Original Article

Effect of High-dose Vitamin D Supplementation on Beta Cell Function in Obese Asian-Indian Children and Adolescents: A Randomized, Double Blind, Active Controlled Study

Shweta Varshney, Rajesh Khadgawat, Monita Gahlot[1], Deepak Khandelwal, Avneet Kaur Oberoi, R. K. Yadav[2], V Sreenivas[3], Nandita Gupta, Nikhil Tandon

Departments of Endocrinology and Metabolism, [1]Dietitics, [2]Physiology, [3]Biostatistics, All India Institute of Medical Sciences, New Delhi, India

Abstract

Objective: Vitamin D deficiency has been found to be associated with insulin resistance. In an attempt to explore this association, we planned a study to investigate the effects of high-dose vitamin D supplementation on beta cell function in obese children and adolescents. **Methods:** A randomized, double blind, active-controlled study was carried out to investigate the effects of high dose (120,000 IU once a month) vitamin D supplementation in comparison to recommended daily allowance (12,000 IU/month) for 12 months. Beta cell function was assessed by disposition index. Inflammatory cytokines and cardiovascular risk factors were also assessed before and after supplementation. **Results:** A total of 189 obese children and adolescents were recruited. The mean serum 25OHD level of the study population was 8.36 ± 5.45 ng/ml. At baseline, 94.7% subjects were vitamin D deficient (<20 ng/mL). After 12 months of supplementation, serum 25OHD level in intervention group was 26.89 ± 12.23 ng/mL, while in control group, it was 13.14 ± 4.67 ng/mL ($P < 0.001$). No significant difference in disposition index as well as other parameters of insulin resistance, sensitivity, inflammatory cytokines, and pulse wave velocity was seen after supplementation. **Conclusion:** Vitamin D supplementation in doses of 120,000 IU per month for 12 months in obese Asian-Indian children and adolescents did not affect beta cell function as well as cardiovascular risk factors.

Keywords: Beta cell function, insulin resistance, obesity, vitamin D supplementation

Introduction

Approximately 10% of the world's children and adolescents are estimated to be carrying excess body fat, with an increased risk for developing chronic diseases. The prevalence of obesity is dramatically higher in economically developed regions, but also rising significantly in most parts of the world.[1] Childhood obesity has been found to be associated with an increase in insulin resistance (IR), impaired glucose tolerance, and metabolic syndrome.[2] Children and adolescents with IR and metabolic syndrome are more likely to develop type 2 diabetes and cardiovascular diseases in comparison to children and adolescents with normal body mass index (BMI).[1] Through various mechanisms, IR is clearly a mediator of future type 2 diabetes and risk of cardiovascular diseases. However, current scientific understanding of natural history and determinants of IR is incompletely understood.[2] Recent literature in adult population has shown a role of vitamin D deficiency in IR and its possible future consequences.[3,4] There is a mechanistic support that vitamin D may influence both, insulin secretion as well as insulin sensitivity, and subsequently incidence of type 2 diabetes. In general, cross-sectional and prospective studies have found associations between vitamin D insufficiency with impaired glucose tolerance and IR, while supplementation of vitamin D in high-risk populations for IR has resulted in improvement in insulin secretion in adults.[4]

In spite of accumulating evidence of the possible association between vitamin D deficiency and IR, vitamin D supplementation studies are limited by relatively small

Access this article online

Quick Response Code:

Website: www.ijem.in

DOI: 10.4103/ijem.IJEM_159_19

Address for correspondence: Prof. Rajesh Khadgawat, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, India.
E-mail: rajeshkhadgawat@hotmail.com

How to cite this article: Varshney S, Khadgawat R, Gahlot M, Khandelwal D, Oberoi AK, Yadav RK, *et al.* Effect of high-dose Vitamin D supplementation on beta cell function in obese Asian-Indian children and adolescents: A randomized, double blind, active controlled study. Indian J Endocr Metab 2019;23:545-51.

sample sizes, lack of randomized active controlled design, and short-term interventions.

The effect of vitamin D supplementation on IR has not been well studied in prospective, active-controlled design in population at high risk for IR. We planned a randomized double blind active controlled study to assess the effect of vitamin D supplementation on beta cell function in obese children and adolescent subjects, using robust parameters which are well validated in both adult as well as pediatric population.

Methods

Study Subjects: The study was conducted as randomized, double-blind, active controlled, single-center study. Obese children and adolescents attending the endocrinology outdoor service for the management of obesity were recruited (between October 2011and September 2015). The inclusion criteria included obese children and adolescents between the ages of 11 and 17 years. Obesity was defined as per International Obesity Taskforce (IOTF) criteria.[5] All parents signed informed consent while assent was taken from all study subjects. The study was approved by the Institutional Ethics Committee. Ethics committee approval is obtained on 28th September 2010 by institutional ethics committee.

Subjects already diagnosed with diabetes mellitus or impaired glucose tolerance were excluded from the study as were subjects taking metformin or any weight reducing drugs. Similarly, subjects taking or had taken vitamin D supplementation in last 6 months in doses exceeding 400 IU/day, using medications known to interact with vitamin D metabolism (steroids, thiazide diuretics, phenytoin, phenobarbitone, and antitubercular drugs) or subjects with clinical features suggestive of osteomalacia or severe vitamin D deficiency were excluded from the study. Subjects with any known systemic illness (endocrine, cardiac, renal, hepatic, or gastrointestinal diseases), syndromic obesity, or with symptoms suggestive of hypothalamic obesity were also excluded. After biochemical screening, additional exclusion criteria were subjects with serum calcium level >1 mg/dL above the upper limit of normal for age, hypercalciuria as per definition, and serum 25OHD level >100 ng/mL.

Sample size calculation: The study sample size has been calculated based on the primary outcome of beta cell function. We anticipated an effect size of 10% change with vitamin D supplementation. With 90% power and 95% confidence interval, sample size was estimated at 125 subjects in each group (total 268 subjects, OpenEpi version 2.3) while with 80% power and 95% confidence interval, 93 evaluated subjects would be required in each group. A 30% dropout was expected and added in final sample size.

Vitamin D supplementation: Each subject was counseled by certified dietician in the presence of parents. A low caloric weight reducing diet was advised to all subjects and encouraged to participate in regular physical activity on a daily basis. After counseling, subjects were randomized into two groups—intervention and control, in the ratio of 1:1 (computer-generated randomized code). The intervention group received 120,000 IU of vitamin D per month, while control group received 12,000 IU vitamin D per month in a single oral dose after meal. Plan of vitamin D intervention is shown in Figure 1. The dose of 120,000 per month was selected to assess effects of high dose of vitamin D supplementation. Ideally, active groups should have been compared with placebo but in view of very high prevalence of vitamin D deficiency in this population in prior studies, recommended daily requirement dose of vitamin D was given to control group in place of placebo.[6]

There were total 12 doses, of which, six were given as directly supervised doses during follow-up visits in the hospital while rest of the six doses were taken at home which were confirmed by telephonic call in the evening. On each visit, subjects were called in the morning in fasting state with the fasting midstream urine sample collected at home. Once blood samples were collected, vitamin D supplementation was given after breakfast.

Both preparations for intervention as well as control (120,000 IU tablets for intervention group and 12,000 IU for the control group, Torrent Pharmaceuticals, Ahmedabad, India) were unlabeled for dose and identical in all physical aspects (color, taste, and external appearance).

Physical examination: Height was measured with wall-mounted Holtain's stadiometer (Holtain Inc., Crymych, Pembs. UK), while weight was measured with the same digital weighing machine. Blood pressure (BP) was measured in the right upper limb in sitting position with a mercury sphygmomanometer after 5-min rest with an appropriate size cuff. BP was measured three times and the mean value was recorded.

Body fat analysis: Total body fat distribution was assessed by dual energy X-ray absorptiometry (DXA) by fan beam technology using pediatric software for age <16 years (DXA, Hologic QDR 4500A, Hologic Inc, Bedford, MA).

Biochemical and hormonal measurements: Fasting venous blood samples were collected on ice, centrifuged immediately using a refrigerated centrifuge, and stored at −20°C until analysis. Complete blood count, liver function tests, renal function test,

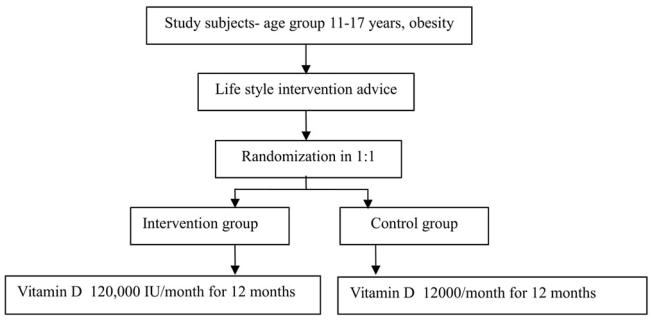

Figure 1: Plan of vitamin D intervention

serum calcium (corrected calcium with serum albumin), phosphate, alkaline phosphatase, uric acid, and blood glucose were measured in all subjects on an automated chemistry analyzer (Roche Hitachi 912 Chemistry Analyzer, GMI Inc., USA). Glycosylated hemoglobin (HbA1c) was measured in whole blood using ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories Inc., CA, US). Serum intact PTH and serum C-peptide were measured on a Roche Elecsys e411 autoanalyzer using an electrochemiluminescent tracer-based immunometricassay. Serum 25OHD was measured on the DiaSorin auto analyzer ("LIASON" DiaSorin, Inc., Stillwater, MN, USA) using a chemiluminescent label. The reproducibility of the assay ranged from 6% to 12% and was within the performance characteristics described by the manufacturer. Our laboratory is registered with the UK-DEQAS vitamin D assay external quality control assessment program and hence met their performance targets regularly (www.deqas.org). Vitamin D deficiency was defined as a serum 25OHD level <20 ng/ml. This was further subdivided into severe, moderate, and mild vitamin D deficiency if serum 25OHD levels were <5, 5 to less than 10, and 10 to less than <20 ng/mL, respectively, while levels between 20 and 30 ng/mL were considered as insufficiency and >30 as vitamin D sufficient.[7] Five samples of oral glucose tolerance test (OGTT) were carried out with sample collection at 0, 30, 60, 90, and 120 min after ingestion of glucose. The dose of glucose was calculated as per recommendations of American Diabetes Association.[8]

Indices of glucose and insulin metabolism: Changes in beta cell function were measured by "Disposition Index" (calculated by the insulin secretion × insulin sensitivity). Insulin secretion was calculated by "insulinogenic index,"[9] while insulin sensitivity was calculated by whole body insulin sensitivity index, also known as "Matsuda Index."[10]

Measurement of cardiovascular risk factors

Biochemical outcomes—inflammatory markers, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and highly sensitive C-reactive protein (hsCRP)—were measured by enzyme-linked immune sorbent assay (ELISA) technique, using commercially available kits [R and D Systems, Minneapolis (MN), USA].

Pulse wave velocity and augmentation index by radial pulse wave analysis were measured noninvasively by SphygmoCor® pulse wave velocity system (AtCor Medical, Sydney, Australia) using standard guidelines.[11]

Statistical analysis: Statistical analysis was carried out using Stata 14.1 (College Station, Texas, U.S.A.). Data is presented as mean ± SD. Comparison between groups was performed using two tailed *t*-tests, Mann--Whitney and Chi-square tests. *P* value ≤ 0.05 was considered as significant.

Results

A total of 189 obese children and adolescents completed the study, of which 120 (63.49%) were boys and 69 (36.51%) were girls [Figure 2]. The baseline characteristics of study subjects are shown in Table 1. Combined history of diabetes, hypertension, or obesity in any family member (any of these three diseases in any family member) was present in 89% of total study subjects.

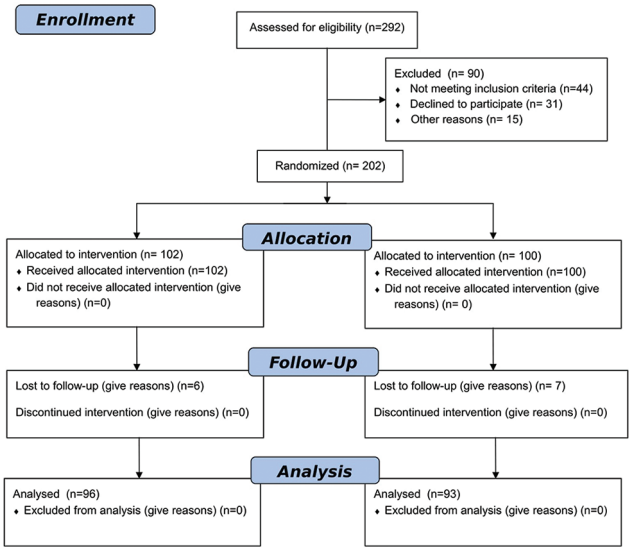

Figure 2: The CONSORT flow diagram of the study

At baseline, 94.7% subjects (boys—94.1%; girls—95.7%,) were vitamin D deficient (serum 25OHD <20 ng/mL). Severe vitamin D deficiency (<5 ng/mL) was seen in 27.8% subjects, while 70.6% had serum vitamin D levels <10 ng/mL. Only four subjects had serum 25OHD level >30 ng/mL [Table 2]. No significant correlation was seen between serum 25OHD and any other biochemical parameters including BMI. However, significant positive correlation was seen between BMI and HOMA-IR (r = 0.34, P < 0.001) and inverse correlation between BMI-disposition index (r = −0.18, P = 0.01).

Impaired fasting glucose (IFG) was seen in 11.5% subjects while none in diabetic range. Ten subjects had both, IFG as well as IGT, while 30 subjects who had IGT but FBG was in normal range. Approximately 67.5% subjects had HbA1c levels in normal range. Of total 189 subjects, 47.6% subjects had at least one of the three parameters abnormal.

Effect of supplementation: The biochemical characteristics of study subjects after 12 months of supplementation are shown in Table 3. Vitamin D sufficiency (>30 ng/mL) could be achieved in 41.2% of subjects in intervention group, while none of the control group subjects reach to this level. In spite of using high dose of vitamin D supplementation in intervention group, 32% subject still remained vitamin D deficient, while 90.2% subjects were still vitamin D deficient in the control group [Table 3].

Baseline serum PTH was not significantly different between the two groups at baseline. After 12 months of vitamin D supplementation, serum PTH decreased in both groups (40.26 ± 13.25 pg/mL in intervention group; P < 0.001 compared with baseline PTH vs. 43.84 ± 15.74 pg/mL in

Table 1: Comparison of anthropometric and biochemical parameters of study subjects at baseline

Parameter (unit)	Intervention group	Control group	*P*
Age (years)	12.89±1.63	13.15±1.54	0.25
BMI (kg/m^2)	29.53±3.27	30.36±4.27	0.13
HbA1c (%)	5.48±0.36	5.53±0.43	0.42
Serum calcium (mg/dL)	9.45±0.54	9.57±0.52	0.12
Serum albumin	4.58±0.82	4.56±0.71	0.17
Serum alkaline phosphatase (IU/L)	615.73±256.95	559.76±299.01	0.11
Serum total cholesterol (mg/dL)	157±28.65	155±23.65	0.63
Serum LDL (mg/dL)	95±23.5	91±21	0.33
Serum HDL (mg/dL)	40±4.09	40±6.95	0.54
Serum VLDL (mg/dL)	22±8.56	22±7.16	0.93
Serum PTH (pg/mL)	57.65±49.16	56.45±60.52	0.88
Serum 25OHD (ng/mL)	8.36±5.45	9.01±5.59	0.42
Total body fat (gm)	23,835.41±6,941.94	23,086.83±5,896.83	0.70
Blood glucose 0 min (mg/dl)	91±7.36	91±7.98	0.89
Blood glucose 30 min	148±31.8	143±27.8	0.21
Blood glucose 60 min	133±33.5	137±29.4	0.33
Blood glucose 90 min	127±31.3	131±26.58	0.41
Blood glucose 120 min	120±24.99	124±20.52	0.32
Serum insulin 0 min (μU/ml)	19.8±10.08	20.67±11.53	0.58
Serum insulin 30 min	165.09±121.62	149.16±115.29	0.37
Serum insulin 60 min	148.38±124	133.93±96.97	0.37
Serum insulin 90 min	121.98±93.87	138.1±108.19	0.28
Serum insulin 120 min	128.31±104.68	115.84±90.63	0.38
Plasma c-peptide 0 (ng/mL)	3.52±1.17	3.33±1.03	0.31
Plasma c-peptide 30	12.88±5.09	11.26±4.60	0.049
Plasma c-peptide 60	13.28±5.51	11.84±3.67	0.06
Plasma c-peptide 90	12.93±4.98	12.36±4.28	0.46
Plasma c-peptide 120	12.92±5.6	12.11±4.26	0.33
Matsuda index	2.68±2.19	2.47±1.48	0.46
HOMA IR	4.44±2.30	4.60±2.48	0.65
Insulinogenic Index	2.81±2.01	2.83±2.38	0.94
Disposition Index	5.48±4.52	5.72±4.42	0.72

Data expressed as means±SD. BMI: Body mass index; HOMA-IR: Homeostasis model of assessment for IR index; LDL: Low density lipoprotein; HDL: High density lipoprotein; PTH: Parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D. *P<0.05

control group; P = 0.03 compared with baseline PTH). However, comparison between serum PTH levels between the two groups after 12 months, no significant difference was observed.

There was no statistically significant difference in blood glucose, serum insulin, and C-peptide levels at any time point after 12 months. Similarly, there was no significant difference in disposition index between the two groups at the end of 12 months. In a sub-group analysis, subjects had moderate to severe vitamin D deficiency at baseline (serum 25OHD level <5 and 5--10 ng/mL) also; there were no significant difference in disposition index between the two groups. Similarly, subjects who showed greater increase in serum 25OHD level (change of >20 ng/mL between baseline and 12 months) also did not show any significant difference in disposition index.

Among pro-inflammatory markers, no significant difference was found in serum hsCRP, IL-6, and TNF-α levels between the two groups at 12 months (data not shown). Similarly, no significant difference was also observed in any of the parameters of pulse wave velocity [Table 4].

There was no significant difference between the two groups when comparing lipid profile at baseline and after the supplementation. Similarly, no significant difference was also observed in total body fat.

Safety of intervention: Vitamin D supplementation in the present doses was found to be absolutely safe. None of the study subject developed symptomatic hypercalcemia. Even in the absence of symptoms, none of the study subject developed both hypercalcemia as well as hypercalciuria at single point of time requiring withdrawal from the study. Similarly, none of the study subjects could reach to very high serum 25OHD level (>100 ng/ml) in any group. The highest serum 25OHD levels seen after supplementation were 54 ng/ml in intervention group and 24 ng/ml in control group. Only seven subjects developed hypercalcemia (intervention group 3; control group 4), however none of them had any symptoms related to hypercalcemia or found to have hypercalciuria

Table 2: The effect of vitamin D supplementation on metabolic parameters in intervention and control group after 12 months

Parameter (unit)	Intervention group	Control group	*P*
BMI (kg/m^2)	29.83±3.60	31.06±4.67	0.06
HbA1c (%)	5.46±0.28	5.58±0.37	0.22
Serum calcium (mg/dL)	9.64±0.52	9.51±0.65	0.17
Serum albumin	4.63±0.69	4.48±0.62	0.18
Serum alkaline phosphatase (IU/L)	505.7±232.73	549.69±246.43	0.26
Serum total cholesterol (mg/dL)	156.82±28.66	154.97±23.65	0.63
Serum LDL (mg/dL)	86±17.6	82±20.97	0.27
Serum HDL (mg/dL)	39±6.92	40±8.27	0.43
Serum VLDL (mg/dL)	24±11.36	23±7.16	0.52
Serum PTH (pg/mL)	40.26±13.25	43.84±15.74	0.12
Serum 25OHD (ng/mL)	26.89±12.23	13.14±4.67	<0.001*
Total body fat (gm)	24,595.5±6,067.91	24,254.68±6,250.99	0.76
Blood glucose 0 min (mg/dl)	90±7.63	90±8.11	0.83
Blood glucose 30 min	139±31.4	133±26.25	0.15
Blood glucose 60 min	134±29.39	132±28.05	0.76
Blood glucose 90 min	119±25.83	118±23.43	0.87
Blood glucose 120 min	115±37.35	114±24.04	0.77
Serum insulin 0 min (µU/ml)	24.77±18.43	23.01±15.05	0.48
Serum insulin 30 min	193.37±148.92	168.75±123.36	0.24
Serum insulin 60 min	172.04±144.45	166.41±151.03	0.79
Serum insulin 90 min	147.54±137.97	147.65±153.23	0.99
Serum insulin 120 min	139.26±134.40	119.27±111.16	0.27
Plasma c-peptide 0 (ng/mL)	3.83±1.87	3.55±1.36	0.37
Plasma c-peptide 30	12.95±6.29	11.21±4.98	0.09
Plasma c-peptide 60	13.53±6.03	12.06±4.42	0.15
Plasma c-peptide 90	12.56±5.92	11.51±4.3	0.30
Plasma c-peptide 120	11.43±5.64	10.92±4.58	0.60
Matsuda index	2.37±2.23	2.35±1.24	0.93
HOMA IR	5.57±4.40	5.17±3.47	0.48
Insulinogenic Index	3.27±3.32	3.96±6.87	0.40
Disposition Index	5.84±4.92	5.85±4.03	0.43

Data expressed as means±SD. BMI: Body mass index; HOMA-IR: Homeostasis model of assessment for IR index; LDL: Low density lipoprotein; HDL: High density lipoprotein; PTH: Parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D. *Significant

Table 3: Vitamin D status of study subjects at baseline and after 12 months of supplementation

Group	<5 ng/ml		<10 ng/ml		<20 ng/ml		<30 ng/ml		>30 ng/ml	
	Before	After	Before	After	Before	After	Before	After	Before	After
Intervention	31.6%	0.0%	71.6%	2.1%	94.7%	32.0%	97.8%	58.8%	2.2%	42.2%
Control	23.9%	0.0%	69.6%	28.3%	94.6%	90.2%	97.8%	100%	2.2%	0.0%

Table 4: Pulse wave velocity in the study subjects intervention and control group

Parameters	Intervention group			Control group			Difference between two groups values at baseline	Difference between the two groups values at 12 months
	Baseline	After 12 months	*P*	Baseline	After 12 months	*P*		
Carotid-Radial*	6.85±1.59	7.03±1.62	0.37	6.55±1.22	6.75±1.27	0.76	0.17	0.27
Radial-Distal*	6.76±1.93	6.72±1.79	0.93	7.35±2.40	7.33±1.92	0.19	0.84	0.5
Carotid-Distal*	6.76±1.04	6.50±0.93	0.07	6.83±1.14	6.75±0.71	0.26	0.64	0.10
Carotid-Femoral*	5.97±1.86	5.98±1.11	1.00	5.93±1.90	6.14±1.44	0.95	0.89	0.46

*All values are in M/s

at the same time, hence continued in the study and did not develop hypercalcemia on next visit. None of the subjects had hypercalciuria at baseline while 5.3% at 6 months and 3.2% had hypercalciuria at 12 months, however none of them were found to have hypercalcemia. There was no significant difference in urinary calcium creatinine ratio at baseline;

however, after 12 months of vitamin D supplementation, urinary calcium creatinine ration was significantly higher in intervention group than in control group (0.08 ± 0.05 vs. 0.05 ± 0.03 $P < 0.01$).

Discussion

Here, we are reporting the effects of vitamin D supplementation in doses of 120,000 IU/month for 12 months in obese Asian-Indian children and adolescents and compared with dose of 12,000 IU/month. Vitamin D supplementation increased circulating serum 25OHD concentrations in both the groups with predominant effect in intervention group. No effect of vitamin D supplementation was observed in any of the parameters of insulin sensitivity and beta cell function in both the groups. Results of our study are in agreement with other vitamin D supplementation studies published.[3,12-22] The study subjects in these studies varied from normal healthy adults,[12] to subjects with high prevalence of IR like nondiabetic obese children,[13-17] adults with metabolic syndrome,[19] subjects with PCOS,[23] and pregnant women.[21]

There are several lines of evidence supporting a role for vitamin D in pancreatic beta cell function. Vitamin D appears to affect the insulin response to glucose stimulation, whereas it does not appear to influence basal insulinemia.[3] Vitamin D deficiency may influence insulin secretion and sensitivity via its effects on intracellular calcium. Vitamin D deficiency results in elevated parathyroid hormone, which in turn is known to elevate intracellular calcium. Pancreatic beta cells depend on an acute intracellular calcium increase for insulin secretion, which may be attenuated with elevated cytosolic calcium.[4]

Gedik and Akalin[24] were first to report the normalization of insulin secretion after 6 months of vitamin D supplementation in four subjects with vitamin D deficiency. Since then, there have been many studies reporting effects of vitamin D supplementation in IR. Results of many of these studies did not show any effect,[12-14,16-24] however, some of the recently published studies have shown significant effect of vitamin D supplementation on parameters of IR. Kelishadi *et al*.[15] studied effect of 300,000 units of vitamin D supplementation in obese adolescents in the age group of 10-16 years and reported decrease in HOMA-IR ($P = 0.02$) 12 weeks after supplementation. Similarly, other studies have also reported improvement in insulin parameters after vitamin D supplementation.[25,26]

The most of the intervention studies have used HOMA as outcome parameter to assess effect of vitamin D supplementation. It is the most widely used parameter of IR because of its convenience of estimation. It gives more information about hepatic IR than whole body insulin sensitivity and does not provide any information about insulin secretion. Vitamin D appears to affect exclusively the insulin response to glucose stimulation, whereas it does not appear to influence basal insulin levels.[3] Vitamin D supplementation has been reported to influence peripheral insulin sensitivity more, as shown by Nagpal *et al*.[27] where three doses of 120,000 IU vitamin D3 fortnightly versus placebo showed significant improvements in a 3-h OGTT-derived insulin sensitivity index, but not indices derived from fasting values.

It would be difficult to compare results of these studies because of many limitations like lack of randomization,[19] placebo-controlled design,[20] use of HOMA only as outcome parameter,[12] small sample size,[14,16] shorter duration of intervention,[14,16] absence of vitamin D deficiency in study subjects at baseline.[13]

Improvements in serum vitamin D levels depends upon baseline vitamin D status with maximum change seen in subjects with severe vitamin D deficiency while subjects with mild deficiency show minimal increase in vitamin D level.[28] Although we used high dose of vitamin D supplementation (120,000 IU per month), still vitamin D deficiency could not be corrected in 32% subjects in intervention and 90% in control group. It has been estimated that non-obese adults require a 100-IU intake to increase serum 25OHD concentrations by 1.0 ng/mL,[29] whereas obese adults require twice the dose to see similar response.[30] In this study, 120,000 per month produced a mean increase of 26.89 ng/mL or 1.0 ng/mL for every 149 IU ingested in obese adolescents. This suggests obese subject require much higher dose of vitamin D to correct deficiency. The requirement of higher doses of vitamin D could be partly due to the decreased bioavailability because of preferred deposition in body fat compartments with subsequent non-availability to body cells.

The duration of intervention has been reported to be one of the important factors affecting IR. Blenchia *et al*. reported no effect of supplementation at 3 months while significant effect was observed 6 months after supplementation.[16] Similarly, Hurst *et al*.[31] also found no significant change until 6 months. Our study had supplementation for 12 months, however it may be possible that longer duration of supplementation (for 24–30 months) may give different results.

Strengths of our study were manifolds. We selected obese Asian-Indian children and adolescent who have been documented to have high IR and low whole body insulin sensitivity.[32] Others were use of high doses of vitamin D supplementation for long duration of intervention, high prevalence of vitamin D deficiency in study subjects, and assessment of both insulin secretion and insulin sensitivity. Apart from insulin-based parameters, we also used serum C-peptide based parameters for assessment for IR.

Limitations of our study were lack of supervision of intake of all doses, lack of a 24-h urine calcium excretion measurement to assess safety, and fairly wider age range. Another important limitation may be the use of 12,000 IU per month in control group which might have blunted the difference between the two groups. This dose was chosen for ethical reasons to give them at least recommended daily dose of vitamin D[6] to prevent

rickets as very high prevalence of VDD have been reported in this population.

Conclusion

Our study did not show any effect of vitamin D supplementation in the dose of 120,000 IU/month, for 12 months, on beta cell functions. At the same time, no effect was also observed on inflammatory cytokines and parameters of arterial stiffness.

Financial support and sponsorship

This study was funded by ICMR through extramural research grant.

Conflicts of interest

There are no conflicts of interest.

References

1. Lobstein T, Baur L, Uauy R. IASO International Obesity Task Force. Obesity in children and young people: A crisis in public health. Obes Rev 2004;5(Suppl 1):4-104.
2. Rosenbloom AL, Joe JR, Young RS, Winter WE. Emerging epidemic of type 2 diabetes in youth. Diabetes Care 1999;22:345-54.
3. Seida JC, Mitri J, Colmers IN, Majumdar SR, Davidson MB, Edwards AL, *et al*. Clinical review: Effect of vitamin D3 supplementation on improving glucose homeostasis and preventing diabetes: A systematic review and meta-analysis. J Clin Endocrinol Metab 2014;99:3551-60.
4. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010;2010:351385.
5. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: International survey. BMJ 2000;320:1-326.
6. Wagner CL, Greer FR. American Academy of Pediatrics Section on Breastfeeding; American Academy of Pediatrics Committee on Nutrition. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 2008;122:1142-52.
7. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. Endoc Rev 2001;22:477-501.
8. Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, *et al.* The 1997 American Diabetes Association and 1999 World Health Organization criteria for hyperglycemia in the diagnosis and prediction of diabetes. Diabetes Care 2000;23:1108-12.
9. Pacini M, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. Best Pract Res Clin Endocrinol Metab 2003;17:305-22.
10. Dalla Man C, Campioni M, Polonsky KS, Basu R, Rizza RA, Toffolo G, *et al.* Two-hour seven-sample oral glucose tolerance test and meal protocol: Minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. Diabetes 2005;54:3265-73.
11. Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, *et al.* Clinical applications of arterial stiffness, Task Force III: Recommendations for user procedures. Am J Hypertens 2002;15:445-52.
12. Mitchell DM, Leder BZ, Cagliero E, Mendoza N, Henao MP, Hayden DL, *et al.* Insulin secretion and sensitivity in healthy adults with low vitamin D are not affected by high-dose ergocalciferol administration: A randomized controlled trial. Am J Clin Nutr 2015;102:385-92.
13. Javed A, Vella A, Balagopal PB, Fischer PR, Weaver AL, Piccinini F, *et al.* Cholecalciferol supplementation does not influence b-cell function and insulin action in obese adolescents: A prospective double-blind randomized trial. J Nutr 2015;145:284-90.
14. Nader NS, Aguirre Castaneda R, Wallace J, Singh R, Weaver A, Kumar S. Effect of vitamin D3 supplementation on serum 25(OH)D, lipids and markers of insulin resistance in obese adolescents: A prospective, randomized, placebo-controlled pilot trial. Horm Res Paediatr 2014;82:107-12.
15. Kelishadi R, Salek S, Salek M, Hashemipour M, Movahedian M. Effects of vitamin D supplementation on insulin resistance and cardiometabolic risk factors in children with metabolic syndrome: A triple-masked controlled trial. J Pediatr (Rio J) 2014;90:28-34.
16. Belenchia AM, Tosh AK, Hillman LS, Peterson CA. Correcting vitamin D insufficiency improves insulin sensitivity in obese adolescents: A randomized controlled trial. Am J Clin Nutr 2013;97:774-81.
17. Shah S, Wilson DM, Bachrach LK. Large doses of vitamin D fail to increase 25-hydroxyvitamin D levels or to alter cardiovascular risk factors in obese adolescents: A pilot study. J Adolesc Health 2015;57:19-23.
18. Yin X, Yan L, Lu Y, Jiang Q, Pu Y, Sun Q. Correction of hypovitaminosis D does not improve the metabolic syndrome risk profile in a Chinese population: A randomized controlled trial for 1 year. Asia Pac J Clin Nutr 2016;25:71-7.
19. Sollid ST, Hutchinson MY, Fuskevåg OM, Figenschau Y, Joakimsen RM, Schirmer H, *et al.* No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes. Diabetes Care 2014;37:2123-31.
20. Oosterwerff MM, Eekhoff EM, Van Schoor NM, Boeke AJ, Nanayakkara P, Meijnen R, *et al.* Effect of moderate-dose vitamin D supplementation on insulin sensitivity in vitamin D-deficient non-Western immigrants in the Netherlands: A randomized placebo-controlled trial. Am J Clin Nutr 2014;100:152-60.
21. Yap C, Cheung NW, Gunton JE, Athayde N, Munns CF, Duke A, *et al.* Vitamin D supplementation and the effects on glucose metabolism during pregnancy: A randomized controlled trial. Diabetes Care 2014;37:1837-44.
22. Salehpour A, Shidfar F, Hosseinpanah F, Vafa M, Razaghi M, Amiri F. Does vitamin D3 supplementation improve glucose homeostasis in overweight or obese women? A double-blind, randomized, placebo-controlled clinical trial. Diabet Med 2013;30:1477-81.
23. Garg G, Kachhawa G, Ramot R, Khadgawat R, Tandon N, Sreenivas V, *et al.* Effect of vitamin D supplementation on insulin kinetics and cardiovascular risk factors in polycystic ovarian syndrome: A pilot study. Endocr Connect 2015;4:108-16.
24. Gedik O, Akalin S. Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. Diabetologia 1986;29:142-5.
25. Yeow TP, Lim SL, Hor CP, Khir AS, Won Mohamud WN, Pacini G. Impact of vitamin D replacement on markers of glucose metabolism and cardiometabolic risk in women with former gestational diabetes—A double-blind, randomized controlled trial. PLoS One 2015;10:e0129017.
26. Asemi Z, Foroozanfard F, Hashemi T, Bahmani F, Jamilian M, Esmaillzadeh A. Calcium plus vitamin D supplementation affects glucose metabolism and lipid concentrations in overweight and obese vitamin D deficient women with polycystic ovary syndrome. Clin Nutr 2015;34:586-92.
27. Nagpal J, Pande JN, Bhartia A. A double-blind, randomized, placebo-controlled trial of the short-term effect of vitaminD3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men. Diabet Med 2009;26:19-27.
28. Khadgawat R, Marwaha RK, Garg MK, Ramot R, Oberoi AK, Sreenivas V, *et al.* Impact of vitamin D fortified milk supplementation on vitamin D status of healthy school children aged 10-14 years. Osteoporos Int 2013;24:2335-43.
29. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 2003;77:204-10.
30. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690-3.
31. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient-A randomised, placebo-controlled trial. Br J Nutr 2010;103:549-55.
32. Khadgawat R, Thomas T, Gahlot M, Tandon N, Tangpricha V, Khandelwal D, *et al.* The effect of puberty on interaction between vitamin D status and insulin resistance in obese Asian-Indian children. Int J Endocrinol 2012;2012:173581.