

Beneficial Effect of Calcium Supplementation on Bone Mineral Density of Calcium Deficient Adolescents

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Abstract: The supplementation of calcium during growth years may help in bone formation. Insufficient intake of calcium does not produce obvious symptoms since the body maintains calcium levels in the blood by taking it from bones. Therefore, the present study was undertaken with an objective to assess the beneficial effect of calcium supplementation on the bone mineral density of calcium deficient adolescents. The feeding trial was performed on select set of adolescent students - 48 female and 65 male subjects. These selected subjects for experimental feeding trial were sub-divided into three groups, comprising one control and two supplemental fed groups COS (Calcium organic supplement), CIS (Calcium inorganic supplement). Statistical analysis of T-Score before and after feeding trial indicated, that for males, a significant difference was observed in calcium organic supplement ($p=0.0006$), while, no significant difference was observed for male's control and inorganic calcium supplement. Female groups indicated significant difference for OCS ($p=0.0137$) and ICS ($p=0.0084$) while no significant difference was observed for control. In conclusion, significant increase in Bone mineral density were observed among male and female subgroups after intervention. Also, Organic supplements had a greater effect on BMD.

Keywords: Adolescence, Bone Mineral Density, Calcium Deficiency, p-DEXA, Osteopenia

1. Introduction

Calcium is a major element in bones. The bones need plenty of calcium throughout childhood and adolescence to reach their peak strength and calcium contents by about age of 30 year. After that, bones slowly lose calcium. Less intake of calcium severely affects the bones and results in poor bone density. This can be quite a serious condition for children and adolescents as it may lead to easy fractures, muscular aches, and spasms, and in extreme cases rickets. Hence, adequate dietary calcium intake is necessary for infants, children and adolescents in order to is necessary for the attainment of peak bone mass to reduce risk of fractures and osteoporosis in the later life. The supplementation of calcium at this stage may help in bone formation. Insufficient intake of calcium does not produce obvious symptoms since the body maintains calcium levels in the blood by taking it from bones. However, over the long term, inadequate intake of calcium (below recommended levels) produces serious health consequences, such as causing low bone mass (osteopenia) and increasing the risks of osteoporosis including bone fractures.

Therefore, the present study was undertaken with an objective to assess the Beneficial effect of calcium supplementation on the bone mineral density of calcium deficient adolescents.

2. Methods and Materials

2.1. Selection of Subjects

A total of 706 adolescent students, 468 males and 238 females, aged 11-16 years were randomly selected from 8th, 9th and 10th standards of different government schools of District Kurukshetra. Separate groups of male and female were formed, both of which were sub classified on the basis of age into two groups - group I (11 to 13 years) and group II (14 to 16 years). Female group I comprised of 97 subjects while Female group II comprised of 141 subjects. Similarly, Male group I comprised of 116 subjects while Male group II comprised of 352 subjects. Calcium intake and anthropometric measurements were done on these 706 subjects. BMD was applied on 409 subjects (those have low

dietary calcium intake). Male group I had 72 and group II 239 subjects, whereas, female group-I and group II had 48 and 50 subject respectively. Among the 409 adolescent students on whom bone mineral density assessment was performed, 42 female and 72 male students were found to be osteopenic while 19 female and 54 male subjects were found to be on borderline. Both borderline and osteopenic subjects were considered for experimental trial. 48 female subjects and 65 male subjects volunteered for blood test and for participation in the experimental feeding trial. The feeding trial was performed on these select set of adolescent students. 48 female and 65 male subjects selected for experimental feeding trial were sub-divided into three groups, comprising one control and two supplemental fed groups COS (Calcium organic supplement), CIS (Calcium inorganic supplement). Distribution of subjects are given in Table 1.

Table 1. Distribution of subjects into sub groups, control, COS (Calcium organic supplement), CIS (Calcium inorganic supplement).

	Male	Female
Control	15	15
COS	22	15
CIS	28	18

Supplements of calcium were given to the subjects of group II and III for a continuous duration of 60 days at the time of recess in schools.

Supplementation: Calcium (inorganic and organic) supplements were given to subjects of group II and III. Supplements were given for the period of 60 days on a daily basis. The inorganic form of calcium supplement was CCM (calcium citrate malate) tablet. It was purchased from the local medical store of Kurukshetra city and given to the subjects of group II. It was given to the subjects of group II over and under the supervision of expert doctors. It contains 250 mg of calcium citrate malate salt. Organic form of calcium supplements was given to the subjects of group III. Calcium rich foods-sesame seeds and mawa were incorporated in til mawa laddoo. One laddoo was given to each subject of group III on a daily basis for 60 days.

2.2. Anthropometric Measurements

Weight measurements were measured for all subjects. Weight was measured in kilograms using methods of Jelliffe [1] and ICMR [2]. These anthropometric measurements were compared with ICMR, NCHS and other values which helped determine growth performance, prevalence and incidence of degree of malnutrition among the adolescent's (both males and females). BMI of these adolescents was also assessed following the classification given by James *et al* [4].

2.3. Bone Mineral Density (BMD) Measurements

The Bone mineral density of subjects was determined using p-DEXA (peripheral dual-energy x-ray absorptiometry) method and measured by Ultrasound Bone Densitometer CM-200. For this measurement, the X-rays were applied on the right leg's heel (calcaneus) bone. Bone Mineral Density,

expressed in grams per centimetre square and T score, was recorded for each of the subjects among all groups before and after the intervention trail.

2.4. Statistical Analysis

Mean and standard deviation were calculated for bone mineral density separately for each group of males and females. Anova was calculated among all the groups and t-test was calculated between each group before and after the intervention trial. The statistical significance was defined as $p < 0.01$ and 0.05 .

3. Results and Discussion

Weight: Weight of subjects is shown in Table 1. Statistically non-significant variation in weight of each subgroups of male and female subjects (OCS and ICS) was noticed with their respective control subgroup as well as on comparing before and after values of male and female OCS and ICS subgroups after completion of intervention trial. In male and female subgroups, the highest per cent increase in weight i.e. 1.40 per cent, was recorded in the subjects of male OCS followed by 1.11 per cent in female ICS and 0.70 per cent in both male ICS and female OCS. Following study disclosed the similar outcome. Rozen *et al* [5] testified no significant changes in anthropometric variables (weight) was observed during the study period within and between the inorganic CS and placebo groups. One more study by Dibba *et al* [6] showed that there are no significant effects of the calcium carbonate supplements on weight. Cadogan *et al* [7] reported that no significant differences in increments in weight was observed between the control and milk supplemented groups.

Table 2. Mean±Standard Deviation and t value of weight of selected subjects before and after feeding trial.

Weight (kg)		Group I (Control)	Group II (COS)	Group III (CIS)
Male	Subjects	15	22	28
	Before	43.33±6.62	35.07±4.55	42.71±9.51
	After	43.35±6.66	35.56±4.6	43.01±8.59
	Mean change	0.02±0.07	0.49±0.13	0.3±0.08
	% change	0.05%	1.40%	0.70%
	t-value	-5.551	-2.383	-4.236
	P(T<=t) one-tail	0.057	0.148	0.438
Female	Subjects	15	15	18
	Before	36.67±5.09	37±2.56	33.4±3.24
	After	36.7±5.12	37.26±2.58	33.77±3.38
	Mean change	0.03±0.05	0.26±0.33	0.37±0.48
	% change	0.08%	0.70%	1.11%
	t-value	-3.010	-2.383	-4.236
	P(T<=t) one-tail	0.394	0.215	0.085

** : The mean difference is significant at 0.01 level.

* : The mean difference is significant at 0.05 level.

Group-I: Control

Group-II: Organic calcium supplement

Group-III: Inorganic calcium supplement

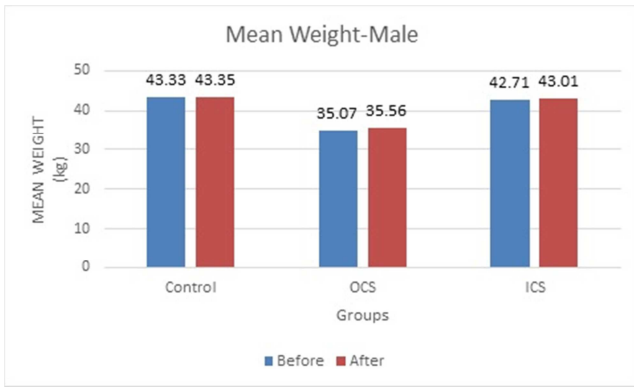


Figure 1. Mean±SD for weight of the selected male subjects before and after feeding trial.

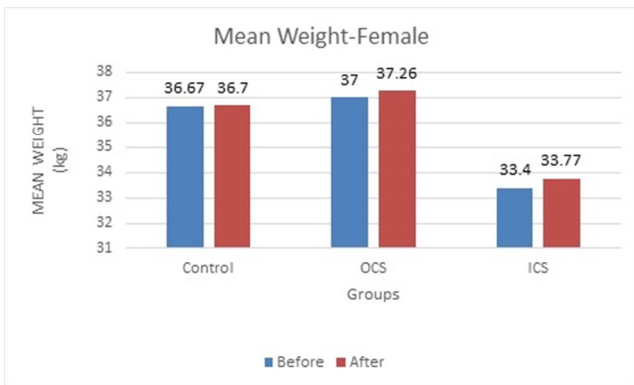


Figure 2. Mean±SD for weight of the selected female subjects before and after feeding trial.

Bone mineral density: Before commencing the dietetic intervention trial with male subgroups (control, Organic Calcium supplement and Inorganic Calcium supplement) the mean bone mineral density (with T-Score in Table 2) of subjects were 0.522 ± 0.03 (-0.64 ± 0.21), 0.455 ± 0.02 (-1.16 ± 1.19) and 0.459 ± 0.02 (-1.22 ± 0.14) respectively, whereas, with female subgroups (control, OCS and ICS) the mean bone mineral density (with T-Score) of subjects were 0.535 ± 0.05 (-0.48 ± 0.29), 0.448 ± 0.01 (-1.18 ± 0.15) and 0.450 ± 0.02 (-1.18 ± 0.09) respectively. After sixty days dietetic intervention trial with male groups (control, OCS and ICS) the mean bone mineral density (with T-Score) of subjects were 0.531 ± 0.05 (-0.61 ± 0.18), 0.523 ± 0.04 (-0.53 ± 0.29) and 0.517 ± 0.15 (-0.88 ± 0.39) respectively, whereas, the mean bone mineral density (with T-Score) of corresponding female subgroups was 0.537 ± 0.04 (-0.47 ± 0.16), 0.535 ± 0.05 (-0.48 ± 0.8) and 0.496 ± 0.04 (-0.52 ± 0.6) gm/cm^2 respectively. The respective percentage increase in mean BMD (with T-Score) of male subjects in control, OCS and ICS were 1.72 (4.68%), 14.94 (54.31%) and 12.64 (27.86%) per cent, while, the percentage increase in mean BMD (with T-Score) of female subjects in control, OCS and ICS were 0.37 (2.08%), 19.41 (59.32%) and 10.22 (55.93%) per cent respectively.

Efficacy of the intervention for increasing T-Score value was found maximum in female subgroup OCS (59.32%) and minimum in male subgroup ICS (27.86%) followed by female subgroup CIS (55.93%) and male subgroup OCS (54.31%) respectively.

Table 3. Mean±Standard Deviation and t-value for Bone mineral density (BMD) and T-Score of selected subjects before and after feeding trial.

Gender		Group I (Control)		Group II (OCS)		Group III (ICS)	
		BMD (gm/cm^2)	T-Score	BMD (gm/cm^2)	T-Score	BMD (gm/cm^2)	T-Score
Males	Subject	15		22		28	
	Before	0.522 ± 0.03	-0.61 ± 0.18	0.455 ± 0.02	-1.16 ± 1.19	0.459 ± 0.02	-1.22 ± 0.14
	After	0.531 ± 0.05	-0.64 ± 0.21	0.523 ± 0.04	-0.53 ± 0.29	0.517 ± 0.15	-0.88 ± 0.39
	Mean change	0.009 ± 0.064	0.03 ± 0.12	0.068 ± 0.12	0.64 ± 0.40	0.058 ± 0.049	0.34 ± 0.83
	Percent change	1.72%	4.68%	14.94%	54.31%	12.64%	27.86%
	t-value	-0.34	-0.60302	-4.44	-5.02312	-2.902	-1.7153
	P(T<=t) one-tail	0.3727	0.284286	0.0006**	0.00026**	0.099	0.062315
Female	Subject	15		15		18	
	Before	0.535 ± 0.05	-0.48 ± 0.29	0.448 ± 0.01	-1.18 ± 0.15	0.450 ± 0.02	-1.18 ± 0.09
	After	0.537 ± 0.04	-0.47 ± 0.16	0.535 ± 0.05	-0.48 ± 0.8	0.496 ± 0.04	-0.52 ± 0.6
	Mean change	0.002 ± 0.016	-0.01 ± 0.27	0.087 ± 0.063	0.7 ± 0.31	0.046 ± 0.049	0.66 ± 0.3
	Percent change	0.37%	2.08%	19.41%	59.32%	10.22%	55.93%
	t-value	-0.36	-0.38623	-3.081	-2.12024	-3.955	-3.30057
	P(T<=t) one-tail	0.3655	0.35633	0.0137*	0.05066	0.0084**	0.01073*

** : The mean difference is significant at 0.01 level.

* : The mean difference is significant at 0.05 level.

Group-I: Control

Group-II: Organic calcium supplement

Group-III: Inorganic calcium supplement

Table 4. Analysis of variance for T-Scores (BMD) level of selected male subjects before and after feeding trial.

Source of Variation	Before					After						
	SS	df	MS	F	P-value	F crit	SS	Df	MS	F	P-value	F crit
Between Groups	0.090	2	0.044792	0.844	0.435	3.145258	1.466	2	0.732975	6.610	0.002**	3.145258
Within Groups	3.290	62	0.053061				6.875	62	0.110882			
Total	3.379	64					8.341	64				

Table 5. Analysis of variance for T-Scores (BMD) level of selected female subjects before and after feeding trial.

Source of Variation	Before						After					
	SS	df	MS	F	P-value	F crit	SS	Df	MS	F	P-value	F crit
Between Groups	0.085	2	0.042674	0.110	0.896	3.204317	4.757	2	2.378667	60.497	1.76E-13**	3.204317
Within Groups	17.443	45	0.387617				1.769	45	0.039319			
Total	17.528	47					6.527	47				

Table 6. Multiple comparison for BMD T-Score of selected male and female subjects after experimental trial (Tukey).

Dependent Variable	Gender	Comparing Groups	Mean Difference	Tukey HSD Q Statistic	P Value	
BMD	Male	Control	OCS	0.013	11.688	0.001**
			ICS	0.007	13.681	0.001**
	Female	Control	OCS	0.049	4.264	0.012*
			ICS	0.010	0.954	0.761

** : The mean difference is significant at 0.01 level.

* : The mean difference is significant at 0.05 level.

Group-I: Control

Group-II: Organic calcium supplement

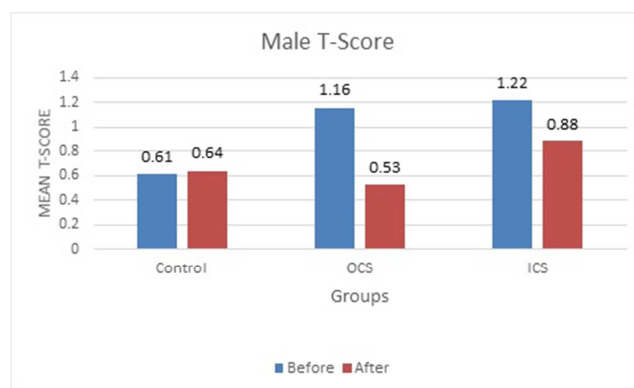
Group-III: Inorganic calcium supplement

Statistical analysis of T-Score before and after feeding trial indicated, that for males, a significant difference was observed in calcium organic supplement ($p=0.0006$), while, no significant difference was observed for male's control and inorganic calcium supplement. While, in females indicated significant difference for OCS ($p=0.0137$) and ICS ($p=0.0084$) while no significant difference was observed for control. Further analysis by post hoc Tukey test indicated significant difference in mean BMD-T Score values of male subgroups OCS ($p=0.001$) and ICS ($p=0.001$) with their control group, while, female subgroup OCS ($p=0.012$) had significant difference with its control group and subgroup ICS non-significant difference with its control group.

Similar findings were reported by following studies. Study by Chan *et al* [8] had reported that the dairy supplement group had higher intake of calcium than control subjects. The dairy group had significantly greater increases in bone mineral density in total body bone mineral ($14.2\% \pm 7.0\%$ vs $7.6\% \pm 6.0\%$) than control subjects. Young girls whose dietary calcium intake was provided primarily by dairy products at or above the recommended dietary allowances had an increased rate of bone mineralization. In another study by Matovic *et al* [9], it is reported that there was a more pronounced increase in bone mass over time in the calcium-supplemented group (1640 mg Ca/d) than in the control group i.e. 750 mg Ca/d. Johnston *et al* [10] also found that calcium supplementation had a positive effect on the rate of increase in bone mineral density at several skeletal sites in pubertal age. The subjects who were given supplements received on average 719 mg more calcium per day than their twins and had an increase of about 3 per cent more mineral in the radius during the three-year study period.

Bone mineral acquisition was significantly greater in the group given milk supplements than in the control group. The intervention group had greater percentage increases of total body bone mineral density (9.6% (SD 1.9%; 95% confidence interval 9.0% to 10.2%) v 8.5% (2.7%; 7.6% to 9.4%); $p=0.017$). Expressed in absolute terms, the respective increases were 0.090 (0.020; 0.084 to 0.096) v 0.081 (0.025;

0.072 to 0.089) g/cm² for total body bone mineral density ($p=0.021$) [7], [15]. Findings by other studies [5], [19] concluded that calcium supplementation of post-menarcheal girls age of 14+.5 year with habitual low calcium intakes <800 mg/day enhances bone mineral acquisition especially in girls > 2 yr past the onset of menarche. Another study carried out by Marwaha *et al* [11], provides information on BMD in peripheral sites (calcaneum) in healthy Indian girls. It was found that the mean BMD values in LSES subjects were 0.358 ± 0.066 g/cm² while those in the USES subjects were 0.389 ± 0.067 g/cm², whereas the mean values of BMD in 12-16-year-old girls from China (study by Afgani *et al* [13], Chan G. M. [14]) was 0.34 ± 0.05 g/cm². In additional study by Marwaha *et al* [12], Comparison of BMD at calcaneum in Indian children with data from healthy Caucasian children in the United Kingdom acquired with the same model of densitometer showed that LSES subjects had lower values and USES subjects had higher values. In the study conducted in China [13], the mean BMD values in 12-16 yr old girls were 0.47 ± 0.06 g/cm²; values which were higher than those of LSES subjects (0.429 ± 0.064 g/cm²) and lower than USES subjects (0.488 ± 0.083 g/cm²) from that study.

**Figure 3.** Mean±SD for BMD -T Score of the selected male subjects before and after feeding trial.

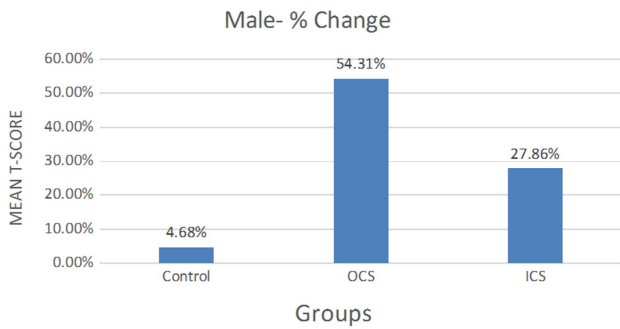


Figure 4. Change in percentage of BMD -T Score of the selected male subjects before and after feeding trial.

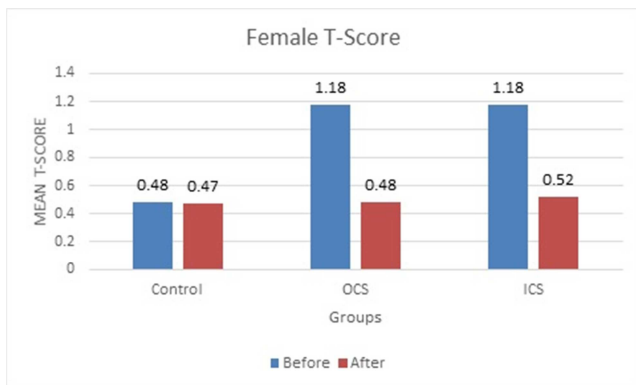


Figure 5. Mean±SD for BMD -T Score of the selected female subjects before and after feeding trial.

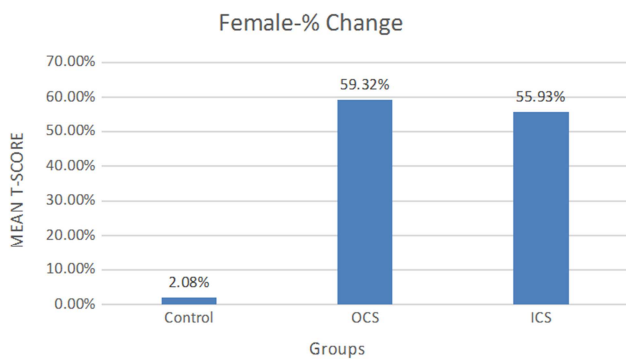


Figure 6. Change in percentage of BMD -T Score of the selected female subjects before and after feeding trial.

4. Conclusion

Significant increase in Bone mineral density were observed among male and female subgroups after intervention. Organic supplements had greater effect on BMD. The respective percentage increase in mean BMD (with T-Score) of female subjects have more than male subjects.

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