CHAPTER 5
Effect of Calcium and Zinc Supplementation on Height Velocity of LSES Premenarchal Girls vs. Unsupplemented HSES Girls

Growth rate during adolescence is determined by a complex interaction of physical, endocrine and nutritional factors, of which nutrition is a key determinant (Bogin 1999). Multiple micronutrient deficiencies have been reported in developing countries resulting in growth faltering as is commonly observed in children especially from low income groups (Bhandari et al, 2001). Meta analysis of zinc supplementation studies indicated beneficial effect on height increment in prepubertal children (Brown et al, 2002) while a study in African children showed no significant effects (Muller et al, 2003). Similarly, while evidence from animal studies and observational studies in children indicate that inadequate dietary intake of calcium may result in linear growth retardation (Bhandari et al, 2001), other studies indicate no effect of calcium supplementation on height increments (Merrilees et al, 2000; Dibba et al, 2000). In the present intervention trial increments in height and weight were also recorded along with bone parameters of the premenarchal girls from LSES. The present chapter examines the effect of one year supplementation with zinc, multivitamin, calcium and vitamin D on height velocity of premenarchal girls from lower socioeconomic stratum and compare it with the height velocity observed in an age-matched cohort of un-supplemented girls from higher socioeconomic stratum.

5.1 Methods

5.1.1 Study Design

From the one year supplementation trial of calcium, zinc and vitamin D on bone mass accrual in premenarchal girls from lower socioeconomic stratum (LSES), height velocity was compared with similar data on age-matched unsupplemented girls from high socio-economic stratum (HSES).
5.1.2 Study population

*Lower socioeconomic stratum (LSES)*

Based on the variability observed in height velocity of girls in an Indian study (Rao et al, 1998), a sample size of 119 was estimated to obtain a power of 0.8 at 5% level of significance to detect a difference of more than 5% in the means. Therefore, 119 girls from the intervention trial (Chapter 4) were randomly selected and age-matched with girls from higher socioeconomic stratum.

Of the 119 girls, 33 were from Ca+D group receiving 500 mg of calcium (Calcium Sandoz, Novartis, Mumbai, India) 6d/wk and vitamin D (30,000IU/3 months); 36 were from Ca+MZn+D group receiving 500 mg of oral elemental calcium (same as Ca+D group) 6d/wk and a tablet (Becosule-Z, Pfizer limited, Mumbai, India) containing multivitamin with zinc along with vitamin D (30,000IU/3 months); and 50 were from the M+D group receiving tablets with the same multivitamin composition but without any minerals along with vitamin D (30,000IU/3 months). Details of the supplements have been described in previous chapter 4, section 4.1.4).

*Higher Socioeconomic stratum (HSES)*

From the available data of yearly health check on 405 girls (8-12 years) from a private school (catering to children from affluent areas i.e. areas without slum clusters, low income housing schemes and those with high land prices as published by Government agencies, minimum yearly fees of around Rs 10000, Indian per capita income 2007-2008, Rs 2021/month), total of 119 girls (8-12 yr) in class 3rd-5th were age-matched with girls in each intervention group and were observed for their growth parameters after 12 months without any supplementation and were treated as the control group (Group C1-age-matched controls for Ca+MZn+D group; C2-age-matched controls for Ca+D group and C3-age-matched controls for M+D group).
5.1.3 Exclusion criteria

Girls were excluded based on factors known to adversely affect bone metabolism. Details of exclusion criteria have been described in Chapter 2 (Section 2.1.2).

5.2 Outcome parameters

Data on anthropometry (height, weight) and pubertal stage (Tanner criteria) were assessed at baseline and at the end of one year, in all the HSES girls (n=119) as per methods described in Chapter 2 (Section 2.2) and similar data were available on LSES girls.

5.3 Statistical Methods

Analyses were performed using Statistical package for Social Sciences (SPSS) software for Windows (version 11.0, 2001, SPSS Inc, Chicago, IL). All the variables were tested for normality using Kolmogorov-Smirnov test before any statistical treatment of the data. Data are presented as mean and standard deviation for normal variables. In order to test the statistical significance of difference of primary outcome measures (height, weight, BMI, height velocity) between the three supplemented groups and the control group, analysis of variance model (ANOVA) was used with post hoc Tukeys’ test to test the pairwise difference between the groups. Paired t-test was used to assess differences in pre and post supplementation parameters. Control and the three supplemented groups were also compared using general linear model adjusting for baseline Tanner stage. P-value less than 0.05 was considered statistically significant.
5.4 Results

5.4.1 Anthropometric parameters in the study population

Table 5.1 describes the baseline anthropometric characteristics of girls from LSES in the three supplemented groups (Ca+MZn+D, Ca+D, M+D) compared with age-matched unsupplemented girls from HSES group (Groups C1, C2 and C3 respectively). The mean age of the study population was 9.9±1.2 years with no significant differences between the groups (p>0.1). At baseline, there were no significant differences observed in height, weight and BMI between the three intervention groups (LSES) (p>0.1) (Table 5.1). However, the mean height, weight and BMI of girls from the three intervention groups were significantly lower compared to their respective age-matched counterparts from HSES (p<0.05).

Low height-for-age Z scores (<-2) as per Indian reference database (Khadilkar et al, 2009) were seen in 19.4%, 33.3% and 32.7% of the girls in Ca+MZn+D, Ca+D and M+D group respectively. For HSES controls, only 1% of the girls had their Z scores below -2 indicating that the prevalence of under nutrition was more in girls from LSES. Weight-for-age Z scores above -2 were observed in only 1% of the girls from HSES.
Table 5.1: Comparison of anthropometric characteristics between supplemented groups (LSES) and age-matched unsupplemented controls from HSES at baseline and endline

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ca+MZ+D (n=36)</th>
<th>C1 (n=36)</th>
<th>Ca+D (n=33)</th>
<th>C2 (n=33)</th>
<th>M+D (n=50)</th>
<th>C3 (n=50)</th>
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<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
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<tr>
<td>Baseline</td>
<td>10.0±1.3</td>
<td>10.0±1.3</td>
<td>10.1±1.5</td>
<td>10.1±1.5</td>
<td>9.6±1.0</td>
<td>9.6±1.0</td>
</tr>
<tr>
<td>Endline</td>
<td>11.2±1.3</td>
<td>11.1±1.3</td>
<td>11.3±1.5</td>
<td>11.2±1.5</td>
<td>10.8±1.0</td>
<td>10.7±1.0</td>
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<tr>
<td><strong>Height (cm)</strong></td>
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</tr>
<tr>
<td>Baseline</td>
<td>129.3±8.2</td>
<td>139.1±10.3</td>
<td>127.1±8.5</td>
<td>138.0±10.7</td>
<td>126.1±7.2</td>
<td>135.1±8.3</td>
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<tr>
<td>Endline</td>
<td>136.8±8.8*</td>
<td>144.5±10.0*</td>
<td>134.0±8.3*</td>
<td>143.8±10.2*</td>
<td>133.2±8.0*</td>
<td>141.1±8.5*</td>
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<tr>
<td><strong>Weight (cm)</strong></td>
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<tr>
<td>Baseline</td>
<td>23.7±5.4</td>
<td>33.6±9.6</td>
<td>21.6±4.8</td>
<td>33.4±10.0</td>
<td>21.4±3.2</td>
<td>29.9±8.1</td>
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<tr>
<td>Endline</td>
<td>28.3±7.4*</td>
<td>37.2±9.9*</td>
<td>25.9±6.2*</td>
<td>38.4±11.0*</td>
<td>25.5±4.5*</td>
<td>34.3±9.3*</td>
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<td><strong>BMI (kg/m^2)</strong></td>
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<tr>
<td>Baseline</td>
<td>14.0±1.8</td>
<td>17.1±3.3</td>
<td>13.2±1.4</td>
<td>17.2±3.1</td>
<td>13.4±1.2</td>
<td>16.2±3.0</td>
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<tr>
<td>Endline</td>
<td>14.9±2.2</td>
<td>17.6±3.3</td>
<td>14.3±1.9*</td>
<td>18.4±3.4*</td>
<td>14.3±1.5*</td>
<td>17.0±3.4*</td>
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<tr>
<td><strong>Height-for-age Z score</strong></td>
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<tr>
<td>Baseline</td>
<td>-1.3±0.9</td>
<td>0.2±1.0</td>
<td>-1.6±1.0</td>
<td>0.1±0.9</td>
<td>-1.4±0.8</td>
<td>-0.0±0.9</td>
</tr>
<tr>
<td>Endline</td>
<td>-1.0±1.0*</td>
<td>0.2±1.0</td>
<td>-1.5±1.0*</td>
<td>0.1±0.9</td>
<td>-1.3±1.0*</td>
<td>-0.1±0.9</td>
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<tr>
<td><strong>Weight-for-age Z score</strong></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>-1.6±0.9</td>
<td>0.0±1.0</td>
<td>-2.1±0.8</td>
<td>0.0±0.9</td>
<td>-1.8±0.7</td>
<td>-0.3±0.9</td>
</tr>
<tr>
<td>Endline</td>
<td>-1.4±1.0*</td>
<td>-0.1±1.0</td>
<td>-1.8±0.9*</td>
<td>-0.1±0.9</td>
<td>-1.6±0.8*</td>
<td>-0.2±1.0</td>
</tr>
<tr>
<td><strong>BMI-for-age Z score</strong></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>-1.3±0.8</td>
<td>-0.1±1.0</td>
<td>-1.7±0.7</td>
<td>0.0±0.9</td>
<td>-1.5±0.7</td>
<td>-0.3±1.0</td>
</tr>
<tr>
<td>Endline</td>
<td>-1.2±0.8*</td>
<td>-0.2±1.0</td>
<td>-1.4±0.8*</td>
<td>0.1±0.9</td>
<td>-1.3±0.7*</td>
<td>-0.3±1.1</td>
</tr>
</tbody>
</table>

All values are Mean±SD. Ca+MZ+D group, Calcium+zinc+Vitamin D; Ca+D+Vitamin D group, Calcium; M+D group, Multivitamin+Vitamin D

C1: Age-matched unsupplemented HSES controls for Ca+MZ+D group; C2: Age-matched unsupplemented HSES controls for Ca+D group; C3: Age-matched unsupplemented HSES controls for M+D group

All parameters in LSES were significantly lower than the HSES group at both baseline and endline (p<0.05)

No significant differences were observed between the three supplemented groups at baseline for all parameters (p>0.1)

*Significantly different from baseline

Pubertal stage in the study population indicated that at baseline, majority of the girls in supplemented LSES group (Ca+MZ+D, Ca+D and M+D groups) were...
in Tanner stage I (61-72%) with none of the girls having attained menarche (Tanner stage V) indicating their premenarchal status. For the age-matched control groups from HSES (C1, C2 and C3 groups), 21-30% of the girls were at Tanner stage I while 4-14% girls had attained menarche. This indicated that though age-matched girls from LSES showed later puberty than HSES girls.

**Figure 5.1 (A, B, C) Distribution of girls as per Tanner stage in the three intervention groups and their age-matched controls at baseline and endline**

(A) Ca+MZn+D group and C1 group

(B) Ca+D group and C2 group

*C1: Age-matched HSES controls for Ca+MZn+D*

*C2: Age-matched HSES controls for Ca+D*
Post interventions, while only 4-14% of the LSES girls had attained menarche, 18-31% of the girls from HSES were seen at Tanner stage V. The mean height, weight and BMI of the girls was significantly higher than the baseline for both LSES and HSES girls (p<0.05) as is expected over a period of one year of natural growth (Table 5.1). When the three intervention groups were compared to their respective age-matched HSES girls, it was observed that the mean height, weight and BMI of the girls from LSES (three supplemented groups) were significantly lower than the age-matched HSES counterparts even at endline (Table 5.1). However, a significant improvement was observed in height-for-age, weight-for-age and BMI-for-age Z scores of the girls from LSES, in each of the three intervention groups compared to baseline (p<0.05) while there was no significant change observed in the Z scores of HSES girls at endline (p>0.1) (Table 5.1).

To examine this further, change in height-for-age Z score in each of the groups was calculated (Table 5.2). It was observed that the change in height Z score was significantly higher in the Ca+MZn+D group and M+D group compared to their respective age-matched controls (C1 and C3 respectively) (p<0.05). Between the three supplemented groups, Ca+MZn+D group showed significantly higher change in height Z score compared to Ca+D and M+D group (p<0.05).
Table 5.2 Change in height-for-age Z score in the study population

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted Change in height-for-age Z score</th>
<th>Change in height-for-age Z score adjusted for Tanner stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca+MZn+D C1</td>
<td>0.24 ± 0.06** -0.05 ±0.04</td>
<td>0.32 ±0.06** -0.02 ±0.05</td>
</tr>
<tr>
<td>Ca+D C2</td>
<td>0.14 ±0.04 0.05 ±0.05</td>
<td>0.18 ±0.06 0.07 ±0.04</td>
</tr>
<tr>
<td>M+D C3</td>
<td>0.10 ±0.04 -0.03 ± 0.03</td>
<td>0.14 ±0.04* 0.01 ±0.03</td>
</tr>
</tbody>
</table>

All values are Mean±SE. Ca+MZn+D group, Calcium+zinc+Vitamin D; Ca+D group, Calcium+Vitamin D; M+D group, Multivitamin+Vitamin D C1: Age-matched unsupplemented HSES controls for Ca+MZn+D group; C2: Age-matched unsupplemented HSES controls for Ca+D group; C3: Age-matched unsupplemented HSES controls for M+D group **Significantly higher than age-matched controls (p<0.01) * Significantly higher than age-matched controls (p<0.05)

Since the girls were in various stages of puberty, considering the influence of Tanner stage on growth, adjustments were made to the change in height-for-age Z score. It was observed that after adjusting for Tanner stage, the change in height Z score was significantly higher in the Ca+MZn+D group compared to age-matched controls (C1), Ca+D and M+D group (p<0.05).

5.4.2 Improvement in Height-for-age Z scores

In order to analyze this further, improvement in linear growth of the girls (LSES, HSES) was assessed by classifying the change in height-for-age Z scores further into three categories:

Class I: Z score above -1, Class II: Z score between -2 to -1, Class III: Z score below -2. “Improvement” was defined by a shift in the Z score from lowest group to successively higher groups; i.e. from III to II, or II to I. “Decline” was defined by a drop in the Z score category from higher to lower category i.e. I to II or III, or from II to III. “No change” was observed if the individual’s Z score remained in the same category (Figure 5.2). It was seen that 22.2% of the girls from Ca+MZn+D group,
9.1% from Ca+D group and 16% girls from the M+D group showed improvement while majority of the girls (95.7%) from HSES showed no change with respect to height-for-age Z scores. This indicates that supplementation with calcium, Vitamin D and multivitamin especially along with zinc may result in catch up growth with respect to height in premenarchal girls from LSES.

Figure 5.2: Change in height-for-age Z scores in supplemented and unsupplemented girls

![Bar chart showing percent girls with improvement and decline]

- Ca+MZn+D group, Calcium+zinc+Vitamin D
- Ca+D group, Calcium+Vitamin D
- M+D group, Multivitamin+Vitamin D
- C group, Control group of age-matched un-supplemented HSES girls (C1, C2, C3 combined)

5.4.3 Height velocity in the study population

To assess the effect of supplementation on height increment, height velocity was calculated for each of the groups (Table 5.3). The mean height velocity was seen to be significantly higher in the Ca+MZn+D group and Ca+D group compared to their respective age-matched controls (C1 and C2 respectively) (p<0.05). For M+D groups, no significant differences were observed when compared to age-matched control groups (C3) (p>0.1)
Further, since height velocity is also known to increase during pubertal growth spurt and the girls in the present study were premenarchal at baseline, height velocity adjusted for Tanner stage was compared between the groups. The analysis revealed that height velocity was significantly higher in the Ca+MZn+D group compared to C1 group (p<0.05). No significant differences were observed in Ca+D and M+D groups compared to their respective age-matched controls (C2 and C3 respectively). Comparison of height velocity in the three intervention groups revealed that Ca+MZn+D group showed significantly higher height velocity compared to both Ca+D and M+D group (p>0.1) indicating the beneficial effect of zinc supplementation on height increment in underprivileged premenarchal girls.

### 5.4.4 Comparison of height velocity with published reference database

Furthermore, the height velocity observed in present study population was compared with published reference database from Taiwan, U.S. and Indian populations. Since for Taiwan and U.S. populations, median height velocity values were reported, Figure 5.3 gives age-wise comparison of median values of the study population with the other studies. Since a higher height velocity was observed in all the three supplemented groups compared to the HSES girls (unsupplemented Group
C), comparison has been shown between LSES (all groups combined), HSES and Caucasian (Berkey et al, 1993) and Taiwanese (Lee et al, 2004) populations. The analysis revealed that while for the HSES group and the Caucasian and Taiwanese population, the peak height velocity was achieved at 11 years, the girls from LSES showed continued increase in height velocity even at age 12 years possibly as a result of supplementation.

Figure 5.3: Comparison of age-wise median whole year height velocity with other published references

When compared to mean height velocity reported by an Indian study in both LSES and HSES girls, it was seen that in both LSES and HSES girls from the published study who were without any supplementation, the peak height velocity was achieved at age of 11 years (Figure 5.4). However, in the present study population of supplemented girls from LSES, the height velocity continued to increase even at age 12. Also, the height velocity of the girls from the present study (LSES and HSES) was comparable to that reported by the Indian study till age 11.
years indicating that supplementation during premenarchal period especially at age 11 and 12 may provide an opportunity for improving height deficits in underprivileged Indian girls.

**Figure 5.4: Comparison of age-wise mean whole year height velocity with other published references**

![Figure 5.4: Comparison of age-wise mean whole year height velocity with other published references](image)

*Data not available for age group 9 for Indian study (LSES, HSES)*

5.5 Discussion

In the present study population of premenarchal girls from LSES, one year supplementation with calcium, multivitamin+zinc showed a significant improvement in height-for-age Z scores as well as higher height velocity compared to age-matched unsupplemented girls from HSES. Ca+MZn+D group showed the maximum height velocity adjusted for pubertal stage compared to age-matched controls (C1 group) as well as Ca+D group and M+D. These findings are in line with those reported in 6-9 years old Chinese children from low income groups where the authors found that the increase in growth parameters as assessed by knee height was highest in the group supplemented with micronutrients along with zinc than those supplemented with micronutrients alone group or zinc alone group (Sandsead et al, 1998). Also, Yang et al (2002) have demonstrated significantly greater height gain in Chinese preschool children with Calcium+zinc supplementation (7.7 cm/yr) as against the control group (6.74 cm/yr) (p<0.05). Sarma et al (2006) in their micronutrient supplementation study (6-16 yr old residential school children supplemented with a micronutrient rich beverage containing calcium, zinc and vitamin D along with other vitamins and minerals) also found greater height velocity in the supplemented group (5.3 cm/yr) compared to the placebo group (4.9 cm/yr).

In the present study population, girls from LSES supplemented with calcium, multivitamin+zinc showed significantly higher height velocity as well as improvement in height-for-age Z scores compared to age-matched controls from HSES. These results are in line with a study by Rosado (1999) in preschool where he observed that those children belonging to low and medium socioeconomic strata on multiple micronutrient supplementations grew about 1 cm more as compared to the placebo group with similar results not observed in children from higher socioeconomic stratum. This indicates that low income groups girls may show beneficial effects of supplementation. The fact that we observed a comparatively lower increase in height velocity in the Ca+D group is in line with results from a meta analysis by Prentice and Bates (1994) where they reported no improvement in height or weight gain with calcium supplementation in children from developing countries with low to medium intakes of dietary calcium.
Further, girls who received calcium with zinc supplementation showed continued higher height velocity even at age 12 as compared to study cohort by Rao et al (1998) where they found the peak height velocity to be around 11 years in Indian girls from LSES. Due to lack of data for further age groups, we are unable to conclude the exact age of peak height velocity in LSES girls in the present study population. However, the fact that the height velocity continued to show an increase even at age 12 could be due to the added effect of supplementation.

The absolute change in height-for-age Z score in all the three groups (Ca+Mzn+D, Ca+D and M+D group) was significantly higher as compared to age-matched group of girls from HSES with no supplementation (Group C1, C2 & C3). Also within the three supplemented groups from LSES, Ca+Mzn+D showed highest improvement in terms of change in height-for-age Z scores (p<0.05) compared to both Ca+D and M+D group. These results are in line with those reported by Sarma et al (2006) in apparently healthy Indian residential school children where they found significant increase in the mean increment of height-for-age Z scores in the supplemented group (-0.04) compared to the placebo group (-0.14) (p<0.05).

In conclusion, zinc supplementation along with multivitamin and vitamin D showed improvement in height velocity as well as height-for-age Z scores indicating beneficial effect of zinc in enhancing growth parameters in underprivileged premenarchal girls especially in the age group of 11-12 years. Supplementation aimed at improving micronutrient status especially in the premenarchal period may provide a window of opportunity for catch-up growth with respect to height deficits in underprivileged premenarchal girls.