

**Association of serum 25-hydroxyvitamin D with bone health measured by calcaneal quantitative ultrasound: a large cross-sectional analysis in children and adolescents**

**Supplementary online content**

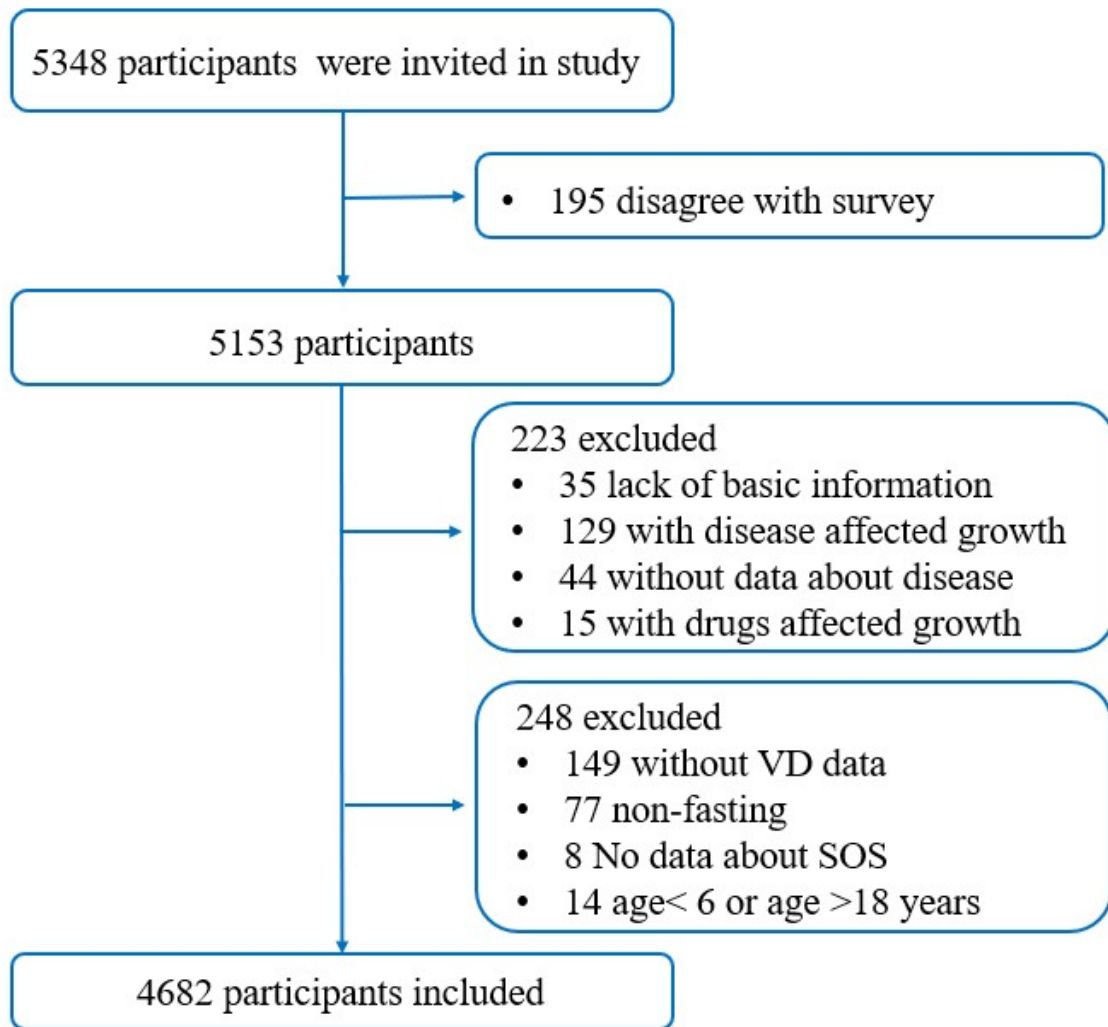
**Supplemental Figure 1.** Flow of participants in current study.

**Supplemental Figure 2.** The association between serum 25-hydroxyvitamin D levels and SOS Z score stratified by age (A), gender (B), stage of puberty (C), and weight status (D).

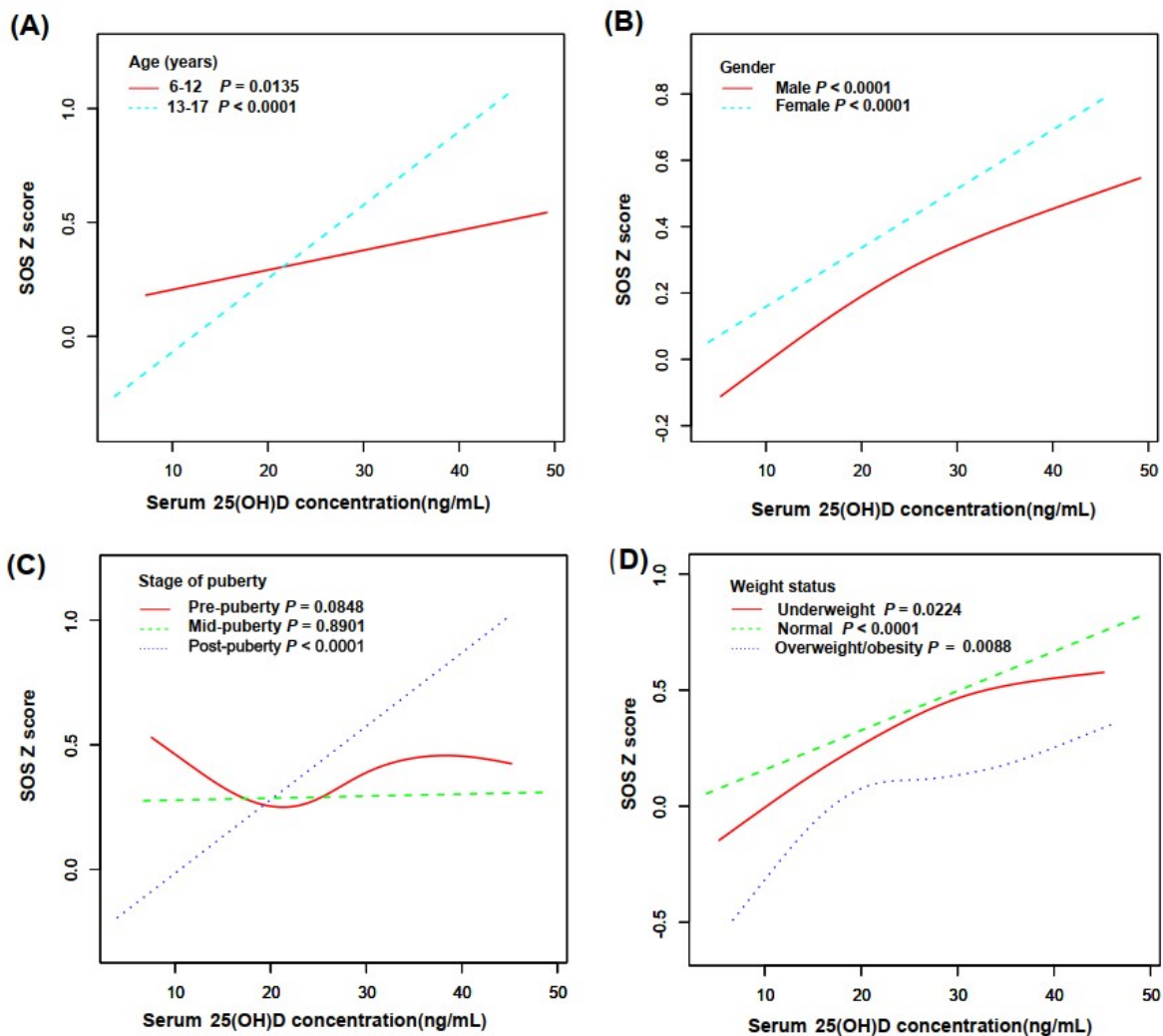
**Supplemental Table 1.** Baseline characteristics of 4682 participants by quartiles of serum 25-hydroxyvitamin D3.

**Supplemental Table 2.** Stratified analyses of potential modification effect for the association between serum 25-hydroxyvitamin D and SOS Z score.

**Supplemental Table 3.** Association between serum vitamin D status and SOS (SOS Z score).



**Supplemental Figure 1. Flow of participants in current study**



**Supplemental Figure 2. The association between serum 25-hydroxyvitamin D levels and SOS Z score stratified by age (A), gender (B), stage of puberty (C), and weight status (D).** Spline smoothing, based on a generalized additive model, was used, the models were adjusted as followed: age, gender, paternal education levels, maternal education levels, household income, pubertal development stages, weight status, sedentary behaviour, nighttime sleep duration, nap duration, levels of MVPA, drinking status, smoking status, vitamin D supplement, calcium supplement, and multivitamin mineral supplement. Analyses that stratified by age (6-12 or 13-18years), gender (male or female), stage of puberty (pre-, mid-, or post-puberty), and weight status (underweight, normal weight, or overweight/obesity), each of the else groups were adjusted for all covariates expect itself.

**Supplemental Table 1. Baseline characteristics of 4682 participants by quartiles of serum 25-hydroxyvitamin D<sub>3</sub>.**

Variables	Q1	Q2	Q3	Q4	P
<b>N</b>	1170	1170	1172	1170	
<b>Age, years</b>	14.7 (12.4-16.2)	12.6 (10.2-14.8)	10.9 (8.8-13.4)	9.4 (7.9-12.0)	<0.001
<b>Gender (female), n (%)</b>	695 (59.4%)	534 (45.6%)	453 (38.7%)	413 (35.3%)	<0.001
<b>Stage of puberty, n (%)</b>					<0.001
Pre-puberty	109 (9.3%)	267 (22.8%)	440 (37.5%)	638 (54.5%)	
Mid-puberty	168 (14.4%)	260 (22.2%)	335 (28.6%)	266 (22.7%)	
Post-puberty	893 (76.3%)	643 (55.0%)	397 (33.9%)	266 (22.7%)	
<b>BMI, kg/m<sup>2</sup></b>	19.2 (17.2-21.7)	18.6 (16.3-21.4)	17.6 (15.4-20.4)	16.8 (15.0-19.6)	<0.001
<b>Weight status, n (%)</b>					0.002
Underweight	76 (6.5%)	97 (8.3%)	114 (9.7%)	123 (10.5%)	
Normal weight	860 (73.5%)	790 (67.5%)	781 (66.6%)	770 (65.8%)	
Overweight	143 (12.2%)	165 (14.1%)	157 (13.4%)	170 (14.5%)	
Obesity	91 (7.8%)	118 (10.1%)	120 (10.2%)	107 (9.1%)	
<b>Sedentary behaviour, hour/d</b>	9.6 (7.7-11.4)	8.2 (6.9-10.1)	7.6 (6.4-9.2)	7.1 (5.9-8.8)	<0.001
<b>nighttime sleep duration, hour/d</b>	8.0 (7.4-8.7)	8.5 (7.9-9.2)	8.8 (8.1-9.3)	9.0 (8.3-9.4)	<0.001
<b>Nap duration, hour/d</b>	0.6 (0.4-0.9)	0.7 (0.4-1.0)	0.6 (0.4-1.0)	0.7 (0.4-1.0)	0.23
<b>Levels of MVPA, n (%)</b>					<0.001
<0.5hour/d	419 (35.8%)	305 (26.1%)	281 (24.0%)	296 (25.3%)	
~1hour/d	527 (45.0%)	530 (45.3%)	540 (46.1%)	530 (45.3%)	
~3hour/d	185 (15.8%)	291 (24.9%)	293 (25.0%)	290 (24.8%)	
>3hour/d	31 (2.6%)	35 (3.0%)	47 (4.0%)	41 (3.5%)	
missing	8 (0.7%)	9 (0.8%)	11 (0.9%)	13 (1.1%)	
<b>Smoking status</b>					0.578
Never	1144 (97.8%)	1142 (97.6%)	1148 (98.0%)	1150 (98.3%)	
<1 cigarette/month	11 (0.9%)	9 (0.8%)	5 (0.4%)	5 (0.4%)	
>= 1cigarette/month	6 (0.5%)	6 (0.5%)	6 (0.5%)	3 (0.3%)	
e-cigarette	2 (0.2%)	2 (0.2%)	4 (0.3%)	0 (0.0%)	
Missing	7 (0.6%)	11 (0.9%)	9 (0.8%)	12 (1.0%)	
<b>Drinking status,</b>					<0.001
Never	978 (83.6%)	1011 (86.4%)	1052 (89.8%)	1098 (93.8%)	
<1 standard drink/ month	134 (11.5%)	126 (10.8%)	85 (7.3%)	56 (4.8%)	
>= 1 standard drink/ month	52 (4.4%)	27 (2.3%)	28 (2.4%)	13 (1.1%)	
Missing	6 (0.5%)	6 (0.5%)	7 (0.6%)	3 (0.3%)	
<b>Vitamin D supplement, n (%)</b>					<0.001
No	1063 (90.9%)	1016 (86.8%)	979 (83.5%)	946 (80.9%)	
Yes	102 (8.7%)	153 (13.1%)	189 (16.1%)	222 (19.0%)	
Missing	5 (0.4%)	1 (0.1%)	4 (0.3%)	2 (0.2%)	
<b>Calcium supplement, n (%)</b>					<0.001
No	1029 (87.9%)	969 (82.8%)	935 (79.8%)	934 (79.8%)	
Yes	136 (11.6%)	200 (17.1%)	234 (20.0%)	234 (20.0%)	
Missing	5 (0.4%)	1 (0.1%)	3 (0.3%)	2 (0.2%)	
<b>Multivitamin mineral supplement, n (%)</b>					0.669
No	1072 (91.6%)	1058 (90.4%)	1054 (89.9%)	1059 (90.5%)	
Yes	89 (7.6%)	107 (9.1%)	111 (9.5%)	103 (8.8%)	
Missing	9 (0.8%)	5 (0.4%)	7 (0.6%)	8 (0.7%)	
<b>Household income, n (%)</b>					<0.001
<120,000 CNY/years	367 (31.4%)	343 (29.3%)	276 (23.5%)	250 (21.4%)	
~250,000 CNY/years	387 (33.1%)	393 (33.6%)	409 (34.9%)	389 (33.2%)	
>=250,000 CNY/years	404 (34.5%)	424 (36.2%)	479 (40.9%)	527 (45.0%)	
Missing	12 (1.0%)	10 (0.9%)	8 (0.7%)	4 (0.3%)	

<b>Paternal education level, n (%)</b>					<0.001
<=9 years	266 (22.7%)	204 (17.4%)	154 (13.1%)	135 (11.5%)	
10~12 years	289 (24.7%)	286 (24.4%)	260 (22.2%)	219 (18.7%)	
13~15 years	324 (27.7%)	329 (28.1%)	317 (27.0%)	339 (29.0%)	
>=16 years	277 (23.7%)	339 (29.0%)	434 (37.0%)	465 (39.7%)	
Missing	14 (1.2%)	12 (1.0%)	7 (0.6%)	12 (1.0%)	
<b>Maternal education level, n (%)</b>					<0.001
<=9 years	313 (26.8%)	244 (20.9%)	199 (17.0%)	165 (14.1%)	
10~12 years	337 (28.8%)	324 (27.7%)	284 (24.2%)	270 (23.1%)	
13~15 years	276 (23.6%)	323 (27.6%)	355 (30.3%)	370 (31.6%)	
>=16 years	230 (19.7%)	277 (23.7%)	329 (28.1%)	356 (30.4%)	
Missing	14 (1.2%)	2 (0.2%)	5 (0.4%)	9 (0.8%)	
<b>25(OH)D, ng/mL</b>	14.6 (12.5-16.1)	19.4 (18.3-20.4)	23.6 (22.4-24.7)	29.5 (27.5-32.2)	<0.001
<b>25(OH)D<sub>2</sub>, ng/mL</b>	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	<0.001
<b>25(OH)D<sub>3</sub>, ng/mL</b>	13.9 (11.9-15.5)	18.9 (17.8-19.8)	23.0 (22.0-24.1)	29.0 (27.0-31.7)	<0.001
<b>SOS, m/s</b>	1550.0 (1532.0-1574.0)	1551.0 (1532.0-1574.0)	1551.0 (1533.0-1573.0)	1555.0 (1534.0-1575.0)	0.708
<b>SOS Z score</b>	0.0 (-0.6-0.8)	0.2 (-0.4-0.9)	0.3 (-0.3-0.9)	0.3 (-0.3-1.0)	<0.001

Abbreviations: CNY, Chinese Yuan; MVPA, moderate to vigorous physical activity

**Supplemental Table 2. Stratified analyses of potential modification effect for the association between serum 25-hydroxyvitamin D and SOS Z score <sup>a</sup>.**

	N	Q1	Q2	Q3	Q4	<i>P</i> trend <sup>b</sup>	Per 1SD increment	<i>P</i> interaction <sup>c</sup>
<b>SOS</b>								
<b>Age, years</b>								
6-12	2762	0(reference)	0.10 (-0.03, 0.23)	0.07 (-0.06, 0.19)	0.17 (0.05, 0.30)	0.01	0.06 (0.02, 0.10)	<0.0001
13-18	1920	0(reference)	0.11 (-0.02, 0.23)	0.35 (0.20, 0.49)	0.48 (0.31, 0.65)	<0.0001	0.19 (0.13, 0.25)	
<b>Gender</b>								
Male	2587	0(reference)	0.07 (-0.05, 0.19)	0.22 (0.10, 0.33)	0.26 (0.14, 0.38)	<0.0001	0.11 (0.06, 0.15)	0.2117
Female	2095	0(reference)	0.16 (0.03, 0.30)	0.15 (0.01, 0.30)	0.31 (0.15, 0.47)	0.0003	0.10 (0.05, 0.16)	
<b>Stage of puberty</b>								
Pre-puberty	1454	0(reference)	-0.11 (-0.33, 0.11)	-0.10 (-0.31, 0.11)	0.03 (-0.17, 0.23)	0.0916	0.05 (-0.01, 0.11)	0.0004
Mid-puberty	1029	0(reference)	0.00 (-0.19, 0.20)	0.06 (-0.13, 0.25)	0.04 (-0.16, 0.24)	0.5669	0.02 (-0.05, 0.10)	
Post-puberty	2199	0(reference)	0.17 (0.05, 0.28)	0.31 (0.18, 0.45)	0.48 (0.33, 0.64)	<0.0001	0.19 (0.13, 0.24)	
<b>Weight status</b>								
Underweight	410	0(reference)	0.06 (-0.28, 0.40)	0.20 (-0.14, 0.54)	0.40 (0.04, 0.76)	0.009	0.17 (0.05, 0.29)	0.6250
Normal weight	3201	0(reference)	0.09 (-0.01, 0.20)	0.18 (0.07, 0.30)	0.29 (0.18, 0.41)	<0.0001	0.11 (0.07, 0.15)	
Overweight/obesity	1071	0(reference)	0.28 (0.10, 0.46)	0.27 (0.08, 0.45)	0.31 (0.11, 0.52)	0.0081	0.12 (0.05, 0.19)	

<sup>a</sup> Generalized linear models were used. Model adjusted age, gender, paternal education levels, maternal education levels, household income, pubertal development stages, weight status, sedentary behaviour, nighttime sleep duration, nap duration, levels of MVPA, drinking status, smoking status, vitamin D supplement, calcium supplement, and multivitamin mineral supplement.

<sup>b</sup> *P* value for trend was test by using the median value with each quartile.

<sup>c</sup> *P* value for interaction was test by using log likelihood ratio test.

**Supplemental Table 3. Association between serum vitamin D status <sup>a</sup> and SOS (SOS Z score) <sup>b</sup>.**

	<b>Deficiency</b>	<b>Insufficiency</b>	<b>Sufficiency</b>	<b><i>P</i> trend <sup>c</sup></b>
<b>N (%)</b>	668 (14.3%)	1258 (26.9%)	2756 (58.9%)	
<b>SOS</b>				
Model 1	0(reference)	3.88 (0.72, 7.03)	7.43 (4.35, 10.50)	<0.0001
Model 2	0(reference)	3.58 (0.43, 6.74)	7.61 (4.51, 10.70)	<0.0001
<b>SOS Z score</b>				
Model 1	0(reference)	0.14 (0.04, 0.24)	0.26 (0.16, 0.36)	<0.0001
Model 2	0(reference)	0.12 (0.02, 0.22)	0.25 (0.15, 0.35)	<0.0001

<sup>a</sup> The definition of vitamin D status based on criteria 2: serum vitamin D  $\leq 15$  ng/ml, 15-20 ng/ml, >20 ng/ml were defined as deficiency, insufficiency, and sufficiency, respectively.

<sup>b</sup> Generalized linear model were used. Model adjusted age, gender, paternal education levels, maternal education levels, household income, pubertal development stages, weight status, sedentary behaviour, nighttime sleep duration, nap duration, levels of MVPA, drinking status, smoking status, vitamin D supplement, calcium supplement, and multivitamin mineral supplement.

<sup>c</sup> *P* value for trend was test by using the median value with each quartile.