is nutrition education and counseling, which influences maternal and neonatal health outcomes. Prenatal malnutrition affects not only the fetus but also the mother's health. Despite the fact that the majority of fetal weight increase happens in the fourth trimester, nutritional impacts do not only occur during this time.[4]

Pregnant women especially young pregnant ladies face many problems in their first pregnancy due to lack of awareness and knowledge. Poor nutrition and improper antenatal care affect the health of these women which lead to malnutrition and many macronutrients and micronutrients ultimately putting them at high risk of maternal mortality and high risk deliveries. World Health Organization recommendations on routine antenatal care (ANC) for pregnant women and adolescent girls provide comprehensive guidance and prioritize woman-centered care to facilitate a positive pregnancy experience. Recognizing that ANC provides a strategic platform, 14 out of the 49 recommendations in the WHO ANC guideline are related to nutrition.[5]

Calcium and Vitamin D_3

A remarkable set of physiological change takes place during pregnancy with the goal of maintaining maternal homeostasis while facilitating the growth and development of the fetus. Calcium and vitamin D are extremely beneficial for both mother and baby during pregnancy span. The insufficient amount of calcium and vitamin D can lead to a number of problems during pregnancy.[6] There is an increased risk of preeclampsia, gestational diabetes mellitus, smallness for gestation infant and pre term birth due to vitamin D deficiency. Extreme deficiency of Vitamin D in pregnancy may lead to severe illness in both fetus and mother. Decreased weight gain and pelvic deformities that prevent normal vaginal delivery in pregnancy is also a cause of Vitamin D deficiency. Maternal vitamin D deficiency is linked with neonatal problems comprising

hypocalcaemia, with or without convulsions rickets and defective tooth enamel.[7] Pregnancy-related calcium deficiency is extremely important for the health of the mother and the developing fetus. Maternal calcium status is highly demanded for fetal growth.[8] The important functions of Calcium intake during pregnancy and infant health are Calcium transfer from mother to fetus, infant growth, bony growth in offspring, developmental origins of osteoporosis.[9]

Recommended daily requirement during pregnancy

A newborn baby's skeleton has between 20 and 30 g of calcium in it. The third trimester is when the fetal skeleton grows the fastest, with the majority of the growth occurring from mid-pregnancy onward.[10] The body uses about 10–20 mEq of calcium every day. Adequate calcium consumption is necessary for women of reproductive age, especially during pregnancy. Depending on age, women should consume 1,000–1,300 mg of calcium each day.[11]

Pregnancy causes a progressive decrease in the mother's serum 25(OH)D due to changes and demands on the fetal body. A daily dose of 600 IU of vitamin D3 should be administered to all expectant mothers. Higher vitamin D dosages may be practical to obtain better outcomes for moms and babies, according to the evidence. Pregnant women who had low levels of vitamin D may have had babies who are more likely to have poor bone mineral content, enamel abnormalities, and attention deficit hyperactivity disorder.[12] There is some evidence to support a relationship between maternal 25(OH)D status and offspring birth weight, bone mass, and serum calcium concentrations.[13] Vitamin D supplementation during pregnancy is a potentially effective technique to help prevent small for gestational age (SGA) and low birth weight (LBW) newborns.[14]

Promoting the consumption of naturally calcium-rich foods

In regions where naturally bioavailable calcium and Vitamin D_3containing foods are available, affordable, and acceptable, promoting the consumption of these foods may be effective in improving calcium and Vitamin D_3 intake. Sunlight exposure is the body's primary source of vitamin D, which is produced from cholesterol derivatives, even if diet may include trace amounts of both vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Some foods are natural sources of vitamin D and some foods are fortified with vitamin D.[15]

Materials and Methods

This interventional study was carried in Peoples Medical College Hospital Nawabshah, Sindh Province.

Sample Size: 200 Primigravida Women of 18 to 26 years of age were selected as study participants by nonprobability Convenience Sampling and were divided in two equal groups, Study group and comparison group fulfilling the criteria. 09 participants form study group were missing at their 2nd visit.

Sample selection: For study purposes women with first pregnancy (Primi-gravida) visiting for antenatal checkup in their end of 2nd trimester or at the start of 3rd trimester (Study Group) and were followed up to the termination of pregnancy and comparison Group were selected at the time of Delivery.

Inclusion Criteria: Age ranging from 18 years to 26 years, Primigravida women, No any chronic disease or illness related to nutrition or absorption disorder and not taking any supplement or multivitamins.

Data collection procedure: After taking informed and written consent from the participant, data was collected on already designed Questionnaire containing all Socio-demographic information, antenatal history, Clinical relevant examination for any signs and symptoms of nutritional

deficiency related illness. ***Clinical Examination:*** Anthropometric measurements were done by taking height and weight and Body Mass Index (BMI) was calculated. Clinical assessment and necessary examination were performed for and signs and symptoms of related nutrition deficiency.

Blood Sample collection: Blood sample were collected in recommended sample bottles for Serum Calcium Level and Vitamin D3 level and were sent to laboratory immediately for analysis. For Serum Calcium Chem reader Smart –N SE 250-N Semi auto Chemistry Analyzer was used and for Serum D3 level ICHROMA-II was Used ichroma™ II was used.

Intervention and follow up:
Proper nutritional counselling and education was given to the study group during their first visit at the end of 2nd trimester of start of 3rd trimester. Booklet was provided for further guidance to maintain nutritional chart and weekly calcium intake was calculated for to measure effective intervention assessment. Signs and symptoms of nutritional deficiencies were also shared to counter any complication. Participants were allowed to maintain their daily calories and RDA for Calcium and Vitamin D_3. Food items rich in Calcium and Vitamin D_3 were recommended and were convinced to take minimum 1300 mg calcium per day and Vitamin D_3 from 600 to 1000 IU per day following the below given chart. Participants were also asked for regular exercise and exposure to sun light minimum 15 to 30 minutes daily. Participants were followed after three months at the termination of pregnancy and all tests were performed again. Following products were daily consumed and were easily available in the local market of adjacent areas of the participants.

Chart No.01: Calcium and Vitamin D3 sources and content[16-18]

Sr. No.	Products	Serving Size (g or mL)	Calcium (mg)	Vitamin D_3 (IU)
1	Milk	240 ml (1 cup)	290	100 IU
2	Yogurt	240 ml (1 cup)	300	-
3	Cheese, butter	85 gm (1/2 Cup)	612	-
4	Chinese spinach	85 gm (1/2 Cup)	114	-
5	Cod liver oil	(1 tablespoon)	-	1360
6	Salmon (fish), cooked	3 ounces	-	570
7	Liver, beef, braised	3 ounces	50	42
8	Soy, Rice, Almond	85 gm (1/2 Cup)	-	100
9	Dates	85gm	33	-
10	Egg	1 large, scrambled	-	44
11	Liver, beef, braised,	3 ounces	-	40
12	Fortified Orange Juice	125 mL (1/2 cup)	-	50
13	Banana	Per (large size)	-	75
14	Exposure to sunlight, UVB (0.5 MED*)		-	3000

* After an average of 5 to 10 minutes (depending on the time of day, season, latitude, and skin sensitivity) of exposure of the arms and legs to direct sunshine, about 0.5 MED of UVB radiation would be absorbed.
IU refers to international units, mL refers to milliliter, mg refers to milligram.

Data Analysis Procedure: After collecting data on questionnaire, calculation daily intake of Calcium and Vitamin D_3 and reports of laboratory investigations from Diagnostic Laboratory, the data was processed by hand sorting techniques, calculator, Microsoft office and using statistical program for social sciences (SPSS Version 25.0) for analysis. Each variable separately and data was analyzed by applying Chi Square test. Final findings were recorded and presented in the form of charts, graphs and tables. Level of significance was set at $P \leq 0.05$.

Results
Total numbers of participants in our study were 200 and were equally divided in 02 groups. 100 were labeled as **Study Group** and 100 were labeled as **Comparison group**. 100 Study subjects were followed for around 03 months with intervention out of them 09 participants didn't continue

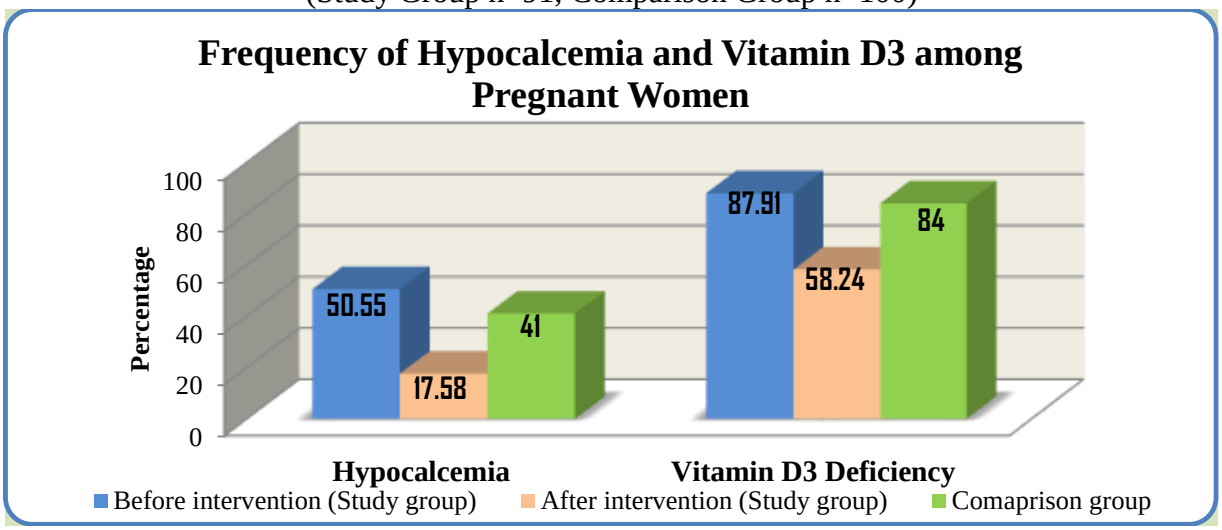
the protocol and made no contact after trial so were labeled as "**Missing".** Data was collected from 100 comparison subjects during the same time interval.

Table No. 01: Socio demographic information of the Study Participants
(Study Group n=91, Comparison Group n=100)

			Study Group		Comparison Group	
Sr. No.	Characteristics	Category	Frequency	Percentage	Frequency	Percentage
1	**Age of the Participants**	18 to 20 years	21	23.08	25	25.0
		21 to 23 year	21	23.08	38	38.0
		24 to 26 years	49	53.84	39	39.0
2	**Residence of Participants**	Urban	41	45.05	49	49.0
		Rural	50	54.94	51	51.0
3	**Educational status**	Primary	20	21.97	35	35.0
		Secondary	62	68.13	56	56.0
		Graduate	09	9.89	09	9.0
4	**Occupation of the Participants**	Employed	16	17.58	12	12.0
		Skilled Person	03	3.29	03	3.0
		House Wife	72	79.12	85	85.0
5	**Socioeconomic Status of Participants**	Lower Middle Class	39	42.85	42	42.0
		Upper Middle Class	52	57.14	58	58.0
6	**Term of Pregnancy**	Preterm (<37week GA)	20	21.97	39	39.0
		Full Term (37week GA)	63	69.23	44	44.0
		Post Term (>37week GA)	08	8.79	17	17.0

The above table shows the sociodemographic information of the participants. Mean age of the participants in study group was 23.11 ± 2.45 with 09 participants missing and for comparison group mean age 22.54 ± 2.51. The age was divided in three class intervals, 18 to 20 years, 21 to 23 years and 24 to 26 years of age. Majority of the participants were in third age interval. In urban setting, 41 participants were in study group and 49 participants were in comparison group. In rural setting 50 participants were in study group and 51 participants were in comparison group. Majority of the participants were house wives in both groups. Participants in lower middle class were 42 in comparison group and 39 in study group while in upper middle class, 58 participants were from comparison group and 52 were in study group. The frequency of the preterm (<37 weeks) participants were 20 and 39 in study group and comparison group respectively, full term 37 weeks) were 63 and 44 in study group and comparison group respectively while post term (>37 weeks) participants in study group were 08 and in comparison group were 17.

Figure No. 01 Frequency of Hypocalcemia and Vitamin D3 in Pregnant Women
(Study Group n=91, Comparison Group n=100)



The frequency of Hypocalcemia was 50.55% before intervention in study group and was decreased to 17.58% after intervention for 03 months as compared to comparison group having 41% hypocalcemia. The frequency of Vitamin D_3 deficiency/ insufficiency was 87.91% before intervention and was reduced to 58.24% after intervention in study group while in comparison group it was 84%.

Table No. 02 Mean and Standard deviation of Serum Calcium level of the Participants
(Study Group n=91, Comparison Group n=100)

Serum Calcium level		Normal: 8.8 to 10.3 mg/dl Hypocalcemia: < 8.8 mg/dl			
		N	Mean Std. Deviation	Minimum	Maximum
Study Group	Serum Calcium (mg/dl) 1st Visit	91	**8.86 ± 1.04**	7.10	10.80
	Serum Calcium (mg/dl) 2nd Visit	91	**9.85 ± 0.72**	8.40	11.10
Comparison Group	Serum Calcium (mg/dl)	100	**8.75 ± 1.11**	7.0	11.10

The above tale shows the mean calcium level of the participants. Mean serum calcium level of study group at the time of 1st visit was **8.86 ± 1.04** mg/dl and at the 2nd visit **9.85 ± 0.72**. For comparison group mean serum calcium level at the time of delivery was **8.75 ± 1.11**.

Table No. 03 Mean and Standard deviation of Serum Calcium level of the Participants
(Study Group n=91, Comparison Group n=100)

Serum Vitamin D3 Level		Normal: >=30 ng/ml Insufficiency: 10-30 ng/ml Deficiency: <10 ng/ml			
		N	Mean Std. Deviation	Minimum	Maximum
Study Group	Serum D3 Level (ng/ml) 1st Visit	91	**17.24 ± 7.16**	9.27	35.76
	Serum D3 Level (ng/ml) 2nd Visit	91	**25.99 ± 8.55**	13.83	52.12
Comparison Group	Serum D3 Level (ng/ml)	100	**14.41 ± 6.65**	9.10	42.12

The above table shows the serum Vitamin D3 level of the participants in our study. Mean Vitamin D3 level of the study group in 1st visit was **17.24 ± 7.16** and in 2nd visit was **25.99 ± 8.55** while in comparison group mean serum Vitamin D3 level was **14.41 ± 6.65**.

Table No. 04 Association of Serum Calcium with outcome of Mother
(Study Group n=91, Comparison Group n=100)

		Health status of Mother (Study Group)		Total	Health status of Mother (Comparison Group)		Total
		Healthy Recovery	Ill health / Need care		Healthy Recovery	Ill health / Need care	
Status Of Calcium	Normal	66	9	75	47	12	59
	Hypocalcemia	7	9	16	16	25	41
Total		73	18	91	63	37	100
*Ill health of mother was measured as APH, PPH, Severe Distress leading to prolonged hospital stay and further management							
There was statistically significant association between serum calcium and health outcome of mother (p-value 0.001)							

The above table shows the association between Serum Calcium levels and health status of mother at the time of delivery. The values were highly statistically significant at Normal Serum Calcium level has significant association with positive maternal outcome (p-value = 0.001).

Table No. 05 Association of Vitamin D3 with Outcome of Mother
(Study Group n=91, Comparison Group n=100)

		Health status of Mother (Study Group)		Total	Health status of Mother (Comparison Group)		Total
		Healthy Recovery	Ill health* / Need care		Healthy Recovery	Ill health / Need care	
Status Of Vitamin D_3	Normal	29	9	38	8	8	16
	Deficiency / Insufficiency	44	9	53	55	29	84
Total		73	18	91	63	37	100
*Ill health of mother was measured as APH, PPH, Severe Distress leading to prolonged hospital stay and further management							
There was statistically significant association between serum Vitamin D_3 level and health outcome of mother (p-value 0.021)							

The above table shows the association between deficiency of Vitamin D3 and Health status of the mother and it was noted that there was statistically significant association between Vitamin D_3 level and maternal health outcome (p-value 0.016)

Table No. 06 Association of Maternal Serum Calcium with Baby outcome
(Study Group n=91, Comparison Group n=100)

		Baby Outcome (Study Group)			Total	Baby Outcome (Comparison Group)			Total
		Healthy Recovery	Ill health* / Need care	Death		Healthy Recovery	Ill health* / Need care	Death	
Status Of Calcium	Normal	65	9	1	75	32	11	0	43
	Hypocalcemia	10	6	0	16	35	14	8	57
Total		75	15	1	91	67	25	8	100
*Ill health is considered as premature, low birth weight, IUGR, Hypoxia, Asphyxia causing prolonged hospital stay and further management.									
There was statistically significant association between serum calcium of mother and baby outcome (p-value 0.042)									

The above table shows the association between Serum Calcium levels of mother and baby outcome at the time of delivery. The values were statistically significant at Normal Serum Calcium level has significant association with healthy baby outcome (p-value = 0.042).

Table No. 07 Association of Maternal Serum Vitamin D_3 with Baby outcome
(Study Group n=91, Comparison Group n=100)

		Baby Outcome (Study Group)			Total	Baby Outcome (Comparison Group)			Total
		Healthy Recovery	Ill health* / Need care	Death		Healthy Recovery	Ill health* / Need care	Death	
Status Of Vitamin D_3	Normal	34	3	1	38	11	4	1	16
	Insufficiency / Deficiency	41	12	0	53	56	21	7	84
Total		75	15	1	91	67	25	8	100
*Ill health is considered as premature, low birth weight, IUGR, Hypoxia, Asphyxia causing prolonged hospital stay and further management.									
There was statistically no significant association between serum Vitamin D_3 level of mother and baby outcome (p-value 0.053) at < 0.05									

The above table shows the association between deficiency of maternal Serum Vitamin D3 and baby outcome and there was statistically significant association between Vitamin D_3 level and maternal health outcome (p-value 0.050).

Discussion

Calcium and Vitamin D_3 are essential for smooth muscle contraction, neural signaling, and skeletal structure. Promoting the consumption of naturally accessible calcium and Vitamin D_3 containing foods may be an effective way to increase calcium and Vitamin D_3 intake in areas where these items are readily available, reasonably priced, and socially acceptable.[19] A healthy, balanced diet is essential for expectant mothers because it must meet both the needs of the developing fetus and the mother's regular nutritional requirements. This allows the mother to maintain her stores of nutrients and those needed for the fetal health. Total serum calcium typically decreases during pregnancy.[20] Sustaining an appropriate level of calcium and phosphorus in the serum is mostly dependent on vitamin D and merely 10 to 15% of dietary calcium and roughly 60% of phosphorus is absorbed in the absence of vitamin D. As a result, vitamin D plays a major role in the development and maintenance of strong bones.[21]

In our study, the frequency of Hypocalcemia was 50.55% before intervention in study group and was significantly decreased to 17.58% after intervention for 03 months as compared to comparison group having 41% hypocalcemia. The frequency of Vitamin D_3 deficiency/ insufficiency was 87.91% before intervention and was reduced to 58.24% after intervention in study group while in comparison group it was 84%. In late pregnancy, total hypocalcaemia affects 56.70% women. The frequency of total hypocalcaemia during pregnancy is greatly influenced by variables such as the number of meals consumed each day, dessert consumption, the composition of the most consumed meal, and monthly income.[22] Pakistan has the highest incidence of adult vitamin D deficiency in South Asia at 73%, which is extremely worrying given the importance of vitamin D to nutrition. Pregnant women in Pakistan, who make up around 79.7% of the population overall who do not obtain adequate vitamin D, are the main group affected. Given that it increases the risk of potentially catastrophic pregnancy problems like gestational diabetes, preeclampsia, and early births, this is a serious cause for concern.[23] This data is in accordance with our study as well.

According to a study by Loa T et al,[24] 84% of young pregnant women in our study had vitamin D insufficiency without intervention, and 29% of them experienced serious or minor pregnancy problems. According to the study's statistics, up to 80% of Asian pregnant women reported having a vitamin D deficit. A lower birthweight, newborn hypocalcemia seizure, poor skeletal, lung, and immunological development, as well as a higher risk of maternal problems such as preeclampsia, reduced glucose tolerance, and cesarean section rate, were all linked to vitamin D deficiency. This is in accordance with our study as well.

A clinical triple-blind trial was carried out on 126 pregnant women who were referred to Tabriz health centers by Alizadeh-Charandabi SM et al[25] The study's findings demonstrated that there were no appreciable variations in average newborn weight, height, or head circumference, gestational age at delivery, type of delivery, or length of pregnancy when vitamin D3 levels were raised during pregnancy. Additionally, there was no discernible correlation between the serum vitamin D3 level and the fate of the baby in our investigation.

Maternal serum Calcium is found directly affecting the health of newborn it was clear from the data that 57% hypocalcemic mothers had poor (38.6%) baby outcome in comparison group compared to study group after intervention where 17.6% participants were hypocalcemic and only 06 had poor baby outcome. In a study by Elsary AY et al,[26] 76% of newborns were hypocalcemic, and 52% of those babies had late hypocalcemia. 38% of people had hypovitaminosis D. Neonate with no history of maternal calcium supplementation (57.9%) or vitamin D supplementation (98.7%) was more likely to have hypocalcemia.

Conclusion

Calcium and Vitamin D_3 intake in many low and middle income countries remains well below the recommended levels. To help improve calcium and Vitamin D_3 intake, there are various interventions that can be executed that include promoting the consumption of foods naturally high in calcium and Vitamin D_3. These interventions are available to policy makers interested in improving calcium and Vitamin D_3 intake especially during pregnancy to reduce feto-maternal morbidity and mortality.

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