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Calcium absorption in postmenopausal Chinese women: a randomized crossover intervention study

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The Ca intake and food sources of Chinese postmenopausal women are quite different from those of their Western counterparts. But, little information on Ca metabolism is available in Chinese populations. We determined true fractional calcium absorption (TFCA), true Ca absorption (= TFCA × Ca intake, V_a), urinary Ca excretion (V_u) and the difference between V_a and V_u (V_{a-u}), in response to three dietary Ca intake levels. Twenty-one healthy postmenopausal Chinese women aged 49–64 years were recruited for this randomized crossover trial from a general community, Guangzhou, China. Subjects were randomly assigned to receive 0, 500 and 1000 mg Ca/d for 5 weeks separated by 2-week washout periods. TFCA using Ca stable isotopes, total urinary Ca excretion and Ca intake were determined after 4 weeks of adaptation. Mean values for total Ca intake (V_i) of the three phases were 391 (SD 197), 880 (SD 130) and 1382 (SD 160) mg/d. On usual diet, TFCA, V_u, V_a and V_{a-u} were 0·57 (SD 0·12), 175 (SD 59) mg/d, 216 (SD 98) mg/d and 41 (SD 99) mg/d, respectively. With the supplementations of 500 and 1000 mg Ca/d, TFCA significantly decreased to 0·52 (SD 0·12) and 0·43 (SD 0·13) ($P<0·001$); whereas urinary Ca ($P=0·003$), V_a and V_{a-u} increased significantly ($P<0·001$). Using a mixed-effects nonlinear regression model, it was estimated that V_{a-u} was approaching a plateau when mean Ca intake reached 1300 mg/d. In conclusion, the present findings suggest postmenopausal Chinese women have high Ca absorption efficiency and a mean Ca intake of about 1300 mg/d is required to maximize the V_{a-u}.

Dietary calcium: True fractional absorption: Stable isotopes: Postmenopausal women: Chinese

Epidemiological data have shown that age-adjusted rates of hip fracture were much higher in the Caucasian populations than in the Asian populations (Gullberg *et al.* 1997; Lau *et al.* 2001), whereas Ca intakes in Asians are only half to three-quarters of that of their Western counterparts (US Department of Agriculture, 1989; Woo *et al.* 1998). In Chinese urban adults, mean Ca intake ranged from 350 to 500 mg/d (Ge *et al.* 1996). Although the differences in Ca intake tend to be small in the middle-aged and elderly populations (US Department of Agriculture, 1989; Woo *et al.* 1998), it remains unclear whether the differences in the fracture rates could be partly due to the discrepancies in the efficiency of Ca utilization.

Ca absorption efficiency plays a key role in optimizing Ca utilization. The limited published data have shown that intestinal true fractional Ca absorption (TFCA) was about 2-fold higher in the Chinese (Kung *et al.* 1998) than in the Western populations (Heaney *et al.* 1989). However, the study by Kung *et al.* (1998) has limitations; and as indicated by Heaney (1999), a 100 mg Ca carrier load was used (as the chloride salt) without accompanying food. Therefore, the study may not provide the desired information on Ca absorption and requirement in Chinese women.

Due to the relatively low level of Ca intake, different dietary habits and Ca sources as compared with White populations, the Chinese might have higher TFCA. About 72 % of the dietary Ca for US adults is derived from dairy products, 11 % from grain products, and 6 % from vegetables and fruits (US Department of Agriculture, 1989). Dietary Ca in the Chinese population is mainly provided by vegetables

Abbreviations: NTx, cross-linked N-telopeptide; PTH, parathyroid hormone; TFCA, true fractional calcium absorption.

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(37 %) and fruits and beans (10 %); while milk and its products supply approximately 24 % of the Ca (Chen, 2003). Ca from different sources varies in bioavailability. Weaver *et al.* (1997) reported that TFCA from several commonly consumed Chinese vegetables is higher than that from milk by almost 10 %. Further, several studies have demonstrated adaptation in Ca absorption and excretion depending on Ca intake levels. Malm (1958) showed that subjects transferred from a high to a low Ca diet were initially in negative balance, but zero balance was achieved after a period of adaptation (1·5 years). Lee *et al.* (1994) also reported higher TFCA in children with habitual lower Ca intake than those with higher Ca intake, using the same Ca test load.

The purpose of the present study was to determine true Ca absorption, and urinary Ca excretion, and to evaluate benefits to the Ca economy at double the Ca intake currently consumed by the majority of postmenopausal Chinese women.

Subjects and methods

Subjects

Twenty-one apparently healthy postmenopausal Chinese women within 10 years of natural menopause were recruited from a community near the Third Affiliated Hospital of Sun Yat-sen University in Guangzhou, China. Eligibility criteria included Guangzhou residents (at least 5 years) of Chinese origin aged between 48 and 64 years, and within 10 years of natural menopause, defined as at least 12 months since the last menstrual cycle. Subjects who were taking hormonal replacement therapy for 3 months or more, and those who had a confirmed medication known to affect bone health, such as malabsorption syndromes, chronic liver or kidney diseases, parathyroid and thyroid diseases, gastric operation or cancer, were excluded from the study. Women who had undergone oophorectomy and/or hysterectomy were also excluded because of the inability to determine menopausal status. Procedures, objectives and requirements of the study were explained in detail to the eligible subjects. The protocol was approved by the Ethical Committee of the Chinese University of Hong Kong, and written informed consent was obtained from each subject.

Study protocol

Subjects participated in a randomized cross-over trial consisting of three successive periods of Ca supplementation of 5 weeks each, separated by 2-week washout periods. During the intervention periods, subjects were supplemented with either 0 mg (no placebo) (A), 500 mg (B) or 1000 mg (C) Ca (as calcium carbonate). Eligible subjects were randomly assigned to one of the three study arms, i.e. A-B-C, B-C-A or C-A-B. Ca supplements (Phase B: half tablet; Phase C: one tablet; 500 mg per tablet) were consumed twice daily (in the morning and evening) within 10 min of completing the relevant meals during the 4-week adaptation period and the subsequent 5 d test period. Subjects were advised to consume their habitual diet and avoid any other supplements including vitamins, minerals, fish oils and herbs during the whole course of the study. TFCA and urinary Ca were determined during the fifth week of each intervention period by using a dual stable-isotope technique. The duplicate diet technique was used to assess habitual Ca intake.

Isotope preparation

Calcium carbonate enriched with ^{42}Ca (enrichment 87 %) or ^{43}Ca (enrichment 52 %) was purchased from Asian Isotopes Ltd (Hong Kong). The Ca isotope solutions for oral (^{42}Ca) and intravenous (^{43}Ca) administration were prepared as previously described by Lee *et al.* (1994). The solution of ^{43}Ca for injection was dispensed into glass bottles, sealed, autoclaved and sterilized. Each 25 ml dose of ^{42}Ca for oral administration was dispensed into a polyethylene tube, sealed and stored at − 20°C until use. The enrichments of the two Ca isotope solutions, as determined by inductively coupled plasma MS were: 14·59 % ^{40}Ca, 84·45 % ^{42}Ca, 0·12 % ^{43}Ca and 0·83 % ^{44}Ca for the oral ^{42}CaCl solution; 30·81 % ^{40}Ca, 0·90 % ^{42}Ca, 51·17 % ^{43}Ca, and 17·11 % ^{44}Ca for the intravenous ^{43}CaCl solution.

Administration of stable isotopes

The subjects were invited to the hospital, after an overnight fast, in the morning (approximately 08.00–09.30 hours) of the first day of the fifth week during each intervention period. About 1·0 mg ^{43}Ca/60 kg body weight in 5 ml normal saline was infused via the antecubital vein over approximately 1–2 min, and flushed with 5 ml normal saline. Approximately 4·0 mg ^{42}Ca/60 kg body weight in 300 ml fresh orange juice (≈ 36 mg Ca), mixed 24 h before administration, was divided into three bottles (100 ml each) and taken orally by the subjects with breakfast, lunch and dinner (within 5 min post-meal) on the same day. The supplements and oral dose were taken with a meal in the morning and evening in the sequence of 'meal → Ca tablet → isotope dose'. A standard breakfast (150 ml milk + 150 g bread + 100 ml orange juice with isotope dose, ~ 200 mg Ca) was provided on the test day and the subjects were sent home after breakfast. Telephone calls were made to remind them to drink the oral isotope with lunch and dinner of a self-selected diet. The exact quantity of oral and intravenous isotope given to each subject was precisely weighed by using a 1/1000 g balance.

Sample collection, preparation and analysis

Subjects were instructed to empty their bladders before the isotope administration. About 40 ml urine was collected at baseline. A 120 h urine specimen in two pools (0–48 h, 48–120 h) was collected during each metabolic phase immediately post-dosing by using acid-washed polyethylene pots. Aliquots of 90 ml were digested using concentrated nitric acid and the residue dissolved in 0·2 M-nitric acid.

The urine digests were purified by using an oxalate precipitation procedure similar to that described by Patterson *et al.* (1999) and Turnlund *et al.* (1993). In brief, approximately 0·5–3·0 ml of digest in solution (containing approximately 1–3 mg Ca) was transferred to an acid-washed tube, and mixed with 0·5 ml saturated ammonium oxalate (pH 8). The pH was then adjusted to ~ 8·0 with 5 % ammonia. The solution was mixed and left to stand overnight. The samples were centrifuged, and the supernatant was discarded. The remaining Ca precipitate

was then washed twice with MilliQ water (5 ml each time). The isotope profile of the purified samples was determined using a Single Focussing Multicollector Mass Spectrometer (Isoprobe; Micromass, Manchester, UK) using a desolvating sample introduction system with a microconcentric nebulizer (Aridus and T1H; Cetac, Omaha, NE, USA). All samples were calibrated against NIST915 with the following accuracies (%relative standard deviation): 44/40, 0·018 %; 42/40, 0·025 %; 43/40, 0·048 %. The total Ca concentration in digested urine was measured using a colorimetric method (Commercial Kit, Randox Laboratories Ltd, Co. Antrim, UK). The average CV for intra- and inter-runs were 0·76 % and 2·04 %, respectively. Isotope profile and total Ca concentration were then used to calculate TFCA.

Calculation of true fractional calcium absorption

The TFCA was calculated in a similar manner to that described by DeGrazia *et al.* (1965). In brief, the TFCA is given by the ratio of the mass of the two stable isotopes measured in urine, expressed as the fraction of the administered dose. This technique assumes that the oral tracer, once absorbed, follows the same kinetics as the intravenous tracer and natural Ca. Fractional absorption was calculated from the isotope mass determined in urine samples collected in the first 120 h post-dosing.

$$\text{Fractional absorption} = \frac{\text{Mass}_{\text{oral label in urine sample}}}{\text{Mass}_{\text{IV label in urine sample}}} \times \frac{\text{Dose}_{\text{IV}}}{\text{Dose}_{\text{oral}}}$$

where

$$\text{Mass}_{\text{oral label in urine sample}} = \frac{Mtot_{\text{Ca}} \times Ca_{\text{fraction oral}} \times MW_{\text{oral}}}{Ca_{\text{fraction NA}} \times MW_{\text{NA}} + Ca_{\text{fraction oral}} \times MW_{\text{oral}} + Ca_{\text{fraction IV}} \times MW_{\text{IV}}}$$

$$\text{Mass}_{\text{IV label in urine sample}} = \frac{Mtot_{\text{Ca}} \times Ca_{\text{fraction IV}} \times MW_{\text{IV}}}{Ca_{\text{fraction NA}} \times MW_{\text{NA}} + Ca_{\text{fraction oral}} \times MW_{\text{oral}} + Ca_{\text{fraction IV}} \times MW_{\text{IV}}}$$

$Mtot_{\text{Ca}}$ is the total Ca mass in urine; $Ca_{\text{fraction oral}}$ is the mole fraction of Ca of the oral dose; $Ca_{\text{fraction IV}}$ is the mole fraction of Ca of the intravenous dose; $Ca_{\text{fraction NA}}$ is the mole fraction of natural Ca in urine; MW_{oral}, MW_{IV} and MW_{NA} are the molecular weights of the oral dose, intravenous dose and natural Ca, respectively.

Calcium intake

Habitual Ca intake was assessed by measuring the Ca content in duplicate food samples collected for 3 d consecutively after the isotope administration during each intervention phase. Wet and dry foods were collected separately, and edible parts of the samples were carefully weighed on the following day. The samples were then homogenized, and combined into one pool. About 20 g of the homogenized sample were ashed at 450°C for 48 h, and dissolved using 0·2 M-HNO_3. The total Ca concentration in digested food samples was measured using the same method for testing total urinary Ca.

In some cases, duplicate diet collections were incomplete due to reasons such as not enough foods was prepared, subjects had forgotten to collect or it was not convenient to collect foods consumed while eating out. All subjects were advised to record in detail the type and quantity of the foods consumed and our staff would check the food records following each day of collection. A total of 4·8 % (energy ratio) of foods were not collected and calculated based on the food record. Energy and other dietary nutrients such as protein, carbohydrates and phosphorus were calculated based on the weighed food data using computerized food tables (US Department of Health, Education and Welfare & Food and Agriculture Organization of the United Nations, 1972; Wang, 1991).

Biochemical analysis

Serum cross-linked N-telopeptide (NTx) was measured by ELISA (Osteomark; Ostex International Inc., Seattle, WA, USA); the intra-assay variability (CV) was 5·1 %. Serum intact parathyroid hormone (PTH) was measured by ELISA (BioSource International Inc., Camarillo, CA, USA); the intra-assay variability (CV) was 6·4 %. Inter-assay variation for NTx and PTH was avoided by analysing all samples from the study on the same plate.

Compliance assessment

Ca tablet consumption during the 5-week intervention periods was assessed by counting the leftover tablets at the end of each of the study phases. All subjects consumed more than 90 % of the supplements during the relevant intervention periods. On the days of testing and sample collection, all subjects, except one during the first phase, took the supplements as instructed. Compliance with the collection of urine and food samples was assessed by face-to-face interview.

Statistical analysis

True absorbed Ca (V_a) was calculated as TFCA × Ca intake. The difference between V_a and urinary Ca (V_u) was calculated and is referred to as V_{a-u}.

A non-linear regression model was initially used to describe the association of Ca retention with Ca intake in previous studies (Institute of Medicine, 1997; Jackman *et al.* 1997). Due to the high correlations of repeated observations from the same individuals, a relevant mixed-effects model was used to fit the association of V_a and V_{a-u} with Ca for the present study, as described by Pinheiro & Bates (1995).

$$V_{a-u} = \frac{a + u_1}{1 + e^{(b - c \times \text{Ca intake})}} + d; \quad V_a = \frac{a + u_1}{1 + e^{(b - c \times \text{Ca intake})}}$$

where a, b, c and d are fixed-effect parameters: a is the range between the minimum and maximum value of V_{a-u} or V_a; b is a determinant of the intercept of the curve; c is the slope; d represents the minimum V_{a-u}; u_1 is a random-effect parameter which follows a normal distribution with a mean of 0 and a variance of σ^2.

The 'proc nlmixed' procedure of the SAS software version 8.2 (SAS Institute Inc., Cary, NC, USA) was used for the data modelling and the calculation of mean (with 95 % CI) prediction of V_{a-u} and V_a in relation to Ca intakes.

Analysis of covariance for repeated measures was used to examine the effects of Ca supplementation on TFCA, urinary Ca excretion, V_a, V_{a-u}, PTH and NTx. The model included possible confounding factors, such as age, years since menopause, body weight, height and BMI, and dietary protein and phosphorus. A linear mixed-effects model was used to test the linear trend of the above parameters in response to Ca intake after adjusting for possible confounding factors. SAS software was used for statistical analysis. All results were considered significant at $P<0{\cdot}05$.

Results

Of the twenty-one participants, two subjects were excluded after the first phase because of the introduction of hormone replacement therapy and menses-like bleeding. One subject dropped out due to personal reasons during the second phase. The remaining eighteen subjects completed the study. None of the subjects suffered from any diagnosed disease or used medication known to affect bone health during the preceding 3 years.

Subjects had a mean age of 54·3 (SD 4·4) years (range 49·2–63·6), mean years since menopause of 5 (SD 3) (range 1–10) and mean BMI of 24·1 (SD 3·0) kg/m^2 (range 19·5–29·3) (Table 1). Of the twenty-one subjects who attended the study at baseline, ten were retirees, seven were clerks, officers, doctors or nurses, and the remaining two were blue-collar workers. Ten subjects had achieved college education; eight and three subjects had attained secondary and primary levels of education, respectively.

Means for TFCA of the three groups were 0·57 (SD 0·12), 0·52 (SD 0·12) and 0·43 (SD 0·13), respectively. There was no significant difference in the habitual dietary Ca intake between the three intervention phases. With the increases in Ca intake through supplementation, the TFCA decreased significantly ($P<0{\cdot}001$), and urinary Ca ($P=0{\cdot}003$), V_a and V_{a-u} increased significantly ($P<0{\cdot}001$) (Table 2).

Non-linear mixed-effects models were used to fit the association of V_{a-u} and V_a with Ca intake. The estimated values of the parameters are shown in Table 4. $V_{a-u} = (524 + u_1)/[1 + e^{(2{\cdot}34 - 0{\cdot}00\,408 \times Ca\,\text{intake})}] - 135$, and $V_a = (633 + u_1)/[1 + e^{(2{\cdot}09 - 0{\cdot}00\,348 \times Ca\,\text{intake})}]$. The random-effect parameter u_1 follows a normal distribution with a mean of zero and a variance of 21 006 (for V_{a-u}) and 21 171 (for V_a).

Figures 1 and 2 show the observed values for individuals and mean predicted values of V_a and V_{a-u} in relation to Ca intake. The mean (with 95 % CI) predicted values of V_{a-u} and V_a at different levels of Ca intake are summarized in Table 3. The present findings demonstrate that the predicted mean V_{a-u} was approaching a plateau (defined as 95 % of the maximum V_{a-u}) when the mean Ca intake reached 1300 mg/d.

Discussion

In this crossover study, we examined true Ca absorption and urinary Ca excretion in postmenopausal Chinese women after adaptation to three levels of Ca supplementation (0, 500 and 1000 mg/d). The decline in TFCA in response to an additional 500 mg/d was not statistically significant (0·57 *v.* 0·52). The statistically significant decline in TFCA following supplementation with 1000 mg/d (0·57 *v.* 0·43) agrees with the previously published inverse relationship between Ca intake and fractional absorption (Heaney & Recker, 1986). As expected, the V_a, V_{a-u} and urinary Ca increased significantly with Ca supplementation.

The TFCA in the subjects consuming their habitual diet (0·57 (SD 0·12)) was similar to that obtained in healthy 9–17-year-old north Chinese girls (0·60 (SD 0·14)) consuming on average 600 mg Ca/d (Lee *et al.* 2002). Other studies also using the double-isotope technique found a mean TFCA of 0·27 (SD 0·10) in Western women with a mean Ca intake of around 800 mg/d (Heaney & Recker, 1986; Heaney *et al.* 1989). The subjects in the present study with a similar level of Ca intake (Phase B) had a TFCA of almost twice this value (0·52 (SD 0·12)). Potential reasons for the observed differences in TFCA are likely to include racial differences, life-long adaptation to low Ca intake especially in childhood, different Ca food sources and potential inter-laboratory errors (Fleming & Heimbach, 1994; Leung *et al.* 1997).

Although the postmenopausal Chinese and the Caucasian women have similar levels of Ca intake after adjusting for body weight, the Ca intake is much lower in the Chinese during childhood (aged 5 years, 200–400 mg/d; Lee *et al.* 1993) and early adulthood than their American counterparts (aged 2–17 years, 921 mg/d; US Department of Agriculture, 1989). Life-long adaptation might thus contribute to the higher TFCA in the Chinese. So far, little is known about the effect of ethnicity on differences in TFCA between Caucasian and Chinese populations, and further studies are needed to address this issue.

Table 1. Baseline characteristics of the subjects (*n* 21)

Parameters	Mean	Median	SD	Minimum	Maximum
Age (years)	54·3	52·7	4·4	49·2	63·6
Years since menopause (years)	5	5	3	1	10
Height (m)	1·54	1·53	0·05	1·45	1·62
Weight (kg)	56·9	55·5	8·0	43·5	72·5
BMI (kg/m^2)	24·1	24·3	3·0	19·5	29·3

Table 2. Means of parameters on calcium metabolism by the calcium intervention doses‡

Parameters§	0 mg/d (*n* 19)		500 mg/d (*n* 18)		1000 mg/d (*n* 20)		*P* value for ANOVA‖	*P* value for trend¶
	Mean	SD	Mean	SD	Mean	SD		
Ca from diet (mg/d)	391	197	380	130	382	160	0·632	–
Total Ca intake (mg/d)	391	197	880	130**	1382	160**†	0·000	–
Urinary Ca (mg/d)	175	59	192	69	214	82*	0·101	0·003
TFCA	0·57	0·12	0·52	0·12	0·43	0·13**†	0·000	<0·001
V_a (mg/d)	216	98	460	128**	594	160**†	0·000	<0·001
$V_{a\text{-}u}$ (mg/d)	41	99	273	107**	376	129**†	0·000	<0·001
Serum parathyroid hormone (pg/ml)	31·0	20·7	26·0	20·8	23·7	19·9*	0·029	0·029
Serum N-telopeptide (nm BCE)	17·4	6·3	16·7	5·6	16·2	5·4*	0·016	0·016
Dietary P (mg/d)	730	195	734	182	748	205	0·879	–
Ca:P ratio (from diet)	0·52	0·16	0·52	0·12	0·50	0·11	0·866	–
Dietary protein (g/d)	52	9	53	11	57	19	0·540	–
Dietary mg (mg/d)	198	56	191	54	206	56	0·867	–
Dietary k (mg/d)	1309	371	1356	380	1321	400	0·670	–

TFCA, true fractional calcium absorption; BCE, bone collagen equivalent.
Mean values were significantly different from those of the 0 mg Ca/d group: *$P<0·05$; **$P<0·01$.
Mean values were significantly different from those of the 500 mg Ca/d group: †$P<0·01$.
‡ For details of procedures, see pp. 161–163.
§ Average daily Ca intake from diet assessed by measuring a 3 d duplicated food sample after isotope administration. Total Ca intake = Ca from diet + Ca from supplements. Urinary Ca is the 5 d average urinary Ca excretion; V_a is the true absorbed Ca fraction (= TFCA × Ca intake). $V_{a\text{-}u}$ is the difference between V_a and urinary Ca.
‖ *P* value from ANOVA for repeated measures. Covariates: age, years since menopause, body weight, height and BMI, and dietary protein and phosphorus (no covariate for the comparison of dietary intakes).
¶ *P* value for coefficients derived from linear mixed-effects models (dependent variables: parameters listed in the table; independent variable: total Ca intake; covariates were the same as those in the ANOVA).

TFCA was measured using a dual-isotope method as performed in previous studies (Abrams *et al.* 1991; Heaney & Recker, 1994; Heaney & Skillman, 1964). We used a similar approach to Eastell *et al.* (1989) in that the oral tracer was divided into three equal parts and then given to the subjects with the three main meals on the test day. The supplement was given as 250 mg (Phase B) or 500 mg (Phase C) twice daily. That the isotope doses were not given in parallel to the Ca supplements on the test day might result in an overestimation of the Ca absorption. Based on the regression equation [TFCA = 0·889 − 0·0537 × ln(Caload) ± 0·095] by Heaney *et al.* (1990), our method, as compared to the approach of equal Ca load and three equal isotope doses given, would overestimate the overall absorption by 3·2 % and 7·2 % at Phases B and C, respectively. The adjusted mean TFCA would thus be 0·50 and 0·40 at Phases B and C, respectively.

Urine Ca excretion is quite high in this population. Two, four and six subjects excreted urinary Ca of more than 250 mg/d during the intervention periods of 0, 500 and 1000 mg Ca/d, respectively. The mean urinary Ca excretion (175 (SD 59) mg/d) in subjects consuming their habitual diet was higher than that reported for Japanese (152 (SD 68) mg/d; Itoh *et al.* 1998) and Caucasian women (median 134, 5th to 95th percentile 55–264, mg/d) (Heaney *et al.* 1999). The difference may be explained by other dietary and non-dietary factors that affect urinary Ca excretion such as sodium and protein intake, age and menopausal status.

Table 3. Predicted value of true absorbed calcium fraction (V_a) and $V_{a\text{-}u}$ in relation to calcium intakes‡

Ca intake (mg/d)	$V_{a\text{-}u}$ (mg/d)		V_a (mg/d)	
	Mean	95 % CI	Mean	95 %CI
300	−6	−43, 31	164	138, 191
400	38	2, 74	210	179, 241
500	88	50, 126	261	226, 296
600	141	99, 183	316	276, 355
700	193	145, 240	370	326, 414
800	240	187, 293	422	374, 469
900	279	221, 337	468	416, 519
1000	310	248, 373	507	451, 561
1100	334	268, 399	538	479, 596
1200	351	283, 418	563	501, 624
1300	363	294, 431§	582	517, 645

‡ $V_{a\text{-}u}$ is the difference between true absorbed Ca fraction and urinary Ca. The predictions of V_a and $V_{a\text{-}u}$ were made based on the non-linear regression model indicated in Figs. 1 and 2, and Table 4.
§ Equal to 95 % (81–109 %) of predicted maximal value of $V_{a\text{-}u}$.

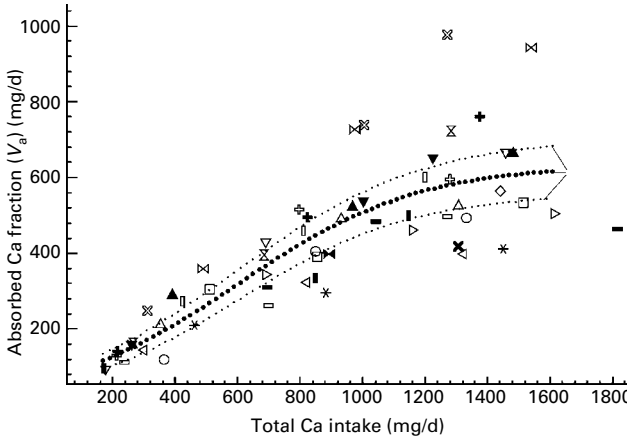

Fig. 1. Observed individual values and predicted values (•••, mean; ···, 95 % CI) of the true absorbed calcium (Ca) fraction in relation to Ca intake. For details of procedures, see pp. 161–163. The same markers represent replicate values for a given woman; the different markers represent twenty-one participants. The predicted values of V_a were derived from a non-linear mixed-effect model: $V_a = (633 + u_1)/[1 + e^{(2·09 - 0·0035 \times \text{Ca intake})}]$. 95 % CI and *P* values of the parameters in the model are presented in Table 4.

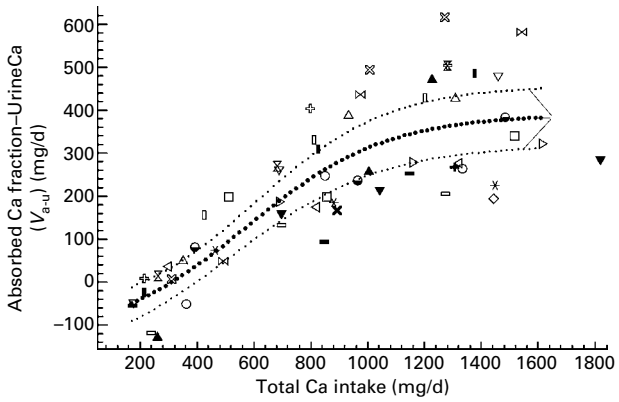

Fig. 2. Observed individual values and predicted values (•••, mean; ···, 95 % CI) of $V_{a\text{-}u}$ in relation to calcium (Ca) intake. For details of procedures, see pp. 161–163. The same markers represent replicate values for a given woman; the different markers represent twenty-one participants. The predicted values of $V_{a\text{-}u}$ were derived from a non-linear mixed-effect model: $V_{a\text{-}u} = (524 + u_1)/[1 + e^{(2{\cdot}339 - 0{\cdot}00\,408 \times \text{Ca intake})}] - 135{\cdot}3$. 95 % CI and *P* values of the parameters in the model are presented in Table 4.

In the present study, we found that the non-linear mixed-effects model could well describe the association of V_{a-u} or V_a with dietary Ca intake. Based on the model, it was predicted that the V_{a-u} was approaching a plateau (defined as 95 % of estimated maximal V_{a-u}) when the mean Ca intake reached 1300 mg/d. Although the association between the observed values of V_a and Ca intake could be well fitted to the non-linear mixed-effects model, it was inappropriate to estimate the Ca intake that produces plateau absorption due to the limited test range of Ca intake in the present study. Moreover, it might not be valid to make any prediction using these models if a given Ca intake is out of the test range.

Very high values of mean V_{a-u} (273 and 376 mg/d) were observed in the subjects in the present study at the phases of 500 and 1000 mg Ca intervention. It seems unphysiologic that so much Ca is accreted to bone on these intakes for a long time (such as over 1 or 2 years). The high apparent retention may be due to the bone remodelling being transient after the short-term sharp changes of Ca intake. Heaney (2001) found that bone balance increased from −20 mg Ca/d at baseline to the maximum value of ~230 mg Ca/d during the transient period, and then decreased to ~20 mg Ca/d after adaptation to 1 year bisphosphonate treatment of osteoporosis. On the other hand, the high V_{a-u} might also be counteracted by an increase in endogenous faecal Ca excretion, and a moderate Ca balance would thus be expected in the subjects with Ca supplementation.

We found a significant negative association between PTH and Ca intake which is in agreement with other studies (Meier *et al.* 2004; Pfeifer *et al.* 2001). Secondary hyperparathyroidism in postmenopausal women and/or the elderly population increases bone resorption. The PTH levels were significantly lower (−23·6 %) during Phase C (+1000 mg Ca/d) compared to Phase A (habitual diet) ($P < 0{\cdot}05$). All but one subject (104 pg/ml) had PTH values in the reference range (12·5–49·1 pg/ml).

Compared to habitual diet, the bone resorption marker, serum NTx, decreased on average by 7 % and 4 % in subjects supplemented with 1000 and 500 mg Ca/d, respectively. Others have reported a more pronounced reduction in urine NTx (25 %) in postmenopausal women with low habitual Ca intake after supplementation with 1200 mg Ca/d for 2 months (Kamel *et al.* 1998).

We recognize that the relatively small sample size of the present study might limit the generalization of the results regarding TFCA, V_a and V_{a-u} in relation to Ca intake in postmenopausal Chinese women. Another limitation is that the foods during test periods were home prepared rather than controlled diets with constant Ca contents, suggesting larger day-to-day variation in Ca intake. Moreover, a 5-week adaptation period was too short to produce a long-term equilibrium regarding Ca kinetics and bone remodelling. A short-term adaptation to high Ca intake might also overestimate Ca absorption efficiency in this population with a habitually low Ca intake.

In conclusion, the present study demonstrates that postmenopausal Chinese women have a high level of Ca absorption efficiency. A minimum mean Ca intake of about 1300 mg/d is required to maximize V_{a-u}.

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Table 4. Parameters of the non-linear mixed-effect model describing the associations of V_a and $V_{a\text{-}u}$ with calcium intake‡

	$V_{a\text{-}u}$ and Ca intake			V_a and Ca intake		
Parameters in the model	Estimate	95 % CI	*P* value	Estimate	95 % CI	*P* value
a	524	357, 691	<0·001	633	557, 708	<0·001
b	2·34	1·76, 2·92	<0·001	2·09	1·90, 2·28	<0·001
c (× 10^{-3})	4·08	2·52, 5·64	<0·001	3·48	2·95, 4·03	<0·001
d	−135·3	−266·2, −4·3	0·043			
σ^2 (× 10^{-3})	21·07	−4·36, 46·49	0·099	21·17	6·66, 35·68	0·006

‡ V_a is the true absorbed Ca fraction (mg/d); $V_{a\text{-}u}$ is the difference between V_a and urine Ca (mg/d). The associations between V_a or $V_{a\text{-}u}$ and Ca intake were fitted into non-linear mixed models of $V_a = \frac{a + u_1}{1 + e^{(b - c \times \text{Ca intake})}}$ and $V_{a-u} = \frac{a + u_1}{1 + e^{(b - c \times \text{Ca intake})}} + d$, respectively; where *a*, *b*, *c* and *d* are the fixed-effect parameters: *a* is the range between the minimum and maximum value of $V_{a\text{-}u}$ or V_a; *b* is a determinant of the intercept of the curve; *c* is the slope; *d* represents the minimum $V_{a\text{-}u}$; u_1 is the random-effect parameter assumed to follow a normal distribution with a mean of 0 and a variance of σ^2.

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