

## Bone mineral status and its relation with dietary estimates of net endogenous acid production in Hong Kong Chinese adolescents

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Diet composition influences net endogenous acid production (NEAP), which may affect bone health. No studies are available to relate dietary estimate of NEAP to bone health in Chinese adolescents. This study examined the association of dietary estimates of NEAP with bone mineral status in Hong Kong Chinese adolescents. Baseline data on 171 boys and 180 girls aged 10–12 years from the Hong Kong Adolescent Bone Health Cohort Study were presented. Weight, height, Tanner stage and dietary intakes by FFQ were collected. NEAP was estimated from diet using Frassetto's method. Bone area (BA), bone mineral content (BMC) and bone mineral apparent density (BMAD) of total hip, lumbar (L1–L4) spine and whole body were estimated by dual-energy X-ray absorptiometry (DXA). No significant association was observed between BMC or BMAD and energy-adjusted NEAP or other nutrients. BA was significantly and positively associated with BMC at all sites in both sexes. Weight was significantly and positively associated with BMC in hip and spine in both sexes. Height was negatively correlated with hip BMC for boys and whole body BMC for girls. Pubertal stage was significantly and positively associated with BMC in all sites in both sexes. Weight and height contributed most of the variability in BMAD at different sites. The results suggest that anthropometric characteristics and pubertal stage are more influential than dietary NEAP in determining bone mineral status of Hong Kong Chinese adolescents. However, the methodological weaknesses regarding the use of DXA and FFQ in the present sample require attention.

### Bone health: Net endogenous acid production: Chinese: Adolescents

Osteoporosis and its associated fractures is a major public health problem worldwide. Hong Kong is no exception to this problem. The age-specific hip fracture rates among Hong Kong Chinese population have increased 2-fold in the past few decades<sup>(1)</sup>. The cost for the acute care of hip fracture totalled HK\$150 million in 1995, and the cost is projected to increase to HK\$960 million in 2010<sup>(2)</sup>. It is therefore important to identify dietary and lifestyle factors that could increase or optimize bone stability and probably reduce the risk of developing osteoporosis later in life.

During adolescence, tremendous skeletal growth occurs, and approximately 90% of adult bone mineral density (BMD) is acquired by the end of adolescence<sup>(3)</sup>. Genetic factors account for an estimated 60–80% of the variability in adult BMD with diet, physical activity and hormonal status being important modifiable factors of bone accrual<sup>(4,5)</sup>. The role of Ca has long been recognized to be important for bone health<sup>(6,7)</sup>. However, there is increasing evidence to highlight the role of overall diet instead of individual nutrient

on bone metabolism and Ca balance<sup>(8–10)</sup>. The interplay among diet composition and its effect on net endogenous acid production (NEAP) and bone health has been reviewed<sup>(11–13)</sup>. It has been hypothesized that diets high in acid-forming components, including several amino acids in protein foods, P and Cl, and low in base-forming components, such as fruit and vegetables, K and Mg, could increase net acid and urinary Ca excretion, which may lead to lower BMD and higher fracture risk.

The diet of Hong Kong Chinese has undergone rapid changes in the past decades. Consumption of meat by Hong Kong population increases markedly whereas daily intake of fruit and vegetables is inadequate<sup>(14)</sup>. Previous studies also revealed that dietary practices and lifestyle of the Hong Kong youth population are worse than are those of their adult counterparts<sup>(15,16)</sup>. Since no studies have been done to explore the relationship between diet composition, NEAP and other determinants of bone health in adolescents of Chinese origin, the aim of this study was to investigate the

**Abbreviations:** BA, bone area; BMC, bone mineral content; BMD, bone mineral density; BMAD, bone mineral apparent density; DXA, dual-energy X-ray absorptiometry; NEAP, net endogenous acid production.

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association of dietary estimates of NEAP with bone mineral status in a group of Hong Kong Chinese adolescents.

## Materials and methods

### Subjects

The subjects were the 171 boys and 180 girls who participated in a longitudinal Hong Kong Adolescent Bone Health Cohort Study. The study was conducted by the Jockey Club Centre for Osteoporosis Care and Control, the Chinese University of Hong Kong. Boys aged 11–12 years and girls aged 10–11 years were recruited from nine primary schools in Hong Kong on a voluntary basis. Subjects suffering from any conditions known to influence bone or receiving prior therapy with a known effect on bone metabolism were excluded from the study. All subjects were measured at baseline and followed up annually. Results of this study were based on baseline data on anthropometry, dietary intake and bone mineral status collected between November 2003 and October 2004. Informed consent was obtained from both the subjects and their parents or guardians. The study was approved by the Ethics Committee of the Chinese University of Hong Kong.

### Anthropometric measurements

Body weight was measured to the nearest 0.1 kg by the Physician Balance Bean Scale (Healthometer, IL, USA) with subjects wearing a light gown. Body height was measured to the nearest 0.1 cm by Holtain Harpenden Stadiometer (Holtain Ltd, Crosswell, UK) with subjects barefoot. Subject's sexual development was assessed by the researcher using the Tanner grading system. This included assessments of the pattern of development of pubic hair in all adolescents and of breast development in girls and penile and testicular size in boys<sup>(17)</sup>. If discrepancies existed among criteria, greater emphasis was placed on the degree of breast development in girls and on testicular and penile size in boys, for determination of Tanner stage.

### Dual-energy X-ray absorptiometry of total hip, lumbar (L1–L4) spine and whole body

Dual-energy X-ray absorptiometry (DXA) was performed by using a Hologic QDR-4500 W densitometer (Hologic, Waltham, Mass., USA) to measure bone area (BA), bone mineral content (BMC), and BMD of total hip, lumbar (L1–L4) spine and whole body. The machine was calibrated daily in accordance with the manufacturer's instructions. Repositioning the subjects, reproducibility of the DXA scan results was within 1.5% for all measured sites. In the present study, bone mineral apparent density (BMAD) rather than BMD was presented to reflect more accurate changes in volumetric bone density during growth. Total hip BMAD was calculated as  $BMC/A^2$ , spine BMAD was calculated as  $BMC/A^{3/2}$  and whole body BMAD was estimated as  $BMC/(A^2/h)$ , where  $A$  is the projected bone area and  $h$  is height<sup>(18,19)</sup>.

### Nutrient analysis

Dietary intake was assessed by a modified version of the FFQ based on data obtained in the Hong Kong Adult Dietary

Survey in 1995<sup>(20)</sup>. The FFQ had been validated with the basal metabolic rate calculation and the 24h Na/creatinine and K/creatinine analysis<sup>(21)</sup>. Subjects were asked about their usual frequency and consumption in the past 12 months from the food list. Standard portion size was listed and a food photo album was provided to assist assessment. Daily nutrient intake was calculated by the Food Processor Nutrition analysis and Fitness software version 7.9 (Esna Research, Salem, USA), with the addition of composition of some local foods based on a food composition table from China<sup>(22)</sup>. Intakes of Na were calculated from food, drinks and the amount of salt used in cooking or at table. To assess the validity of the reported energy intakes, the intake divided by basal metabolic rate was calculated<sup>(23)</sup>. A ratio of less than 1.35 was used to indicate potential under-reporters of energy intake.

### Dietary estimates of net endogenous acid production

Several algorithms have been developed to estimate NEAP from diet<sup>(24)</sup>. Frassetto *et al.* calculated the estimated NEAP from the diet's protein:K ratio<sup>(25)</sup> whereas Remer *et al.* estimated NEAP from average intestinal absorption rates of ingested protein and other minerals as well as an anthropometry-based estimate for organic acid excretion<sup>(26)</sup>. Each has its rationale and limitations<sup>(27)</sup>.

In this study, Frassetto's method was adopted to allow for the particular dietary focus of the present study. The method calculates the diet's protein:K ratio expressed as g/mEq and exclusively estimates the diet-dependent net acid load to the metabolic system. The rationale and algorithm of this method have been described previously and the estimated NEAP could account for about 70% of the variation in renal net acid excretion<sup>(25)</sup>.

### Statistical analysis

Data analysis was performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). Data are presented as means and standard deviations, unless indicated otherwise. Normality of the data was checked by histograms. Log transformation was applied for intakes of vitamins C, D and K for analysis. The independent *t* test and non-parametric Mann–Whitney *U* test were used to compare characteristics between boys and girls. Analysis of covariance (ANCOVA) was used to compare the nutrient intakes between boys and girls after adjustment for daily energy intake.

Multiple regression analysis was conducted to examine the contribution of estimated NEAP in explaining the variance of BMC and BMAD in each measured site. Either BMC or BMAD was the dependent variable. For the model with BMC, the following predictor variables were entered into a multiple linear regression analysis: BA, weight, height, pubertal stage (stage I as reference category), dietary protein, Ca and K intakes and estimated NEAP. The same set of predictor variables was entered for the regression model of BMAD, except for BA. Protein, Ca and K intakes and estimated NEAP were adjusted for dietary energy intake by the residual method<sup>(28)</sup>. Statistical tests were considered significant if  $P < 0.05$  (two-sided).

## Results

### Subject characteristics

The anthropometric and bone characteristics of the study population are presented in Table 1. These characteristics differed significantly between boys and girls, with boys being older and having higher weight, height and BMI than girls. Boys had significantly higher hip and whole body BMC but lower spine and whole body BMAD than girls.

### Daily nutrient intakes and estimated net endogenous acid production

Table 2 shows the daily nutrient intakes and the estimated NEAP of the study population. There was no significant difference in the energy-adjusted intakes of most nutrients and estimated NEAP between boys and girls, except for intakes of vitamins C and D. In all, 13.5% of the boys ( $n$  23) and 8.3% of girls ( $n$  15) were found to be potential under-reporters, but no significant sex difference was observed. Subjects with higher BMI were more likely to be under-reporters (details not shown).

### Factors predicting bone mineral content or bone mineral apparent density at different sites

No significant association was observed between BMC and energy-adjusted NEAP or other nutrients (Table 3). BA was significantly and positively associated with BMC at all sites in both boys and girls. A significant positive association was found between weight and BMC in hip and spine in both sexes. Height showed negative correlation with BMC at all sites, but significant difference was only observed in hip for boys and in whole body for girls. Pubertal stage was significantly and positively associated with BMC in different sites in both sexes. The model accounted for 80–90% of the

variability in BMC of different measured sites in boys and 85–94% in girls. No significant association was observed between BMAD and energy-adjusted NEAP or other nutrients (Table 4). Weight and height contributed most of the variability in BMAD at different sites. The models explained about 17–54% of the variability in BMD at different sites in boys, and 23–62% in girls.

## Discussion

The results of this study suggested that anthropometric characteristics and pubertal stage were the more influential factors than dietary NEAP and dietary intake of protein, Ca and K in determining bone mineral status of Hong Kong Chinese adolescents.

Anthropometric measurement and pubertal stage were the main predictors for BMC and BMAD in the study population. The results were supported by previous studies<sup>(29,30)</sup>. Cheng *et al.*<sup>(29)</sup> examined the effects of puberty, physical activity, physical fitness and Ca intake on bone mineral acquisition in 179 healthy Hong Kong Chinese adolescents aged 12–16 years. Pubertal stage overrode all external factors in determining bone mass accretion in their studied subjects. Carter and colleagues<sup>(30)</sup> investigated the effect of Ca intake on BMC in 227 children aged 8–17 years in Saskatoon, and found that over 90% of the variability in total body and spine BMC were accounted for by BA, weight and height. The results illustrated the importance of adjusting for size-related variables in multivariate analyses of BMC before examining the effect of dietary intake as proposed by Prentice *et al.*<sup>(31)</sup>. Prentice and colleagues suggested that the relationship between BMC and BA at various sites may change when adjustments are made to account for differences in body size between individuals over and above differences in bone size. Where weight and height were not allowed for, BA considered as part of

**Table 1.** Anthropometric and bone characteristics of the study population (Mean values and standard deviations for 171 boys and 180 girls)

	Boys		Girls		P value*
	Mean	SD	Mean	SD	
Age (years)	11.7	0.4	10.7	0.4	<0.0001
Weight (kg)	40.9	9.3	35.7	8.0	<0.0001
Height (cm)	147.6	7.7	143.0	7.5	<0.0001
BMI (kg/m <sup>2</sup> )	18.6	3.3	17.3	2.7	<0.0001
Tanner stage†					–
I	44 (25.7)		99 (55.3)‡		
II	102 (59.7)		59 (33)		
III	25 (14.6)		19 (10.6)		
IV	0 (0)		2 (1.1)		
Total hip BMC (g)	19.51	4.35	17.01	4.05	<0.0001
L1–L4 spine BMC (g)	27.66	6.05	27.39	7.24	0.7097
Whole body BMC (g)	1163.08	215.58	1061.10	222.25	<0.0001
Total hip BMAD (g/cm <sup>3</sup> )	0.027	0.004	0.027	0.003	0.4651
L1–L4 spine BMAD (g/cm <sup>3</sup> )	0.084	0.009	0.090	0.010	<0.0001
Whole body BMAD (g/cm <sup>3</sup> )	0.091	0.010	0.094	0.009	0.0034

BMC, bone mineral content; BMAD, bone mineral apparent density; L1–L4, lumbar regions.

\* Gender difference by independent *t* test.

†  $n$  (%).

‡ One girl refused assessment of pubertal development.

**Table 2.** Estimated net endogenous acid production (NEAP) and daily nutrient intakes for 171 boys and 180 girls\*  
(Mean values and standard deviations; medians and interquartile ranges (IQR))

	Boys				Girls				P value†	P value‡
	Mean	SD	Median	IQR	Mean	SD	Median	IQR		
Energy (kJ/d)	9128.2	2084.2			8251.8	1994.3			<0.0001	–
Protein										
(g/d)	94.3	27.9			82.0	23.2			<0.0001	0.0502
(g/kJ)	0.0103	0.0017			0.0099	0.0014				
Fat										
(g/d)	81.7	24.0			73.1	21.0			0.0004	0.8883
(g/kJ)	0.0089	0.0013			0.0088	0.0013				
Carbohydrate										
(g/d)	266.9	62.6			247.4	65.9			0.0048	0.1318
(g/kJ)	0.0294	0.0035			0.0300	0.0033				
Na										
(mg/d)	3725.5	1200.6			3505.9	1121.5			0.0773	0.9020
(mg/kJ)	0.4131	0.1188			0.4355	0.1315				
K										
(mg/d)	2121.7	672.1			1976.3	706.3			0.0492	0.0740
(mg/kJ)	0.2310	0.0442			0.2377	0.0520				
Mg										
(mg/d)	249.1	69.2			223.8	63.6			0.0004	0.5166
(mg/kJ)	0.0274	0.0048			0.0272	0.0044				
Ca										
(mg/d)	608.2	224.8			546.0	201.1			0.0065	0.7787
(mg/kJ)	0.0665	0.0203			0.0662	0.0188				
P										
(mg/d)	1153.9	337.1			1034.7	292.4			0.0004	0.8938
(mg/kJ)	0.1259	0.0202			0.1251	0.0166				
Ca:P	0.53	0.15			0.53	0.13			0.9073	–
Vitamin C§										
(mg/d)			99.7	72.7–135.0			94.8	70.6–155.8	0.9945	0.0118
(mg/kJ)			0.0109	0.0083–0.0150			0.0122	0.0094–0.0181		
Vitamin D§										
(µg/d)			2.62	1.71–3.80			2.54	1.63–3.59	0.6966	0.0370
(µg/kJ)			0.0003	0.0002–0.0004			0.0003	0.0002–0.0004		
Vitamin K§										
(µg/d)			139.3	93.7–215.9			139.4	88.1–209.9	0.6543	0.8205
(µg/kJ)			0.0155	0.0105–0.0235			0.0168	0.0115–0.0282		
Estimated NEAP										
(g/mEq per d)	1.8	0.3			1.7	0.3			0.0051	0.0812
(g/mEq per kJ) ( × 10 <sup>3</sup> )	0.02	0.06			0.022	0.07				

\* For details of procedures see Materials and methods.

† Two samples *t* test.

‡ Analysis of covariance (ANCOVA) adjusted for energy intake.

§ The intake of these nutrients was not normally distributed. Vitamins C, D, K are log transformed for comparison in ANCOVA.

|| Mann–Whitney *U* test.

**Table 3.** Regression models of energy adjusted estimated net endogenous acid production (NEAP) and nutrients in predicting bone mineral content (BMC) at hip, lumbar region L1–L4 spine and whole body for boys (*n* 171) and girls (*n* 179)\*

	Hip BMC			L1–L4 spine BMC			Whole body BMC		
	$\beta$	<i>P</i> value	<i>r</i> <sup>2</sup>	$\beta$	<i>P</i> value	<i>r</i> <sup>2</sup>	$\beta$	<i>P</i> value	<i>r</i> <sup>2</sup>
<b>Boys</b>									
Estimated NEAP (g/mEq)	-1.4362	0.3461	0.0055	-3.5001	0.1571	0.0124	-89.17	0.1625	0.0121
Ca (mg)	0.0002	0.8085	0.0004	0.0010	0.4662	0.0033	0.0197	0.5684	0.0020
Protein (g)	0.0230	0.4075	0.0043	0.0680	0.1335	0.0139	1.3527	0.2436	0.0084
K (mg)	-0.0005	0.6671	0.0012	-0.0030	0.1192	0.0150	-0.0581	0.2401	0.0086
Bone area (cm <sup>2</sup> )	0.8364	<0.0001	0.5654	0.7077	<0.0001	0.4252	1.1533	<0.0001	0.5381
Weight (kg)	0.1324	<0.0001	0.2034	0.1588	<0.0001	0.1255	-1.7981	0.1987	0.0102
Height (cm)	-0.0681	0.0450	0.0247	-0.0054	0.9236	0.0001	-2.5391	0.0837	0.0185
Pubertal stages									
II v. I	-0.3073	0.3543	0.0053	-0.0812	0.8796	0.0001	-16.49	0.2314	0.0089
III v. I	0.9504	0.0658	0.0209	2.3519	0.0052	0.0476	21.12	0.3222	0.0061
Constant	2.8248			-6.3697			171.50		
<i>R</i> <sup>2</sup>	0.8595			0.8095			0.9014		
<b>Girls</b>									
Estimated NEAP (g/mEq)	0.9454	0.3030	0.0063	-1.7657	0.2490	0.0079	-15.92	0.6035	0.0016
Ca (mg)	0.0004	0.6498	0.0012	-0.0006	0.7074	0.0008	0.0094	0.7617	0.0005
Protein (g)	-0.0042	0.8319	0.0003	0.0377	0.2539	0.0077	-0.3567	0.5872	0.0018
K (mg)	0.0005	0.4730	0.0031	-0.0014	0.1874	0.0103	-0.0070	0.7506	0.0006
Bone area (cm <sup>2</sup> )	0.7927	<0.0001	0.4880	0.8477	<0.0001	0.5213	1.2688	<0.0001	0.7064
Weight (kg)	0.1487	<0.0001	0.1876	0.2192	<0.0001	0.1548	-3.1387	0.0106	0.0383
Height (cm)	-0.0224	0.5055	0.0026	-0.0255	0.6524	0.0012	-3.7751	0.0011	0.0616
Pubertal stages									
II v. I	0.5135	0.1177	0.0145	0.4712	0.3896	0.0044	8.8260	0.4193	0.0039
III v. I	0.5278	0.2912	0.0066	1.1884	0.1640	0.0115	-5.0004	0.7658	0.0005
IV v. I	4.8612	0.0001	0.0863	4.5655	0.0254	0.0294	103.44	0.0120	0.0369
Constant	-7.6836			-12.13			158.04		
<i>R</i> <sup>2</sup>	0.8501			0.8699			0.9443		

$\beta$ , Parameter estimate; *r*<sup>2</sup>, partial *R*<sup>2</sup>.

\*For details of procedures see Materials and methods.

**Table 4.** Regression models of energy adjusted estimated net endogenous acid production (NEAP) and nutrients in predicting bone mineral apparent density (BMAD) at hip, lumbar region L1–L4 spine and whole body for boys (*n* 171) and girls (*n* 179)\*

	Hip BMAD			L1–L4 spine BMAD			Whole body BMAD		
	$\beta$	<i>P</i> value	<i>r</i> <sup>2</sup>	$\beta$	<i>P</i> value	<i>r</i> <sup>2</sup>	$\beta$	<i>P</i> value	<i>r</i> <sup>2</sup>
<b>Boys</b>									
Estimated NEAP (g/mEq)	0.0008	0.7783	0.0005	-0.0073	0.3230	0.0060	-0.0013	0.8243	0.0003
Ca (mg)	$-2 \times 10^{-6}$	0.2717	0.0075	$1 \times 10^{-6}$	0.7170	0.0008	$5 \times 10^{-7}$	0.8891	0.0001
Protein (g)	$7 \times 10^{-6}$	0.8839	0.0001	0.0002	0.2505	0.0081	$1 \times 10^{-5}$	0.9567	$2 \times 10^{-5}$
K (mg)	$1 \times 10^{-6}$	0.5131	0.0027	$-1 \times 10^{-5}$	0.3135	0.0063	$4 \times 10^{-7}$	0.9361	$4 \times 10^{-5}$
Weight (kg)	0.0001	0.0031	0.0526	0.0004	<0.0001	0.1093	-0.0007	<0.0001	0.3203
Height (cm)	-0.0004	<0.0001	0.2748	-0.0003	0.0108	0.0394	-0.0001	0.2964	0.0067
Pubertal stages									
II v. I	-0.0008	0.1723	0.0115	$-4 \times 10^{-5}$	0.9790	$4 \times 10^{-6}$	-0.0012	0.3740	0.0049
III v. I	0.0006	0.5332	0.0024	0.0066	0.0091	0.0412	0.0011	0.5773	0.0019
Constant	0.0736			0.1244			0.1370		
<i>R</i> <sup>2</sup>	0.3939			0.1752			0.5441		
<b>Girls</b>									
Estimated NEAP(g/mEq)	0.0007	0.6846	0.0010	-0.0057	0.2326	0.0084	-0.0017	0.5870	0.0018
Ca (mg)	$3 \times 10^{-7}$	0.8520	0.0002	$-1 \times 10^{-6}$	0.8801	0.0001	$-2 \times 10^{-6}$	0.5270	0.0024
Protein (g)	$2 \times 10^{-5}$	0.5191	0.0025	0.0001	0.1753	0.0108	$-5 \times 10^{-5}$	0.4946	0.0028
K (mg)	$-1 \times 10^{-7}$	0.9277	$4 \times 10^{-5}$	$-4 \times 10^{-6}$	0.2119	0.0092	$-1 \times 10^{-6}$	0.7240	0.0007
Weight (kg)	0.0002	0.0002	0.0786	0.0006	<0.0001	0.1229	-0.0008	<0.0001	0.3404
Height (cm)	-0.0003	<0.0001	0.1853	-0.0001	0.4843	0.0029	-0.0001	0.2087	0.0093
Pubertal stages									
II v. I	0.0005	0.4665	0.0031	0.0014	0.4209	0.0038	0.0007	0.5566	0.0021
III v. I	0.0007	0.4541	0.0033	0.0019	0.4751	0.0030	-0.0008	0.6383	0.0013
IV v. I	0.0079	0.0008	0.0642	0.0119	0.0614	0.0206	0.0049	0.2459	0.0080
Constant	0.0622			0.0885			0.1470		
<i>R</i> <sup>2</sup>	0.2379			0.2759			0.6199		

$\beta$ , Parameter estimate; *r*<sup>2</sup>, partial *R*<sup>2</sup>.

\*For details of procedures see Materials and methods.

bone stability provided information about the overall size of the individual and the size of the bone being scanned.

The present findings failed to show an association between bone variables and dietary Ca intake. The results were in line with those reported by Cheng *et al.* (29) and Cvijetic *et al.* (32), in which no significant effect of Ca intake on bone mass accretion or bone stiffness was found. The effect of increasing Ca and dairy product intake for promoting child and adolescent bone mineralization, especially for non-white populations however remains controversial (33). Instead, there is increasing evidence to show that bone development is influenced by the overall diet and not just by a single nutrient. Nutrients may interact with each other and with other genetic and environmental factors, and these complex interactions are likely the main reason for inconsistent results in studies of the role of single nutrients in bone health. Therefore, quantifying the acid–base contents of diets generally consumed by populations is useful for identifying the diets' effects on bone health (34). As NEAP is difficult to measure directly, we applied the simple algorithm by Frassetto *et al.* (25) to estimate the NEAP from the diet's protein:K ratio.

Our data do not support the growing interest in the importance of acid–base homeostasis to skeletal integrity. No significant association was observed between estimated NEAP and BMC or BMAD in our subjects. The result was in line with that of the study by Ginty *et al.* in which no evidence for a negative association between bone mineral status and indirect estimates of renal net acid excretion was found in 212 adolescent boys and girls (35). However, previous studies examining dietary acidity and bone variables in children (9) and women (36,37) showed different results. Alexy *et al.* (9) showed that children with higher dietary acid load had significantly less cortical area and BMC. However, the influence of dietary acid load on the growing bone was not very strong and could only be unmasked after adjustment for the stronger protein-anabolic impact on bone (9). In women, it was shown that diets with a lower protein content but higher K content (i.e. lower acidity or higher alkalinity) were associated with greater bone mass and a tendency to less bone resorption (36,37). Higher K content could simply be a marker of higher fruit and vegetable intake. The beneficial role of fruit and vegetable intake on bone might be related to the alkalinizing effect (38), or factors secondary to the fruit and vegetable intake, such as a healthier lifestyle (39), the presence of vitamins C and K (8,40) and other plant-based compounds (41,42). Therefore, it was proposed that the balance between the amount of protein in the diet and the dietary acid load in part determines whether the diet as a whole has a net anabolic or catabolic effect on bone (43). High protein intake by Hong Kong children and low intake of fruits and vegetables have long been documented (14,44). The mean protein intake of the sample studied was also higher than the Chinese dietary reference intake (45). Considering the tracking effect of diet from childhood to adulthood, the effect of high protein intake and low fruit and vegetable intake on the long-term bone health of Hong Kong Chinese adolescents warrants attention.

We failed to show any association between bone variables and estimated NEAP or nutrient intakes in our adolescent subjects. Issues on the methodological weaknesses should be considered in interpreting the results. The DXA method used for the measurements may not have been accurate enough to

specifically identify the association as it provides only two-dimensional projection that is affected by the subject's size (46,47). Its analyses in the growing years require adjustment for bone size, which itself is also a parameter of bone stability. Thus, architectural bone structures responsible for bone strength cannot be determined specifically with this kind of analysis. We tried to minimize the problem of size effects by using BMAD (47); the results however remained the same. Second, the use of FFQ in children requires attention. Correlation between FFQ and other diet-assessment methods (e.g. food weighed dietary record) is small-to-modest (48). Poor agreement has been observed in estimating protein intake in children and adolescents by FFQ as compared with food weighed dietary record, and FFQ is primarily used for ranking individual diet, not for quantifying individual intake (49,50). We tried to minimize the reporting errors by using the set of photographs and by adding enough items for better discrimination of individual consumption. However, careful interpretation of results is needed. Although the subjects were not recruited on a random basis, they were recruited in nine primary schools of various school types and different geographic locations in Hong Kong. Their anthropometric and dietary data were also comparable to those reported previously for Hong Kong adolescents (51,52). Therefore, we speculated that the present findings could reflect the current dietary practice of adolescents of similar age in Hong Kong.

In conclusion, the findings of this study suggest that anthropometric characteristics and pubertal stage are the more influential factors than dietary NEAP in determining bone mineral status of Hong Kong Chinese adolescents. However, issues on the methodological weaknesses regarding the use of DXA and FFQ in the present study require attention.

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