

Original Research

Associations of Serum Mineral levels of Copper, Magnesium and Calcium with Skeletal Muscle mass and Strength in Chinese children, aged 6-11: A Cross-Sectional Study

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Background: Skeletal Muscle Mass (SMM) and strength are crucial for children's health, and low SMM can lead to adverse health outcomes. A deficiency of essential minerals can cause poor growth, fractures or osteoporosis. The study aimed to explore the relationship between serum Calcium (Ca), Copper (Cu), and Magnesium (Mg) levels and SMM in Chinese children. **Methods:** 466 primary school children were recruited for a cross-section study aged 6-11 in Guangzhou City, South of China, from December 2015 to February 2017. The study conducted whole-body dual-energy x-ray absorptiometry (DXA) scans using the Hologic discovery W to obtain the Total skeletal mass (TSM) and appendicular skeletal mass (ASM) were calculated, which were used as parameters to calculate variables of Skeletal muscle mass (SMM). **Results:** Of the 466 primary school children, 266 were boys and 200 girls with mean ages of 8.00±1.00 years for boys and 8.08±0.97 years for girls. An increase in mean Cu serum concentration between the T1 and T3 in girls resulted in 3.19% (P- trend =0.034, P-diff =0.083), 3.57% (P- trend =0.037, P-diff=0.083), 2.44% (P- trend =0.018, P=diff=0.060) and 2.25% (P- trend =0.024, P-diff= 0.072) for ASM/Height, ASH/ Weight, TSM/ Height and TSM/ Weight respectively. No significant mean difference was observed between serum Cu and ASM, TSM, ASM/ AFM and TSM/TFM (P- trend >0.05). A significant mean difference in serum Mg levels and ASM/ AFM for the total and boys' group was observed, with 6.68% and 9.44% (P- trend =0.042 and 0.041) respectively. No significant mean differences were seen between all the variables of serum Ca levels and all the variables of skeletal muscle mass and strength. **Conclusions:** Serum copper levels influence the development of SMM in girls, but not in boys. Serum Ca and Mg level in girls and boys do not influence SMM development among children aged 6-11.

Keywords: Skeletal muscle mass; muscle strength; copper; magnesium; calcium; children

1. INTRODUCTION

Skeletal Muscle mass (SMM) and strength are essential parameters in determining children's health throughout their stages of development and through adulthood. Similar to its properties in adulthood and aging, low skeletal

muscle mass and strength contribute to adverse health outcomes in childhood. Several studies have shown that children and adolescents with low skeletal muscle mass and strength have an increased risk of developing metabolic dysfunction and cardiovascular diseases.⁽¹⁻³⁾

Several macro and micro minerals are essential for maintaining healthy skeletal muscles and strength from childhood to adulthood. Copper (Cu), Magnesium (Mg), and Calcium (Ca) are essential for the normal development of the skeleton.⁽⁴⁾ Copper is as an essential trace element vital to all living organisms' health. Collagen is the primary structural material in bone. Its deficiency can lead to fractures, skeletal abnormalities, and osteoporosis.^(5,6) A study found that children with average bone mineral density (BMD) have higher serum levels of Cu than children with low BMD. The incidence of normal BMD increases as the serum level of Cu increases.⁽⁷⁾ Serum Cu concentrations were reported to have a positive correlation with the bone of the lumbar spine as measured using dual-energy X-ray absorptiometry and quantitative computerized tomography in premenopausal females⁽⁸⁾ and with bone density at total hip and spine in postmenopausal women.⁽⁹⁾

Magnesium (Mg) minerals play a significant role in bone structural formation and function of the human body.^(10,11) Studies have shown that presenting an average level of serum Mg concentration may not represent the normalcy of Mg in the body and bone.⁽¹²⁾ In a UK population of the EPIC-Norfolk cohort study, inconsistent result was observed between associations of serum Mg concentration groups and skeletal muscle mass indices was observed.⁽¹²⁾ These results may not be surprising considering the tight homeostatic control of serum Mg and the fact that less than 1% of total body Mg is present in the blood.⁽¹³⁾

Calcium (Ca) is an essential mineral of bones and teeth and plays a vital role as the second messenger in cell signalling pathways. Circulating serum Ca concentrations are strongly controlled by the parathyroid hormone (PTH) and vitamin D at the expense of the skeleton, with decrease in dietary calcium intakes.⁽¹⁴⁾ Serum Ca levels between subjects with average muscle mass and low muscle mass and strength showed no difference in serum Ca levels and variables of muscle mass between the study population, irrespective of definitions.⁽¹⁵⁾

However, plasma minerals concentration of Cu, Mg, and Ca may be essential nutrients that can prevent and treat loss of muscle mass and strength,⁽¹⁶⁾ but they still need to be studied. Having reviewed the available literature on SMM and strength, we discovered that information about childhood SMM and strength and their relationship with serum mineral concentration in children needs improvement. Therefore, this study aims to explain the associations of Cu, Mg, and Ca serum mineral levels and skeletal muscle mass and strength in Chinese children.

2. METHODS

2.1 Study population

A cross-section study aged 6-10 in Guangzhou City, South of China, from December 2015 to February 2017. The study recruited 465 primary school children, comprising of 265 boys and 200 girls aged 6-11 (Figure 1). The recruitment process involved two procedures described in the literature.⁽¹⁶⁾ Firstly, an invitation letter was sent to 1394 children from primary schools, including detailed inclusion and exclusion criteria. Among them, 315 children agreed to participate in the study. Subsequently, an advertisement and referral were created, recruiting 206 additional children, which made 521 participants for the study. However, 55 participants were excluded from the study, including 12 twins, 22 preterm births and 12 other medical condition, and 8 lacks completed data. The guardian or parent of all the participants received and signed written consent before enrolment for this study. The sample size was calculated using an infinite population of cross-sectional study formula ($(n) = N/1+N*d^2$).

2.2 Data collection

2.2.1 Anthropometric measurement

When measuring the physical development of children, a trained professional will measure their standing height using a portable stadiometer (Leicester Health Meter, Child Growth Foundation, London, UK, range 60-207 cm), accurate to the nearest 0.1cm. Weight was measured using Tanita MC-780A (Tanita Corporation, Tokyo, Japan), accurate to the nearest 0.1 kg. Both measurements are taken twice or thrice for each child and then averaged. The measuring device is calibrated at the start and end of each day, following the manufacturer's instruction. The children's Total skeletal mass (TSM), appendicular skeletal mass (ASM),

appendicular fat mass (AFM) and Total fat mass (TFM), and used as parameters in calculating the Skeletal muscle mass (SMM) were calculated using whole-body dual-energy x-ray absorptiometry (DXA) scans. The Hologic discovery W (Hologic Inc., Waltham, MA, USA), is used for scans, and the same technical staff complete the analysis. The Skeletal muscle mass (SMM) is

calculated using various parameters, including $TSM \text{ kg} / \text{Height}(h) \text{ m}^2$, $TSM \text{ kg} / \text{Weight}(W) \text{ kg} \times 100$, $TSM \text{ kg} / \text{AFM} \times 100$, $ASM \text{ kg} / \text{Height} (h) \text{ m}^2$ and $ASM \text{ kg} / \text{Weight} (W) \text{ kg} \times 100$, $ASM \text{ kg} / \text{TFM} \times 100$. The Body mass index (BMI) was calculated as $\text{Weight} (\text{kg})$ divided by $\text{Height} (\text{m}^2)$, using the WHO reference 2007 standard to calculate z score for BMI.

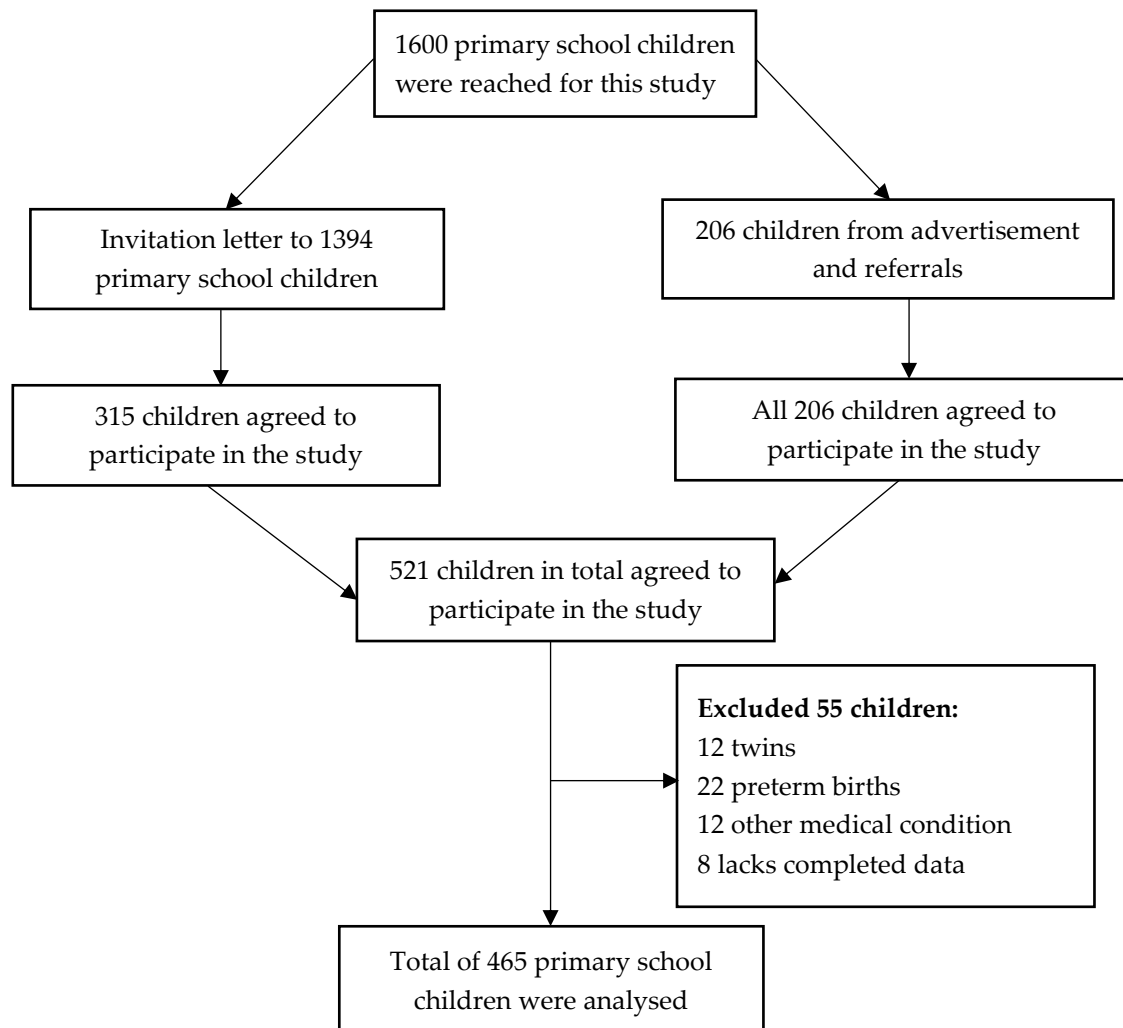


Figure 1. Sample size determination flow diagram for selection of study population

2.2.2 Muscle strength measurement (LHGS and RHGS)

Handgrip strength dynamometry is a reliable and accurate method of measuring muscle strength in children at school,^(17,18) The Jamar® Plus Hand Dynamometer (JAMAR® Hydraulic Hand Dynamometer, Sammons Preston, Bolingbrook, IL, USA) was used to measure handgrip strength, following the manufacturers' recommended testing posture. A trained hand therapist took all measurement. Children were made to take a sitting position, with both feet flat on the floor, shoulder adducted and a rotated elbow flexed at

90 degrees, with forearm and wrist occupying a neutral position. Measurements were taken from the dominated and non-dominated hands of all participants, and the handle was calibrated on the second position for all measurements. After familiarizing themselves with the Dynamometer, the children were instructed to squeeze the dynamometer as hard as possible for up to 4 to 5 seconds. Three alternating measurements were taken between the dominated and non-dominated hand, with a minute of rest between each measurement. The mean value of the measurement was recorded and used for analysis.

2.2.3 Blood/Serum mineral level measurement of Mg, Cu and Ca

A trained medical practitioner collected blood specimen from all participants in the morning hours. Children were made to fast overnight for not more than 10 hours. Each sample was stored for 30 minutes at 3000 rpm to obtain separate plasma. The plasma specimens were analysed after duplication of each sample for four hours and stored at 80°C. Plasma mass spectrometry (ICP-MS) NexION 350X (PerkinElmer Inc., USA) was used to establish each mineral's plasma concentration levels (Mg, Ca, and Cu). Polyatomic interferences were overcome using a pressurized collision cell operated in kinetic energy discrimination mode. In each batch, 7–9 blank samples were used to confirm that the analytical system was free of analyte contamination or interfering substances. The mean concentrations of each mineral (Mg, Ca, and Cu) in the blank samples were 0.001 mg/L, 0.148 mg/L, and 0.007 µg/L, respectively. We used scandium as an internal standard for Mg and Ca and Germanium for Cu. The coefficients of variation of mixing plasma sample controls and standardized controls were Mg “6.30% and 1.45%” Ca “5.88% and 5.62%” and Cu “4.21% and 1.88%”.

2.3 Assessment of Covariates

Vital information for all parents and children was collected through a face-to-face interview by a trained research assistant, including maternal age, educational level, height, weight, children's sex, gestational age at birth, and birth weight. Dietary quality scores for primary school children were calculated using the recommendation of the New Chinese dietary guidelines for healthy eating patterns and food-based dietary recommendations in 2018.⁽¹⁹⁾ Ten food groups were included in the dietary quality scores; eight were considered adequate food groups and two were moderate groups.⁽²⁰⁾ The various food groups and their recommended intake were based on the Chinese Food Guide Abacus (2016), which was specifically designed for children aged 6-12 years. The daily dietary intake was as follows: Cereals 250-400g, fruits 200-350g, vegetables 300-500g, fish 40-75g, whole grains 50-150g, nuts 25-35g, eggs 40-50, Milk 300g, lean meat 40-75g, cooking oil 25-30g, salt <6g and water 1500-1700 mL.⁽¹⁹⁾ A dietary quality score was calculated by calculating recommended and reported intake ratio for each food group. The group scores were taken at 1 and 0. All food groups that were adequately consumed are scores as 1,

while those that were not consumed are score as 0.⁽²¹⁾ The dietary score on a scale from 0 to 10, a higher score depicts healthy food consumed.⁽²²⁾ All children's physical activity levels were assessed using a three-days structured questionnaire, participants were made to recall activities on two working days and one day during weekends. Each day was divided into 96 blocks as described.⁽²³⁾ Combining the metabolic equivalent scores MET, kcal·kg⁻¹·h⁻¹, of each physical activity and multiply by its duration per day (hour (h)/day(d)) to calculate the level of physical activities.⁽²⁴⁾

2.4 Statistical analysis

Analyses were performed using the SPSS software version V.21.0, (SPSS Inc., Chicago, IL, USA). Differences in baseline characteristics for boys and girls were analysed separately. Primary data were presented as means and standard deviations (SD) for normally distributed continuous variables, or medians and interquartile ranges (IQRs) for non-normally distributed continuous variables and percentages for categorical variables. The independent t-test was used to examine gender differences for normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables by gender. Normality was used to assess normal distribution. The calculated z scores of both dependent and independent variables using Pearson's correlation coefficient.

The direction and strength of the associations were assessed through analyses of covariance (ANCOVA) using the tertiles of dietary pattern mineral intake and plasma concentration level, and the adjusted mean, standard deviation, and standard error were reported. The dominant and non-dominated hands' skeletal muscle mass and handgrip strength were entered as the independent variables and dietary mineral intake and serum mineral levels of calcium, magnesium, copper as the dependent variable. Model 1, univariate analysis without adjustment; model 2; adjusted for covariates including age, sex, and BMI; model 3, adjusted for covariates in model 2 plus physical activity, household income, maternal and paternal education, mode of delivery, and usage of calcium and multivitamin supplement. Mean and standard (mean±SD) for analysis of variance were reported in Model 1, while mean and standard error (mean±SE) for covariance analysis were reported in model 2 and 3. The level of statistical significance was set at a p-value <0.05.

3. RESULTS

Table 1 presents the basic characteristics of the study subjects. Of the 465 primary school children, 265 boys and 200 girls. The mean ages of the boys and girls were 8.00 ± 1.00 and 8.08 ± 0.97 years, respectively. The BMI of boys was higher than girls with 16.3 and 15.2 kg/m² respectively ($p < 0.001$). Moreover, boys had higher levels of physical activity, dietary energy, and

protein intake than girls. The mean plasma concentration of Cu, Mg, and Ca were 1.25 ± 0.31 mg/L, 21.24 ± 4.43 mg/L, and 91.22 ± 16.81 mg/L. Additionally, compared with the girls, the boys had higher values in all the parameters of skeletal muscle mass (ASM, ASM/H, ASM/W, ASM/AFM, TSM, TSM/H, TSM/W, TSM/TFM) and handgrip strength (LHGS, RHGS) with all values having a $p < 0.001$.

Table 1. Basic Characteristics of study participants (Mean and standard deviations; number, percentages, IQR and P-values)

Characteristics	Total (N=465)	Boys (N=265)	Girls (N=200)	p-value
Age, years	8.03±0.99	8.00±1.00	8.08±0.97	0.406
BMI, kg/m ²	15.82±2.72	16.28±3.04	15.22±2.08	< 0.001*
Weight, kg	26.50±7.10	27.34±7.97	25.38±5.55	0.003*
Height, cm	128.73±8.06	128.88±8.28	128.54±7.79	0.656
Physical activity, Met*h/day	39.96±4.29	40.70±4.45	38.98±3.85	< 0.001*
Mode of delivery, (%)				0.092
Normal	233(50.11)	124(46.6)	109(54.5)	
Cesarean	232(49.90)	142(53.4)	91(45.5)	
Maternal education (%)				0.177
Primary/ no education	13(2.8)	9(3.4)	4(2.0)	
Secondary education	165(35.5)	91(34.3)	74(37.0)	
Graduate	247(53.1)	136(51.3)	111(55.5)	
Post graduate and above	39(8.4)	28(10.6)	11(5.5)	
NA	1(0.2)	1(0.4)	0(0.0)	
Paternal education (%)				0.761
Primary/no education	7(1.5)	5(1.9)	2(1.0)	
Secondary education	180(39.0)	101(38.4)	79(39.7)	
Graduate	212(45.9)	117(44.5)	95(47.7)	
Post graduate and above	62(13.4)	39(14.8)	23(11.6)	
NA	1(0.2)	1(0.4)	0(0.0)	
Household Income, Yuan*month ⁻¹ (%)				0.701
≤12000	155(33.3)	92(34.7)	63(31.5)	
>12000	228(49.0)	127(47.9)	101(50.5)	
NA	82(17.6)	46(17.4)	36(18.0)	
Calcium supplements (%)				0.213
No	276(59.2)	151(56.8)	125(62.5)	
Yes	190(40.8)	115(43.2)	75(37.5)	
Multivitamin supplements (%)				0.821
No	387(83.1)	220(82.7)	167(83.5)	
Yes	79(17.0)	46(17.3)	33(16.5)	
Dietary energy intake, Kcal/day	1436.23±43.15	1511.97±447.67	1335.88±394.80	< 0.001*
Dietary protein intake, g/day	64.94±22.80	67.36±23.63	61.72±21.28	0.008*
Dietary calcium intake, mg/day	511.45±212.00	522.39±215.28	496.95±207.21	0.200
Dietary magnesium intake, mg/day	262.40±92.01	268.08±91.21	254.88±92.76	0.126
Dietary copper intake, mg/day	3.13±2.25	3.20±2.31	3.05±2.16	0.478
Serum concentration for calcium, mg/L	91.22±16.81	91.01±16.11	91.50±17.73	0.763

Table 1 (continued)

Characteristics	Total (N=465)	Boys (N=265)	Girls (N=200)	p-value
Serum concentration for magnesium, mg/L	21.24±4.43	21.18±4.00	21.33±4.94	0.736
Serum concentration for copper, mg/L	1.25±0.31	1.27±0.28	1.65±0.35	0.068
ASM, kg	7.48±1.76	7.85±1.91	6.97±1.41	<0.001*
ASM/Height ² , kg/m ²	4.46±0.63	4.66±0.65	4.18±0.48	<0.001*
ASM/Weight, %	0.28±0.03	0.29±0.28	0.28±0.28	<0.001*
ASM /AFM, %	2.24±0.82	2.41±0.87	2.00±0.67	<0.001*
TSM, kg	18.07±3.71	18.88±3.95	16.98±2.98	<0.001*
TSM/Height, kg/m ²	10.80±1.24	11.26±1.27	10.21±0.91	<0.001*
TSM/ Weight, %	0.69±0.06	0.70±0.59	0.68±0.56	<0.001*
TSM/TFM, %	2.69±0.79	2.84±0.83	2.49±0.68	<0.001*
Left handgrip strength, kg (LHS)	9.83±2.69	10.31±2.84	9.18±2.33	<0.001*
Right handgrip strength, kg (RHS)	10.59±2.88	11.07±3.06	9.95±2.49	<0.001*

BMI, body mass index; ASM, appendicular skeletal mass; TSM, total skeletal mass; AFM, appendicular fat mass; TFM, total fat mass; Physical activity is represented by metabolic equivalent (MET) hours per day, LHGS; left handgrip strength, RHGS; right handgrip strength. The * entries represent a significance of $p < 0.05$

Table 2 shows analysis of sex-specific tertiles using ANCOVA linear regression models; there was a statistically significant linear relationship with an increase in mean copper serum concentration between the first and third tertiles in girls resulted in 3.19% (P-trend =0.034, P-diff -0.083), 3.57% (P-trend =0.037, P-diff-0.083), 2.44% (P-trend =0.018, P-diff=0.060) and 2.25% (P-trend =0.024, P-diff-0.072) for ASM/Height kg/m², ASM/ Weight %, TSM/ Height kg/m² and TSM/ Weight respectively, no significant linear relationship in girls for ASM kg, TSM kg, ASM/ AFM% and TSM/TFM% (P-trend >0.05). In both the total and the boy's groups, there was no statistically significant mean difference in copper concentration between tertiles for parameters of skeletal muscle mass. We also observed no significant association in all groups for serum copper levels and muscle strength (LHGS and RHGS) (P-trend>0.05).

Table 3 shows linear regression with ANCOVA analysis between serum magnesium levels and parameters of skeletal muscle mass and strength; the association was only seen in ASM/ AFM% for total and boys' group with mean difference of 6.68% and 9.44% (P-trend =0.042, 0.041 and P-diff=0.124, 0.123) respectively, no significant mean difference with no significant association was observed in the other variables of skeletal muscle mass and in both LHGS and RHGS (P-trend>0.05).

In the relationship between serum calcium and skeletal muscle mass and strength, as displayed in Table

4, no significant main differences were observed between plasma Ca levels and all the variables of skeletal muscle mass and strength across all groups (ASM, ASM/H, ASM/W, ASM/AFM, TSM, TSM/H, TSM/W, TSM/ TFM, LHGS, and RHGS) (P-trend>0.05).

4. DISCUSSION

Copper, magnesium, and calcium are essential for the normal development of the skeleton.⁽⁴⁾ This is the first study to investigate the association between Cu, Mg, and Ca serum mineral levels with skeletal muscle mass and strength in healthy children aged 6-11. In the present study, we found a statistically significant linear relationship between serum Cu and parameters of SMM in girls, but not in boys, which may be as a result of their growth and development situation and nutritional lifestyle. Similar studies in determining the association between serum Cu, Mg, and Ca with bone health.

Consistence with this study, a study indicates a high degree of linear correlations between the serum Cu concentrations and the bone density of the lumbar spine as measured using dual-energy X-ray absorptiometry in premenopausal females and indicates the involvement of serum Cu in bone health and premenopausal female osteopenia.⁽⁸⁾ It was further suggested that serum Cu may be useful as a cheap and straightforward method indicative of bone mineral density in postmenopausal females.⁽⁸⁾ Another study also illustrated a positive correlation between serum Cu

Table 2. Association between Serum Copper and parameters of Skeletal muscle mass and strength

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=145)	T3(n=144)	Diff%	P-Diff	P-trend	T1(n=82)	T3(n=80)	Diff%	P-Diff	P-trend	T1(n=63)	T3(n=63)	Diff%	P-Diff	P-trend
ASM, kg															
Model 1	7.27±1.50	7.52±1.97	3.35	0.451	0.239	7.54±1.62	7.88±2.13	4.57	0.213	0.248	7.05±1.28	6.78±1.24	-3.85	0.533	0.271
Model 2	7.4±0.08	7.47±0.08	0.83	0.797	0.583	7.77±0.11	7.88±0.10	1.41	0.764	0.466	6.97±0.11	6.87±0.12	-1.49	0.728	0.525
Model 3	7.47±0.08	7.41±0.08	-0.79	0.790	0.611	7.88±0.11	7.82±0.11	-0.86	0.899	0.675	6.97±0.12	6.81±0.12	-2.26	0.650	0.357
ASM/H², kg/m²															
Model 1	4.35±0.53	4.48±0.70	3.07	0.129	0.072	4.52±0.57	4.66±0.72	3.16	0.048	0.162	4.23±0.45	4.13±0.45	-2.51	0.255	0.204
Model 2	4.43±0.03	4.42±0.03	-0.27	0.820	0.774	4.62±0.04	4.64±0.04	0.36	0.498	0.757	4.24±0.04	4.11±0.04	-2.96	0.060	0.043*
Model 3	4.44±0.03	4.41±0.03	-0.76	0.596	0.434	4.64±0.04	4.62±0.04	-0.25	0.503	0.844	4.22±0.04	4.09±0.04	-3.19	0.083	0.034*
ASM/W, %															
Model 1	0.28±0.03	0.28±0.03	-1.54	0.270	0.189	0.29±0.02	0.29±0.03	-1.61	0.542	0.272	0.28±0.03	0.27±0.03	-4.36	0.034	0.012*
Model 2	0.28±0.00	0.28±0.00	-0.30	0.913	0.742	0.29±0.00	0.29±0.00	0.51	0.518	0.658	0.28±0.00	0.27±0.00	-2.98	0.056	0.041*
Model 3	0.28±0.00	0.28±0.00	-0.71	0.701	0.460	0.29±0.00	0.29±0.00	0.12	0.505	0.925	0.28±0.00	0.27±0.00	-3.15	0.083	0.037*
ASM /AFM, %															
Model 1	2.26±0.83	2.17±0.84	-3.88	0.615	0.363	2.51±0.85	2.37±0.88	-5.78	0.382	0.289	2.12±0.67	1.88±0.66	-11.12	0.140	0.050*
Model 2	2.24±0.05	2.22±0.05	-0.77	0.926	0.813	2.38±0.07	2.41±0.07	1.20	0.936	0.786	2.09±0.07	1.92±0.07	-8.35	0.148	0.065
Model 3	2.23±0.05	2.22±0.05	-0.60	0.984	0.861	2.37±0.08	2.41±0.08	1.59	0.800	0.743	2.08±0.07	1.91±0.07	-7.73	0.259	0.105
TSM, kg															
Model 1	17.63±3.16	18.2±4.13	3.20	0.405	0.195	18.24±3.33	19±4.47	4.13	0.252	0.227	17.08±2.69	16.6±2.53	-2.78	0.661	0.365
Model 2	17.94±0.16	18.05±0.16	0.60	0.760	0.628	18.79±0.21	18.97±0.2	0.95	0.687	0.542	16.93±0.23	16.78±0.24	-0.91	0.879	0.649
Model 3	18.06±0.16	17.94±0.16	-0.70	0.693	0.582	19.01±0.21	18.83±0.21	-0.95	0.642	0.563	16.89±0.24	16.66±0.24	-1.40	0.772	0.499
TSM/H²,															
Model 1	10.56±1.05	10.88±1.38	3.03	0.063	0.029	10.96±1.05	11.27±1.42	2.89	0.056	0.112	10.28±0.92	10.13±0.82	-1.50	0.394	0.334
Model 2	10.77±0.05	10.72±0.05	-0.45	0.614	0.483	11.2±0.06	11.21±0.06	0.02	0.797	0.977	10.32±0.07	10.06±0.07	-2.51	0.029	0.014*
Model 3	10.77±0.05	10.7±0.05	-0.63	0.459	0.344	11.22±0.07	11.19±0.07	-0.25	0.720	0.772	10.28±0.07	10.03±0.07	-2.44	0.060	0.018*
TSM/W, %															
Model 1	0.69±0.06	0.68±0.06	-1.43	0.284	0.152	0.71±0.05	0.7±0.06	-1.85	0.181	0.153	0.69±0.05	0.66±0.06	-3.22	0.080	0.027*
Model 2	0.69±0.00	0.69±0.00	-0.43	0.772	0.491	0.7±0.00	0.7±0.00	0.08	0.950	0.914	0.68±0.00	0.67±0	-2.42	0.028	0.013*
Model 3	0.69±0.00	0.69±0.00	-0.49	0.692	0.457	0.7±0.00	0.7±0.00	0.03	0.859	0.972	0.68±0.00	0.67±0	-2.25	0.072	0.024*

Table 2 (continued)

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=145)	T3(n=144)	Diff%	P-Diff	P-trend	T1(n=82)	T3(n=80)	Diff%	P-Diff	P-trend	T1(n=63)	T3(n=63)	Diff%	P-Diff	P-trend
TSM/TFM %															
Model 1	2.72±0.78	2.62±0.83	-3.87	0.484	0.260	2.96±0.78	2.8±0.85	-5.25	0.288	0.234	2.61±0.64	2.36±0.69	-9.27	0.138	0.048*
Model 2	2.7±0.05	2.67±0.04	-0.83	0.920	0.728	2.82±0.06	2.85±0.06	0.95	0.919	0.764	2.58±0.06	2.4±0.06	-6.95	0.101	0.044*
Model 3	2.69±0.05	2.67±0.05	-0.80	0.947	0.748	2.81±0.07	2.84±0.07	1.03	0.772	0.765	2.56±0.06	2.4±0.06	-6.32	0.208	0.078
Left handgrip strength, kg															
Model 1	9.62±2.56	9.76±2.76	1.48	0.447	0.653	9.97±2.66	10.34±2.84	3.70	0.266	0.412	9.32±2.36	8.72±2	-6.38	0.268	0.134
Model 2	9.73±0.17	9.77±0.17	0.37	0.776	0.879	10.17±0.23	10.4±0.23	2.25	0.657	0.487	9.18±0.23	8.89±0.23	-3.16	0.593	0.385
Model 3	9.81±0.17	9.7±0.17	-1.07	0.828	0.673	10.31±0.25	10.3±0.24	-0.14	0.932	0.967	9.07±0.24	8.88±0.24	-2.15	0.498	0.574
Right handgrip strength, kg															
Model 1	10.33±2.7	10.57±2.98	2.33	0.475	0.478	10.68±2.8	11.14±3.07	4.32	0.371	0.339	10.03±2.51	9.54±2.3	-4.82	0.424	0.271
Model 2	10.43±0.18	10.59±0.18	1.60	0.718	0.510	10.89±0.25	11.21±0.24	2.98	0.643	0.349	9.87±0.25	9.74±0.26	-1.37	0.752	0.709
Model 3	10.49±0.18	10.52±0.18	0.30	0.921	0.905	11.05±0.26	11.09±0.25	0.33	0.994	0.922	9.77±0.26	9.71±0.27	-0.60	0.589	0.879

Analysis of covariance (ANCOVA). Copper; Cu, Mean, SD; standard deviation, SE; standard error, ASM, appendicular skeletal mass; TSM, total skeletal mass; AFM, appendicular fat mass; LHGS kg; left handgrip strength, RHGS kg; right handgrip strength. Model 1, univariate analysis without adjustment; model 2; adjusted for covariates including age, sex and BMI; model 3, adjusted for covariates in model 2 plus physical activity, household income, maternal and paternal education, mode of delivery and usage of calcium and multivitamin supplement. Model 1 is analysis of variance, presented as mean±SD, model 2 and 3 are analysis of covariance presented as mean±SE. %Diff.: percentage difference = (T3 – T1) /T1 × 100%. P-diff: P-diff for group difference; P-trend: P-trend for linear trend. The italic shows statistical significance*: p < 0.05.

Table 3. Association between Serum Magnesium and parameters of Skeletal muscle mass and strength

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=144)	T3(n=146)	Diff%	p-Diff	p-trend	T1(n=83)	T3(n=85)	Diff%	p-Diff	p-trend	T1(n=63)	T3(n=63)	Diff%	p-Diff	p-trend
ASM, kg															
Model 1	7.33±1.68	7.59±1.99	3.62	0.361	0.199	7.6±1.77	8.04±2.23	5.81	0.324	0.134	6.97±1.47	6.97±1.36	-0.01	0.636	0.997
Model 2	7.39±0.08	7.52±0.08	1.79	0.298	0.228	7.76±0.1	7.97±0.1	2.70	0.241	0.154	6.94±0.11	6.94±0.11	0.08	0.707	0.972
Model 3	7.4±0.08	7.49±0.08	1.22	0.605	0.418	7.76±0.1	7.96±0.1	2.51	0.388	0.193	6.96±0.12	6.87±0.12	-1.26	0.708	0.604
ASM/H², kg/m²															
Model 1	4.4±0.58	4.5±0.72	2.43	0.252	0.150	4.56±0.59	4.73±0.77	3.66	0.255	0.099	4.17±0.48	4.19±0.5	0.53	0.528	0.791

Table 3 (continued)

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=144)	T3(n=146)	Diff%	p-Diff	p-trend	T1(n=83)	T3(n=85)	Diff%	p-Diff	p-trend	T1(n=63)	T3(n=63)	Diff%	p-Diff	p-trend
Model 2	4.42±0.03	4.46±0.03	0.92	0.549	0.314	4.62±0.04	4.67±0.04	1.12	0.613	0.328	4.15±0.04	4.19±0.04	0.75	0.564	0.611
Model 3	4.42±0.03	4.44±0.03	0.63	0.799	0.505	4.63±0.04	4.67±0.04	0.88	0.739	0.455	4.16±0.04	4.16±0.04	0.1	0.701	0.947
ASM/W, %															
Model 1	0.28±0.03	0.28±0.03	-0.17	0.984	0.887	0.29±0.03	0.29±0.03	-0.57	0.780	0.696	0.28±0.03	0.28±0.03	0.68	0.854	0.708
Model 2	0.28±0.00	0.28±0.00	0.84	0.597	0.355	0.29±0.00	0.29±0.00	1.22	0.551	0.280	0.28±0.00	0.28±0.00	0.42	0.655	0.777
Model 3	0.28±0.00	0.28±0.00	0.63	0.783	0.497	0.29±0.00	0.29±0.00	1.02	0.681	0.382	0.28±0.00	0.28±0.00	-0.11	0.671	0.942
ASM /AFM, %															
Model 1	2.19±0.73	2.24±0.86	2.20	0.830	0.617	2.35±0.74	2.41±0.96	2.33	0.799	0.685	1.96±0.64	2.02±0.66	3.04	0.885	0.622
Model 2	2.16±0.05	2.28±0.05	5.97	0.193	0.071	2.29±0.07	2.5±0.07	9.05	0.126	0.043	1.98±0.07	2.02±0.07	2.22	0.814	0.641
Model 3	2.15±0.05	2.3±0.05	6.68	0.148	0.051	2.3±0.07	2.52±0.07	9.44	0.123	0.041	1.99±0.07	2.03±0.07	1.97	0.700	0.69
TSM, kg															
Model 1	17.71±3.54	18.37±4.18	3.71	0.254	0.130	18.31±3.66	19.36±4.69	5.74	0.230	0.087	16.94±3.21	16.99±2.8	0.29	0.682	0.925
Model 2	17.85±0.15	18.19±0.15	1.94	0.172	0.111	18.65±0.20	19.15±0.20	2.68	0.143	0.080	16.87±0.23	16.94±0.23	0.41	0.764	0.835
Model 3	17.86±0.15	18.14±0.15	1.54	0.369	0.213	18.64±0.20	19.15±0.20	2.70	0.192	0.081	16.93±0.24	16.79±0.24	-0.83	0.769	0.685
TSM/Height², kg/m²															
Model 1	10.66±1.13	10.94±1.43	2.60	0.105	0.058	11.03±1.11	11.44±1.51	3.68	0.116	0.040	10.16±0.94	10.25±0.92	0.88	0.563	0.576
Model 2	10.7±0.05	10.82±0.05	1.14	0.190	0.071	11.15±0.06	11.28±0.06	1.16	0.332	0.139	10.12±0.07	10.24±0.07	1.13	0.425	0.276
Model 3	10.7±0.05	10.81±0.05	1.02	0.284	0.113	11.15±0.06	11.28±0.06	1.15	0.350	0.155	10.14±0.07	10.2±0.07	0.59	0.628	0.570
TSM/ W, %															
Model 1	0.69±0.06	0.69±0.06	0.09	0.995	0.926	0.7±0.05	0.7±0.06	-0.43	0.905	0.742	0.67±0.06	0.68±0.05	1.03	0.783	0.491
Model 2	0.69±0.00	0.69±0.00	1.11	0.178	0.072	0.7±0.00	0.7±0	1.31	0.232	0.088	0.67±0.00	0.68±0.00	0.8	0.502	0.417
Model 3	0.69±0.00	0.69±0.00	1.08	0.216	0.089	0.7±0.00	0.71±0	1.35	0.232	0.089	0.68±0.00	0.68±0.00	0.4	0.515	0.693
TSM/TFM %															
Model 1	2.67±0.75	2.69±0.82	0.78	0.972	0.823	2.82±0.73	2.82±0.89	0.06	0.917	0.989	2.45±0.71	2.52±0.68	2.68	0.852	0.595
Model 2	2.63±0.04	2.74±0.04	4.10	0.218	0.086	2.76±0.06	2.92±0.06	5.89	0.177	0.063	2.47±0.06	2.52±0.06	1.95	0.610	0.585
Model 3	2.63±0.05	2.75±0.05	4.39	0.185	0.074	2.76±0.06	2.93±0.06	6.23	0.157	0.055	2.5±0.06	2.52±0.06	0.98	0.458	0.788
Left handgrip strength, kg															
Model 1	9.67±2.67	9.97±2.82	3.16	0.604	0.332	10.12±2.86	10.57±3.02	4.48	0.594	0.309	9.06±2.27	9.17±2.24	1.23	0.946	0.781
Model 2	9.73±0.16	9.93±0.16	2.06	0.617	0.390	10.28±0.23	10.56±0.23	2.76	0.449	0.379	9.03±0.23	9.13±0.23	1.17	0.946	0.749
Model 3	9.71±0.17	9.91±0.17	2.04	0.681	0.408	10.22±0.23	10.56±0.23	3.35	0.501	0.302	9.11±0.24	9.16±0.24	0.52	0.845	0.891
Right handgrip strength, kg															
Model 1	10.34±2.89	10.64±2.95	2.93	0.574	0.372	10.78±3.08	11.17±3.12	3.62	0.594	0.412	9.74±2.50	9.99±2.56	2.59	0.847	0.568

Table 3 (continued)

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=144)	T3(n=146)	Diff%	p-Diff	p-trend	T1(n=83)	T3(n=85)	Diff%	p-Diff	p-trend	T1(n=63)	T3(n=63)	Diff%	p-Diff	p-trend
Model 2	10.41±0.18	10.6±0.18	1.84	0.628	0.442	10.95±0.24	11.17±0.24	1.96	0.819	0.528	9.71±0.25	9.96±0.25	2.54	0.724	0.492
Model 3	10.38±0.18	10.58±0.18	1.95	0.580	0.428	10.88±0.24	11.18±0.24	2.74	0.656	0.394	9.79±0.26	9.97±0.26	1.89	0.852	0.626

Analysis of covariance (ANCOVA). Magnesium; Mg, Mean, SD; standard deviation, SE; standard error, ASM, appendicular skeletal mass; TSM, total skeletal mass; AFM, appendicular fat mass; LHGS kg; left handgrip strength, RHGS kg; right handgrip strength. Model 1, univariate analysis without adjustment; model 2; adjusted for covariates including age, sex and BMI; model 3, adjusted for covariates in model 2 plus physical activity, household income, maternal and paternal education, mode of delivery and usage of calcium and multivitamin supplement. Model 1 is analysis of variance, presented as mean±SD, model 2 and 3 are analysis of covariance presented as mean±SE. %Diff.: percentage difference = (T3 – T1) / T1 × 100%. P-diff: P-diff for group difference; P-trend: P-trend for linear trend. The italic shows statistical significance*: p < 0.05.

Table 4. Association between Serum Calcium and parameters of Skeletal muscle mass and strength

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=145)	T3(n=144)	Diff%	p-Diff	p-trend	T1(n=82)	T3(n=80)	Diff%	p-Diff	p-trend	T1(n=63)	T3(n=63)	Diff%	p-Diff	p-trend
ASM, kg															
Model 1	7.49 ±1.86	7.51 ±1.77	0.33	0.440	0.904	7.93 ±2.01	7.98 ±1.93	0.66	0.346	0.861	6.91 ±1.47	6.85 ±1.27	-0.96	0.930	0.788
Model 2	7.48 ±0.93	7.31 ±0.93	-2.25	0.189	0.125	7.92 ±0.94	7.64 ±0.95	-3.45	0.115	0.067	6.90 ±0.91	6.83 ±0.91	-1.15	0.670	0.625
Model 3	7.46 ±0.94	7.34 ±0.93	-1.71	0.392	0.253	7.88 ±0.97	7.71 ±0.97	-2.08	0.333	0.294	6.93 ±0.94	6.79 ±0.92	-2.02	0.581	0.405
ASM/Height, kg/m²															
Model 1	4.46 ±0.69	4.48 ±0.64	0.33	0.201	0.841	4.70 ±0.70	4.70 ±0.69	0.12	0.227	0.955	4.16 ±0.54	4.14 ±0.42	-0.58	0.934	0.776
Model 2	4.45 ±0.34	4.41 ±0.34	-1.02	0.531	0.263	4.70 ±0.34	4.60 ±0.34	-2.01	0.209	0.078	4.14 ±0.35	4.13 ±0.35	-0.13	0.635	0.932
Model 3	4.45 ±0.35	4.41 ±0.35	-1.04	0.528	0.263	4.69 ±0.36	4.61 ±0.35	-1.84	0.318	0.132	4.15 ±0.35	4.11 ±0.34	-0.83	0.606	0.585
ASM/Weight, %															
Model 1	0.28 ±0.03	0.28 ±0.03	-0.81	0.491	0.491	0.29 ±0.03	0.29 ±0.03	-2.13	0.291	0.147	0.27 ±0.03	0.27 ±0.02	0.48	0.392	0.794
Model 2	0.28 ±0.02	0.28 ±0.02	-0.77	0.638	0.395	0.29 ±0.02	0.29 ±0.02	-1.61	0.355	0.154	0.27 ±0.02	0.27 ±0.02	-0.20	0.595	0.892
Model 3	0.28 ±0.02	0.28 ±0.02	-0.79	0.685	0.395	0.29 ±0.02	0.29 ±0.02	-1.52	0.440	0.208	0.28 ±0.02	0.27 ±0.02	-0.86	0.572	0.573
ASM /AFM, %															
Model 1	2.20 ±0.81	2.19 ±0.79	-0.76	0.572	0.862	2.39 ±0.82	2.32 ±0.84	-2.97	0.465	0.605	1.96 ±0.72	1.96 ±0.62	0.30	0.641	0.961
Model 2	2.22 ±0.61	2.22 ±0.61	0.21	0.976	0.948	2.39 ±0.66	2.40 ±0.67	0.38	0.957	0.931	1.99 ±0.53	1.96 ±0.53	-1.58	0.808	0.740
Model 3	2.23 ±0.62	2.22 ±0.62	-0.35	0.989	0.916	2.40 ±0.69	2.39 ±0.69	-0.35	0.903	0.941	2.02 ±0.54	1.94 ±0.53	-3.70	0.690	0.444
TSM, kg															
Model 1	18.05 ±3.86	18.20 ±3.78	0.85	0.395	0.725	18.95 ±4.09	19.24 ±4.11	1.54	0.319	0.641	16.88 ±3.21	16.79 ±2.73	-0.56	0.984	0.858

Table 4 (continued)

Dependent Variables and models	Total(n=434)					Boys(n=245)					Girls(n=189)				
	T1(n=145)	T3(n=144)	Diff%	p-Diff	p-trend	T1(n=82)	T3(n=80)	Diff%	p-Diff	p-trend	T1(n=63)	T3(n=63)	Diff%	p-Diff	p-trend
Model 2	18.02 ±1.85	17.76 ±1.85	-1.41	0.287	0.245	18.93 ±1.83	18.54 ±1.85	-2.07	0.231	0.178	16.85 ±1.87	16.74 ±1.86	-0.63	0.877	0.749
Model 3	17.99 ±1.86	17.82 ±1.85	-0.97	0.527	0.428	18.83 ±1.88	18.67 ±1.87	-0.88	0.400	0.583	16.91 ±1.92	16.67 ±1.88	-1.47	0.762	0.471
TSM/Height, kg/nf															
Model 1	10.79 ±1.33	10.89 ±1.33	0.87	0.135	0.523	11.27 ±1.34	11.38 ±1.41	0.94	0.197	0.597	10.17 ±1.04	10.17 ±0.84	-0.05	0.977	0.977
Model 2	10.77 ±0.58	10.75 ±0.58	-0.15	0.974	0.818	11.27 ±0.56	11.20 ±0.57	-0.68	0.587	0.391	10.11 ±0.59	10.17 ±0.59	0.53	0.739	0.611
Model 3	10.77 ±0.58	10.74 ±0.58	-0.27	0.864	0.672	11.27 ±0.59	11.19 ±0.59	-0.67	0.679	0.422	10.15 ±0.59	10.13 ±0.58	-0.12	0.966	0.909
TSM/ Weight, %															
Model 1	0.69 ±0.06	0.68 ±0.06	-0.37	0.324	0.717	0.70 ±0.05	0.69 ±0.06	-1.44	0.275	0.274	0.67 ±0.06	0.67 ±0.05	0.93	0.456	0.534
Model 2	2.68 ±0.53	2.66 ±0.54	-0.65	0.915	0.990	0.70 ±0.03	0.70 ±0.03	-0.42	0.860	0.590	0.67 ±0.04	0.67 ±0.04	0.40	0.696	0.687
Model 3	0.69 ±0.04	0.69 ±0.04	-0.16	0.954	0.795	0.70 ±0.04	0.70 ±0.04	-0.53	0.807	0.523	0.67 ±0.04	0.67 ±0.04	-0.23	0.889	0.821
TSM/TFM %															
Model 1	2.66 ±0.79	2.62 ±0.77	-1.71	0.254	0.625	2.84 ±0.78	2.73 ±0.84	-4.03	0.250	0.382	2.43 ±0.75	2.44 ±0.60	0.68	0.496	0.893
Model 2	2.68 ±0.53	2.66 ±0.54	-0.65	0.724	0.783	2.84 ±0.56	2.81 ±0.57	-0.87	0.855	0.782	2.47 ±0.50	2.44 ±0.50	-0.95	0.658	0.791
Model 3	2.69 ±0.55	2.66 ±0.54	-1.17	0.753	0.626	2.84 ±0.59	2.80 ±0.58	-1.36	0.693	0.681	2.50 ±0.50	2.43 ±0.49	-3.07	0.589	0.394
Left handgrip strength, kg															
Model 1	9.89 ±2.71	9.84 ±2.80	-0.48	0.749	0.880	10.46 ±2.84	10.53 ±2.99	0.62	0.498	0.886	9.14 ±2.36	8.96 ±2.23	-1.96	0.851	0.654
Model 2	9.89 ±1.97	9.59 ±1.98	-2.99	0.317	0.204	10.45 ±2.07	10.11 ±2.08	-3.20	0.488	0.307	9.16 ±1.85	8.93 ±1.85	-2.57	0.690	0.476
Model 3	9.86 ±2.02	9.62 ±2.01	-2.44	0.457	0.315	10.31 ±2.16	10.20 ±2.16	-1.03	0.657	0.759	9.27 ±1.90	8.81 ±1.86	-5.04	0.352	0.169
Right handgrip strength, kg															
Model 1	10.63 ±2.98	10.60 ±3.00	-0.32	0.758	0.919	11.29 ±3.04	11.21 ±3.22	-0.70	0.404	0.871	9.78 ±2.70	9.80 ±2.39	0.15	0.799	0.974
Model 2	10.63 ±2.11	10.33 ±2.11	-2.84	0.338	0.225	11.26 ±2.18	10.75 ±2.19	-4.53	0.302	0.138	9.81 ±2.01	9.76 ±2.01	-0.47	0.664	0.897
Model 3	10.60 ±2.16	10.36 ±2.14	-2.21	0.496	0.359	11.13 ±2.27	10.85 ±2.27	-2.52	0.583	0.442	9.90 ±2.10	9.66 ±2.05	-2.43	0.659	0.522

Analysis of covariance (ANCOVA). Serum Calcium; Ca, Mean, SD; standard deviation, SE; standard error, ASM, appendicular skeletal mass; TSM, total skeletal mass; AFM, appendicular fat mass; LHGS kg; left handgrip strength, RHGS kg; right handgrip strength. Model 1, univariate analysis without adjustment; model 2; adjusted for covariates including age, sex and BMI; model 3, adjusted for covariates in model 2 plus physical activity, household income, maternal and paternal education, mode of delivery and usage of calcium and multivitamin supplement. Model 1 is analysis of variance, presented as mean±SD, model 2 and 3 are analysis of covariance presented as mean±SE. %Diff.: percentage difference = (T3 – T1) / T1 × 100%. P-diff: P-diff for group difference; P-trend: P-trend for linear trend. The italic shows statistical significance*: p < 0.05.

concentration and bone density at total hip and spine in postmenopausal women. Serum Cu could have an adjourning factor and a protective agent for bone loss in all healthy women.⁽⁹⁾ It was concluded that serum copper had an independent role in bone density in all women.⁽⁹⁾ Several studies have shown that Cu deficiency in children can lead to metabolic bone diseases, including osteoporosis, later in life.⁽²⁵⁾ A study found that children with normal BMD have higher serum levels of Cu than children with low BMD and that the incidence of normal BMD increases as the serum level of Cu increases.⁽⁷⁾ By contrast, the boys in our study had a decrease mean in serum Cu levels with skeletal muscle mass and no significant relationship. Furthermore, the relationship between serum Cu and muscle strength across all age categories has yet to be investigated, and this study found no statistically significant linear relationship between serum Cu and handgrip strength in children. Thus, adequate serum Cu concentration is essential for skeletal muscle growth during childhood and for bone health in adulthood.

In this study, the association between serum Mg levels and skeletal muscle mass is less clear, while no association was found between serum Mg and muscle strength. Consistent with our study, a study also found an inconsistent result between the associations of serum Mg concentration groups and skeletal muscle mass indices in a UK population of 14,340 middle to older-aged men and women participating in the EPIC-Norfolk cohort study.⁽¹²⁾ These results may not be surprising considering the tight homeostatic control of serum Mg and the fact that less than 1% of total body Mg is present in the blood.⁽¹³⁾ Homeostatic control is significant in the impact of serum Mg on bone health. It makes it less likely that concentration serum Mg outside the average level represents an extreme dietary intake of Mg, and more likely that results from pathological issue or diuretic medication.⁽¹³⁾ Studies have shown that low dietary intake for an extended period may present severe Mg deficiency, with low concentrations in bone and muscle of individuals due to long-term compensatory release of Mg to maintain serum concentration; therefore, presenting an average level of serum Mg concentration may not represent normalcy of serum magnesium in the body and bone.⁽¹²⁾ This may partly explain the inconsistent relationship between serum Mg concentrations and variables of SMM.

In contrast to our results, some studies found a significant relationship between serum Mg and skeletal

mass; a significant positive correlation existed between muscle quality and serum Mg.⁽²⁶⁾ In a multiple regression analysis of 310 patients, serum Mg was significantly and independently associated with muscle quality and other significant and independent variables of age, gender, duration of hemodialysis, and diabetes.⁽²⁷⁾ Another study described that serum Mg concentrations were significantly associated with indices of muscle performance, such as grip strength, lower-leg muscle power, knee extension torque, and ankle extension strength, in older persons.⁽²⁷⁾

It is essential to understand and appreciate the magnitude of the differences seen in dietary intake analyses to understand inconsistency. They also consider that the effect of age on skeletal muscle mass and strength has already been well-established. The results for serum Mg are less patent, potentially due to the tight homeostatic control of blood Mg concentrations.

The present study found no significant mean difference across tertiles (T1-T3) (i.e., no association) of serum Ca and skeletal muscle mass and strength (P -trend >0.05) in children aged 6-11 years. Accordingly, a study compared serum Ca levels between subjects with average muscle mass and low muscle mass defined using different methods; subsequently, no difference was observed between serum Ca levels and muscle mass in the study population irrespective of definitions.⁽¹⁵⁾ On the other hand, using the Korean Genome and Epidemiology Study (KoGES) national cohort that observes a large portion of regional populations each year, a study investigated the change in muscle mass over ten years according to Ca intake and serum Ca levels among Korean adults aged 50 years and older. It determined whether the relationship was independent of muscle-associated factors;⁽²⁸⁾ the results show that low serum Ca levels significantly predicted muscle loss but not weight loss.⁽²⁹⁾ This study included only a few subjects with serum Ca levels beyond the reference range, while results remained unchanged even after excluding such subjects. Therefore, low serum Ca levels may predict muscle loss among Korean adults aged 50 years and older.⁽²⁹⁾

The discrepancy in these studies might result from estimating muscle mass using the BIA method. At the same time, dual-energy X-ray absorptiometry (DXA) has been preferred as a standard method, for estimating appendicular muscle mass, was used in the present study. Furthermore, the study did not measure muscle

strength, while the current study did investigate muscle strength using handgrip strength. Moreover, some factors determining circulating Ca concentrations were not included, but the current study involved all the factors in estimating serum Ca. Lastly, FFQ did not collect information on Ca supplementation, although physiological studies have suggested no material differences in the metabolic actions of dietary Ca and that obtained from supplements. However, given that supplementary calcium intake may cause temporary hypercalcemia, the functional role between dietary and supplementary Ca differs.

The results of the present study recommended that Chinese girls should be encouraged to improve dietary Ca intake before puberty to help achieve maximum peak bone mass.⁽³⁰⁾ Therefore, recommending increased dietary calcium intake may be worthwhile for preserving muscle mass and strength.

Our study has several strengths. To the best of our knowledge, this is the first study assessing the association between dietary minerals intake and serum mineral levels of Cu, Mg and Ca with parameters of skeletal muscle mass and strength in children; the use of DXA scan for the estimation and assessment of both skeletal muscle mass and muscle strength. We also use ICP-MS for the measure blood elements and adjust multiple potential covariates, which ensured accuracy in estimating the associations.

Several limitations in this study were acknowledged. A cross-section study design could not establish a temporal sequence of the observed association between the parameters. Also, the analyses of the relation between micronutrient concentrations and muscle mass and strength cannot presume causality, only association. Therefore, we cannot establish the directionality of the reported associations, which propel a more robust prospective study and clinical trials to confirm the association. The study cannot rule out the possibility of residual confounding by unknown risk factors, most importantly genetic predispositions. Furthermore, thirteen food items were excluded from the complete calculation of dietary intake, though some of these food items, such as thyme, eugenol, saffron, and rosemary, are usually consumed in small quantities, infrequently or not consumed at all in the Chinese pediatric population; so, the absence of these foods' items may not have played a significant impact. Moreover, the children included in the study had a relatively narrow age; hence, the results may need

to be better deduced for children of all age groups. The daily intake of dietary minerals of Cu, Mg, and Ca did not explain the total amounts from the supplements and may lead to underestimation of their usual intake. Nevertheless, blood serum indicators cover the contribution of these elements from both dietary intake (foods and/or supplements) and environment. It might attenuate the effect of the absence of these data. Lastly, the sample population of this study was drawn from the same ethnicity, race, and social class; hence, the results cannot be readily generalized to other populations.

However, more work is needed to confirm the results obtained in this study and also to establish using an interventional study and a clinical trial of a large cohort from childhood through into old age to have a complete understanding of the relationship between serum concentration and supplementation minerals with skeletal muscle mass and strength. This study could be a gateway for more research in this field, which tried to highlight the critical importance of micro and macronutrient in maintaining a healthy bone.

5. CONCLUSION

The concentration of serum copper (Cu) affects the skeletal muscle mass (ASM/Height kg/m², ASH/Weight %, TSM/ Height kg/m², and TSM/ Weight%) in girls but not boys. The impact of serum magnesium (Mg) concentration on skeletal muscle mass is uncertain, as there seems to be no association between serum magnesium and handgrip strength. Additionally, serum calcium concentration doesn't affect the growth skeletal muscle mass and strength in children aged 6-11 years

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Conflict of Interest

The authors declare no conflict of interest.

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