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Calcium and Vitamin D for Adolescent Idiopathic Scoliosis – A Further In-depth Review Using Finite Element Analysis (FEA) for a Randomized Double-blinded Placebo-controlled Trial

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Introduction

Adolescent Idiopathic Scoliosis (AIS) is a complex three-dimensional spinal deformity mostly affecting girls during the peri-pubertal period. The prevalence ranges from 2 to 4% depending on gender and ethnicity. The higher the latitude, the higher the prevalence of AIS [1]. When the deformity becomes severe, serious morbidities can occur including early back degeneration, cardiopulmonary compromise, grossly deformed torso and associated psychosocial issues which could pose serious health threats for these young subjects [2].

One important health threat that deserves special attention is osteopenia as defined by Bowden et al. for children having a bone mineral density age and gender-adjusted Z-score < -1 [3]. Thirty to 38% of AIS girls were osteopenic as compared to 16% in the general population [4]. Osteopenia could persist across puberty [4] and was found to be an independent and significant prognostic factor for curve progression in AIS [5]. Osteopenia was also found to be associated with low dietary calcium (Ca) intake at a median of 400mg/day among AIS girls. The low Ca intake was significantly correlated with age-adjusted peripheral volumetric bone mineral density (vBMD) measured by peripheral quantitative computed tomography (pQCT), as well as axial and peripheral areal BMD (aBMD) measured by Dual-Energy X-ray Absorptiometry (DXA) [6].

Low Ca intake when coupled with low Vitamin D (Vit-D) status can seriously affect a child's bone health. Without Vit-D, only 10 to 15% of dietary Ca is absorbed [7]. 1,25-$(OH)_2$Vit-D can increase intestinal Ca absorption to 30 to 40% [7], and prevent muscle weakness [8]. Likewise, Vit-D insufficiency or disturbance in Vit-D physiology is associated with osteopenia, deranged muscle function [8] and ligamentous laxity [9] which

could potentially predispose AIS subjects to curve progression. Vit-D and AIS also share other similarities. First, it has been reported that higher latitude is associated with higher prevalence of both Vit-D insufficiency [10] and AIS [1]. Second, Vit-D insufficiency or dysfunction is associated with raised serum bone-alkaline-phosphatase (bALP) [9] and our group has reported bALP level was elevated in AIS subjects [11].

Given that serum 25(OH)Vit-D level was also found to be low at a mean level of 41·6 nmol/L among AIS subjects [12], it will be logical to evaluate whether Ca plus Vit-D (Ca+Vit-D) supplementation could improve bone health and prevent curve progression in AIS. To the best of our knowledge, no study in this area has been reported in the literature. This study aimed at evaluating the therapeutic effect and its determinants of Ca+Vit-D supplementation on improving bone health and preventing curve progression in immature AIS patients with low bone mass.

Methods

This was a randomized double-blinded placebo-controlled trial recruiting AIS girls (11-14 years old, Tanner stage < IV) with femoral neck areal bone mineral density (aBMD) Z-scores < 0 and Cobb angle ≥ 15°. 330 subjects were randomly allocated to either Group1 (placebo), Group2 (600mg Calcium+400 IU Vit-D3/day) or Group3 (600mg Calcium+800 IU Vit-D3/day). The subjects, their guardians, the investigators and the caring orthopaedic surgeon were all blinded to the grouping throughout the entire study. The treatment period was two years. Regular clinic follow up for the scoliosis and

assessment were conducted with bracing prescribed and weaned according to a standard clinical protocol [2].

At baseline and the 24-month time-point, serum 25(OH)Vit-D level was assayed with liquid chromatography tandem mass spectrometry. Dietary calcium intake was assessed with validated Food Frequency Questionnaire. Areal bone mineral density (aBMD) and bone mineral content (BMC) at bilateral femoral necks were measured with Dual-Energy X-ray Absorptiometry (DXA). Volumetric BMD (vBMD), gross bone morphometry and trabecular bone micro-architecture were measured with High Resolution Peripheral Quantitative Computed Tomography (XtremeCT or HR-pQCT) at the non-dominant distal radius.

Finite Element Analysis (FEA) on HR-pQCT images was conducted using the software provided by the manufacturer (μFE Element Analysis Solver v.1.15; Scanco Medical, Switzerland) [13]. The subject-specific Finite Element Model (FEM) was constructed from a three-dimensional (3D) segmented bone image in form of a number of small elements. Cube and tetrahedron are commonly used as the element shape in three-dimensional bone structure. In this study, the FEM contained eight-node brick elements with an element size of $82 \times 82 \times 82\ \mu m^3$ which was the same as the voxel size of HR-pQCT images. It was assumed that bone tissue is an isotropic and linear material with a Young's modulus of 10 GPa and a Poisson's ratio of 0.3 [13]. Poisson's ratio is calculated as the ratio of transverse strain to strain along the loading direction. After establishment of the FEM, the amount and direction of the loads and the boundary conditions were assigned [14]. In this linear FEM, uniaxial compression test with 1% strain along the axial direction was performed. Stiffness, failure load and apparent modulus were

calculated in simulation. Failure load was determined when 1 mm^3 elements in the model had an effective strain greater than 7000 microstrain [15].

To evaluate spinal deformity, Cobb angle was measured using the standard method on whole spine standing postero-anterior radiographs taken at routine clinic visits once every 6 months before skeletal maturity and brace weaning, followed by once every 8 to 12 months thereafter.

For the first study objective, changes in bone parameters during the 2-year treatment period were compared between the study groups to evaluate the treatment effects of Ca+Vit-D supplementation on bone health.

For the second objective, Cobb angle taken without brace at baseline and at the latest follow-up were evaluated for the entire cohort and the SRS (Scoliosis Research Society) guideline on scoliosis outcome study [16] was followed for inclusion of subjects who have completed the 2-year treatment into the latest follow-up analysis. Hence, those who satisfied the followings were included in the latest follow-up curve outcome analysis:

1. they have completed the 2-year treatment and
2. Cobb angle of the major curve at baseline $\leq 40°$ and
3. brace was already stopped or never been braced and
4. Risser sign ≥ 4 and YSM > 2 years at the latest follow-up

Curve outcome analysis at the latest follow-up was made with reference to the modified SRS bracing outcome criteria [16]:

1. Percentage of patients with Cobb increase $\geq 6°$

2. Percentage of patients with Cobb reaching surgical levels (Cobb > 55° at our institute)
3. Percentage of patients with final Cobb > 45°

The spread of data was tested for normality. For data that was normally distributed, the numerical data was expressed as "mean ± standard deviation (SD)". Otherwise, medians and inter-quartile ranges were given with specification. Subject baseline characteristics were compared between groups with ANOVA. Analysis on changes in bone parameters between groups was conducted with ANCOVA using the corresponding baseline value as a covariate. Analyses on blood and curve severity parameters were conducted using the same approach. McNemar test was used for paired comparison on Vit-D status between baseline and the 2-year time-point. Chi-square test was used to compare curve progression between groups with reference to the modified SRS bracing outcome criteria mentioned earlier [16]. To further validate the treatment effect, logistic regression was conducted to adjust for known determinants of curve progression namely YSM at baseline, initial Cobb angle and bracing treatment. SPSS 20 for Windows was used for all statistical analyses. P value of $< 0{\cdot}05$ was considered statistically significant. The "Intention To Treat" principle was followed for data analysis on those who have completed the 2-year treatment.

Results

From April-2010 to November-2012, a total of 1037 consecutive AIS subjects noted to be eligible at initial screening were evaluated and finally, 330 suitable subjects were randomised. Details of the trial profile are depicted in Figure 1. The baseline characteristics of maturity, anthropometric, lifestyle, biochemical, HR-pQCT and FEA parameters are shown in Table 1. No significant difference was noted between Group 1, 2 and 3 (all with p>0·05). Distribution of curve types for the three study groups is shown in Table S1 (Appendix).

270 (81·8%) subjects completed the 2-year treatment (Figure 1). The overall treatment compliance was 83·3±12·9% (Group 1: 84·5±11·8%, Group 2: 82·5±14·0%, and Group 3: 82·8±12·8%, p= 0·519).

Prevalence of Vit-D insufficiency at baseline and at the 2-year time-point, the changes on serum 25(OH)Vit-D levels, HR-pQCT and FEA parameters from baseline to the 2-year time-point are shown in Table 2. The gain in Average vBMD and all FEA parameters of stiffness, failure load and apparent modulus at the non-dominant distal radius were significantly greater in Group 3 than the placebo group (all with p<0·05, Table 2). Changes in Trabecular vBMD, Trabecular Bone Volume to Tissue Volume Ratio and Trabecular Number were different between groups demonstrating improvement in bone health with Ca+Vit-D supplementation in both treatment groups (all with p<0·05, Table 2). Likewise, the increase in Trabecular Separation was significantly greater in the placebo group than the treatment groups (p<0.05).

Outcome on curve deformity was assessed according to the modified SRS bracing outcome criteria at the latest follow-up as mentioned earlier (N=132, Table 3). Outcome

182 analysis with respect to curve progression in Cobb angle ≥ 6° showed that 21·7% in
183 Group 3 and 24·4% in Group 2 progressed as compared with 46·7% in Group 1. Logistic
184 regression analysis was conducted to control confounding from curve severity (Cobb
185 angle of the major curve at baseline), maturity (YSM at baseline) and bracing history.
186 When Group 3 was compared with Group 1, the adjusted odds ratio for curve progression
187 ≥ 6° was 0·343 (p=0·028).

188 Using the same logistic regression approach, within-group analysis showed that in
189 Group 3, increase in FEA parameters of failure load and apparent modulus during the 2-
190 year treatment were independent and significant protective factors against curve
191 progression (p=0.043 and 0.034 respectively) while that for stiffness reached borderline
192 statistical significance (p=0.054).

193 For the subgroup with baseline serum 25(OH)Vit-D ≤ 50 nmol/L (N=103), 16.2% in
194 Group 3 (p=0.003 Vs Group 1) and 22·6% in Group 2 (p=0.028 Vs Group 1) had curve
195 progression ≥ 6° as compared with 48·6% in Group 1. In contrast, for the subgroup with
196 baseline serum 25(OH)Vit-D > 50 nmol/L (N=29), no between-group difference was
197 noted on curve progression with 40% progressed in Group 1 as compared with 44.4% in
198 Group 3 (p>0.05) (Table 3).

199 Concerning dietary calcium level, for the subgroup with baseline dietary calcium
200 intake ≤ 1000 mg/day (N = 109), 19.0% in Group 3 (p=0.001 Vs Group 1) and 15·6% in
201 Group 2 (p=0.001 Vs Group 1) had curve progression ≥ 6° as compared with 54.3% in
202 Group 1. In contrast, for those with baseline dietary calcium intake > 1000 mg/day (N =
203 23), no between-group difference was noted on curve progression (Table 3).

The number of subjects having Cobb angle > 45° for Group 1, 2 and 3 were 2 (4·4 %), 1 (2·4 %) and 0 (0%) respectively. No subject had Cobb angle > 55° at the latest follow-up for all three study groups.

Discussion

In this 2-year randomised double-blinded placebo-controlled trial, we found strong evidences of improvement in bone health and therapeutic control of curve progression with Ca+Vit-D supplementation for AIS girls having Z-score of femoral neck BMD < 0.

After 2-year of treatment, the prevalence of Vit-D insufficiency was reduced to 30·8% in Group 2 ($p<0·001$) and 19·3% in Group 3 ($p<0·001$) whereas for Group 1, the prevalence was 62·6% ($p= 0·035$). This improvement in Vit-D status was also reflected in the rise in serum 25(OH)Vit-D levels with a mean increase of 20·4 nmol/L in Group 2 and 28·0 nmol/L in Group 3 as compared with 6·3 nmol/L in Group 1 (all with $p<0·001$) (Table 2).

In alignment with the improvement in Vit-D status, increase in bone density, bone quality and bone strength parameters were also demonstrable in the treatment group (Table 2) providing strong evidences of improvement in bone health with Ca+Vit-D supplementation. Improvement in HR-pQCT and FEA parameters could carry significant clinical implication. Nikodem pointed out primary HR-pQCT parameters were correlated with mechanical properties [17]. It has been a long-existing quest to investigate if

227 improvement in bone quality and bone strength is accompanied by improvement in curve
228 control in AIS.

229 Using the SRS bracing outcome criterion of Cobb increase ≥ 6° at the latest follow-
230 up, the rate of curve progression was 24·4% in Group 2 and 21·7% in Group 3 as
231 compared with 46·7% in Group 1 ($p<0·05$, Table 3). Logistic regression analysis further
232 showed the therapeutic effects of Ca+Vit-D supplementation to prevent curve progression
233 was independent of YSM (maturity) at baseline, initial Cobb angle (curve severity) at
234 baseline and the bracing history. Using the same logistic regression model for within-
235 group analysis in Group 3, the therapeutic effects of preventing curve progression was
236 correlated with increase in FEA parameters, namely failure load and apparent modulus,
237 thus providing strong evidences of the role of derangement in bone health on disease
238 progression in AIS.

239 Apart from FEA parameters, other determinants of the treatment effect of Ca+Vit-D
240 supplementation were also noted in this study. Firstly, Ca+Vit-D supplementation was
241 noted to be significantly more effective for those with low baseline serum 25(OH)Vit-D
242 levels ≤ 50 nmol/L (ie Vit-D insufficiency). Likewise, Ca+Vit-D supplementation was
243 significantly more effective for those with low baseline dietary calcium intake ≤ 1000
244 mg/day.

245 In conclusion, this study demonstrated improvement in bone health and control of
246 curve progression with Ca+Vit-D supplementation for AIS girls having Z-score of
247 femoral neck aBMD < 0. The recruitment criteria of confining those with Z-score of
248 femoral neck aBMD < 0 should be considered when evaluating the external validity of
249 this study. Given the suboptimal 25(OH)Vit-D levels detected in this study and the

association between AIS and low bone mass, Vit-D status, bone mineral density and bone quality should be assessed for newly diagnosed AIS subjects. For those with low BMD, Ca+Vit-D supplementation should be started early for achieving healthy bone status and preventing curve progression from reaching bracing or surgical thresholds especially for those with low Vit-D levels and low dietary calcium intake at baseline. Whether the same therapeutic effects can be achieved with enhanced Ca+Vit-D levels through food fortification and lifestyle modification and the mechanism behind the therapeutic effects of Ca+Vit-D supplementation in AIS warrant further studies.

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Abbreviation

AIS: Adolescent Idiopathic Scoliosis

aBMD: areal bone mineral density

BMC: bone mineral content

BV/TV: Trabecular Bone Volume to Tissue Volume Ratio

Ca+Vit-D supplementation: Calcium plus Vitamin D supplementation

DXA: Dual Energy X-ray Absorptiometry

FEA: finite element analysis

FEM: finite element model

HR-pQCT: High Resolution Peripheral Quantitative Computed Tomography

vBMD: volumetric bone mineral density

YSM: Year-since-Menarche

278 Figure 1 The Trial Profile

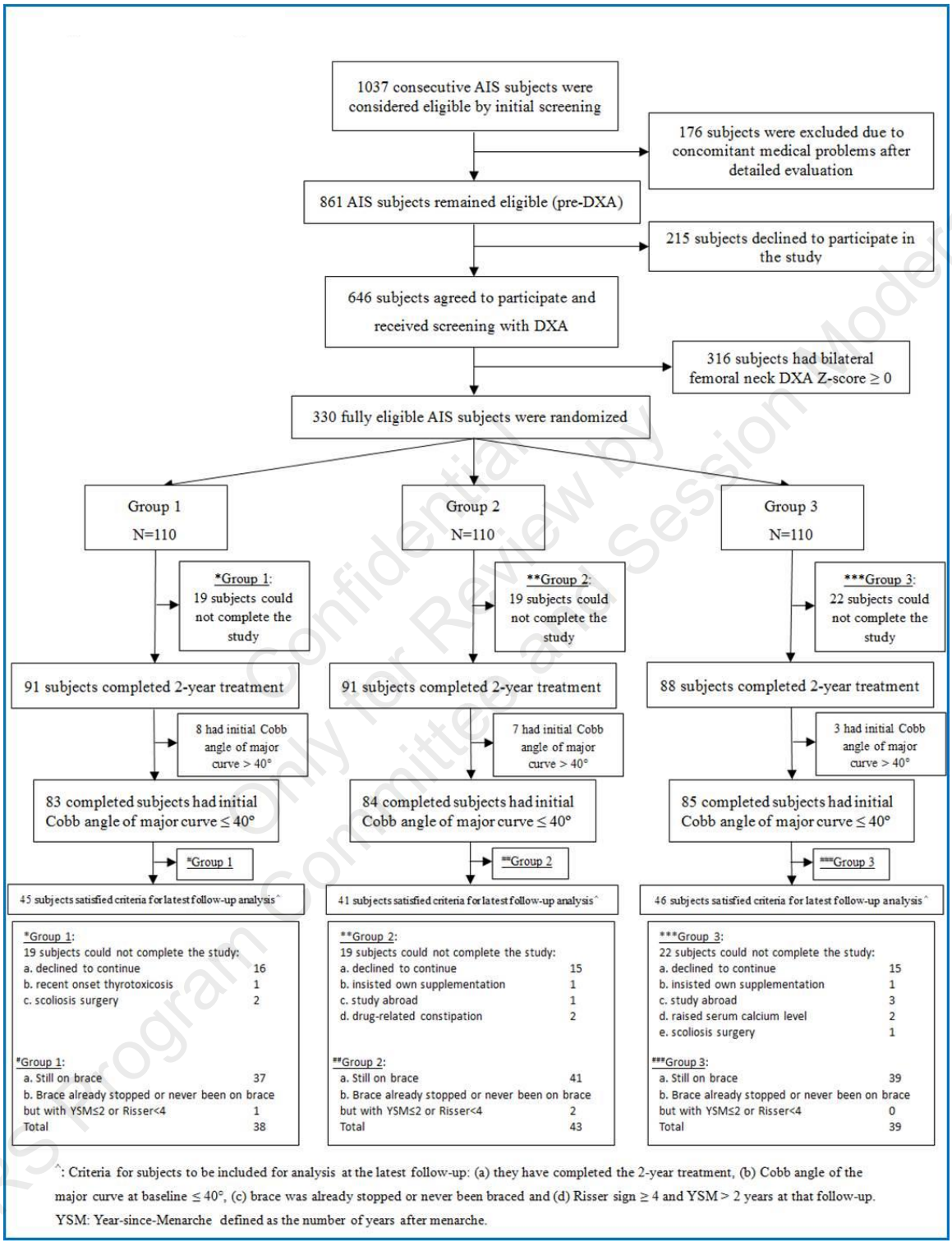

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Table 1: Baseline characteristics of maturity, anthropometric, lifestyle, biochemical, HR-pQCT and Finite Element Analysis (FEA) parameters for the three study groups

	mean ± SD at baseline		
	Group 1, N=110	Group 2, N=110	Group 3, N=110
Age (year)	13·03 ± 0·86	12·92 ± 0·91	12·81 ± 0·89
Tanner (breast)	2·88 ± 0·70	2·84 ± 0·75	2·75 ± 0·74
Risser sign	2·01 ± 1·60	1·92 ± 1·65	1·58 ± 1·68
Body mass (Kg)	40·41 ± 6·14	40·50 ± 5·73	40·33 ± 5·80
Standing height (cm)	153·8 ± 6·6	154·0 ± 6·1	153·4 ± 6·2
Armspan (cm)	153·4 ± 6·9	153·0 ± 7·0	152·5 ± 7·5
Physical activity level (Baecke score)	7·30 ± 0·93	7·08 ± 0·91	7·14 ± 0·82
Dietary Vit-D intake (IU/day)*	116·5 (125·6)	109·8 (138·4)	110·1 (132·9)
Dietary calcium intake (mg/day)*	603·9 (539·8)	618·6 (523·9)	578·1 (361·9)
Serum 25(OH)Vit-D (nmol/L)	41·4 ± 13·3	42·3 ± 14·3	39·4 ± 15·4
Adjusted serum calcium (mmol/L)	2·28 ± 0·09	2·29 ± 0·09	2·28 ± 0·09
Cobb angle of major curve (°)	26·0 ± 9·1	26·3 ± 8·5	25·8 ± 9·1
Number of subjects treated with bracing (%)	85 (77·3%)	90 (81·8%)	86 (78·2%)
HR-pQCT parameters			
Cortical Area in mm^2	23·067 ± 10·977	21·898 ± 11·300	20·404 ± 11·075
Trabecular Area in mm^2	150·400 ± 29·557	147·664 ± 29·847	151·595 ± 27·963
Cortical Thickness in mm	0·417 ± 0·210	0·414 ± 0·234	0·368 ± 0·216
Cortical Perimeter in mm	55·039 ± 4·675	54·425 ± 4·582	54·798 ± 4·080
Average vBMD in mg HA/cm^3	243·155 ± 43·719	240·847 ± 52·171	233·448 ± 46·457
Cortical vBMD in mg HA/cm^3	675·261 ± 79·615	669·159 ± 84·519	656·724 ± 82·802
Trabecular vBMD in mg HA/cm^3	143·225 ± 23·481	140·619 ± 25·299	140·204 ± 25·856
Trabecular Bone Volume to Tissue Volume Ratio	0·119 ± 0·020	0·117 ± 0·021	0·117 ± 0·022
Trabeculae Number /mm	1·689 ± 0·215	1·692 ± 0·229	1·675 ± 0·233
Trabecular Thickness in mm	0·071 ± 0·008	0·069 ± 0·009	0·070 ± 0·008
Trabecular Separation in mm	0·531 ± 0·082	0·535 ± 0·101	0·539 ± 0·086
Finite Element Analysis (FEA) parameters			
FEA: stiffness (kN/mm)	45198 ± 9421	43118 ± 9800	43901 ± 10302
FEA: failure load (N)	1895 ± 366	1825 ± 403	1849 ± 423
FEA: apparent modulus (MPa)	1666 ± 382	1644 ± 450	1648 ± 414

HR-pQCT: High-resolution Peripheral Quantitative Computed Tomography (XtremeCT)
SD: standard deviation
*: median (inter-quartile range)
vBMD: volumetric bone mineral density
Between-group comparison on baseline characteristics: all with p>0·05

Table 2

Prevalence of Vit-D insufficiency and mean changes in serum 25(OH)Vit-D, HR-pQCT and Finite Element Analysis (FEA) parameters from baseline to 2-year time-point for Group 1, Group 2 and Group 3[§]

		Gp 1 N=91	Gp 2 N=91	Gp 3 N=88	p-value	
					Gp 1 Vs Gp 2	Gp 1 Vs Gp 3
Proportion with Vit-D insufficiency	Baseline (N=330) [#]	74·5%	70·0%	75·5%	0·274	0·500
	2-year time-point (N=270) [#]	62·6%	30·8%	19·3%	<0·001^	<0·001^
	p-value for within-group comparison between Baseline Vs 2-year time-point [##]	0·035^	<0·001^	<0·001^		
		Changes from baseline to 2-year time-point[§] mean ± SD			p	
		Gp 1 N=91	Gp 2 N=91	Gp 3 N=88	Gp 1 Vs Gp 2	Gp 1 Vs Gp 3
Serum 25(OH)Vit-D (nmol/L) [###]		6·3 ± 15·3	20·4 ± 19·6	28·0 ± 23·3	<0·001^	<0·001^
HR-pQCT parameters						
Cortical Area in mm^2 [###]		17·698 ± 4·793	19·175 ± 6·731	19·923 ± 6·935	0·244	0·050
Trabecular Area in mm^2 [###]		-9·370 ± 15·019	-10·030 ± 18·120	-10·031 ± 20·253	0·698	0·771
Cortical Thickness in mm [###]		0·320 ± 0·100	0·339 ± 0·151	0·361 ± 0·145	0·442	0·098
Cortical Perimeter in mm [###]		0·819 ± 2·042	0·717 ± 2·481	0·814 ± 2·753	0·558	0·858
Average vBMD in mg HA/cm^3 [###]		59·595 ± 25·897	69·723 ± 41·090	70·189 ± 33·286	0·040^	0·032^
Cortical vBMD in mg HA/cm^3 [###]		113·374 ± 36·436	126·563 ± 46·540	124·400 ± 49·758	0·131	0·360
Trabecular vBMD in mg HA/cm^3 [###]		-6·224 ± 11·861	-0·673 ± 14·575	0·474 ± 10·929	0·004^	0·001^
Trabecular Bone Volume to Tissue Volume Ratio [###]		-0·005 ± 0·010	-0·001 ± 0·012	0·000 ± 0·009	0·004^	0·001^
Trabeculae Number /mm [###]		-0·133 ± 0·138	-0·089 ± 0·161	-0·077 ± 0·157	0·035^	0·015^
Trabecular Thickness in mm [###]		0·003 ± 0·007	0·004 ± 0·005	0·004 ± 0·007	0·495	0·348
Trabecular Separation in mm [###]		0·052 ± 0·055	0·033 ± 0·058	0·029 ± 0·058	0·027^	0·008^
FEA parameters						
		Gp 1 N=83	Gp 2 N=78	Gp 3 N=72		
FEA: stiffness (kN/mm) [###]		13455 ± 4670	15786 ± 5701	16520 ± 5563	0.048^	0.001^
FEA: failure load (N) [###]		533 ± 193	622 ± 243	658 ± 252	0.094	0.002^
FEA: apparent modulus (MPa) [###]		465 ± 220	591 ± 353	588 ± 289	0.020^	0.028^

[§]: Changes from baseline to 2-year time-point refers to the parameter at 2-year minus that at baseline

297 [#]: p-value from Chi-square for between-group comparison
298 [##]: p-value from McNemar test for within-group comparison on the Vit-D status between baseline and 2-year time-point
299 for the 270 subjects who have completed the 2-year treatment
300 [###]: p-value from ANCOVA
301 ^: p-value < 0·05
302

303 Table 3 Spinal deformity parameters and curve progression at the latest follow-up

At latest follow-up# (N =132)				
	Group 1, N=45	Group 2, N=41	Group 3, N=46	p-value*
Cobb angle at baseline	22.2 ± 6.3	24.0 ± 6.7	24.6 ± 7.2	0.230
Age at baseline (year)	13.3 ± 0.8	13.3 ± 0.7	13.2 ± 0.8	0.897
YSM at baseline (year)	0.95 ± 0.95	1.28 ± 0.94	1.18 ± 1.08	0.294
Interval from baseline to latest follow-up (months)	43.2 ± 9.9	43.1 ± 11.0	41.7 ± 10.6	0.747
At latest follow-up# (N =132)				
	Cobb## progression < 6°	Cobb## progression ≥ 6°	p-value**	
Group 1, N=45	24 (53·3%)	21 (46·7%)		
Group 2, N=41	31 (75·6%)	10 (24·4%)	Group 2 Vs Group1: p=0·032^	
Group 3, N=46	36 (78·3%)	10 (21·7%)	Group 3 Vs Group1: p=0·012^	
Logistic Regression Analysis### at latest follow-up# (N = 132)				
	Adjusted odds ratio***	95% CI for adjusted odds ratio	p-value for regression coefficient of the dummy variable	
Group 2 Vs Group 1	0·366	0·138 – 0·966	0·042^	
Group 3 Vs Group 1	0·343	0·132 – 0·892	0·028^	
At latest follow-up# for subjects with baseline serum 25(OH)Vit-D ≤ 50 nmol/L (N = 103)				
	Cobb## progression < 6°	Cobb## progression ≥ 6°	p-value**	
Group 1, N=35	18 (51·4 %)	17 (48·6 %)		
Group 2, N=31	24 (77·4 %)	7 (22·6%)	Group 2 Vs Group1: p=0·028^	
Group 3, N=37	31 (83·8 %)	6 (16·2 %)	Group 3 Vs Group1: p=0·003^	
At latest follow-up# for subjects with baseline serum 25(OH)Vit-D > 50 nmol/L (N = 29)				
	Cobb## progression < 6°	Cobb## progression ≥ 6°	p-value**	
Group 1, N=10	6 (60.0 %)	4 (40.0 %)		
Group 2, N=10	7 (70.0 %)	3 (30.0 %)	Group 2 Vs Group1: p=0.639	
Group 3, N=9	5 (55.6 %)	4 (44.4 %)	Group 3 Vs Group1: p=0.845	
At latest follow-up# for subjects with baseline dietary calcium intake ≤ 1000 mg/day (N = 109)				
	Cobb## progression < 6°	Cobb## progression ≥ 6°	p-value**	
Group 1, N=35	16 (45.7 %)	19 (54.3 %)		
Group 2, N=32	27 (84.4 %)	5 (15.6 %)	Group 2 Vs Group1: p=0.001^	
Group 3, N=42	34 (81 %)	8 (19.0 %)	Group 3 Vs Group1: p=0.001^	
At latest follow-up# for subjects with baseline dietary calcium intake > 1000 mg/day (N = 23)				
	Cobb## progression < 6°	Cobb## progression ≥ 6°	p-value**	
Group 1, N=10	8 (80.0 %)	2 (20.0 %)		
Group 2, N=9	4 (44.4 %)	5 (55.6 %)	Group 2 Vs Group1: p=0.170	
Group 3, N=4	2 (50.0 %)	2 (50.0 %)	Group 3 Vs Group1: p=0.520	

304

305 YSM: Year-since-menarche = chronological age – age of menarche

306 [#]: Criteria for subjects to be included for analysis at the latest follow-up: (a) they have completed the 2-year treatment,
307 (b) Cobb angle of the major curve at baseline ≤ 40°, (c) brace was already stopped or never been braced and (d) Risser
308 sign ≥ 4 and YSM > 2 years at that follow-up.

309 [*]: p-value from 1-way ANOVA

310 [##]: Cobb angle measured at the major curve

311 [**]: p-value from Chi-square

312 [###]: Logistic regression analysis was done with "Progression in Cobb angle ≥ 6°" as the dichotomous dependent
313 variable while "Bracing History", "Cobb angle of the major curve at baseline" and "YSM at baseline" as the
314 independent variables. Exposure variable has three values, namely Group 1, Group 2 and Group 3. Two dummy
315 variables were created, namely DummyGroup_2_1 (Group 2 Vs Group 1 using Group 1 as the reference group) and
316 DummyGroup_3_1 (Group 3 Vs Group 1 using Group 1 as the reference group)

317 [***]: adjusted odds ratio for curve progression in Cobb angle ≥ 6° from Baseline to the latest follow-up with Group 1 as
318 the reference group

319 [^]: p<0·05

320

References

1. Grivas TB, Vasiliadis E, Savvidou O, et al. Geographic latitude and prevalence of adolescent idiopathic scoliosis. Stud Health Technol Inform. 2006;123:84-9.
2. Weinstein SL, Dolan LA, Cheng JC, et al. Adolescent idiopathic scoliosis. Lancet. 2008 May 3;371(9623):1527-37.
3. Bowden SA, Robinson RF, Carr R, et al. Prevalence of vitamin D deficiency and insufficiency in children with osteopenia or osteoporosis referred to a pediatric metabolic bone clinic. Pediatrics. 2008 Jun;121(6):e1585-90.
4. Cheng JC, Guo X, Sher AH. Persistent osteopenia in adolescent idiopathic scoliosis. A longitudinal follow up study. Spine. 1999 Jun 15;24(12):1218-22.
5. Hung VW, Qin L, Cheung CS, et al. Osteopenia: a new prognostic factor of curve progression in adolescent idiopathic scoliosis. The Journal of bone and joint surgery. 2005 Dec;87(12):2709-16.
6. Lee WTK, Cheung CSK, Tse YK, et al. Generalized Low Bone Mass of Girls with Adolescent Idiopathic Scoliosis is Related to Inadequate Calcium Intake and Weight Bearing Physical Activity in Peripubertal Period. Osteoporos Int. 2005;16(9):1024-35.
7. Holick MF, Garabedian M. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, ed. *Primer on the metabolic bone diseases and disorders of mineral metabolism*. 6th ed. Washington, DC: American Society for Bone and Mineral Research 2006:129-37.
8. McCarthy EK, Kiely M. Vitamin D and muscle strength throughout the life course: a review of epidemiological and intervention studies. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2014 Oct 3.

9. Zaleske DJ, Doppelt SH, Mankin HJ. Metabolic and Endocrine Abnormalities of the Immature Skeleton. In: Morrissy RT, ed. *Lovell and Winter's Pediatric Orthopaedics*. Philadelphia: J.B. Lippincott Company 1990:203-61.
10. Fraser DR. Vitamin D-deficiency in Asia. The Journal of steroid biochemistry and molecular biology. 2004 May;89-90(1-5):491-5.
11. Cheung CS, Lee WT, Tse YK, et al. Generalized osteopenia in adolescent idiopathic scoliosis--association with abnormal pubertal growth, bone turnover, and calcium intake? Spine. 2006 Feb 1;31(3):330-8.
12. Lam TP, Ng BKW, Lee KM, et al. Serum 25(OH)vitamin D level and Its correlation with bone mineral density in girls with Adolescent Idiopathic Scoliosis (AIS). Scoliosis. 2015;10(Suppl 1):O7.
13. Hansen S, Brixen K, Gravholt CH. Compromised trabecular microarchitecture and lower finite element estimates of radius and tibia bone strength in adults with turner syndrome: a cross-sectional study using high-resolution-pQCT. J Bone Miner Res. 2012 Aug;27(8):1794-803.
14. Taylor M, Prendergast PJ. Four decades of finite element analysis of orthopaedic devices: where are we now and what are the opportunities? Journal of biomechanics. 2015 Mar 18;48(5):767-78.
15. Mueller TL, Christen D, Sandercott S, et al. Computational finite element bone mechanics accurately predicts mechanical competence in the human radius of an elderly population. Bone. 2011 Jun 01;48(6):1232-8.
16. Richards BS, Bernstein RM, D'Amato CR, et al. Standardization of criteria for adolescent idiopathic scoliosis brace studies: SRS Committee on Bracing and Nonoperative Management. Spine (Phila Pa 1976). 2005 Sep 15;30(18):2068-75; discussion 76-7.

17. Nikodem A. Correlations between structural and mechanical properties of human trabecular femur bone. Acta of bioengineering and biomechanics / Wroclaw University of Technology. 2012;14(2):37-46.

Appendix

Table S1

Distribution of curve types for Group 1, 2 and 3

Curve Type	Study Group Number of subjects (% within the Study Group)			Total	p-value[##]
	Group 1	Group 2	Group 3		
Single Thoracic Curve	24 (21.8%)	16 (14.5%)	19 (17.3%)	59 (17.9%)	0.751
Double Thoracic Curve	10 (9.1%)	6 (5.5%)	6 (5.5%)	22 (6.7%)	
Double Curve	44 (40.0%)	49 (44.5%)	46 (41.8%)	139 (42.1%)	
Thoracolumbar Curve	16 (14.5%)	18 (16.4%)	24 (21.8%)	58 (17.6%)	
Lumbar Curve	10 (9.1%)	15 (13.6%)	10 (9.1%)	35 (10.6%)	
Triple Curve & Misc	6 (5.5%)	6 (5.5%)	5 (4.5%)	17 (5.2%)	
Total	110 (100.0%)	110 (100.0%)	110 (100.0%)	330 (100.0%)	