True fractional calcium absorption in Chinese children measured with stable isotopes (^{42}Ca and ^{44}Ca)

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True fractional Ca absorption (TFCA) was compared in children with different habitual Ca intakes using a double-label stable-isotope technique. Chinese children aged 7 years from Hongkong (*n* 22) and Jiangmen (*n* 12) participated in the study. An oral administration of 8 mg ^{44}Ca in 100 g chocolate milk was given shortly after an intravenous injection of 0·75 mg ^{42}Ca. Ca isotopic ratios were determined in urine samples collected 24 h later using thermal-ionization mass spectrometry. There was no significant difference in TFCA between Jiangmen and Hongkong children ($P = 0·16$). TFCA of a lower-Ca-intake group (Ca $\leqslant$ 500 mg/d, *n* 19) with mean Ca intake 359 mg/d was 63·1 (SD 10·7)% and that of a higher-Ca-intake group (Ca > 500 mg/d, *n* 15) with mean Ca intake 862 mg/d was 54·8 (SD 7·3)%; the difference in TFCA was significant ($P = 0·016$). Serum levels of 25-hydroxycholecalciferol of the children were adequate (33·7 (SD 7·7) ng/ml). The present study indicates that growing children accustomed to a low-Ca diet appear to be able to enhance their absorptive capacity. If it is assumed that dietary Ca absorption by Chinese children resembles their TFCA from a single meal of chocolate milk, then the recommended dietary allowance (RDA) for Ca for Chinese children would be lower than the US RDA (800 mg/d), which is based on an estimated 40% Ca absorption as reported for Caucasian children. A comparative absorption study is necessary to determine whether there is any difference in TFCA between Caucasian and Chinese children.

Calcium: Childhood: Double-label stable-isotope technique

In China and Hongkong there has been an increasing concern that children with dietary Ca intakes below the US recommended dietary allowance (RDA; National Research Council, 1989) would have a lower Ca retention which might result in a reduced peak bone mass (Matkovic *et al.* 1990; Matkovic, 1991, 1992; Anderson, 1992). Children in China do not usually consume milk and have an average Ca intake of about 300 mg/d (Ho, 1988; Lee *et al.* 1993*b*). Habitual Ca intakes of 5-year-old children from Jiangmen, Guangdong Province, China, are below 300 mg/d due to the low consumption of milk and milk products after 1 year of age (Lee *et al.* 1993*b*). On the other hand, the eating habits of

Chinese children in Hongkong are more westernized, and the majority of children continue to consume milk and milk products through at least 5 years of age, resulting in an average Ca intake of about 600 mg/d (Lee *et al.* 1993*a*). However, there are also some Hongkong children who gradually reduce the amount of milk consumed from age 1 to 5 years so that by 5 years of age their Ca intake is about 300 mg/d (Lee *et al.* 1993*a, b*). There has been no information on Ca absorption in Chinese children with different levels of Ca intake, and the capacity of these children to adapt to low Ca intake by increasing their efficiency of absorption so as to obtain adequate Ca is not known.

Although the true fractional Ca absorption (TFCA) in humans can be determined using radioisotopes of Ca (DeGrazia *et al.* 1965; Roth & Werner, 1985), the inherent potential hazards of ionizing radiation limit its use in infants and children. Recently, with the development of stable inorganic isotope methodology, a safe and accurate method of measuring TFCA in infants and children has been made possible. The use of stable Ca isotopes in several recent studies has been shown to be an easy and a safe alternative to obtain TFCA data in infants and children under normal and pathological conditions (Yergey *et al.* 1987; Hillman *et al.* 1988; Miller *et al.* 1988).

The present study was designed to examine the hypotheses that children with lower Ca intakes can compensate by enhancing TFCA, and that there is a negative relationship between habitual Ca intake and absorption in children. This is the first report of the TFCA of 7-year-old Chinese children on self-selected diets using a double-label stable-isotope technique.

<center>MATERIALS AND METHODS</center>

<center>*Subjects*</center>

Chinese children aged 7 years from Hongkong (*n* 22) and from Jiangmen, China (*n* 12) took part in the study. The cities of Hongkong and Jiangmen are close, both being located in the geographic region of Guangdong Province in Southern China, and children from both cities are ethnic Cantonese. Hongkong children (twelve boys and ten girls) were randomly selected from a cohort study of growth and nutrition (Leung & Lui, 1990; Lee *et al.* 1993*a*). There is a wide range of Ca intake among Hongkong children depending on whether or not milk is included in the diets. Accurate dietary intake records of these twenty-two Hongkong children had been kept since infancy. Children from Jiangmen in China, on the other hand, seldom consume milk after 1 year of age. Average Ca intake of 5-year-old Jiangmen children was found to be below 300 mg/d (Lee *et al.* 1993*b*); therefore, the pattern of milk intake among children in Jiangmen is similar to that of children in other regions of Southern China (Ho, 1988). Twelve Jiangmen children (six boys and six girls) from a primary school in Jiangmen were randomly selected for the study. All children fulfilled the selection criteria that they were healthy, growing normally and without any previous major illness or fractures of bones.

<center>*Weight and height measurement*</center>

Unclothed weights of children from Hongkong and Jiangmen were measured using a Seca electronic scale (Vogel & Halke GmbH, Hamburg, Germany) and a beam balance (Model: TGT-100; Lichepai, Guangdong, China) respectively. Standing heights of all children were measured without shoes using a stadiometer (Technical Services Unit, The Chinese University of Hongkong).

<center>*Dietary assessment*</center>

Habitual dietary intakes of all children were assessed before the absorption test. Food intake of the Hongkong group was assessed by a research dietitian (W. T. K. L.) using the

method of dietary history, and cross-checked with a quantitative food-frequency questionnaire and 24 h recall (Burke, 1947; Marr, 1971; Bingham, 1987; Jain, 1989). Details of the procedures have been described by Lee *et al.* (1993 *a*). The dietary assessment method has been a uniform approach to evaluate food intake of the Hongkong cohort since weaning (Leung & Lui, 1990; Lee *et al.* 1993 *a*).

Dietary assessment of Jiangmen children was conducted using a quantitative food-frequency questionnaire (Lee *et al.* 1993 *a*) which was similar to the one administered in the Hongkong cohort for cross-checking dietary information. The questionnaire was modified for use in the Hongkong Chinese population. The food-frequency questionnaire consisted of food and beverage items commonly consumed by local Chinese. Non-milk foods such as dark-green leafy vegetables, cereals, fish and shell fish, beans and nuts, etc. which had been identified as significant sources of Ca among Chinese children (Lee *et al.* 1993 *a*) were also listed in the questionnaire in addition to milk and milk products. The foods and beverages in the questionnaire are expressed in common serving size or natural units. Standard household measures like Chinese rice bowls, spoons and Chinese soup spoons, glasses, teacups were used to facilitate portion size description. The parent administered the questionnaire which was subsequently reviewed by a nutritionist. The parent was requested to estimate the frequency in choosing the food items in the questionnaire either per d, per week, or per month.

Nutrient intake was estimated using a computerized food table with food items compiled from appropriate sources (Tung *et al.* 1961; Department of Health, Education & Welfare, 1972; Church & Church, 1975; Paul & Southgate, 1978; Institute of Health, 1980; Watt & Merrill, 1983), food manufacturers and food chemists.

Preparation and administration of stable isotopes

The doses of oral ^{44}Ca and intravenous ^{42}Ca required for administration to children were estimated according to Yergey *et al.* (1987) (0·2–0·5 mg ^{44}Ca/kg body weight and 0·02–0·1 mg ^{42}Ca/kg body weight). Two enriched Ca isotopes: ^{42}Ca (83·20 atom %) and ^{44}Ca (96·40 atom %) in the form of $CaCO_3$ (Technical and Optical Equipment, Tottenham, London) were used as previously described (Fairweather-Tait *et al.* 1989). The ^{44}Ca solution was prepared by dissolving the $CaCO_3$ in concentrated HCl (Aristar grade; 2·5 g $CaCO_3$ in 5 ml HCl), adjusting the pH to 4·0 with 1 M-NaOH (Aristar grade) and making up the solution with deionized distilled water to a final volume of 316 ml. Each dose of approximately 4·3 ml ^{44}Ca was dispensed into a polyethylene tube, sealed and stored at −20°. The ^{42}Ca solution for injection was prepared in the similar manner but the final pH was adjusted to 6·0 for intravenous injection. Each 2 ml dose was sealed in a glass ampoule and autoclaved. Samples underwent routine sterility testing in the pharmacy of the Prince of Wales Hospital, Hongkong. The final concentrations of the oral dose of ^{44}Ca solution and intravenous dose of ^{42}Ca solution were 1·83 and 0·359 mg/ml respectively. The exact quantities of isotopes given to each subject were precisely weighed with an electronic scale accurate to 0·001 mg.

Following an overnight fast, ^{42}Ca was administered slowly into the antecubital vein of each subject and then flushed with 5 ml normal saline (9 g NaCl/l). ^{44}Ca was mixed in 100 g chocolate milk when the child was being given the injection. The Ca concentration of the test meal (100 g chocolate milk) was 120 mg/100 g as determined by atomic absorption spectrometry (Nordin, 1976). The ^{44}Ca-enriched chocolate milk was taken by the subject immediately after the injection. The child was not allowed to eat for 2 h after the test. A standard breakfast consisting of one 75 g sponge cake and a pack of fruit juice (250 ml) was given 2 h later. A 500 ml urine sample was collected starting exactly 24 h after the test to

determine the ratios of isotopes present in the urine. The urine was collected in an acid-washed bottle until the volume of urine reached the 500 ml mark.

Separation and purification of urine samples

Microwave digestion. The 500 ml urine sample was placed in an acid-washed beaker and evaporated to approximately 30 ml in volume and then heated slowly to dryness in a polyethylene tube at 80°. The dehydrated urine sample was stored at $-20°$ before transporting to Norwich for mass spectrometric analysis. The dehydrated urine sample was reconstituted with 15 ml quartz-distilled water and then digested in concentrated HNO_3 (1·5 ml urine to 5 ml HNO_3, Aristar grade) to release bound Ca in the organic matrix of urine using a microwave oven (CEM Model MD5-2000 Microwave Sample Preparation System; CEM Corporation, Matthews, NC, USA). Before microwave digestion the mixture of each urine sample and concentrated HNO_3 was kept in a digestion vessel overnight (to release gases produced on breaking down of organic constituents). Twelve vessels of urine samples were loaded into the microwave oven at a time. The process of digestion was enhanced by heating the sample in the vessel under controlled pressure. In the initial 30 min the urine sample was heated at 80–90 % of power to bring up the pressure to 70 lb/in²; then the pressure of the vessel was gradually increased to 85 lb/in² and maintained at this level under 100 % full power for 1 h.

Anion-exchange columns. Generation of a stable ionic beam from the Ca sample is of paramount importance for an accurate and precise determination of isotopic ratios of Ca by thermal-ionization mass spectrometry (Mueller & Walker, 1987; Tackett & Ellefson, 1987). From a rapid check of the impurities present in the samples using the fast-atom-bombardment mass spectrometer (FABMS; MS902; Kratos Analytical Instruments, Manchester), we found that the most troublesome impurity interfering with the stability of the Ca signal was ^{40}K. In order to separate and purify the Ca from the digested urine two separate sets of anion-exchange-resin columns were used to eliminate inorganic contaminants, especially ^{40}K. The ion-exchange resin used was Dowex 50 W-hydrogen, 8 % cross-linking, 100–200 dry mesh resin (Aldrich Chemical Co., Dorset). The first set of columns was washed with 2 M-HNO_3 (Aristar), followed by 0·2 M-HNO_3. The digested urine samples were evaporated to dryness, redissolved in 0·2 M-HNO_3 and then applied to the columns which were subsequently washed with 0·2 M-HNO_3 before eluting the samples with 2 M-HNO_3. The resin in the second set of columns was washed with 5·5 M-HCl (Aristar), followed by quartz-distilled water. The eluates from the previous set of columns were evaporated to dryness, redissolved in 0·04 M-HCl before loading into the second set of columns. The columns loaded with Ca samples were flushed with quartz-distilled water; subsequently, the purified Ca samples were eluted in 1 ml fractions with 1·8 M-HCl. The appearance of maximum concentration of Ca was found in the 10th–12th fractions as determined by FABMS. Therefore, the Ca fractions were collected after discarding the first 10 ml of the eluate. The samples were then evaporated to dryness under a 1 kW lamp in a laminar flow cabinet in the clean room.

Thermal-ionization mass spectrometry (TIMS). The isotopic ratios of the enriched urine samples were determined by TIMS (THQ; Finnegan-Mat GmbH, Bremen, Germany). Each dried sample was dissolved in 50 μl 0·04 M-HCl. A standard solution ($CaCl_2$) was made by replacing the nitrate ions of a Ca standard solution ($Ca(NO_3)_2$; SpectroSol, BDH, Poole, Dorset) with chloride ions using HCl, Aristar grade. Using the double-filament technique (Moore, 1984; Heumann, 1988), 5 μl of each sample and a Ca standard were adsorbed onto a separate evaporation filament by glowing the filament and the sample to dull red before the sample and the standard were loaded into the mass spectrometer. Ca

masses of 40, 41, 42 and 44 of a stable ion beam generated from the sample were sequentially scanned and monitored. The element interferences from ^{40}K and ^{41}K were corrected by subtracting the ratios from the pooled isotopic ratios. Each sample was scanned ten times to obtain a mean and a relative standard deviation (one block of data). The final results of $^{40}Ca:^{44}Ca$ and $^{40}Ca:^{42}Ca$ were obtained by determining the mean of five blocks of data (fifty scans). The relative standard deviation (RSD) of Ca ratio determination in the present study was 0·1–0·3 %.

Calculation of TFCA. The calculation of TFCA (%) was based on the assumption that both intravenously and orally administered Ca isotopes were metabolized at the same rate once the state of equilibrium was achieved. The percentage absorption from the oral dose was determined according to the following equation (Yergey *et al.* 1987):

$$TFCA\% = \frac{(na\,^{44}Ca)\,(^{42}Ca\,i.v.) \times \Delta\%XS\,^{44}Ca \times 100}{(na\,^{42}Ca)\,(^{44}Ca\,oral) \times \Delta\%XS\,^{42}Ca},$$

where na is the natural abundance of the two isotopes, i.v. and oral are the exact dose administered, and $\Delta\%XS$ is the degree to which a particular ratio differs from the natural ratios.

Measurement of serum concentration of 25-hydroxycholecalciferol (25-OHD)

Serum concentration of 25-OHD was measured using a competitive protein assay as described previously (Woo *et al.* 1990; Chan *et al.* 1992) in the Hongkong study children before the absorption study (during the autumn month of October). Twenty children agreed to have blood taken. Venous blood (2 ml) was drawn and serum was separated and stored at $-70°$ until analysis. 25-OHD was extracted with acetonitrile and separated using a SepPak C-18 cartridge (Waters Associated, Milford, MA, USA). The extract was then analysed by competitive-protein-binding assay using a commercial kit (Amersham International, Amersham, Bucks.).

Statistical methods

The non-parametric Mann–Whitney U test was used to compare group mean differences between various Ca-intake groups owing to the small sample size and some observed skewness in the data. The non-parametric Kruskal–Wallis test was used to test the overall mean differences in baseline dietary intake and body size among three groups of children with different Ca intakes. Two-sided significance level was set at $P < 0·05$ for test statistics. Statistical analysis was performed by SPSS/PC, Version 4.0, SPSS Inc., Chicago, IL, USA. The power of the statistics was tested by Power, Version 1.3, 1985 (Lavel University, Ste. Foy, Quebec, Canada).

Ethical considerations

The study protocol was approved by the Ethics Committees of The Faculty of Medicine, The Chinese University of Hongkong and the AFRC Institute of Food Research. Informed consent was obtained from all the parents.

RESULTS

Table 1 shows the comparisons of dietary intake and body size of Hongkong and Jiangmen children at 7 years of age. The mean Ca intake of Hongkong children was 693 (SD 410) mg/d with a wide range of variation (185–1641 mg/d) because the subjects included children who consumed milk regularly (*n* 14) and those who did not drink milk

Table 1. *Mean dietary intakes and body size of 7-year-old Hongkong and Jiangmen children**

(Mean values and standard deviations)

Study group...	Hongkong (n 22)			Jiangmen (n 12)			Statistical significance of difference between means†: P =
	Mean	SD	Range	Mean	SD	Range	
Ca (mg/d)	693	410	185–1641	381	103	172–552	0·0062
Ca:energy (mg/MJ)	85·3	34·2	32–164	81·3	14·3	53–103	0·83
P (mg/d)	1002	327	545–1803	683	180	436–1062	0·0017
Ca:P	0·66	0·22	0·30–1·2	0·56	0·12	0·39–0·79	0·17
Energy (KJ/d)	7724	1874	5644–12949	4678	1021	3226–6899	< 0·0001
Protein (g/d)	77	19	49–117	58	17	38–88	0·0049
Wt (kg)	20·9	2·57	17·2–27·4	21·3	3·2	17·8–27·9	0·75
Height (m)	1·19	0·04	1·15–1·29	1·20	0·04	1·14–1·26	0·87

* For details of subjects, see p. 884 and pp. 887–888.
† Mann–Whitney U test.

(n 8). In contrast, the mean Ca intake of Jiangmen children was 381 (SD 103) mg/d with a narrow range of 172–552 mg/d. There was only one girl with Ca intake slightly above 500 mg/d (525 mg/d). Food habits of the Jiangmen group were characterized by low intake of milk and milk products, and dark-green leafy vegetables were the principal source of dietary Ca. In fact, the low-milk-drinking Hongkong children had low Ca intake (359 (SD 94) mg/d) comparable with their counterparts in Jiangmen (366 (SD 92) mg/d; $P = 0·93$). Children from Hongkong consumed more animal products and snack foods than Jiangmen children, e.g. chicken wings, burgers and sugary drinks, etc. Dietary intakes of energy ($P < 0·0001$), P ($P = 0·0017$) and protein ($P = 0·00049$) in Hongkong children were, therefore, significantly higher than those of Jiangmen children. Although the Ca intake of Hongkong children was higher than that of Jiangmen children ($P = 0·0062$), Ca intake corrected for energy intake (Ca:energy; mg/MJ) was not different between the two groups ($P = 0·83$). There were no significant differences in weight ($P = 0·75$) and height ($P = 0·87$) between the Hongkong group and Jiangmen group (Table 1).

TFCA and Ca intake of children at 7 years old

TFCA values of twelve Jiangmen children and twenty-two Hongkong children were 63·1 (SD 11·4) and 57·4 (SD 9·1) % respectively. However, the mean difference in TFCA between Jiangmen and Hongkong children was not statistically significant ($P = 0·16$). If the observed mean and SD for TFCA of the Jiangmen group and Hongkong group reflect the population means, the observed statistical power was only 0·45 (with type I error rate (α) 0·05, two-sided). Therefore, it is possible that the mean TFCA of Jiangmen children may be significantly higher than that of Hongkong children if the sample size increases.

All the study children from both Hongkong and Jiangmen were ethnic Cantonese from Southern China and they were of the same age. Although the two dietary assessment methods were slightly different, a similar quantitative food-frequency questionnaire with a list of milk and non-milk food items identified as the substantial sources of Ca among Chinese children was used to estimate Ca intake among all the study children. Both dietary assessment methods together revealed that the levels of Ca intake for low-milk-drinking Jiangmen and Hongkong children were not significantly different ($P = 0·93$). Thus, the slight difference in the dietary assessment methodology did not appear to affect the

Table 2. *Classification of 7-year-old Hongkong and Jiangmen children into groups A–E according to country of origin and calcium intake**†

Classification group	n	Location(s)	Ca intake (mg/d)
A	19	Hongkong + Jiangmen	⩽ 500
B	15	Hongkong + Jiangmen	> 500
C	8	Hongkong	⩽ 500
D	11	Jiangmen	⩽ 500
E	14	Hongkong	> 500

* For details of subjects, see p. 884, pp. 887–888 and Table 1.
† One child from Jiangmen with Ca intake at 550 mg/d (> 500 mg/d) was excluded from the classification due to inadequate sample size.

Table 3. *Mean dietary intake, body size and true fractional calcium absorption (TFCA) of 7-year-old Hongkong and Jiangmen children classified according to calcium intake into groups A (Ca ⩽ 500 mg/d) and B (Ca > 500 mg/d)**

(Mean values and standard deviations)

Study group...	A (n 19)			B (n 15)			Statistical significance of difference between means†: $P =$
	Mean	SD	Range	Mean	SD	Range	
Ca (mg/d)	363	91	172–500	862	394	505–1641	< 0·0001
Energy (KJ/d)	5355	1324	3226–8134	8290	1957	5751–12949	0·0001
Ca:energy (mg/MJ)	70·4	20·1	31·8–103·3	101·1	28·9	70·5–164·1	0·0047
P (mg/d)	716	163	436–1041	1109	340	724–1803	0·0001
Ca:P	0·52	0·13	0·3–0·79	0·76	0·17	0·5–1·2	0·0001
Protein (g/d)	61·6	18·3	37·9–114·7	81·6	17·6	56·3–117·1	0·0024
Wt (kg)	19·8	2·3	17·2–24·3	21·5	2·6	17·7–27·9	0·13
Height (m)	1·20	0·04	1·14–1·26	1·19	0·04	1·15–1·30	0·92
TFCA (%)	63·1	10·7	48·1–84·6	54·8	7·3	46·0–68·2	0·016

* For details of subjects, see p. 884 and Table 1.
† Mann–Whitney U test.

estimation of Ca intake to a great extent. As a result, the study children from both Hongkong and Jiangmen may be grouped together in order to compare their TFCA with respect to their habitual Ca intakes. Ca intake at 500 mg/d recommended by Food and Agriculture Organization/World Health Organization Expert Group (1962) was used as a cut-off point to allocate children into group A or B. Table 2 shows the grouping of children according to the country of origin and the level of Ca intake. Group A consisted of nineteen children (nine boys and ten girls) with a habitual Ca intake ⩽ 500 mg/d, whereas group B consisted of fifteen children (nine boys, six girls) with a habitual Ca intake > 500 mg/d. Mean dietary intake, body size and TFCA of groups A and B are given in Table 3. Nutrient intakes of regular-milk-drinking children (group B) were significantly higher than those of the low-milk-drinking children (group A; Table 3). The differences in nutrient intakes between groups A and B may be due to the fact that a majority of the children in group B, comprising Hongkong children, consumed more foods rich in energy, protein and P. In addition, a higher consumption of milk in group B might also contribute to the difference in dietary intake. Fig. 1 shows the distribution of TFCA for the study children in groups

Table 4. *Comparisons of dietary intakes, weight and height of 7-year-old Hongkong and Jiangmen children classified according to location and calcium intake into groups C (Hongkong, Ca ≤ 500 mg/d), D (Jiangmen, Ca ≤ 500 mg/d) and E (Hongkong, Ca > 500 mg/d)*

(Mean values and standard deviations)

Study group*...	C (n 8)			D (n 11)			E (n 14)			Statistical significance of difference between means†: $P =$
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	
Ca (mg/d)	359	94	185–470	366	92	172–500	884	399	505–1641	< 0·0001
Energy (KJ/d)	6561	870	5645–8133	4477	782	3224–5854	8389	1992	5751–12951	< 0·0001
Ca:energy (mg/MJ)	55·2	16·3	31·8–79·6	81·3	15·1	53·3–103·3	102·5	29·4	70·5–164·2	0·0008
P (mg/d)	809	151	545–1041	649	141	436–898	1112	353	724–1803	0·0002
Ca:P	0·45	0·10	0·30–0·59	0·57	0·12	0·39–0·79	0·78	0·16	0·50–1·2	0·0001
Protein (g/d)	70·6	20	49·2–114·7	55·1	14·9	37·9–86·2·	81·1	18	56·3–117·1	0·0032
Wt (kg)	19·8	2·3	17·2–23·6	20·7	2·6	17·8–24·3	21·5	2·6	17·7–27·4	0·32
Height (m)	1·20	0·04	1·15–1·25	1·20	0·04	1·14–1·26	1·20	0·04	1·14–1·30	0·88

* For details of subjects, see p. 884 and Table 1.
† Kruskal–Wallis test.

Table 5. *Comparisons of mean true fractional calcium absorption (TFCA) of 7-year-old Hongkong and Jiangmen children classified according to location and Ca intake into groups C (Hongkong, Ca ⩽ 500 mg/d), D (Jiangman, Ca ⩽ 500 mg/d) and E (Hongkong, Ca > 500 mg/d)*

(Mean values and standard deviations)

		TFCA (%)			Ca intake (mg/d)	
Study group*†	n	Mean	SD	Range	Mean	SD
C	8	62·1	9·95	55·1–84·6	359	94
D	11‡	63·7	11·7	48·1–82·7	366	92
E	14	54·7	7·6	46·0–68·2	884	399

* For details of subjects, see p. 884 and Table 1.

† Mean values for TFCA were compared by Mann–Whitney U test: group C v. group E, $P = 0.076$; group D v. group E, $P = 0.033$; group C v. group D, $P = 0.93$.

‡ One Jiangmen child with a Ca intake of 550 mg/d was not included in the analysis.

A and B. The mean TFCA for group A with a Ca intake ⩽ 500 mg/d was 63·1 (SD 10·7)% which was significantly higher than that of group B with a Ca intake > 500 mg/d (54·8 (SD 7·3)%; $P = 0.016$; Table 3).

All the study children from the two groups (A and B) were further re-allocated to groups C–E with respect to their place of origin and the level of Ca intake (Table 2). Eight Hongkong children with Ca intake ⩽ 500 mg/d were re-allocated into group C. Eleven Jiangmen children with Ca intake ⩽ 500 mg/d were re-allocated into group D. Fourteen Hongkong children with Ca intake > 500 mg/d were re-allocated into group E. One child from Jiangmen with Ca intake > 500 mg/d was excluded from the analysis because of an inadequate sample size. The mean Ca intakes of groups C, D and E were 359 (SD 94), 366 (SD 92) and 884 (SD 399) mg/d respectively. The overall differences in dietary intake, weight and height of children in groups C, D and E were tested by the Kruskal–Wallis test (Table 4). There were significant differences between the three groups in nutrient intakes but not in body weight ($P = 0.32$) and height ($P = 0.88$) (see Table 4). Table 5 compares the differences in TFCA between groups C, D and E by using the Mann–Whitney U test. TFCA values of groups C and E were 62·1 (SD 9·95) and 54·7 (SD 7·6)% respectively; there was no significant difference in TFCA between groups C and E ($P = 0.076$). If the observed means and standard deviations for TFCA for groups C and E reflect the population means, the observed statistical power was only 0·5 (with type I error rate (α) 0·05, two-sided). Therefore, it is possible that the mean TFCA for group D may be significantly higher than that for group E if the number of subjects increases. The TFCA for group D (63·73 (SD 11·7)%) was significantly higher than that for group E (54·7 (SD 7·6)%; $P = 0.033$). There was no significant difference in TFCA and mean Ca intake between groups C and D ($P = 0.93$ for both groups), which supports the previous attempt to combine ethnically similar study children with the same age and comparable low Ca intakes for TFCA comparison.

Although TFCA in the low-Ca-intake children was found to be significantly higher than that for the high-Ca-intake children, there was no significant negative association between TFCA and Ca intake ($r -0.28$, $P = 0.11$, n 34). The non-significant association might be explained by the fact that Ca intake may not be linearly related to TFCA (Heaney *et al.* 1975; Eastell *et al.* 1989), or it may be due to a small sample size. On the other hand, there was a borderline significant negative relationship between TFCA and current protein
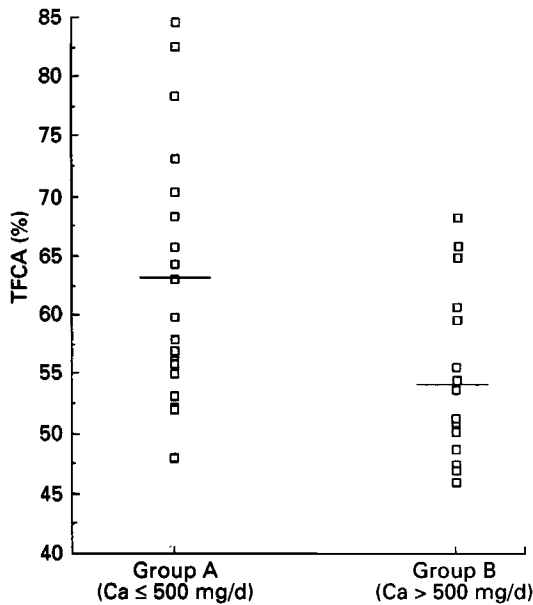
Fig. 1. Distribution of true fractional calcium absorption (TFCA) and Ca intakes of thirty-four 7-year-old Hongkong and Jiangmen children classified according to Ca intake into groups A (Ca ≤ 500 mg/d) and B (Ca > 500 mg/d). For details of subjects, see p. 884. ——, Mean value.

intake (r −0·33, $P = 0·056$) which might be due to the fact that protein intake was highly correlated with Ca intake in these children (r 0·68, $P < 0·0001$). Otherwise, TFCA was not related to any dietary variables of carbohydrate ($P = 0·35$), fat ($P = 0·29$), P ($P = 0·067$), body weight ($P = 0·65$) and height ($P = 0·99$). In addition, there was a large inter-subject variation in Ca absorption in both the low- and high-Ca-intake groups (Fig. 1).

Serum level of 25-OHD in twenty Hongkong study children

The mean serum 25-OHD level in twenty Hongkong study children was 33·7 (SD 7·7, range 19·0–48·4) ng/ml. The serum 25-OHD levels of children with Ca intakes less than and greater than 500 mg/d were 32·9 (SD 7·0, n 7) and 34·1 (SD 7·9, n 13) ng/ml respectively. There was no significant difference in serum 25-OHD level between the lower- and higher-Ca-intake groups ($P = 0·66$). There was also no correlation between the serum 25-OHD level and baseline TFCA (r −0·29, $P = 0·21$). Using serum 25-OHD level at 10 ng/ml as a diagnostic index of vitamin D deficiency (Grindulis et al. 1986), the vitamin D nutritional status of the Hongkong study children was within the normal range.

DISCUSSION

Ca moves across the intestine in both directions; the Ca absorbed from the gut could be secreted as endogenous Ca into the gut lumen and subsequently reabsorbed from it. Net fractional Ca absorption determined by traditional balance studies fails to differentiate the endogenous Ca from the dietary source. However, the technique of doubly labelled isotopes measures TFCA by giving one isotope orally and the other one intravenously which can correct for the endogenous Ca secreted into the intestine. The determination of TFCA using double-label isotopes only requires a sample of body fluids, e.g. urine and blood, collected 24 h after dosing. The measurement does not depend on the absolute quantities of the two labelled isotopes but the ratio of the two isotopes in the body fluids.

Thus, the technique is not subject to inherent errors incurring in a metabolic balance study. Furthermore, the use of stable isotopes to determine fractional Ca absorption was found to be highly correlated with the technique of radioisotopes (Yergey *et al.* 1987; Eastell *et al.* 1989). The test is less time consuming, taking a few minutes to administer the tracers, whereas a balance study needs several weeks to complete. TIMS is a more accurate and sensitive technique than the FABMS for measuring inorganic isotopes (Fairweather-Tait *et al.* 1989). It has a precision of 0·3–0·5%; therefore, lower doses can be used, so that the cost of isotopes can be reduced. Furthermore, for studies in infants and children who have a smaller exchangeable Ca pool, doses can be further scaled down (Eastell *et al.* 1989). In the present study chocolate milk was used instead of ordinary milk as a carrier for ^{44}Ca isotope. Some nutrition textbooks state that Ca in chocolate milk is less readily available for absorption than unflavoured cow's milk due to the presence of oxalate in chocolate. However, Recker *et al.* (1988), using radioisotopes to compare fractional absorption of Ca from chocolate milk, whole milk, yoghurt, cheese and $CaCO_3$ in healthy subjects, found that there were no significant differences in the relative absorbability between chocolate milk and any of these tested dairy products as well as $CaCO_3$ suggesting that the absorbability of Ca from chocolate milk is comparable with that of unflavoured cow's milk. There is a limitation in the present study: the use of chocolate milk as a single carrier for the ^{44}Ca might give a result representing how well the Ca from chocolate milk is absorbed but may not be representative of the Ca absorption from the whole diet. Despite this factor, the absorption test was standardized throughout the present study, and it was still possible to use chocolate milk as a single Ca carrier in order to differentiate between higher absorbers with low Ca intake and lower absorbers with high Ca intake.

The present study shows that the study children with a mean Ca intake of about 360 mg/d had a significantly higher TFCA (63%) than that of children with mean Ca intake 860 mg/d (55%). The results indicate that the study children with low Ca intakes were able to adapt to the habitual diets with Ca intake below the RDA suggested for most developed countries (National Research Council, 1989; Department of Health, 1991; German Society of Nutrition, 1991). Physiologically, it is vitally important that the growing Chinese children accustomed to a non-milk diet were able to enhance the efficiency of the Ca absorptive mechanism in order to compensate for the low Ca intake.

The success of physiological adaptation depends on the systemic mediation of parathyroid hormone and vitamin D in response to a low habitual Ca diet (Hegsted *et al.* 1952; Norman, 1990). During growth the active process of bone remodelling leads to an increased demand for Ca in skeletal development, the increased production of parathyroid hormone and 1,25-dihydroxycholecalciferol may ultimately stimulate the synthesis of Ca-binding protein, intestinal Ca absorption would be enhanced to compensate for the low-Ca diet, and urinary Ca excretion may be reduced as well (Norman, 1990). In this way the body may adapt to achieve a positive Ca balance for maintaining Ca homeostasis appropriate for skeletal mineralization. Classical Ca balance studies in low-Ca-intake children, adolescents and adults have together demonstrated that urinary excretion is reduced (Hegsted *et al.* 1952; Begum & Pereira, 1969; Matkovic, 1991). In the current study the serum 25-OHD level of the Hongkong children was within the normal range. In fact, serum concentrations of 25-OHD have been determined in eighteen randomly selected children aged 7 years from the same school in Jiangmen during December of the same year. The assay of serum 25-OHD concentration used the same technique as that employed in the current study and the assay was performed in the same laboratory. The mean serum 25-OHD for the Jiangmen children was 31·1 (SD 7·4, range 22·6–49·4) ng/ml which was not significantly different from the mean value for the twenty Hongkong study children (33·7 (SD 7·7) ng/ml; $P = 0.170$) (W. T. K. Lee, S. S. F. Leung, D. M. Y. Leung, H. S. Y.

Tsang, J. Lau and J. C. Y. Cheng, unpublished results). These findings suggest that the vitamin D status of children from both Hongkong and Jiangmen was adequate, which may be an important factor for the enhancement of Ca absorption to occur. Two recent studies of infants from Hongkong and Guangdong, China, showed that serum 25-OHD levels in young Chinese children from both regions were comparable (20–26 ng/ml; Leung *et al.* 1989, 1993). Hongkong and Jiangmen share the same sub-tropical climate and there is plenty of sunshine throughout the year. Study children in this age-group actively engage in outdoor activities; therefore, they should obtain adequate vitamin D through regular exposure to the sun. Thus, the variation in TFCA amongst the study children was unlikely to be related to the difference in vitamin D nutritional status. Although serum 1,25-dihydroxycholecalciferol level was found to be raised in healthy low-Ca-intake adults (Norman *et al.* 1981), our findings do not show a significant difference in serum 25-OHD level between the lower- and higher-Ca-intake children ($P = 0.66$) nor was there a correlation between serum 25-OHD concentration and baseline TFCA ($r -0.29$, $P = 0.21$, n 20).

Almost all the Jiangmen children in the current study were breast-fed up to 10 months of age. Only one girl was formula-fed from birth to 15 months, and five children were supplemented with formula milk up to about 12 months of age because the mothers could not produce adequate amounts of breast milk. After 1 year of age, five children would occasionally consume a few teaspoons of condensed milk or powdered milk while the rest of the children seldom consumed milk. Thus, the major sources of dietary Ca for Jiangmen children were dark-green vegetables, cereals and bean products. Hence, the habitual Ca intake of these twelve children from 1 to 7 years was about 300 mg/d due to the low intake of milk and milk products. All the Hongkong children studied, however, were formula-fed during infancy. Fourteen children (group E) with a current Ca intake > 500 mg/d had continued to drink milk since infancy. The remaining eight children (group C) with a current Ca intake ≤ 500 mg/d gradually reduced the frequency and quantity of milk intake by age 1 year; two of these eight children stopped using milk at age 2 and 4 years respectively, while the remaining six children drank milk occasionally. Thus, the discrepancy in habitual Ca intake in the study children had already occurred at younger ages. In addition, both Hongkong and Jiangmen are located in soft-water regions. In Hongkong, the mean level of Ca in tap water was 9·9 mg/ml (The Water Authority of Hongkong, unpublished results); therefore, the contribution of Ca from drinking water is probably not substantial.

A nutritional adaptive mechanism may operate in children in response to different Ca exposures. The current study shows that the study children were able to adapt by increasing the rate of Ca absorption to a moderate extent in response to a low-Ca diet. However, it is interesting to note that even in children on a high Ca intake, the absorption rate was still as high as 55%. Published values for TFCA and the extent of adaptation to low-Ca diets in Asian and Caucasian children are limited. The findings of the present study agree with earlier balance studies in low-Ca-intake Indian children (Begum & Pereira, 1969) and Sri Lankan children (Nicholls & Nimalasuriya, 1939) in that children could adapt to a Ca intake below 300 mg/d and were able to maintain positive Ca retention. The mean Ca retention of twenty-eight rural Indian children (Begum & Pereira, 1969) subsisting on a diet as low as 200 mg/d could reach about 60%, and the mean urinary Ca loss was less than 35 mg/d. On the other hand, Nicholls & Nimalasuriya (1939) showed that Sri Lankan children accustomed to dietary Ca levels of less than 300 mg/d were able to absorb over 60% of Ca from the diet, and the mean total urinary Ca excretion was 16 mg/d. Matkovic (1991) reviewed ninety-nine metabolic balance studies in children aged 2–8 years; the author observed that the mean urinary Ca loss dropped from 117 to about 60 mg/d when

mean Ca intakes declined from 1600 to 470 mg/d. These early studies demonstrated that growing children on habitually-low-Ca diets had a low urinary Ca excretion in addition to a relatively higher Ca retention so that a positive Ca balance could be maintained to facilitate skeletal mineralization.

Abrams et al. (1993) employed the dual-tracer technique to compare TFCA of ten healthy Caucasian girls with a mean age of 7·5 (SD 1·4) years; the results were used for comparison with those of children with juvenile rheumatoid arthritis. ^{44}Ca (0·5 mg/kg) or ^{46}Ca (0·5 μg/kg) was labelled in milk (amount of milk used was not mentioned in the report) and taken orally with a standard breakfast. ^{42}Ca (0·1–0·35 mg/kg) was infused immediately after the breakfast. TFCA of the healthy girls was 30·4 (SD 8·4)%, and the mean dietary Ca intake of the ten subjects was 940 (SD 470) mg/d. The higher percentages of TFCA found in the present study when compared with those of Abrams et al. (1993) may be due to adaptive or ethnic differences in the efficiency of Ca absorption, or slight variations in the study protocols. Miller et al. (1988) and Smith et al. (1987) used a double-isotope technique to determine TFCA from ^{44}Ca-enriched $CaCO_3$ and calcium citrate–malate in Caucasian children and adolescents aged 10–17 years. The TFCA from $CaCO_3$ and calcium citrate–malate were 26 and 36% respectively, which were remarkably lower than those from the present study. In the current study the subjects were younger than those in the studies of Miller et al. (1988) and Smith et al. (1987). In addition, oral ^{44}Ca was administered in chocolate milk as a tracer carrier and the test meal was given to subjects in a fasted state. In contrast, Miller et al. (1988) and Smith et al. (1987) labelled Ca salts with ^{44}Ca (total dose 250 mg Ca) and the oral tracer was taken simultaneously with a standard breakfast. Thus, it is inconclusive whether the discrepancy in TFCA between the results of our own study and those of Miller et al. (1988) and Smith et al. (1987) was due to adaptive or ethnic differences or may be attributed to the variations in the study design.

Eastell et al. (1989) used a dual-isotope technique to estimate Ca absorption by labelling dietary Ca in individual meals rather than in a fixed Ca carrier. The authors observed a negative correlation between dietary Ca and the amount of Ca absorbed. TFCA also varied from meal to meal during the day which may be attributed to the variation in Ca contents of the meals. Thus, in future studies it would be more appropriate to label different meals during the day in order to obtain a more accurate estimate of Ca absorption based on the entire diet.

The value for fractional Ca absorption is one of the key factors in estimating Ca requirements in children (National Research Council, 1989; Department of Health, 1991). In the USA the recommended Ca intake for children aged between 1 and 10 years was 800 mg/d based on an assumption that Ca absorption rate in children is 40% (National Research Council, 1989). The RDA for Ca for Chinese children based on the mean TFCA extrapolated from the present study may be lower than the US RDA. To investigate any difference in Ca absorption between Caucasian and Chinese children would necessitate a further absorption study in Caucasian children with the same protocol as that of the present study. Also, it would be worthwhile to assess Ca absorption in Chinese children living in temperate regions of Northern China in order to determine the extent of variations in Ca absorption which might be influenced by environmental factors such as variations in dietary intakes, vitamin D nutritional status and the frequency of exposure to sunshine.

In conclusion, this is the first Ca absorption study using the technique of doubly-labelled stable isotopes coupled with TIMS to measure TFCA among Chinese children who had a wide range of Ca intakes. The results of the study indicate that growing Chinese children accustomed to a habitually-low-Ca diet were able to adapt to enhance the efficiency of Ca absorption. The vitamin D nutritional status of the study children was within the normal range. The mean TFCA of the Chinese children was above 50%. Further study is necessary

to determine whether there is any difference in Ca absorption between Caucasian and Chinese children.

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