Impact of Supplementation of Milk Fortified with Vitamin D in School Children

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Introduction

- Vitamin D deficiency is a public health issue worldwide including India that affects each stage of the life cycle and crosses sex, economic, educational and ethnic classification, with huge potential human and economic cost implications.

- India is located between 8.4 and 34.6 N latitude with majority of its population living in regions experiencing ample sunlight throughout the year.

INTRODUCTION

- Poor vitamin D status is possibly resulted from the poor sun exposure, dark skin complexion, environmental pollution, vegetarian food habits, absence of fortification and poor intake of vitamin D supplements.

- Evidence of low vitamin D status and its association with the greater risk of poor skeletal health ,autoimmune disorder , diabetes , insulin resistance , obesity and cancer, is growing.

- In view of these observations , Identification and prioritization of feasible and prudent public health measures for the prevention of vitamin D deficiency are urgently required .

Overcoming Vitamin D deficiency

- Sunlight / consumption of foods rich in vitamin D
- Supplementation
- Fortification

Dietary advice or supplementation will not be effective in India

A) Foods rich in vitamin D are very few
B) Indians being vegetarian by nature, do not consume these foods
C) Proportion of population consuming vitamin D supplement is very small.

- It is therefore important that food fortification strategy which has proven to be effective, should be adopted to improve vitamin D status of general public.

IOF position statement:
Vitamin D recommendations for older adults.

- Average vitamin D requirement for older adults is 20 to 25 µg/day (800 to 1,000 IU/day).
- Repletion dose will vary according to:
  - starting level
  - BMI
  - effective sun exposure
  - other unidentified factors.
- Intake lower than 20 µg/day (800 IU/day) *may be* adequate for individuals with regular effective sun exposure.

Osteoporos Int (2010) 21:1151–1154

Vitamin D:
Recommendations

- **Intake adjusted upward to as much as 50 µg/day (2,000 IU/day) in:**
  - **Obese**
  - **Those with osteoporosis**
  - **Limited sun exposure (institutionalized, homebound)**
  - **Malabsorption**
  - ***In non-European populations known to be at high risk for vitamin D deficiency such as those in the Middle East and South Asia, or immigrants from such regions living in Europe***

Osteoporos Int (2010) 21:1151–1154

Vitamin D:
Recommendations

- **In high-risk individuals, we recommend measuring the serum 25OHD level.**
- **The required dose to reach 75 nmol/L can be estimated from the measured level.**
- ***Each 2.5 µg (100 IU) of added vitamin D will increase the serum 25OHD level by about 2.5 nmol/L (range 1.75– 2.75 nmol/L) or 1.0 ng/ml (range 0.7 to 1.1 ng/ml).***
- **Serum 25OHD levels should be retested after about 3 months**

Osteoporos Int (2010) 21:1151–1154

Overcoming Vitamin D deficiency

- Sunlight
- Supplementation
- Fortification

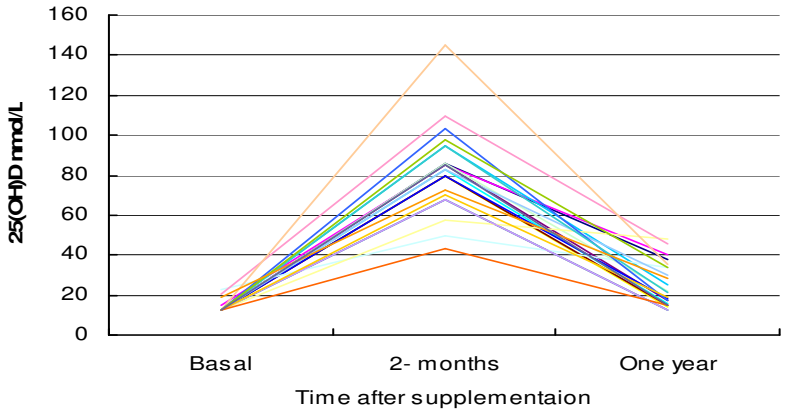
D3 Supplementation in Adults

60,000 IU D3 weekly + calcium daily for 8 weeks

Table 1. Change in serum Ca, 25-hydroxy vitamin D (25(OH)D) and intact PTH (iPTH) after cholecalciferol (1500 μg (60 000 IU)/week) and Ca (1g/d) supplementation*

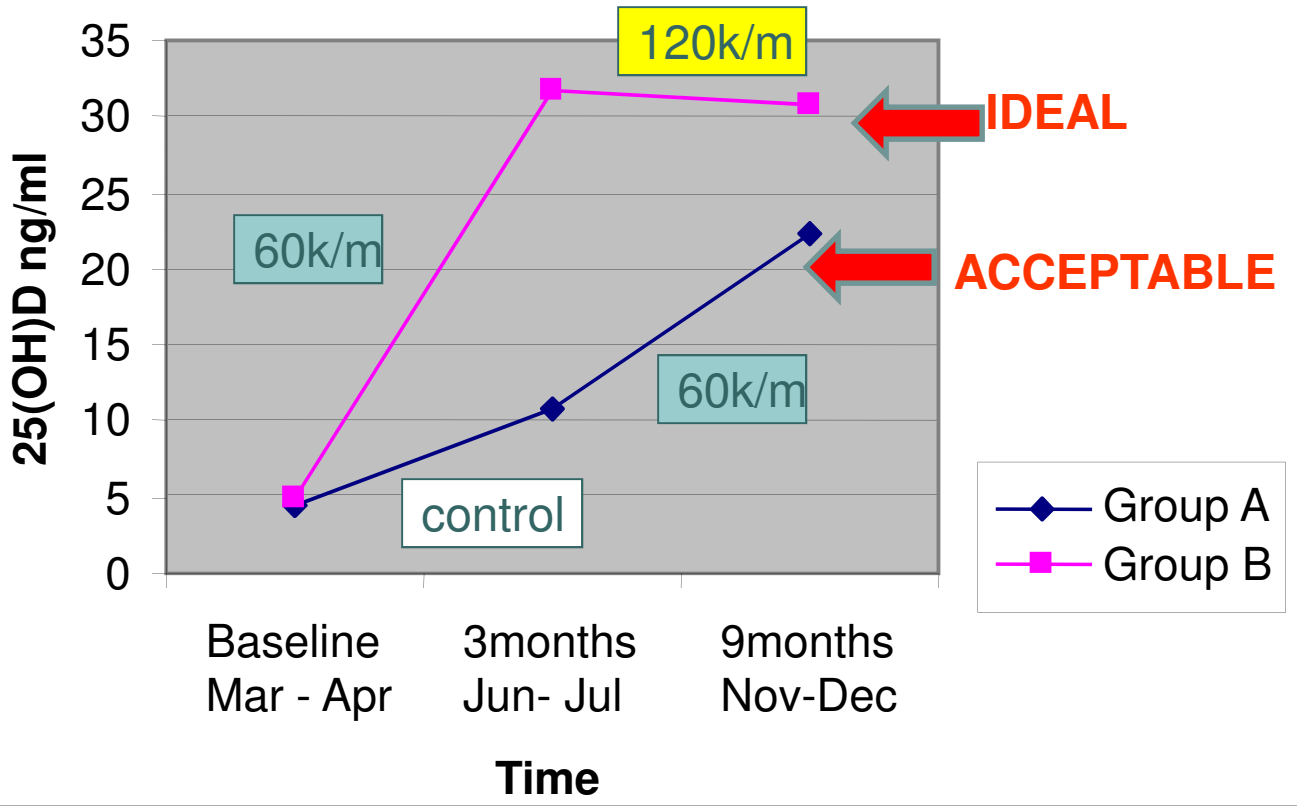
(Mean values and standard deviations)

	Baseline (pre-supplementation)		After 8 weeks of supplementation			
			8 weeks		12 months	
Parameters	Mean	SD	Mean	SD	Mean	SD
Serum total Ca (mmol/l)	2·27	0·22	2·22	0·17	2·34	0·17
Serum inorganic P (mg/dl)	1·32	0·13	1·42[a]	0·13	1·29[b]	0·13
Serum alkaline phosphatase (IU/l)	300	118	253	78	252	100
25(OH)D (nmol/l)	13·5[a]	3·0	82·4[b]	20·7	24·7[a]	10·9
Serum iPTH(ng/l)	54[a]	40	29[b]	20	72[a]	32
Supranormal PTH (*n*, %)	7 out of 23 (30·4 %)		0 out of 23 (nil)		14 out of 23 (60·1 %)	



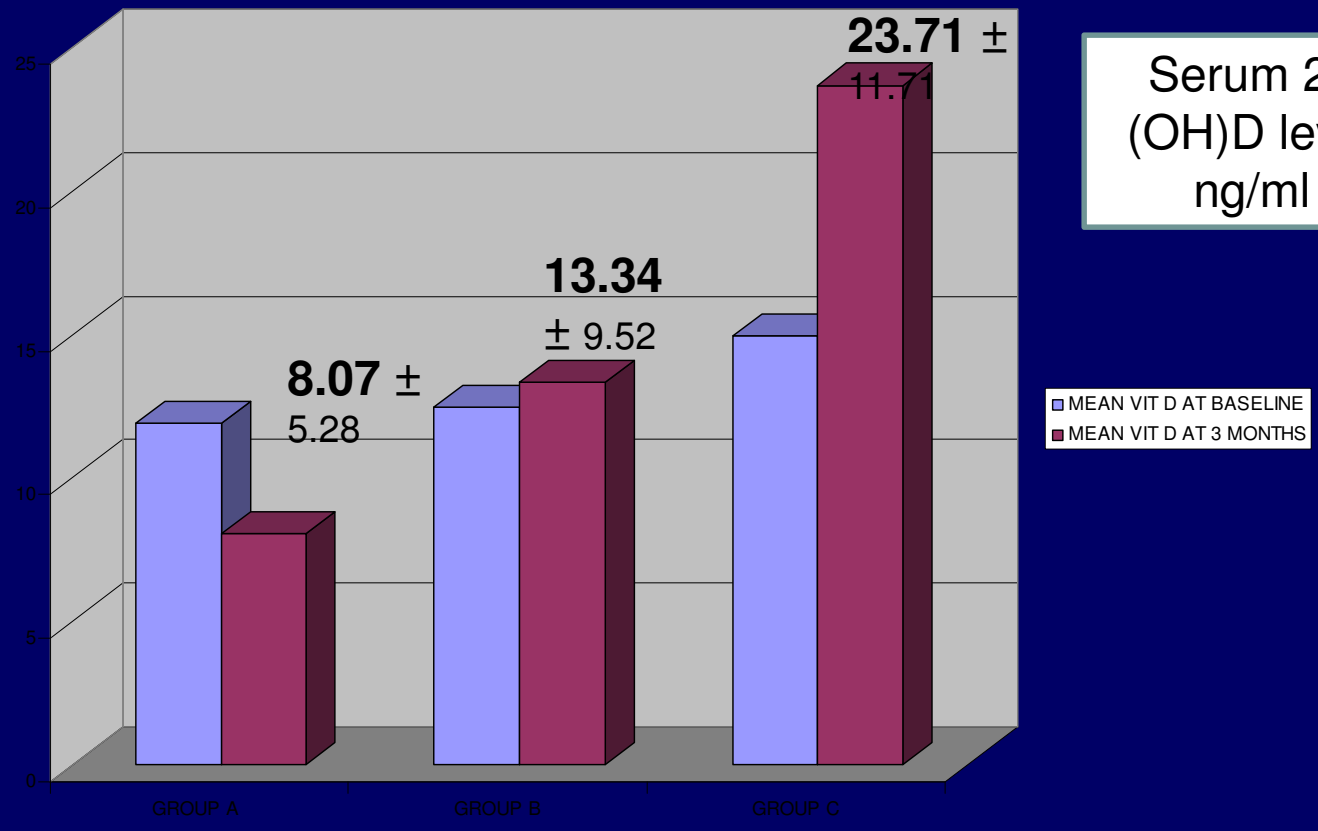
Goswami et al, 2008

D3 Supplementation in Adults
60000 IU per month



Malhotra, et al, 2009; Arch Osteoporosis

Postmenopausal women: D3 500 vs 1000 IU/d + calcium 1000 mg



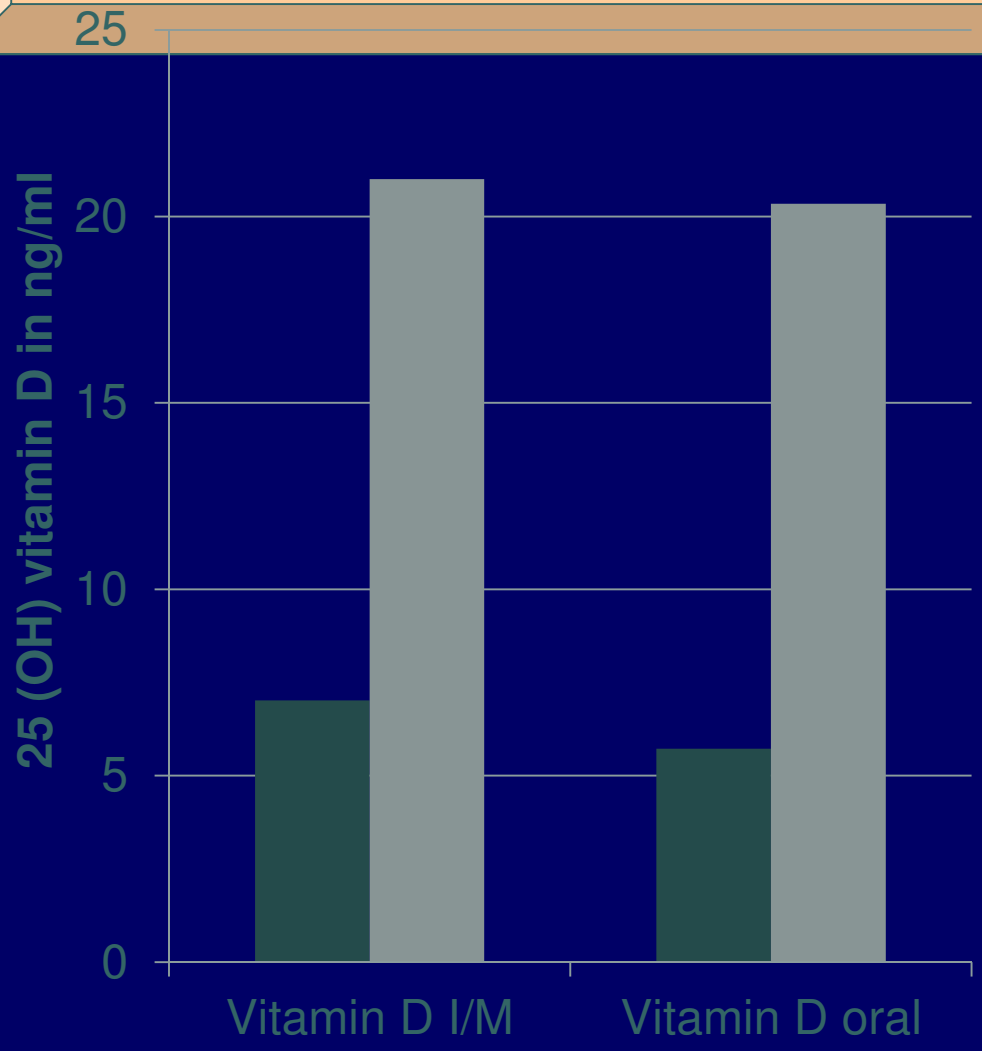
Serum 25 (OH)D level ng/ml

Calcium alone	D 500 iu/d	D 1000 iu/d

Agarwal, Mithal et al 2010

D3 Supplementation in Adults

parenteral once vs oral weekly



- 300000 units I/M vitamin D single dose
- Equivalent dose of oral vitamin D weekly for 5 weeks
- 25 (OH) D level after 6 weeks

Gupta, et al.
Poster ANZMS-IOF

Effect of Vitamin D supplementation in schoolgirls

60,000 IU D3: Monthly vs. 2-Monthly

	0	6 months	12 months
Baseline: 93.7 % school girls were vitamin D deficient			
Despite 1 year of supplementation, Vitamin D sufficiency: 67.8% LSES and 31.9% USES			
USES@			
2 monthly D3	29.1 ±1.54	39.5±1.24*	38.2 ±2.13 #
1 monthly D3	30.8 ±1.39	46.8 ±1.45*	49.9 ±2.01 #

@ Mean diff between 1 and 2 monthly gps stat. sig.

* = P<0.05 for baseline vs. 6 month; # = P<0.05 for baseline vs. 12 month;
$ = P<0.05 for 6 months vs. 12 month

Supplementation in Pregnancy

	Group A (no vitamin D) (n = 14)	Group B (one dose of 60 000 U vitamin D) (n = 35)	Group C (two doses of 120 000 U vitamin D each) (n = 35)	P-value
Baseline 25OHD (nmol/l)	25.8 (18.9–30.7)*	33.4 (22.6–47.7)	40.1 (26.9–58.4)	< 0.01
25OHD at delivery (nmol/l)	23.8 (17.2–32.6)	30.9 (24.8–48.1)	53.4 (41.2–88.0)†	< 0.001
Serum calcium at delivery (mmol/l)	2.31 ± 0.18	2.28 ± 0.27	2.29 ± 0.21	0.41
Increment of 25OHD (nmol/l)	0.4 (−6.5–16.8)	−2.1 (−10.7–13.1)	13.4 (0.2–42.0)‡	< 0.01
25OHD > 80 nmol/l at delivery (nmol/l)	1/14 (7%)	2/35 (5.7%)	12/35§ (34.2%)	0.003
25OHD at delivery conducted in winter (nmol/l)	14.9 (n = 1)	26.9 (22.9–33.3) (n = 11)	43.7 (35.3–62.0) (n = 14)	< 0.01§
25OHD > 80 nmol/l at delivery in winter	0/1	0/11	3/14 (21%)	0.23§

Anthropometric indices: Differences persisted at 9 months

Parameter	Group A	Group B	Group C	p
Head circ (cm)	33.6 ± 0.8	34.4 ± 0.6	34.5 ± 0.9	0.000
Length (cm)	49.4 ± 2.4	50.1 ± 0.9	50.3 ± 0.9	0.000
Weight (kg)	2.8 ± 0.4	3.0± 0.4	3.1± 0.4	0.003

Sahu et al, EJCN, 2009

Conclusion- Supplementation

- Enough evidence to recommend supplementation for vulnerable groups-
  - Older adults
  - Children
  - Pregnancy
  - Optimal regimens need to be determined- studies underway

Types of fortification

- **Mass fortification**:

  To fortify foods that are widely consumed by the general population

  Usually mandatory

  Best option when majority of the population has an unacceptable risk, in terms of public health, of becoming deficient in specific micronutrients.

Types of fortification Cont…

- **Target fortification**

  Foods aimed at specific population subgroups are fortified eg young children/ elderly, pregnant women

  May be mandatory or voluntary

- **Market-driven fortification**

  To allow food manufacturers to voluntarily fortify foods available in the market place.

  Always voluntary, but governed by regulatory limits

Vitamin D Fortification

- Vitamin D2 or D3 can be added to foods.
- Very sensitive to oxygen and moisture, and both interact with minerals.
- Dry stabilized form of vitamin D, which contains an antioxidant (usually tocopherol) that protects activity even in the presence of minerals, is generally used

Experience with vitamin D fortification of specific foods

1. Milk and other dairy products: including dried milk powder and evaporated milk. Yogurt…

2. Margarine (in EU, 30% of the RDI for vitamin D comes from margarine)

3. Orange juice, cereals bread, mushrooms also work! *(AJCN-2010)*

An updated Systemic review and Meta analysis of the efficacy of Vitamin D food Fortification

Lucidia J Black

- 14 out of 16 RCTs [1513 subjects] had a significant beneficial effect of food fortification on 25(OH)D concentrations.
- Subgroup analyses showed a reduction in heterogeneity and significant treatment effect when 4 trials that used milk as the fortified food source were combined.
- When combined in a random effects analysis a mean individual intake of 11µg/d (440 IU/day) from fortified foods increased 25 (OH)D by 19.4 nmol/L(7.76 ng / ml) corresponding to a 1.2 nmol/L increases in 25(OH)D for each 1µg ingested

Characteristics and result of included trials on vitamin D fortified foods and serum 25-hydroxyvitamin D concentrations

Characteristics and results of included trials on vitamin D–fortified foods and serum 25-hydroxyvitamin D concentrations[1]

	Characteristics					Dietary source	
Study and location	Population	IG	CG	Age	BMI	IG	CG
		n	*N*	*y*			
Chee et al (14) [Malaysia (3 °7′ N)]	Postmenopausal women	91	82	59 ± 3[3]	IG: 23.6 ± 3.4 CG: 24.1 ± 3.7	Skim milk powder	Usual diet
Daly et al (22) [Australia (37 °47′ S)]	Ambulatory men ≥50 y old	76	73	61.9 ± 7.7	IG: 26.3 ±3.2 CG: 26/5 ± 3.0	Fortified milk	Usual diet
de Jong et al (24) [Netherlands (51 °58′ N)]	Elderly persons	37	34	78.8	IG: 24.3 ± 2.3 CG: 24.1 ± 3.2	2 Nutrient-dense fruit- and dairy-based products	Regular products
Johnson et al (20) [USA (45 °25′ N)]	Persons ≥60 y old	33	CG1: 34 CG2: 33	NR	NR	Fortified cheese	CG1: Unfortified cheese CG2: No cheese
Keane et al (15) [Ireland (53 °22′ N)]	Elderly persons	18	24	78.1	NR	Fortified milk	Unfortified milk
Lau et al (16) [China (22 °17′ N)]	Postmenopausal women	95	90	56.9	NR	Milk powder	No intervention
McKenna et al (17) [Ireland (53 °22′ N)]	Younger adults	52	50	22.6 (17–54)[6]	NR	Fortified skim milk	Unfortified skim milk
Natri et al (23) [Finland (60 °10′ N)]	Women 25–45 y old	IG1:11 IG2:10	CG1: 9 CG2: 11	29.1	IG1: 22.3 ± 1.4 IG2: 23.6 ± 1.3 CG1: 22.1 ± 0.6 CG2: 23.1 ± 0.8	IG1: Fortified wheat bread IG2: Fortified rye bread	CG1: Regular wheat bread and 10 μg vitamin D supplement CG2: Regular wheat bread
Tangpricha et al (19) [USA (42 °22′ N)]	Persons 19–60 y old	14	12	29.0 ± 9.0	NR	Fortified orange juice	Unfortified orange juice

Characteristics and result of included trials on vitamin D fortified foods and serum 25-hydroxyvitamin D concentrations

Dietary source (continued)		Absolute mean change in serum 25(OH)D					
Daily intake from fortified food (vitamin D/calcium)	Duration	IG	CG	Assay	Fasting sample	Season of sample	Jadad score[2]
μg/g		*nmol/L*	*nmol/L*				
IG: 10/1.2	24 mo	17.3 ± 13.3	2.8 ± 13.1[4]	RIA	Y	NR	2
IG: 20/1.0	24 mo	4.2 ± 20.0	14.4 ± 20.3	RIA	Y	NR	3
IG: 10/NR	4 mo	35 ± 18	5 ± 9	CPBA	Y	NR	2
IG: 15/NR	2 mo	−6.0 ± 11.49	CG1: 3.5 ± 7.29 CG2: 0.75 ± 10.05[5]	RIA	Y	Winter	4
IG: 5/0.8 CG: 0.1/0.6	12 mo	22.25 ± 10.90	6.75 ± 10.92[5]	CPBA	NR	Late winter	4
IG: 6/0.8	24 mo	23.2 ± 13.2[4]	Not estimable	CPBA	NR	NR	3
IG: 3.4/0.44 CG: 0.9/0.36	5 mo	15 ± 21.1	31.0 ± 24.2[4]	RIA	NR	Late winter (baseline) and summer (end of study)	2
IG1: 10/NR IG2: 10/NR	3 wk	IG1: 16.3 ± 21.89 IG2: 14.9 ± 19.61	CG1: 19.5 ± 30.3 CG2: −0.3 ± 13.27[5]	RIA	Y	February–March	1
IG: 25/0.35	3 mo	57.0 ± 26.19	22.3 ± 17.32[5]	CPBA	NR	Spring	4

Efficacy of food fortification on serum 25-hydroxyvitamin D concentrations: a systematic review

O'Donell et al. AJCN 2008

- The individual treatment effects ranged from 14.5 (95% CIs: 10.6, 18.4) nmol/L to 34.5 (95% CIs:17.64, 1.36) nmol/L.
- The lower the baseline D level, the more the increment

Effect of fortification in reducing bone loss

- Studies from Asia have demonstrated the safety and efficacy of milk fortification with vitamin D
- However the powdered milk was fortified to a higher level than that in the US and Canada [i.e. 10 µg/day] }]k vitamin D and 1200 mg/d

Chee WSS, Suriah AR, Chan SP, Zaitun Y, Chan YM. The effect of milk supplementation on bone mineral density in postmenopausal Chinese women in Malasia. Osteoporos Int 2003;14:828–34.

Lau EMC, Lynn H, Chan YH, Woo J. Milk supplementation prevents loss in postmenopausal Chinese women over 3 years. Bone 2002;32: 536–40.

Fortification studies in schoolgirls- Beijing

2 year :
Group 1: Ca fortified milk
(330 mg Ca)
Group 2: Ca fortified milk + 8 µg (320 IU) Vit D
Group 3: Control

Post supplementation 25(OH)D:
Group 1: 17.9 ± 9 nmol/L
Group 2: 47.6 ± 23.4 nmol/L
Group 3: 19.4 ± 10.2 nmol/L

Effects of school milk intervention on cortical bone accretion in Beijing girls[1]

	Adjusted percentage difference in outcome at 24 mo relative to control group[2]	
	Mean (95% CI)	*p*
Periosteal diameter		
Ca milk group	0.8 (0.2, 1.4)	0.004
CaD milk group	1.3 (0.7, 1.9)	<0.001
Pooled interventions	1.0 (0.6, 1.4)	<0.001
Medullary diameter		
Ca milk group	−7.4 (−10.1, −4.7)	<0.001
CaD milk group	−5.2 (−7.8, −2.6)	<0.001
Pooled interventions	−6.3 (−7.5, −5.1)	<0.001
CCT		
Ca milk group	5.4 (3.2, 7.7)	<0.001
CaD milk group	4.5 (2.5, 6.5)	<0.001
Pooled interventions	4.9 (3.1, 6.7)	<0.001
Length of 2nd metacarpal		
Ca milk group	0.9 (0.5, 1.3)	<0.001
CaD milk group	1.0 (0.6, 1.4)	<0.001
Pooled interventions	0.9 (0.5, 1.3)	<0.001

Fraser, 2006

Effect of fortification on reducing fracture rates

- Fortification effective in preventing rickets; eradicating bone disease in primary hyperparathyroidism
- Reduction of fracture rates in elderly possibly requires *supplementation at much higher levels [800-1200 IU/day or 30-40 mcg/day]* compared to that achieved with fortification alone

Vitamin D fortification in the United States and Canada: current status and data needs[1–4]

Mona S Calvo, Susan J Whiting, and Curtis N Barton

Vitamin D Fortification: Planning

- Safety limit
- Technological limit
- Cost limit

A Randomized Double Blind Controlled Trial to Investigate the Effects of Vitamin D fortified milk on Serum Vitamin D levels in school children, aged 10-14 years

Maj Gen (Retd) Raman Kr Marwaha, Dr Rajesh Khadgawat, Col M K Garg, Dr Nandita Gupta, Dr Neena Mehan, Rekha Ramot, Avneet Kaur

Objectives

- **Primary objective of the study:**

To investigate the impact of Vitamin D fortified milk on serum 25(OH) D levels.

- **Primary outcome measures:**

Changes in vitamin D status as measured by serum 25 (OH)D levels.

Study Design: A double blind randomized controlled trial

Inclusion – apparently healthy school children from three schools of Delhi

Exclusion

- Subjects with past/current history of renal stones
- Any known systemic, endocrine or metabolic disorder
- Subjects receiving any medications known to interact with vitamin D metabolism (steroids, thiazide diuretics, phenytoin, phenobarbitone, and antitubercular drugs)
- Subjects taking or had taken vitamin D supplementation in last six months
- Additional exclusion criteria - subjects with hypercalcemia, hypercalciuria or S.25(OH)D level more than 100 ng/ml

- **Hypercalcemia -** serum calcium values exceeding age and sex specific 95th centile values for Indian children

Marwaha RK, Khadgawat R et al Clin Biochem 2010;43:1216-9

- **Hypercalciuria** - spot urinary calcium:creatinine ratio >0.21

Metz MP Ann Clin Biochem 2006; 43:398-401

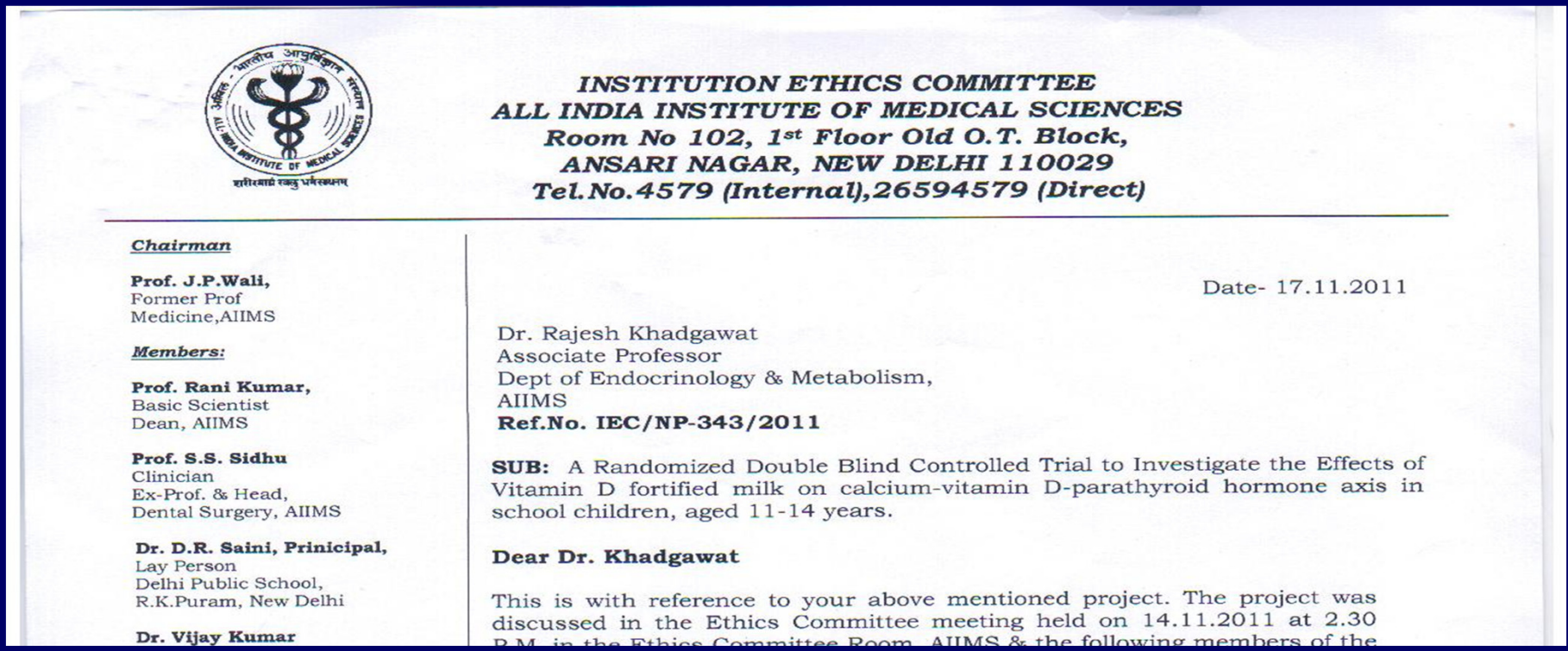
Planned Intervention

- **Group A** – no fortification, control group, received 200 ml of unfortified milk daily for 12 weeks
- **Group B** – 200 ml of milk fortified with 600 IU of vitamin D daily for 12 weeks
- **Group C** - 200 ml of milk fortified with 1000 IU of vitamin D daily for 12 weeks

Randomized by computer generated block randomization plan

Ethics Permission

- Ethics permission - Ethics committee, AIIMS
- Study subjects - consent of the school authorities, parents/guardians and verbal assent from children
- **Clinical Trial Registration Number** - CTRI/2012/02/002429



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Dept of Endocrinology & Metabolism,
AIIMS
Ref.No. IEC/NP-343/2011

SUB: A Randomized Double Blind Controlled Trial to Investigate the Effects of Vitamin D fortified milk on calcium-vitamin D-parathyroid hormone axis in school children, aged 11-14 years.

Dear Dr. Khadgawat

This is with reference to your above mentioned project. The project was discussed in the Ethics Committee meeting held on 14.11.2011 at 2.30 P.M. in the Ethics Committee Room, AIIMS & the following members of the

Sample Size Calculation

- When the sample size in each group is 169, a 5% level Chi-square test will have 90% power to detect a difference in proportion of subjects with S.25(OH)D levels more than 20 ng/ml after supplementation with fortified milk (1% subjects in group A, at least 5% subjects in group B and 10% subjects in group C will have S.25(OH)D >20 ng/ml).
- Assuming a 35% dropout, we require 260 subjects in each group, thus a total of 780 subjects would be required.
- Sample size was calculated with nQuery Advisor 1.0.

Serum 25 (OH) vitamin D

- S.25(OH)D - DiaSorin 'LIASON' chemiluminescence based immunoassay (DiaSorin Inc, USA).
- Our laboratory - registered with vitamin D external quality assessment scheme (www.deqas.org)

Vitamin D deficiency – Lips criteria ***Lips P Endocr Rev 2001;22:477-501***

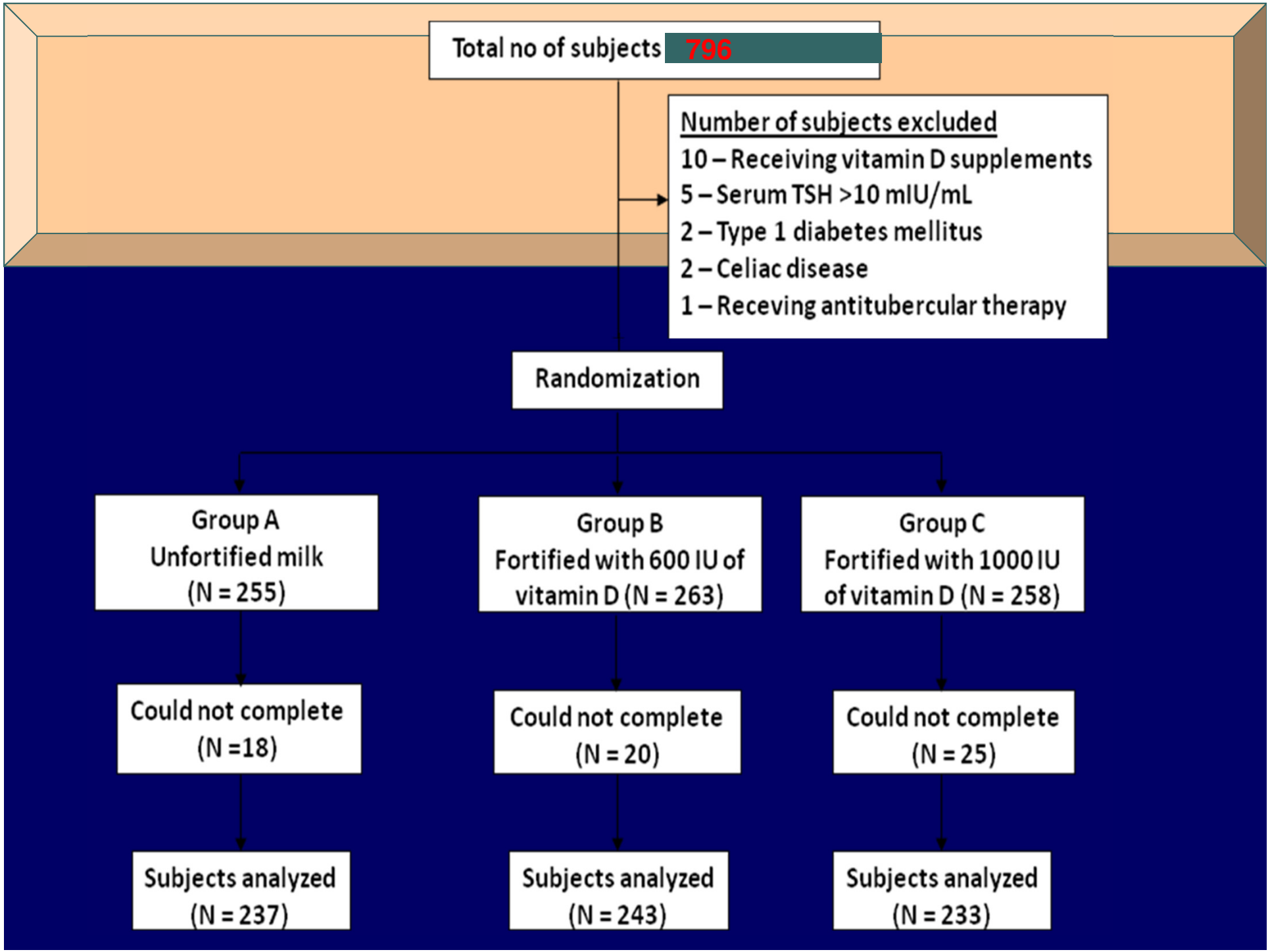
- **Deficiency - less than 20 ng/ml**
  - Severe - <5 ng/ml
  - Moderate 5-<10 ng/ml
  - Mild - 10 -<20 ng/ml

Process of Vitamin D3 Fortification of Milk

- Spray dried and water soluble form of Vitamin D3, best suitable for milk fortification was used.
- Fortified milk (elaichi and strawberry flavours) was manufactured by Gopaljee Dairy, Delhi
- Efficacy of fortification – pre and post fortification evaluation of milk with HPLC –(approx. 10-15%) variation from labeled dose.

Adherence to Intervention

- All subjects received 200 ml milk daily for 12 weeks after morning school prayers under supervision.
- School holidays- calculated additional tetra packs of milk were provided to be taken at home under parent's supervision for all preplanned holidays.
- Absenteeism from school- absenteeism >7days in a month with no milk consumption were excluded from the study


Total no of subjects 796
Number of subjects excluded
10 – Receiving vitamin D supplements
5 – Serum TSH >10 mIU/mL
2 – Type 1 diabetes mellitus
2 – Celiac disease
1 – Receving antitubercular therapy
Randomization
Group A
Unfortified milk
(N = 255)
Group B
Fortified with 600 IU of
vitamin D (N = 263)
Group C
Fortified with 1000 IU
of vitamin D (N = 258)
Could not complete
(N =18)
Could not complete
(N = 20)
Could not complete
(N = 25)
Subjects analyzed
(N = 237)
Subjects analyzed
(N = 243)
Subjects analyzed
(N = 233)

Statistical Analysis

- Descriptive statistics-
  - Mean and SD
- Student's-*t test and* Analysis of variance (ANOVA)
- Multiple linear regression analysis
- p value of <0.05 -statistically significant.
- Analysis – Stata 11.0

STUDY DESIGN

- 713/794 subjects completed the study with mean age of 11.75 ± 1.1 years (300 boys; 413 girls).
-
- Subjects were recruited from three schools.
- No significant difference in number of subjects, age, BMI and pubertal status or sex ratio was observed among three schools.
- All subjects were randomized into three groups –
- Group A (n=237) - non-fortified milk
- Group B (n=243) - milk fortified with 600 IU
- Group C (n=233) - milk fortified with 1000 IU

Vitamin D status at baseline

- Baseline Serum 25(OH) D levels were similar in all the three schools.
- Similarly, no significant differences were noted in anthropometry and other biochemical parameters in the three schools

Baseline parameters of study subjects

Parameter	Control	600 IU Group	1000 IU Group	Total
Age	11.74 ± 1.05	11.75 ± 1.08	11.75 ± 1.14	
BMI	18.94 ± 3.33	18.84 ± 3.66	18.62 ± 3.50	18.80± 3.50
S. calcium	10.2±0.7	10.19±0.7	10.3±0.7	10.2±0.7
S. Phosphate	5.06±0.5	5.1±0.5	5.12±0.5	5.09±0.5
SAP	270±90	267±82	263±87	267±87
Ur.Ca : Cr	0.04±0.05	0.05±0.07	0.05±0.06	0.05±0.05

Results: Characteristics of Subjects at Baseline

Vit D deficiency - 92.3%
- **Severe 8.27%**
- **Moderate 33.24%**
- **Mild 50.77%**

Vitamin D sufficiency (>30ng/ml) 0.7%

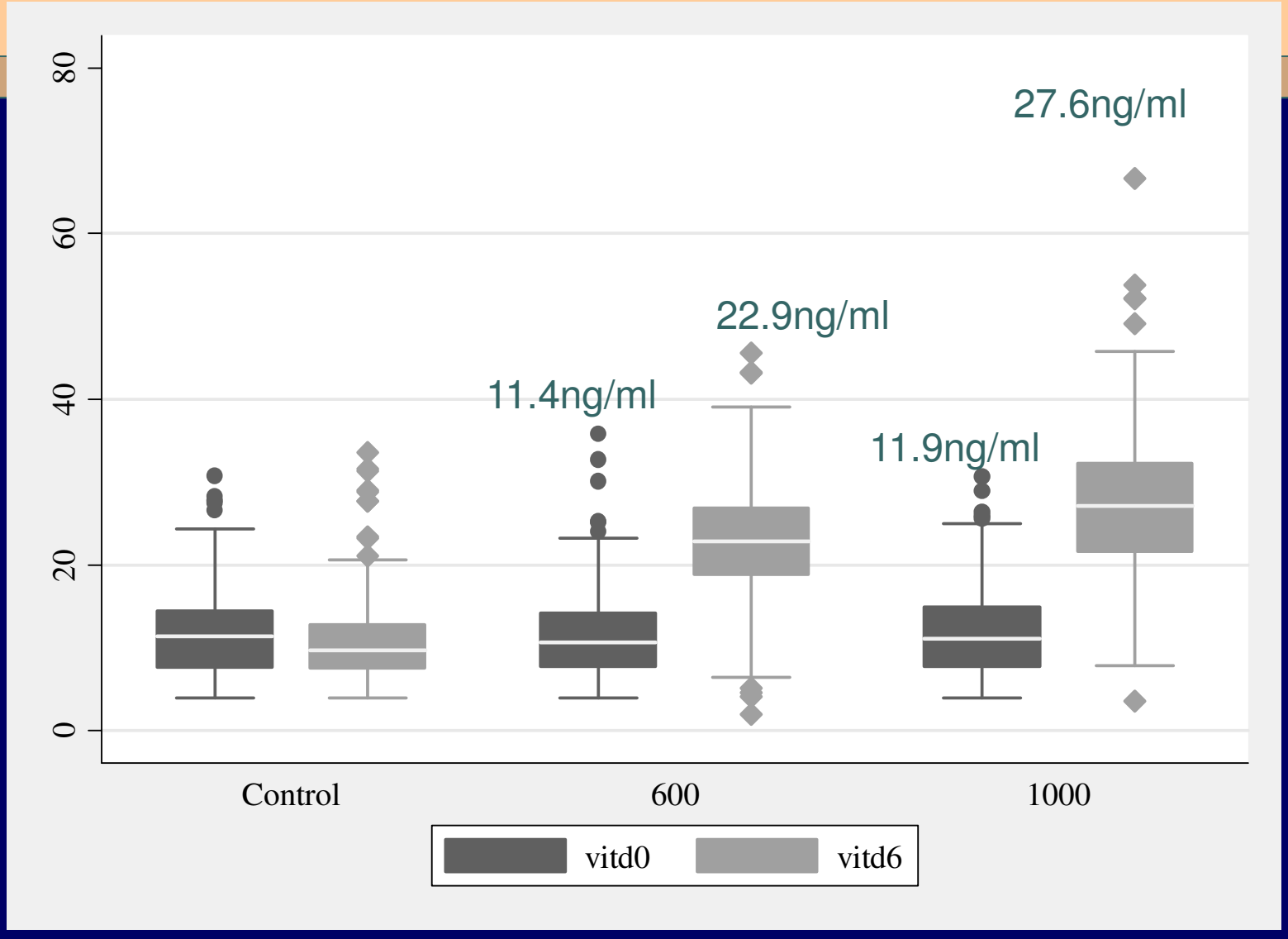
Parameter	Group A (No fortification)	Group B (600 IU)	G (1	
Age (in years)	11.74 ± 1.05	11.75 ± 1.08	11	
BMI (Kg/meter^2)	18.94 ± 3.33	18.84 ± 3.66	18	
Serum calcium (mg/dl)	9.8 ± 0.6	9.7 ± 0.7	9.	
Serum phosphate (mg/dl)	5.06 ± 0.5	5.1 ± 0.5	5.1	± 0.5
Serum alk phosphatase (IU/L)	270 ± 90	267 ± 82	263 ± 87	267 ± 87
Urine calcium creatinine ratio	0.04 ± 0.05	0.05 ± 0.07	0.05 ± 0.06	0.05 ± 0.05
S.25(OH)D (ng/ml)	**11.74 ± 5.2**	**11.4 ± 5.22**	**11.94 ± 5.63**	**11.69 ± 5.36**
S.25(OH)D <5 ng/ml (n/%)	19 (8.01%)	23 (9.46%)	17 (7.29%)	59 (8.27%)
S.25(OH)D 5-<10 ng/ml (n/%)	76 (32.06%)	82 (33.74%)	79 (33.90%)	237 (33.24%)
S.25(OH)D 10-<20 ng/ml (n/%)	**127 (53.58%)**	**126 (51.85%)**	**109 (46.78%)**	**362 (50.77%)**
S.25(OH)D 20-30 ng/ml (n/%)	14 (5.90%)	9 (3.7%)	27 (11.58%)	50 (7.01%)
S.25(OH)D >30 ng/ml (n/%)	1 (0.42%)	3 (1.23%)	1 (0.42%)	5 (0.7%)

Baseline Serum Vitamin D status of study subjects

Parameter	Control	600 IU Group	1000 IU Group	Total
S. 25 (OH) D(ng)	**11.74 ± 5.2**	**11.4 ± 5.2**	**11.94±5.6**	**11.69±5.3**
<5 (%)	8.02%	9.43%	7.3%	8.26%
5-10 (%)	32.07%	34.02%	33.91%	33.33%
10-20 (%)	53.59%	51.64%	46.78%	50.7%
20-30 (%)	5.91%	3.69%	11.59%	7.0%
>30 (%)	0.42%	1.23%	0.43%	0.7%

S.25 (OH) vitamin D – ng/ml

The mean improvement in serum vitamin D level after fortification



Vitamin D status after fortification

S. 25 Vitamin D levels >20 ng/mL

- Control group - 5.9%
- Group B (600IU/D) - 69.95%
- Group C (1000 IU/D) - 81.11%

S. 25 vitamin D sufficiency (>30 ng/mL)

- Control group – 1.26%
- Group B (600IU/D) – 12.34%
- Group C (1000 IU/D) – 36.05%

The mean % change in serum vitamin D level

- Control group - 5.25% (SE 4.42; CI 3.47-13.97%)
- Group B (600IU/D) + 137.97% (SE 7.96; CI 122.28-153.65%)
- Group C (1000 IU/D) +177.29% (SE 9.79; CI 158-196.58%).

Percentage increase in Serum 25 (OH)D in supplementation groups (B+C)

S. 25(OH)D	Mean	SD
< 5	429.11 %	198.2
5-10	209.38 %	106.9
10-20	95.75 %	58.2
20-30	43.76 %	36.4
>= 30	-29.07 %	40.4
Total	157.22 %	
	138.3	

The % increase in serum 25(OH)D levels following fortification is

Improvement in Serum vitamin D level after fortification

	Group A (Control)		Group B (600 IU/D)		Group C (1000 IU/D)	
	Before	After	Before	After	Before	After
< 5	8.01%	6.75%	9.46%	1.23%	7.29%	0.42%
5-10	32.06%	46.83%	33.74%	2.05%	33.90%	0.84%
10-20	53.58%	40.5%	51.85%	26.74%	54.50%	17.59%
20-30	5.90%	4.64%	3.70%	57.61%	11.58%	45.06%
≥30	0.42%	1.26%	1.23%	12.34%	0.42%	36.05%

Vitamin D improvement in pre and post pubertal children

- There was no significant difference in vitamin D improvement in pre and post pubertal children

Safety of intervention

- Not a single case of hypercalcemia or hypercalciuria as evaluated by serum calcium and Uca/cr ratio was observed in the study.
- No other adverse outcome was observed

Strength of the study

- A large cohort with only 10% drop out rate
- No adverse effects were reported
- First clinical trial in Indian children and possibly second in the world
- Pubertal status was evaluated

Weakness of the study

- Duration of trial was short (3months).
- PTH assays could not be done at baseline and following fortification.

Conclusion

We conclude that supplementing milk fortified with vitamin D to children is an effective and safe method of addressing the major public health issue of vitamin D deficiency in children.

Efficacy of vitamin D loading doses on serum 25-hydroxy vitamin D levels in school going adolescents: an open label non-randomized prospective trial

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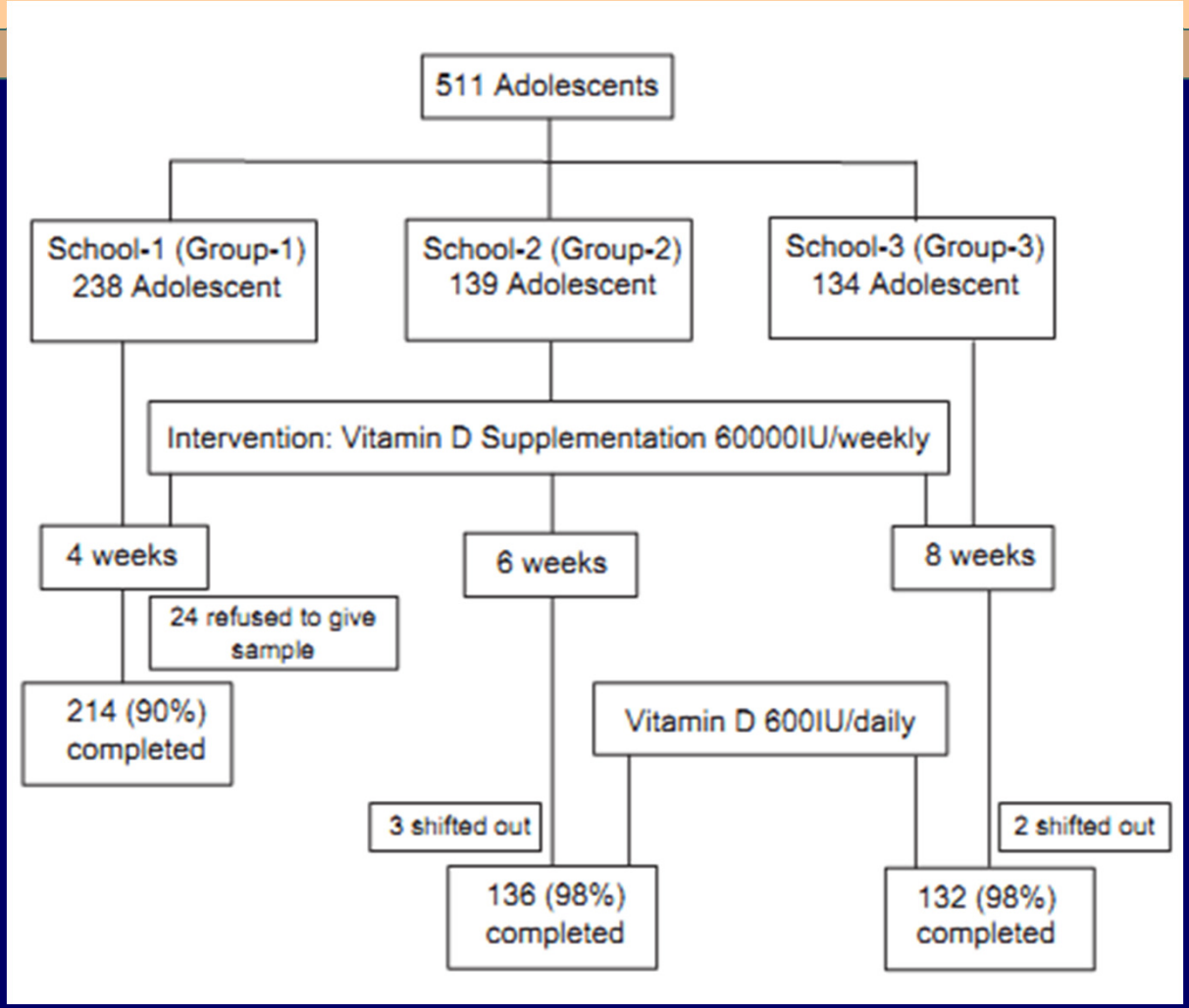
STUDY DESIGN



GROUP-1

Parameters	Baseline	After 4 weeks	p-Value
S. Calcium, mmol/L	2.41±0.12 (2.39–2.43)	2.45±0.19 (2.43–2.48)	0.006
S. Phosphate, mmol/L	1.59±0.17 (1.57–1.61)	1.61±0.27 (1.58–1.65)	0.165
S. ALP, U/L	262±70 (214–246)	229±79 (218–239)	<0.0001
S. 25OHD levels, ng/ml	8.7±4.5 (8.2–9.4)	49.8±14.5 (16.7–51.7)	<0.0001
S. 25OHD levels, nmol/L	21.8±11.3 (20.2–23.2)	152.6±36.3 119.3–129.1)	
25OHD >20 ng/ml (>50 nmol/L)	5 (2.3%)	211 (98.6%)	<0.0001
25OHD >30 ng/ml (>75 nmol/L)	1 (0.5%)	196 (91.6%)	<0.0001

- Serum calcium and 25OHD levels showed significant increase, in contrast to significant decline in serum ALP from baseline with no notable change in serum phosphate levels.

- The increase in serum levels of 25OHD was 554%±268% (95%CI 517-590%, range 169-1623) from the baseline.

Group-2

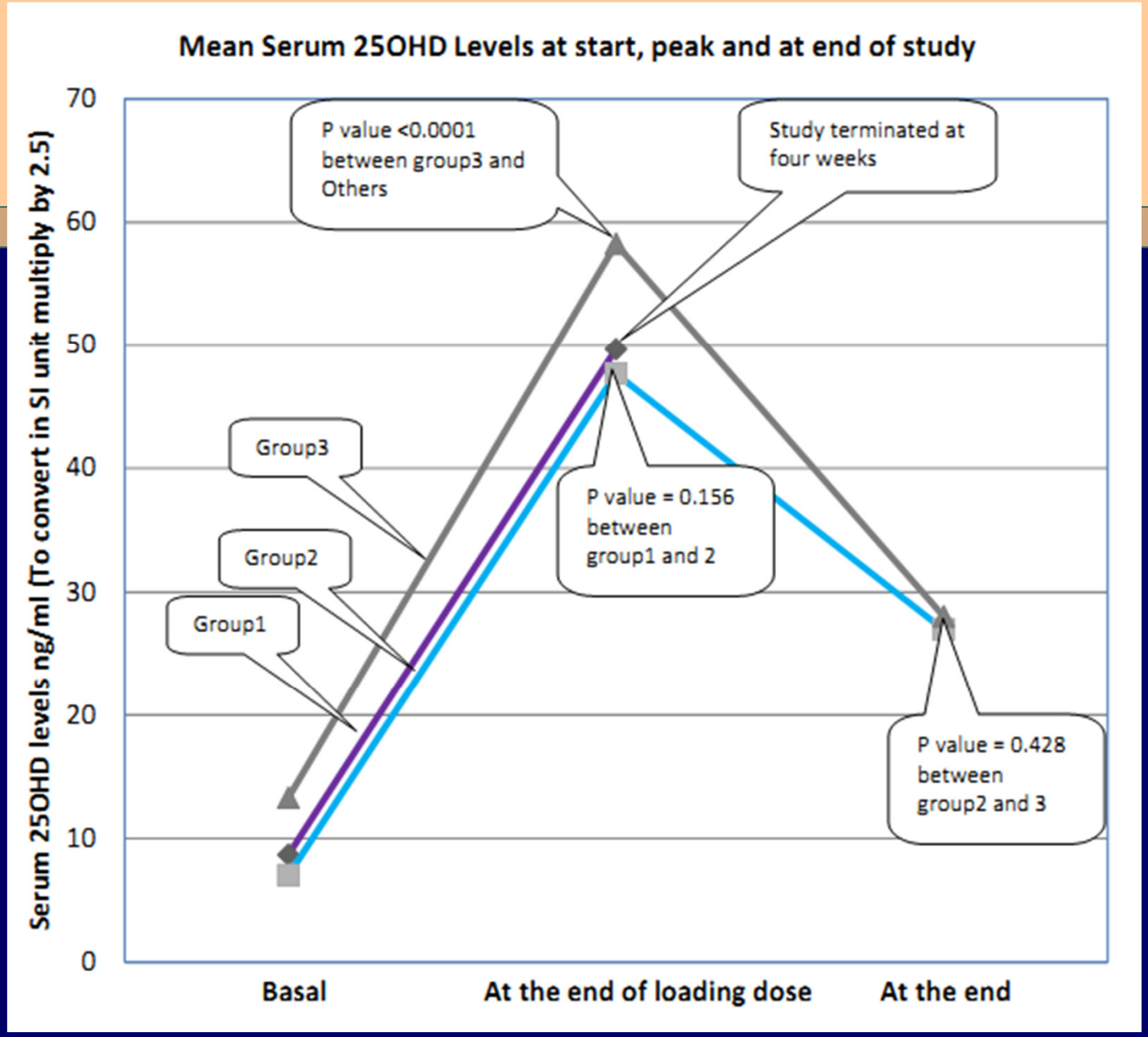
Parameters	Baseline	After 6 weeks	At end of study
S. Calcium, mmol/L	2.53±0.12 (2.51–2.55)	2.6±0.15 (2.57–2.63)	2.51±0.17 (2.44–2.58)
p-Value		<0.0001	<0.0001
S. Phosphate, mmol/L	1.65±0.19 (1.62–1.68)	1.75±0.23 (1.71–1.80)	1.68±0.26 (1.63–1.73)
p-Value		<0.0001	0.003
S. ALP, U/L	431±124 (410–452)	275±92 (258–291)	248±83 (233–263)
p-Value		<0.0001	<0.0001
U Ca:Cr ratio	0.06±0.05	0.07±0.06	0.07±0.06
p-Value		0.68	0.99
S. 25OHD, ng/mL	7.0±3.0 (6.5–7.5)	47.7±11.0 (23.7–83.9)	27.0±6.6 (25.8–29.1)
S. 25OHD, nmol/L	17.5±7.5 (16.3–18.9)	119.3±27.5 (114.5–123.8)	67.5±16.5 (67.2–65.6)
p-Value		<0.0001	<0.0001
25OHD >20 ng/mL (>50 nmol/L)	1 (0.7%)	136 (100.0%)	114 (83.8%)
p-Value		<0.0001	<0.0001
25OHD >30 ng/mL (>75 nmol/L)	0	129 (94.9%)	32 (23.5%)
p-Value		<0.0001	<0.0001

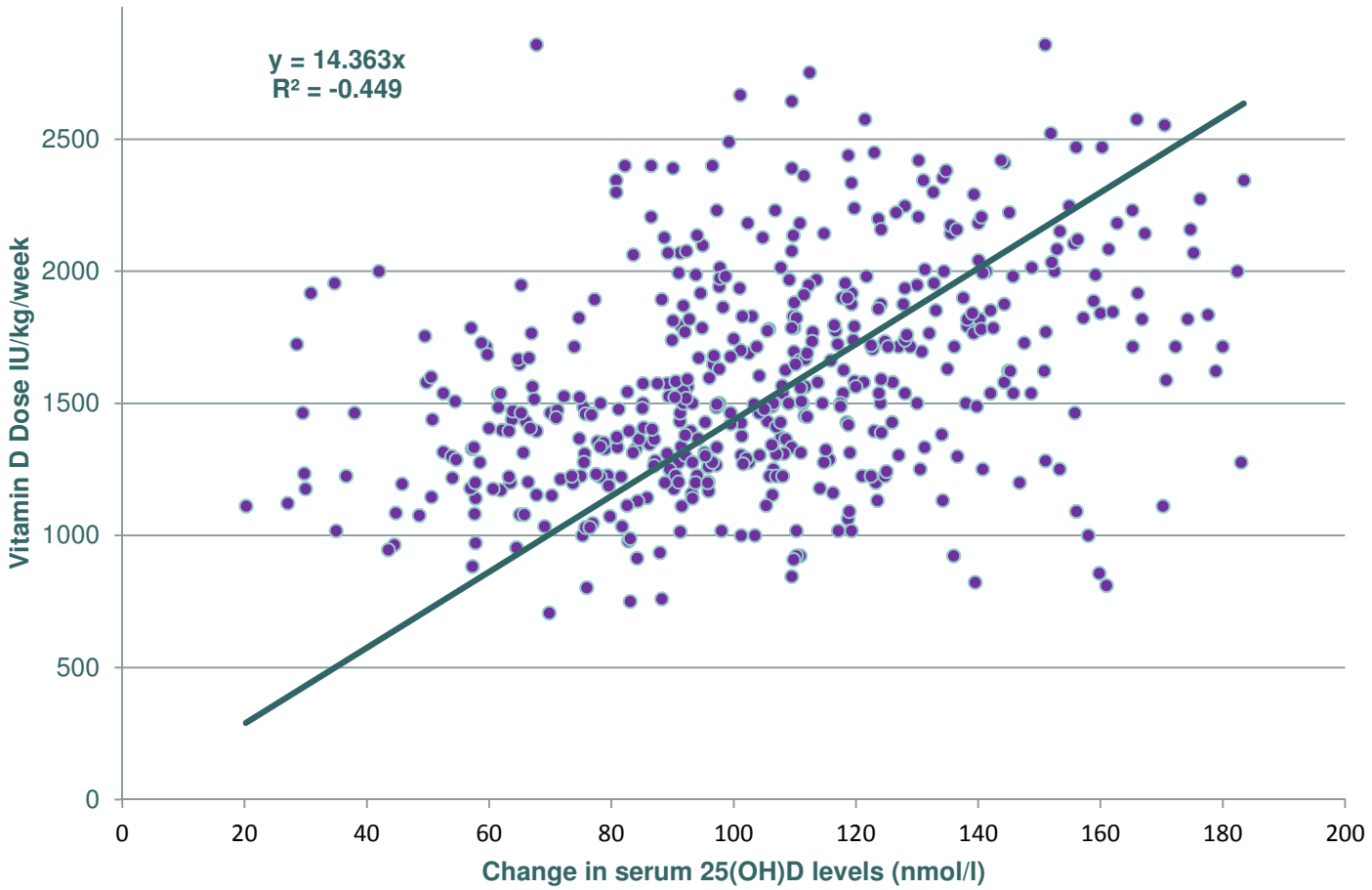
- Serum calcium, phosphates and 25OHD levels increased, whereas serum A decreased significantly after 6 weeks of supplementation. These parameters decrease significantly after 12 weeks of maintenance therapy
- The increase in serum levels of 25OHD was 672± 308% (95%CI 620-724, range 123-1579) from the baseline.

Group-3

Parameters	Baseline	After 8 weeks	At end of study
S. Calcium, mmol/L	2.40±0.19 (2.36–2.42)	2.65±0.06 (2.64–2.66)	2.56±0.11 (2.54–2.58)
p-Value		<0.0001	<0.0001
S. Phosphate, mmol/L	1.55±0.20 (1.51–1.58)	1.61±0.19 (1.57–1.64)	1.45±0.22 (1.41–1.49)
p-Value		0.006	0.001
S. ALP, U/L	228±89 (214–248)	230±84 (233–263)	178±90
p-Value		0.479	<0.0001
U Ca:Cr ratio	0.04±0.05	0.11±0.12	0.07±0.06
p-Value		0.001	0.001
S. 25OHD, ng/mL	13.0±5.7 (12.3–14.3)	58.2±14.3 (55.8–60.7)	28.0±8.7 (26.5–29.6)
S. 25OHD, nmol/L	32.5±14.3 (30.8–35.7)	145.5±35.8 (139.5–151.8)	70.0±21.8 (61.2–70.4)
p-Value		<0.0001	<0.0001
25OHD >20 ng/mL (>50 nmol/L)	19 (14.4%)	132 (100.0%)	113 (85.6%)
p-Value		<0.0001	<0.0001
25OHD >30 ng/mL (>75 nmol/L)	3 (2.3%)	130 (98.5%)	34 (25.8%)
p-Value		<0.0001	<0.0001

- Serum calcium, phosphates and 25OHD levels increased significantly, whereas serum ALP showed no change after 8 weeks of supplementation. These parameters decreased significantly after 12 weeks of maintenance therapy
- The increase in serum levels of 25OHD was 424± 276% ((95%CI 377-472%, range 126-1497) from the baseline.


Mean Serum 25OHD Levels at start, peak and at end of study
Serum 25OHD levels ng/ml (To convert in SI unit multiply by 2.5)
70
60
50
40
30
20
10
0
P value <0.0001 between group3 and Others
Study terminated at four weeks
Group3
Group2
Group1
P value = 0.156 between group1 and 2
P value = 0.428 between group2 and 3
Basal
At the end of loading dose
At the end



Dose to achieve 75 nmol/L = 15 (75-serum 25OHD in nmol/L)
Dose to achieve 30 ng/ml = 36 (30 – serum 25OHD in ng/ml)

Correlation of change of 25OHD levels with various parameters

Parameters	Correlation Coefficient	P-value
Age	-0.033	0.472
BMI	-0.274	<0.0001
Sex	0.247	0.329
Serum Calcium	-0.030	0.545
Serum Phosphates	-0.015	0.747
Serum ALP	-0.107	0.019
Serum 25OHD	-0.048	0.289
Duration	0.112	0.014
Dose IU/kg/week	0.399	<0.0001

Mean change in serum 25OHD was negatively correlated with BMI, ALP and positively correlated vitamin D dose IU/kg/week.

CONCLUSIONS

- Loading dose of 60,000 IU of vitamin D3/wk in granular form with plain milk for 4-8 weeks is an effective and safe strategy of achieving vitamin D sufficiency (>75nmol/L) in apparently healthy school adolescents aged 10-15 years.
- currently recommended maintenance dose of 600 IU of D3/day keeps the serum 25OHD levels above 50 nmol/L (20ng/ml), but is inadequate to maintain above 75nmol/L (30ng/ml).

ACKNOWDLEGEMENTS

- School management, Principals, teachers, parents and students for making this study possible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- Dr Rajesh Khadgawat- Associate professor, Department of Endocrinologyand metabolism, AIIMS
- Col MK Garg- Advisor medicine & Endocrinology, Department of Endocrinology R&R Army Hospital.
- Dr Nandita Gupta- Professor, Dept of endocrinology and metabolism, AIIMS

Thank You.....

INTRODUCTION

- Current Endocrine Society treatment guidelines suggest treatment with 2000 IU/d of vitamin D for at least 6 weeks or 50,000 IU of vitamin D once a week for at least 6 weeks to achieve a blood level of 25-hydroxy vitamin D (25OHD) above 75nmol/L (30ng/ml), followed by maintenance therapy of 600-1000 IU/day in children 1-18 years of age
- However, data in this population is sparse
- This study was planned to evaluate the efficacy of loading doses of 60000IU/week of vitamin D3 with 200 ml of unfortified milk for a period of 4, 6 and 8 weeks, followed by maintenance dose of 600 IU/day through 200 ml of fortified milk, in terms of change in serum 25OHD levels.

Vitamin D3 fortification of milk

- We used Vitamin D3 100 SD/S form for these trials.
- Spray dried and water soluble form of Vitamin D3, best suitable for milk fortification was used.
- **We calculated the quantity of Vitamin D3 100 SD/S per batch as follows:**
  - Overages @ 60% added to compensate for the shelf life losses. E.g. for 600 IU packs, actual quantity added is 1060 IU.
  - Calculated quantity of Vitamin D3 100 SD/S was weighed and added to a 2 litres of processed milk along with a small quantity sugar from the batch. A low speed stirrer was used for mixing the ingredients.
  - This 2 litre milk is added to the batch tank containing processed milk and was kept under continuous stirring for 15-20 minutes to ensure homogenous mixing.
  - The fortified flavored milk is then fed to the packaging station for packing into 200 ml packs.