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 (\(\equiv\) TFCA \(\times\) Ca intake, \(V_u\)), urinary Ca excretion (\(V_a\)) 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, \(V_u\) 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 42Ca (enrichment 87%) or 43Ca (enrichment 52%) was purchased from Asian Isotopes Ltd (Hong Kong). The Ca isotope solutions for oral (42Ca) and intravenous (43Ca) administration were prepared as previously described by Lee et al. (1994). The solution of 43Ca for injection was dispensed into glass bottles, sealed, autoclaved and sterilized. Each 25 ml dose of 42Ca 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 % 40Ca, 84·45 % 42Ca, 0·12 % 43Ca and 0·83 % 44Ca for the oral 42CaCl solution; 30·81 % 40Ca, 0·90 % 42Ca, 51·17 % 43Ca, and 17·11 % 44Ca for the intravenous 43CaCl 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 43Ca/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 40 mg 42Ca/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 vacuum filtered and dried overnight in a vacuum oven.

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The remaining Ca precipitate was vacuum filtered and dried overnight in a vacuum oven.
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 TH; 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.

\[
\text{Fractional absorption} = \frac{\text{Massoral label in urine sample}}{\text{MassIV label in urine sample}} \times \frac{\text{DoseIV}}{\text{Doseoral}}
\]

where

\[
\text{Massoral label in urine sample} = \frac{M_{\text{tot} \text{Ca}} \times C_{\text{fraction oral}} \times MW_{\text{oral}}}{C_{\text{fraction NA}} \times MW_{\text{NA}} + C_{\text{fraction oral}} \times MW_{\text{oral}} + C_{\text{fraction IV}} \times MW_{\text{IV}}}
\]

\[
\text{MassIV label in urine sample} = \frac{M_{\text{tot} \text{Ca}} \times C_{\text{fraction IV}} \times MW_{\text{IV}}}{C_{\text{fraction NA}} \times MW_{\text{NA}} + C_{\text{fraction oral}} \times MW_{\text{oral}} + C_{\text{fraction IV}} \times MW_{\text{IV}}}
\]

\(M_{\text{tot} \text{Ca}}\) is the total Ca mass in urine; \(C_{\text{fraction oral}}\) is the mole fraction of Ca of the oral dose; \(C_{\text{fraction IV}}\) is the mole fraction of Ca of the intravenous dose; \(C_{\text{fraction NA}}\) is the mole fraction of natural Ca in urine; \(MW_{\text{oral}}, MW_{\text{IV}}\) and \(MW_{\text{NA}}\) 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-HNO3. 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 (NTxs) was measured by ELISA (Osteomark; Ostex International Inc., Seattle, WA, USA); the intra-assay variability (CV) was 5.4 %. 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 NTxs 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.

Statistical analysis

 True absorbed Ca \((V_a)\) was calculated as TFCA \times Ca intake. The difference between \(V_a\) and urinary Ca \((V_{a-u})\) 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).

\[ V_{a-u} = \frac{a + u_1}{1 + e^{b - c \times \text{Ca intake}}} + d; \quad V_a = \frac{a + u_2}{1 + e^{b - d \times \text{Ca intake}}} \]
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 $\sigma^2$.

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 intake.

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 $P<0.05$.

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 ($P<0.001$), and urinary Ca ($P=0.003$), $V_a$ and $V_{a-u}$ increased significantly ($P<0.001$) (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.

$$V_a = \frac{(633 + u_1)/\left[1 + e^{(2.09 - 0.00348 \times \text{Ca intake})}\right]}{1 + e^{(2.34 - 0.00408 \times \text{Ca intake})}}.$$  

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)

<table>
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<tr>
<th>Parameters</th>
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<th>Median</th>
<th>sd</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
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<tr>
<td>Age (years)</td>
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<td>52.7</td>
<td>4.4</td>
<td>49.2</td>
<td>63.6</td>
</tr>
<tr>
<td>Years since menopause (years)</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>10</td>
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<td>Height (m)</td>
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<td>1.53</td>
<td>0.05</td>
<td>1.45</td>
<td>1.62</td>
</tr>
<tr>
<td>Weight (kg)</td>
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<td>55.5</td>
<td>8.0</td>
<td>43.5</td>
<td>72.5</td>
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<td>24.1</td>
<td>24.3</td>
<td>3.0</td>
<td>19.5</td>
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</tr>
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</table>
Heaney and 7.2% at Phases B and C, respectively. The adjusted approach of equal Ca load and three equal isotope doses would overestimate the overall absorption by 3.2%. The prediction of the Ca absorption was given as 250 mg (Phase B) or 500 mg (Phase C) twice divided into three equal parts and then given to the subjects.

To predict the Ca supplements on the test day might result in an overestimation of the Ca supplements. Based on the regression equation \( \text{TFCA} = 0.889 - 0.0537 \times \ln(\text{Caload}) \), 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: \(175 \pm 59 \) and \(192 \pm 69 \) for the Ca supplements on the test day might result in an overestimation of the Ca absorption. Based on the regression equation \( \text{TFCA} = 0.889 - 0.0537 \times \ln(\text{Caload}) \). The mean urinary Ca excretion 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 \( \text{TFCA} = 0.889 - 0.0537 \times \ln(\text{Caload}) \) 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; 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.

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 \( \text{TFCA} = 0.889 - 0.0537 \times \ln(\text{Caload}) \) 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.

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### 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†|
|--------------|---------------|-----------------|-----------------|----------------|----------------|
| Ca from diet (mg/d) | 391 ± 197 | 380 ± 130 | 382 ± 160 | 0.632 | – |
| Total Ca intake (mg/d) | 391 ± 197 | 880 ± 130** | 1392 ± 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 |
| \( V_{u} \) (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) | 92 ± 17 | 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.
§ 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. † For details of procedures, see pp. 161–163.
††† For details of procedures, see pp. 161–163. The same markers represent twenty-one participants. The predicted values of \( V_{a} \) were derived from a non-linear mixed-effects model (dependent variables: parameters listed in the table; independent variable: total Ca intake; covariates were the same as those in the ANOVA).

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

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<th>95% CI</th>
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<td>1200</td>
<td>351</td>
<td>283, 418</td>
<td>563</td>
<td>501, 624</td>
</tr>
<tr>
<td>1300</td>
<td>363</td>
<td>294, 4315</td>
<td>582</td>
<td>517, 645</td>
</tr>
</tbody>
</table>

‡ \( V_{u} \) is the difference between true absorbed Ca fraction and urinary Ca. The predictions of \( V_{a} \) and \( V_{u} \) 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 \( V_{u} \).
The values of the parameters in the model are presented in Table 4.

Using these models if a given Ca intake is out of the test range. To the limited test range of Ca intake in the present study, it was inappropriate to estimate the Ca intake that produces plateau absorption due to non-linear mixed-effects model.

In the present study, we found that the non-linear mixed-effects model could well describe the association of V\textsubscript{a-u} or V\textsubscript{a} with dietary Ca intake. Based on the model, it was predicted that the V\textsubscript{a-u} was approaching a plateau (defined as 95 % of estimated maximal V\textsubscript{a-u}) when the mean Ca intake reached 1300 mg/d. Although the association between the observed values of V\textsubscript{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\textsubscript{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/d 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\textsubscript{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). 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<0.05). 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\textsubscript{a} and V\textsubscript{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\textsubscript{a-u}.

### Acknowledgements

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### Table 4. Parameters of the non-linear mixed-effect model describing the associations of V\textsubscript{a} and V\textsubscript{a-u} with calcium intake‡

<table>
<thead>
<tr>
<th>Parameters in the model</th>
<th>V\textsubscript{a} and Ca intake</th>
<th>V\textsubscript{a-u} and Ca intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>524</td>
<td>633</td>
</tr>
<tr>
<td>95 % CI</td>
<td>357, 691</td>
<td>557, 708</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>b</td>
<td>2.34</td>
<td>2.09</td>
</tr>
<tr>
<td>95 % CI</td>
<td>1.76, 2.92</td>
<td>1.90, 2.28</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>c (× 10\textsuperscript{-3})</td>
<td>4.08</td>
<td>3.48</td>
</tr>
<tr>
<td>95 % CI</td>
<td>2.52, 5.64</td>
<td>2.95, 4.03</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>d</td>
<td>−135.3</td>
<td>4.38</td>
</tr>
<tr>
<td>95 % CI</td>
<td>−296.2, −4.3</td>
<td>9.03, 5.04</td>
</tr>
<tr>
<td>P value</td>
<td>0.043</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>σ\textsuperscript{2} (× 10\textsuperscript{-3})</td>
<td>21.07</td>
<td>21.17</td>
</tr>
<tr>
<td>95 % CI</td>
<td>−4.36, 46.49</td>
<td>6.66, 35.68</td>
</tr>
<tr>
<td>P value</td>
<td>0.099</td>
<td>0.006</td>
</tr>
</tbody>
</table>

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

FIG. 2. Observed individual values and predicted values (●●●, mean; …, 95 % CI) of $V_{a-u}$ 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 $V_{a-u}$ were derived from a non-linear mixed-effect model: $V_{a-u} = \frac{(524 + u) \cdot \gamma}{1 + e^{(3.339 - 0.0245 \times \text{Ca intake})}} - 135.3$. 95 % CI and P values of the parameters in the model are presented in Table 4.
References


