Calcium absorption measured by stable calcium isotopes ($^{42}$Ca & $^{44}$Ca) among northern Chinese adolescents with low vitamin D status

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ABSTRACT

An adequate calcium intake and vitamin-D status is important for bone mineralization in adolescents. In Northern China, calcium intake and plasma vitamin-D level of adolescents is low due to low consumption of dairy foods and inadequate sunshine exposure. True fractional calcium absorption (TFCA) in Chinese adolescents has never been performed.

This study aims to evaluate nutritional adaptation namely, TFCA and urinary calcium excretion among Chinese adolescents in northern China.

Key words: calcium absorption, calcium intake, 25-hydroxyvitamin D, puberty, Chinese

INTRODUCTION

In China, calcium intakes of children are low due to their non-dairy based diets. Calcium intakes of school children are usually below 500 mg/d (Lee et al. 1993, Institute of Nutrition & Food Hygiene, 1992). Results of both cross-sectional and follow-up studies (Lee et al. 1993, Johnson et al. 1992, Lee et al. 1994a, Lee et al. 1995a, Lee et al., 1996) have together suggested that a persistently higher calcium intake in childhood and adolescence may help to promote higher bone mass in adulthood. Maximization of peak bone mass by life-long adequate calcium intake is recognized as the best protection against the development of osteoporosis in later life (Institute of Medicine 1997). Until recently, there have been limited studies to examine nutritional adaptation among children accustomed to low calcium diets coupled with a low vitamin D status. Whether children with low calcium intake and low vitamin D status are able to adapt to optimise skeletal growth is largely unknown.

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Our previous study has indicated that growing children with normal plasma vitamin D status accustomed to low calcium diets were able to enhance calcium absorption and reduce urinary calcium excretion (Lee et al. 1994b). The figure for true fractional calcium absorption (TFCA) (54 – 63 %) was found significantly higher then those of US children (25-34%) consuming a calcium diet of 925 mg/d (Abrams & Stuff 1994). This result implies that there is a difference in calcium handling between the Chinese and American children (Lee et al. 1995b). In fact, our previous study was conducted in Southern China where there is plenty of sunshine throughout the year. Children could obtain most of their vitamin D by regular exposure to the sun. Adequate circulating vitamin D seems to allow nutritional adaptation to occur (Lee et al. 1994b, 1995b). Nonetheless, the climate in Northern China is totally different from that in the South, where children experience long cold winters and a lack of sunlight exposure leads to sub-optimal vitamin D status. Among Beijing school children, the average plasma 25(OH)-vitamin D₃ (25-OHD) level in winter is alarmingly low (13.4 nmol/L) and nearly 50% of school children have plasma 25-OHD level below the lower normal limit of 12.5 nmol/L (Du 1998). Plasma 25-OHD level and calcium intakes were found to be strong predictors on bone mass in Beijing children (Du 1998). The prevalence of nutritional rickets in North China is still high among children under 3 years of age (6–44%) and adolescents (7-24%), which is attributable to low sunshine exposure and poor source of vitamin D in foods (Wu et al. 1991, Du 2001). It is not known whether these children with poor vitamin D status and subsisting on low calcium diets are able to absorb adequate calcium for bone mineralization.

Objective
The objective of the present study was to determine whether adolescents living in northern China with lower vitamin D status are still capable of enhancing calcium absorptive efficiency.

MATERIALS AND METHODS
Sixteen students (12 girls, 4 boys) aged 9 to 16 years were recruited from schools in Beijing during December, 1999. All the students were healthy, free from any metabolic diseases that may interfere with calcium and vitamin D metabolism, and without any history of bone fractures in the previous 12 months. Vitamin D status of Beijing adolescents is lowest during winter due to low sunshine exposure (Du 1998).

In vivo TFCA was determined by the dual stable isotope technique which is non-radioactive and safe to be applied on children and adolescents (Yergey et al. 1987, Hillman et al. 1988). The use of dual stable isotopes technique is based on the validated method developed by DeGrazia et al (1965) and Hillman et al. (1988), and modified by the author (WTKL) (Lee et al. 1994b, 1995b). After an overnight fast, ⁴⁴Ca (0.5 mg/kg) was given orally by mixing with 120 ml cow’s milk 24-h in advance of the study. The milk isotope was sub-divided into three small doses and were administrated with the three main meals of the day. Sub-dividing up the oral tracer into three small doses to be given with each of the three meals in a day was found to best represent the overall absorption of calcium (Eastell et al. 1989). ⁴²Ca solution for injection was prepared, sterilised and dispensed aseptically into a vial in the Clinical Pharmacological Research Center Laboratory, Peking Union Medical College Hospital. ⁴⁴Ca (0.05 mg/kg) was given intravenously via the antecubital vein by a nurse immediately after the first oral dose. 5 ml of venous blood was drawn for the measurement of 25-OHD and serum calcium concentrations immediately before the i.v. injection of ⁴²Ca solution. Details of dosing the subjects can be found elsewhere (Lee et al. 1994b, 1995b). 24-h urine collection started exactly 24-h after the isotopes were administered in order to determine the recovery of the two isotopes in the urine.

Calcium intake was assessed by a quantitative food frequency questionnaire specifically designed for use among Chinese children and adolescents (Lee et al. 1996), weight and height were measured by standard methods as described elsewhere (Lee et al. 1994b). Plasma 25-OHD was measured by a competitive protein assay (Amersham, Bucks., U.K.) (Lee et al. 1994b). Serum calcium and 24-h urinary calcium concentrations were analysed by automated analysis (Beckman Synchron CX3 Clinical System, CA, USA.). Informed consent was obtained from the parent. The research protocol was approved by the Human Research Ethics Committee, The University of Newcastle.

Mass spectrometric analysis for isotopic recovery in urine
The organic matters of the urine were removed by digestion upon heat with concentrated nitric acid. Calcium was precipitated from the digested sample by using ammonium oxalate (Fairweather-Tait 1995). All the samples were analysed by Thermal Ionization Mass Spectrometry using a Finnegan MAT 261 Magnet Sector Mass Spectrometer (Finnegan-Mat GmbH,
Bremen, Germany). The calculation of TFCA was based on the fact that both intravenously and orally administered calcium isotopes are metabolised at the same rate once achieving equilibrium. TFCA was determined according to the equation developed by Yergey et al. (1987).

**Statistical analysis**

Descriptive statistics (mean, SD and range) were calculated for all variables. Linear regression analysis was employed to study the relation between different variables and TFCA. The level of significance was set to \( P \leq 0.05 \), 2-tailed. Statistical analysis was performed by using SPSS 8.0 for Windows (SPSS Inc. Chicago, IL, USA).

**RESULTS**

Characteristics of the 16 subjects are shown in Table 1. Mean age of the subjects was 12.2 ± 2.1 years. Mean calcium intake of the subjects was 603 ± 158 mg/d. Milk and dairy products contributed to 54% of the daily total calcium intake (317 ± 133 mg/d), whereas vegetables, beans and bean products contributed to 9% and 5% of the total daily calcium intake of these adolescents.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the 16 subjects</th>
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<tbody>
<tr>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age (y)</td>
<td>12.2 ± 2.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>41.2 ± 9.8</td>
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<tr>
<td>Height (cm)</td>
<td>1.51 ± 0.09</td>
</tr>
<tr>
<td>Calcium Intake (mg/d)</td>
<td>603 ± 158</td>
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</table>

Mean true fractional calcium absorption (TFCA), serum 25-OHD, serum calcium and 24-h urinary calcium of the 16 subjects are depicted in Table 2. There were no association between TFCA and age (\( r = -0.11, p=0.73 \)), weight (\( r = 0.10, p = 0.75 \)), height (\( r = 0.16, p=0.93 \)), dietary calcium (\( r = 0.13, p = 0.69 \)) or 24-h urinary calcium excretion (\( r = -0.17, p = 0.59 \)). Again, there was a significant negative correlation between TFCA and plasma 25-OHD (\( r = -0.73, p = 0.008 \)).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>True fractional calcium absorption (TFCA), plasma 25-OHD, serum calcium and 24-h urinary calcium of the 16 subjects</th>
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<tbody>
<tr>
<td>Mean ± SD</td>
<td>Range</td>
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<tr>
<td>TFCA (%)</td>
<td>57.4 ± 15.4</td>
</tr>
<tr>
<td>25-OHD (nmol/L)</td>
<td>34.3 ± 12</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.3 ± 0.15</td>
</tr>
<tr>
<td>24-h urinary calcium (mg/d)</td>
<td>87.5 ± 59.2</td>
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</table>

The subjects in the study are predominantly girls; therefore, the results are also analysed and presented by grouping all the girls in one group. The characteristics of the adolescent girls are shown in Table 3. Mean calcium intake of the girls was 591 ± 164 mg/d. Mean TFCA, serum 25-OHD, serum calcium and 24-h urinary calcium of the 12 girls are depicted in Table 4. There were no association between TFCA and age (\( r = -0.11, p=0.73 \)), weight (\( r = 0.10, p = 0.75 \)), height (\( r = 0.16, p=0.93 \)), dietary calcium (\( r = 0.13, p = 0.69 \)) or 24-h urinary calcium excretion (\( r = -0.17, p = 0.59 \)). Again, there was a significant negative correlation between TFCA and plasma 25-OHD (\( r = -0.73, p = 0.008 \)).

<table>
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<th>Table 3</th>
<th>Characteristics of the 12 Beijing adolescent girls</th>
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<tbody>
<tr>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age (y)</td>
<td>12.4 ± 2.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>42.1 ± 9.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.52 ± 0.09</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>591 ± 164</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>True fractional calcium absorption (TFCA), serum 25-OHD, serum calcium and 24-h urinary calcium of the 12 Beijing adolescent girls</th>
</tr>
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<tbody>
<tr>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>TFCA (%)</td>
<td>60.4 ± 14.4</td>
</tr>
<tr>
<td>25-OHD (nmol/L)</td>
<td>30.54 ± 9.835</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.29 ± 0.16</td>
</tr>
<tr>
<td>24-h urinary calcium (mg/d)</td>
<td>79.9 ± 49.6</td>
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</table>
DISCUSSION

The present study was piloted to evaluate true fractional calcium absorption in relation to vitamin D status and urinary calcium excretion in a group of adolescents with poor vitamin D status living in northern China. The number of subjects in this pilot study was limited to 16 due to the high costs of the isotopes (HK$3,600 per subject in 1999) and the laboratory cost for treating urine samples (HK$400 per subject).

Traditionally, calcium absorption has been determined by balance studies. However, this approach fails to differentiate the endogenous calcium from the dietary source, and hence underestimates the true fraction of calcium absorbed. Furthermore, the balance technique is tedious, labour intensive, and has inherent problems of incomplete urine and fecal collection. Although TFCA can be determined by using radioisotopes of calcium (DeGrazia et al. 1965), the inherent potential hazards of ionizing radiation limit its use in growing children. The recent development of a dual stable isotope technique provides a safe, non-radioactive and accurate method of measuring in vivo TFCA in infants and children (Hillman et al. 1988, Lee et al. 1994b, Abrams et al. 1997). Most importantly, the dual stable isotopes technique can correct for the endogenous calcium secreted into the gut and greatly improves the accuracy of the estimation of TFCA. By collecting a urine sample 24 hours after the absorption test, TFCA can be evaluated by determining the ratio of the dual stable isotopes recovered in the urinary sample using a thermal ionisation mass spectrometer (Lee et al. 1994b, 1995b; Abrams et al. 1997). The dual stable isotopes technique is less time consuming, at one day compared to 3-4 weeks with the traditional balance method. Thus, the new technique also improves subject compliance (Lee et al. 1995b; Abrams et al. 1997). In fact, the dual stable isotope technique is currently adopted by international nutrition expert groups (Department of Health, 1991, Institute of Medicine, 1997) as the gold standard to determine in vivo calcium bioavailability. The determination of in vivo calcium bioavailability is essential to establish calcium requirement and thus recommended dietary allowances (RDAs) for calcium (Department of Health, 1991).

The mean TFCA of the 16 subjects was higher than the US counterparts (25–34%) with calcium intake at 925 mg/d (Abrams & Stuff, 1994). In addition, the mean TFCA from the present study was comparable to the author’s previous calcium absorption study among 7-year-old children in Hong Kong (TFCA: 54–63%, dietary calcium intake: 862 mg/d and plasma 25-OHD 83.25 nmol/L). The findings from the present study and our previous studies (Lee et al. 1994b, 1995b) confirmed that there is a difference in TFCA between the Chinese and Caucasian children and adolescents. An adequate vitamin D status is important for enhancing calcium absorption (Lee et al. 1993), the mean serum level of 25-OHD of the subjects in the present study was close to the lower normal limit of 27.5 nmol/L.

It is interesting to find that there was a strong inverse correlation between TFCA and plasma 25-OHD, the explanation for this phenomenon is uncertain and it has never been reported before. It is speculated that adolescents with escalated demand for bone mineralization during pubertal bone growth may have adapted to a habitually lower vitamin D status by increasing the rate of conversion of 25-OHD in the kidney to the active metabolite of vitamin D₃ (1,25(OH)₂D₃). Further study is necessary to look into the profile of calcium homeostasis in this group of adolescents in order to understand the mechanism of nutritional adaptation. Mean 24-hour urinary calcium excretion was lower than 100 mg/d implied that there was higher renal conservation of calcium in this group of adolescents in order to retain sufficient calcium for bone growth.

Results of the current study will provide some new scientific data to revise the Recommended Dietary Allowances (RDAs) for children and adolescents in China. Furthermore, countries in South East Asia are in a joint effort to establish local RDAs figures based on research studies on local populations (Tee, 1998). Findings from this study will provide some reference data for the formulation of RDAs in Asian children and adolescents subsisting on non-dairy based diets.

Standard deviations of the outcomes given in Table 2 were wide among the 16 subjects with inter-subject coefficient of variations (CV = SD/Mean x 100%) of TFCA, 25-OHD and 24-h urinary calcium at 26.8%, 35% and 67% respectively. These discrepancies may be due to a wide age range (9 to 17 years old), individual variation in calcium intake (402–847 mg/d) and calcium handling. In our previous TFCA study, the studied children (n=34) were all aged 7, and the CV of TFCA was 13–17%. Furthermore, subjects in our previous study were stratified into two groups based on calcium intake (< 500 mg/d and > 500 mg/d) prior to group mean comparison (Lee et al. 1994b). On the other hand, inter-subject CV of TFCA was also wide in other studies conducted in USA (Abrams & Stuff 1994; Abrams et al. 1995; O’Brien et al. 1996) (21–39%), and the age range of the subjects in those studies were also large (5 to 17 years or 8 to 16 years).

The inter-subject CV of plasma 25-OHD in the
present study was 35% which might be attributable to the extent of sunshine exposure and oral vitamin D intake. Such a wide inter-subject CV was also observed in previous studies among Black and White girls aged 5–17 years in USA (17–39%) (Abrams & Stuff 1994; Abrams et al. 1995; O’Brien et al. 1996). On the other hand, the inter-subject CV of 24-h urinary calcium excretion was even greater in the present study (67.7%) which is related to dietary intake of sodium and protein and most importantly the stage of pubertal growth — urinary calcium concentration markedly reduces in peripubertal period but not in post-pubertal period (Abrams & Stuff 1994; Abrams et al. 1995). Such a large inter-subject CV of 24-h urinary calcium was also observed in other studies (53–78%) (Abrams & Stuff 1994; Abrams et al. 1995).

In conclusion, the study demonstrated that growing individuals with sub-optimal vitamin D status are still capable of enhancing TFCA and reducing urinary calcium absorption to allow for adequate calcium for bone growth and mineralization. The research findings from this study will provide scientific data as regard to nutritional adaptation in growing children and adolescents, in particular missing data on intestinal absorption, urinary calcium excretion among children and adolescents accustomed to non milk based diet and poor vitamin D status.

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REFERENCES