Bioavailability of calcium of fresh cheeses, enteral food and mineral water. A study with stable calcium isotopes in young adult women

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True fractional Ca absorption from six foods was measured in twelve normal healthy women, aged 20–29 years. The tested foods were commercially available fresh cheese, fresh cheese prepared by new technology and rich in Ca, similar cheese with added Fe, enteral food, mineral water alone and combined with a spaghetti meal. The aim of the study was to investigate: (1) Ca absorption from a new Ca-rich fresh cheese and to compare it with that from the traditional commercial type of fresh cheese; (2) the effect of Fe enrichment of the new cheese on Ca absorption; (3) Ca absorption from the mineral water and the enteral product and to compare it with that from the dairy products; (4) the effect of a meal combined with the mineral water on Ca absorption. All test foods were consumed by all subjects according to a design with two Latin squares. Each treatment of 2 d was followed by a wash-out period of 2 weeks. Ca absorption was measured using a double stable-isotope (^{44}Ca and ^{48}Ca) extrinsic labelling technique. Mean fractional Ca absorption from the new fresh cheese was not significantly different from that from the traditional type (37·7 (SD 10·2)% *v.* 42·2 (SD 11·6)%). The addition of Fe to the new cheese did not significantly influence Ca absorption. Ca-absorption values from the mineral water (37·0 (SD 9·8)%) and from the enteral product (42·6 (SD 11·4)%) were not significantly different from those from the dairy products (37·7–42·2%, SD 10·2–11·6%). The co-ingestion of a spaghetti meal with the mineral water significantly enhanced Ca absorption from 37 (SD 9·8)% to 46·1 (SD 11·7)%. It is concluded that a new process leading to a fresh cheese with a higher Ca concentration does not alter Ca bioavailability compared with the standard technology and for a constant Ca supply. Thus this new fresh cheese would probably provide more Ca than the standard one. The fractional Ca-absorption values for mineral water and the enteral product indicate that these products can make an interesting contribution to Ca supply for populations with a low Ca intake and patients with specific diseases respectively.

Fresh cheese: Mineral water: Enteral product: Calcium absorption: Bioavailability.

An adequate intake of Ca throughout life is important for Ca and bone homeostasis (Heaney *et al.* 1982; Cumming, 1990) and probably also for the prevention of several chronic diseases, e.g. hypertension, colon cancer and coagulation disorders (McCarron *et al.* 1990). Osteoporosis is responsible for hip and wrist fractures and vertebral compressions with serious health outcomes in developed countries, in terms of morbidity and mortality (Delmas, 1992). Bone mass is related to the eventuality of osteoporotic fractures (Cummings *et al.* 1993) and positively related to Ca intake (Ramsdale *et al.* 1994). Several Ca-supplementation studies have shown positive effects on bone density (Dawson-Hughes *et al.* 1990; Prince *et al.* 1991; Johnston *et al.* 1992; Reid *et al.* 1993). A large-scale intervention study in France showed a reduced incidence of hip fractures in elderly people supplemented with vitamin D and Ca (Chapuy *et al.* 1992). An adequate intake of Ca-rich foods is the natural way to meet Ca requirements. In Western countries dairy products

contribute on average about 70% to the intake of Ca (Schaafsma, 1991). Other important sources of dietary Ca in Western diets are vegetables, drinking water and possibly certain Ca-rich mineral waters. Since Ca availability for absorption among different foods may vary widely (Heaney *et al.* 1988; Heaney & Weaver, 1990), it is important to assess Ca absorption from different foods.

At present, manufactured foods are widely consumed. For the manufacturer the assessment of the nutritional properties of foods becomes essential beside microbiological and chemical qualities. The aims of the present study were fourfold: (1) to investigate Ca absorption from Ca-rich fresh cheese, developed according to new technology, and to compare it with Ca absorption from a traditional commercial type of fresh cheese; (2) to investigate the effect of Fe enrichment of the new product on Ca absorption; (3) to investigate Ca absorption from a Ca-carbonated mineral water and an enteral food and to compare it with that from dairy products; (4) to investigate the effect of a meal combined with the mineral water on Ca absorption.

Fresh cheeses are widely consumed in various countries, particularly by children, but also by aged people, for whom fresh cheese may be a good Ca source. Applying new technology, which maintains the high natural Ca content of milk, two new fresh cheeses were developed, one with added Fe. This new technology is presently applied in various Western countries. It largely prevents the loss of Ca in the whey. It has been shown that Ca reduces Fe absorption (Cook *et al.* 1991; Hallberg *et al.* 1991, 1992). To our knowledge the effects of Fe on Ca absorbability have not been assessed.

Ca-carbonated mineral waters may be a good non-energy source of Ca. Not all people like dairy products and those people with lactose intolerance (non-Caucasians) may develop intolerance symptoms on milk consumption. Aged people may have higher Ca requirements than younger adults (Heaney *et al.* 1978; National Institute of Health Consensus Conference, 1984) and Ca-carbonated mineral water could be a good source of Ca, particularly if people are on a low-energy diet. In a recent study Halpern *et al.* (1991) found that the bioavailability of Ca from mineral water was at least as good as that from milk. However, in the radio Ca absorption test they used, Ca intake from milk was much higher than that from the mineral water (550 *v.* 63 mg) which will have favoured the fractional absorption from the mineral water, since fractional absorption decreases with Ca intake (Heaney *et al.* 1990).

An interesting question is to what extent Ca absorption is influenced by a concomitant meal. Major determinants of Ca absorbability are the amount of free ionic Ca and, to a lesser extent, the amounts of soluble Ca complexes in the intestine during digestion and pre-absorption (Pak & Avioli, 1988). Since the mineral water tested in the present study was bicarbonated, Ca solubility could have been enhanced by a lower gastric pH, initiated by the ingestion of a meal.

Patients unable to be nourished orally may require gastric feeding in a clinical environment. The special dietary use envisaged for the tested enteral food is nutrition by tube feeding (lactose-free and residue-free) in normal catabolic situations, designed to provide 418·4 kJ (100 kcal)/100 ml, for prescriptions ranging from 4184 to 8368 kJ (1000–2000 kcal)/d. Until now it has not been possible to cover recommended daily allowances for Ca with this kind of product, because of the instability of Ca in solution at concentrations over 300 mg/l. A new technological approach, consisting of switching from Ca-caseinate to $CaCO_3$ and $Ca_3(PO_4)_2$, allowed a fortification up to 500 mg/l. The Ca bioavailability from this entirely formulated product was compared with that from the dairy products.

Young women were selected for this study according to the following consideration. Promotion of an adequate intake of Ca is an important public health objective to decrease

the risk of osteoporosis, notably for women. Adulthood in women until menopause is normally associated with a steady state in Ca metabolism. Before adulthood a positive Ca balance exists and after menopause women are in a negative Ca balance (Riggs & Melton, 1986; Riggs *et al.* 1986; Recker *et al.* 1992). Since the purpose of the present study was to compare the Ca absorption from different foods, we preferred test subjects who were considered to be in a steady state with their Ca metabolism and who formed a rather homogeneous group in order to reduce inter-individual variability in Ca absorption levels and to increase the sensitivity of our results.

METHODS

Subjects

Twelve normal healthy premenopausal women (mean age 24 years, mean body weight 66 kg, mean height 1·73 m and mean habitual Ca intake 0·75 g/d) were selected for the study. Normal health was assessed at pre-study screening. This included a medical history, physical examination, vital signs (including blood pressure and heart rate), electrocardiography and routine clinical laboratory tests. Habitual daily Ca intake of the subjects from dairy products was assessed by a validated dietary questionnaire (Hulshof *et al.* 1989). Only subjects with a typical Dutch food pattern according to the Dutch National Food Consumption Survey 1987–8 (Anonymous, 1988) were selected. Some characteristics of the subjects are summarized in Table 1. The study protocol was approved by the TNO external Medical Ethics Committee and all subjects signed informed consent forms.

Study design

Each subject received six treatments according to two completely randomized Latin square designs (Cochran & Cox, 1957). Each treatment consisted of the ingestion, in the morning, of one of the extrinsically ^{44}Ca-labelled test foods after an overnight fast. Between treatments a wash-out period of 2 weeks was applicable. The dairy foods tested were obtained from Danone, BP 63, 126 Rue Jules Guesde, 93302 Levallois Perret Cedex, France. These products were a commercially available fresh cheese (Petits Gervais aux fruits), containing 720 mg Ca/kg, a new fresh cheese, processed by new technology and containing 1600 mg Ca/kg and the new fresh cheese with Fe (15 mg/kg). The enteral food was Enterogil 500 (Jacquemaire Santé, Diepal NSA, 383 Rue Philippe Veron, 69654 Villefranche sur Saône Cedex, France), containing 500 mg Ca/l. The mineral water was Ferrarelle (Italaquae SpA, Via Appia Nuova 700, 00179 Roma, Italy) and contained 440 mg Ca/l. Volumes of the test foods and mineral water were adjusted to provide a carrier amount of approximately 150 mg Ca. The meal combined with the mineral water was a spaghetti meal and it consisted of 150 g spaghetti plus 20 g tomato sauce. The small amount of Ca in this meal (approximately 31 mg) was thus combined with the 150 mg Ca from the mineral water. The alternative possibility of reducing the volume of mineral water to obtain the carrier amount of 150 mg for the combined mineral water and spaghetti meal was rejected, because we wanted to evaluate the effect of the meal combined with the same volume of mineral water as in the test of mineral water alone. The amounts and composition of the test foods, based on chemical analysis, are presented in Table 2. The foods were analysed according to standard methods of analysis (Ca and Fe by atomic absorption spectrophotometry, P by colorimetry, carbohydrates by HPLC and N by the Kjeldahl method). Samples for Ca, P and Fe analysis were obtained by dry ashing 2 g of the dairy products and the Enterogil on a Pt crucible at 450°. The ashes were solubilized in a solution of 14·4 M-HNO_3–11·7 M-$HClO_4$ (2:1 v/v) and diluted in deionized water to a volume of 100 ml. For Ca and Fe measurements, standard solutions obtained from Merck

Table 1. *Characteristics of the test subjects at pre-study screening*

Subject no.	Age (years)	Weight (kg)	Height (m)	Ca intake (g/d)
1	20	67·7	1·71	0·50
2	25	54·2	1·67	0·51
3	21	58·6	1·62	0·45
4	24	70·4	1·71	0·70
5	28	63·0	1·81	0·46
6	22	86·2	1·79	1·10
7	21	58·5	1·75	0·66
8	29	64·7	1·71	2·44
9	23	62·2	1·72	0·44
10	28	63·0	1·74	0·26
11	21	76·2	1·71	0·56
12	28	65·3	1·78	0·92

Table 2. *Compositions of the six test foods (g or mg nutrient per test meal)*

Test food and meal size	Protein (g)		Fat (g)	Carbohydrate (g)	Ca (mg)	P (mg)	Fe (mg)
	Vegetable	Animal					
Petits Gervais aux fruits (strawberry) 208 g	0	13·9	16·8	37·6	150	200	< 0·1
New fresh cheese 94 g	0	4·6	5·7	14·5	150	113	< 0·1
New fresh cheese with added Fe 94 g	0	4·6	5·7	14·5	150	113	1·4
Enterogil* 300 g	5·1	6	9	42	150	150	3
Mineral water 340 g	0	0	0	0	150	0	0
Mineral water (340 g) and spaghetti (150 g) plus tomato sauce (20 g)	7	0	0	42	181	85	0

* Protein: potassium caseinate 20·4 g/l, soyabean protein isolate 17·4 g/l.

(Titrisol no. 9943, Merck, Darmstadt, Germany) were used as a reference. Data on the chemical composition of the spaghetti meal were obtained from the Dutch Food Composition Table (Erp-Baart, 1994).

Subjects were requested to adhere to their habitual diet and lifestyle during the week preceding any of the absorption tests and during the wash-out periods. Subjects arrived at the metabolic ward on the evening before the test (day 0; 22.00 hours) and from that time they were not allowed to consume any food. Only low-Ca mineral water (10 mg/l) was allowed. Fasting was continued up to 4 h after the test foods or drinks and stable-isotopes administration (in the morning of day 1 between 08.00 and 09.00 hours). Before administration of the isotopes a basal urine sample was collected. Collection of urine was continued for 24 h until the next morning (day 2). Blood samples (10 ml) were drawn just before administration of the isotopes and in the morning of day 2 before breakfast. These samples were taken for a parallel study not reported in the present paper. During their stay in the metabolic ward, food intake of the test subjects was strictly controlled. Standard meals were provided, containing approximately 850 mg Ca/d according to *The Dutch Food*

Table 3. *Composition of the standard meals after administration of the calcium isotopes**

Lunch
4 slices of brown bread
20 g Edam cheese
30 g meat product
30 g honey or marmalade
250 ml skimmed milk
20 g margarine
1 cup of coffee
Dinner
200 g soup (free selection of type)
One selected ready-to-eat dinner
150 g yoghurt with fruits
Evening
1 cup of coffee
20 g biscuits
200 ml apple juice or orange juice
25 g nuts

* After completion of the 24 h urine collection a normal (Dutch) breakfast was served with tea or coffee.

Composition Table (Erp-Baart, 1994). The composition of the standard meals is given in Table 3. The mineral water was drunk gradually during the meal.

Measurement of calcium absorption

Ca absorption was measured using a double-isotope (^{44}Ca and ^{48}Ca) extrinsic-labelling technique, as described by Eastell *et al.* (1989). The validity of this technique, specifically the use of an extrinsic tag, is discussed by Sandström *et al.* (1993) and Fairweather-Tait *et al.* (1989). The conclusion is that in general good agreement of extrinsic with intrinsic labelling of Ca is found. Moreover, our tested products concern fresh cheeses, with a physico-chemical form of Ca that mixes easily with the added Ca isotope. Since the dry matter content of fresh cheeses is only about 240 g/kg, these products have a consistency which is comparable with that of yoghurt. Moreover the pH of fresh cheeses ranges from 4·4 to 4·6. So Ca in these products will be even more soluble than in milk (Le Graet & Brulé, 1993). Therefore there can be no doubt that extrinsic Ca will equilibrate rapidly with the intrinsic soluble Ca in fresh cheeses.

From measurements of Ca isotope ratios (^{44}Ca:^{43}Ca and ^{48}Ca:^{43}Ca) in a urine sample collected before dose administration and in a 24 h urine sample following dose administration, the fractional Ca absorption was measured. ^{44}Ca was used as the oral tracer and ^{48}Ca as the intravenously injected tracer. Fractional absorption (FA) was computed according to the formula:

$$FA = \frac{\Delta\%\ \text{excess}\ ^{44}\text{Ca in urine}}{\Delta\%\ \text{excess}\ ^{48}\text{Ca in urine}} \times \frac{\text{na}\ ^{44}\text{Ca}}{\text{na}\ ^{48}\text{Ca}} \times \frac{\text{dose}\ ^{48}\text{Ca}}{\text{dose}\ ^{44}\text{Ca}},$$

where na is natural abundance of the Ca isotopes. The $\Delta\%$ excess of the isotopes is equivalent to the excess of each isotope in urine relative to the baseline value, according to the following equation:

$$\Delta\%\ \text{excess}\ ^{44}\text{Ca} = \frac{100 \times (\text{measured}\ ^{44}\text{Ca}:^{43}\text{Ca} - \text{na}\ ^{44}\text{Ca}:^{43}\text{Ca})}{\text{na}\ ^{44}\text{Ca}:^{43}\text{Ca}}$$

Table 4. *Isotope abundances* (%) of the calcium isotopes administered in the present study*

	^{48}Ca-enriched label	^{44}Ca-enriched label
^{40}Ca	9·79	2·89
^{42}Ca	0·06	0·06
^{43}Ca	0·01	0·01
^{44}Ca	0·22	97·00
^{46}Ca	< 0·01	< 0·01
^{48}Ca	89·92	0·02

* According to specifications given by Eurisotop and confirmed by inductively coupled plasma mass spectrometry analysis at the TNO laboratories.

and

$$\Delta\,\%\ \text{excess}\ ^{48}\text{Ca} = \frac{100 \times (\text{measured}\ ^{48}\text{Ca}:^{43}\text{Ca} - \text{na}\ ^{48}\text{Ca}:^{43}\text{Ca})}{\text{na}\ ^{48}\text{Ca}:^{43}\text{Ca}}.$$

Isotope ratios in urine were measured by inductively coupled plasma spectrometry (ICP-MS) after protein- and oxalate precipitation. Details and validation of the method, including repeatability and accuracy, have been published (Luten *et al.* 1993) and are summarized below. The ICP-MS used in this study was a Perkin-Elmer/Sciex Elan Model 500 (Sciex, Division of MOS Health Group Ltd, Toronto, Canada). For solution nebulization, a Meinhard glass nebulizer was used with a sample flow rate of 1·5 ml/min controlled with a Gilson peristaltic pump. All measurements were carried out in isotope ratio peak-hopping mode. Typical conditions for operations were: plasma power 1·2 kW, reflected power 5 W, coolant Argon-flow rate 18 litres/min, auxiliary flow rate 2·0 litres/min, nebulizer flow rate 1·0 litres/min, dwell time 60 ms, measuring time 5 s, one measurement per peak, three repeats per integration.

For the isolation of Ca, 45 ml 35 ml/l TCA was added to 15 ml urine for deproteinization. The solution was centrifuged (10 min, 3000 rev./min, at 4°). The pH of 5 ml of the supernatant fraction was adjusted to $5{\cdot}0 \pm 0{\cdot}5$ with NH_3. The Ca was concentrated by precipitating as oxalate by adding 10 ml of a saturated ammonium oxalate solution, mixing, centrifuging and discarding the supernatant fraction. The oxalate was redissolved in 15 ml 1·2 M-HCl. ICP-MS analysis of isotope ratios was executed in duplicate and all values were adjusted to deviations from natural ratios as measured in a standard Ca solution containing 10 mg Ca/l.

The repeatability of the measurements was very good: the CV for the isotopic ratios was less than 1 %.

Adopted natural ratios were 15·452 for ^{44}Ca:^{43}Ca and 1·385 for ^{48}Ca:^{43}Ca (De Bièvre, 1984). The difference between the actual isotope ratio and the natural ratio was rather small (< 4 %) and may have been due to mass discrimination of the ICP-MS. Both baseline urine samples (for each treatment) and the isotope-enriched (24 h) urine samples were analysed on the Ca-isotope ratios.

^{44}Ca and ^{48}Ca isotopes were obtained from Eurisotop (F-91194 Saint-Aubin Cedex, France) as $CaCO_3$. Specifications of both isotopes are given in Table 4.

$^{48}CaCO_3$ was converted into the chloride salt, the pH adjusted to 5 and diluted with saline (9 g NaCl/l). After filtration the solution was distributed into 10 ml sterilization bottles and autoclaved for 20 min at 121°. A similar procedure was followed for the $^{44}CaCO_3$, except that distilled water was used for dilution instead of saline. The Ca

Table 5. *Fractional absorption (%) of calcium from the test products*
(Mean values, standard deviations and ranges for twelve subjects)

Treatment	Mean	SD	Range
A Petit Gervais aux fruits	42·2	11·6	28–63
B New fresh cheese	37·7	10·2	22–58
C New fresh cheese + iron	38·8	11·0	17–59
D Enterogil	42·6	11·4	23–61
E Mineral water	37·0**	9·8	25–54
F Mineral water + spaghetti	46·1	11·7	33–68

** Mean value was significantly different from that for treatment F, $P < 0·01$.

concentrations of the solutions used for injection (^{48}Ca) and extrinsic labelling (^{44}Ca) were 174 and 1275 mg/l respectively. From these solutions 1 ml was used for intravenous injection (corresponding to approximately 1·5 mg ^{48}Ca at an enrichment of about 90%, see Table 4) and 10 ml for oral administration (about 13 mg ^{44}Ca per test).

Test foods and drinks were labelled with approximately 10 ml of the ^{44}Ca solution at least 2 h before administration to allow equilibration of the extrinsic label with intrinsic Ca. The exact amount of the label delivered to the test food or drink was measured by weighing the injection syringe before and after delivery of the solution. A similar procedure was followed for the intravenous administration. Before and after bolus injection blood pressure and heart rates were recorded.

Values for fractional Ca absorption were treated by ANOVA. Differences between groups were evaluated by Student's t test, using within-person pooled error variance, as obtained from the ANOVA. The possible relationship between habitual Ca intake, age and Ca absorption was investigated by computing linear correlation.

RESULTS

All subjects completed the study. Enrichment values for the urinary $^{44}Ca:^{43}Ca$ ratio ranged between 2·80 and 12·45% and those for the urinary $^{48}Ca:^{43}Ca$ ratio between 14·11 and 39·02%. Table 5 shows the mean values for fractional Ca absorption for the six treatments. Mean values ranged from 37·0% (mineral water) to 46·1% (mineral water + spaghetti meal).

Our results indicate that the bioavailability of Ca from fresh cheeses, prepared by new technology, does not differ significantly from that from the traditional fresh cheese. Moreover Fe addition to this new product did not influence Ca absorption. Ca absorption values from the mineral water and the enteral food (Enterogil) were not significantly different from those from the dairy products. The co-ingestion of a spaghetti meal with the mineral water significantly improved Ca absorption from 37 to 47%, a rise of 24% ($P < 0·01$).

Mean overall Ca absorption per subject over the six treatments did not show a significant correlation ($P > 0·05$) with habitual Ca intake (results not shown).

Mean values of 24 h urinary Ca excretion after the treatments showed a significant positive correlation with mean values of Ca absorption (r 0·61; $P < 0·025$, see Fig. 1). Values of urinary Ca excretion are given in Table 6. From this table it appears that treatment means of urinary Ca excretion did not vary substantially; only the relatively low value of 109 mg Ca/d after the treatment with mineral water was apparent and was significantly different from that for the mineral water + spaghetti meal ($P < 0·05$).

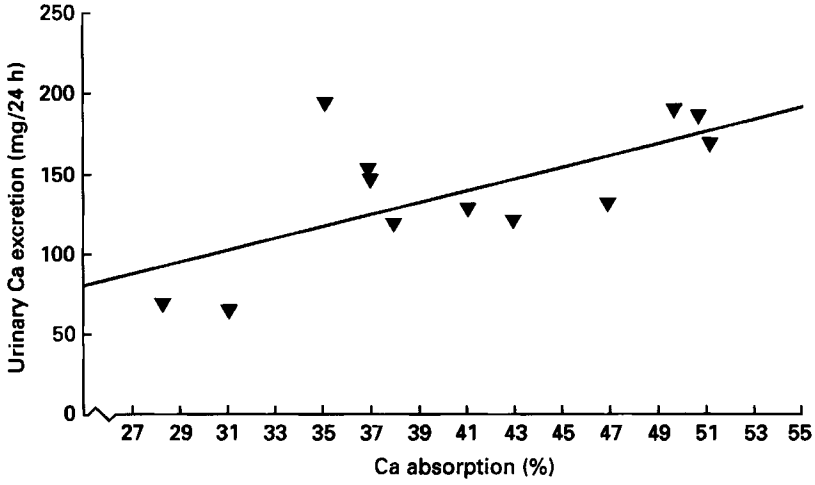

Fig. 1. Urinary calcium excretion *v.* calcium absorption in young adult women consuming six different test diets. Values are means for all six diets. For details of procedures and diets see pp. 895–899.

Table 6. *Urinary calcium excretion (mg/24 h) by women following consumption of six test foods*

(Mean values, standard deviations and ranges for twelve subjects)

Treatment		Mean	SD	Range
A	Petit Gervais aux fruits	146	53	45–231
B	New fresh cheese	142	65	58–243
C	New fresh cheese + iron	135	47	55–207
D	Enterogil	151	59	62–264
E	Mineral water	109*	47	16–193
F	Mineral water + spaghetti	149	52	54–237

* Mean value was significantly different from that for treatment F, $P < 0.05$.

DISCUSSION AND CONCLUSION

Our results for true fractional Ca absorption compare favourably with those reported by others using comparable stable- or radio-isotope techniques. Eastell *et al.* (1989) found a fractional absorption of 48 (SE 3)% from a 100 mg $CaCl_2$ solution, using the urinary ratio method in twenty-one women. This is somewhat higher than the value we obtained with mineral water (37 (SE 3)%), but in our study the carrier amount of Ca was 150 mg. Other groups of investigators found lower fractional absorption values than observed in the present experiment. Recker *et al.* (1988) reported mean values of Ca absorption from dairy products for postmenopausal women ranging from 22·4 to 26·7%, using a carrier amount of 250 mg Ca. This percentage of absorption corresponds to absolute amounts of about 60 mg Ca, which is identical to our overall mean value of about 40% of 150 mg Ca. Heaney *et al.* (1988) reported a fractional Ca absorption of 27·6% from milk and of only 5·1% from spinach in healthy adults, using a carrier of 200 mg Ca.

Our results demonstrate that co-ingestion of a meal together with mineral water improves Ca absorption by 24%. This confirms results obtained by Heaney *et al.* (1989) who showed that co-ingestion of a meal improved the absorption from Ca salts by 10–30%.

Also, Wood *et al.* (1987) found that the efficiency of Ca absorption was enhanced by about 20% by co-ingestion of glucose or a glucose polymer. The beneficial meal effect may be related to stimulation of gastric acid secretion, to formation of soluble Ca complexes and to a decreased gastric emptying rate, allowing better dissolution of poorly soluble test materials. The positive effect of the co-ingestion of a meal with the mineral water on the Ca absorption was associated with an increased urinary Ca excretion. This could reflect the increased Ca absorption (Harvey *et al.* 1988; Matkovic *et al.* 1990), but it might also be due in part to an increase in protein and Na intake (Schaafsma *et al.* 1987). However, it is not likely that the small amounts of protein (approximately 7 g) and Na (approximately 200 mg) in the spaghetti meal could account for all of the 40 mg increase in urinary Ca. Kerstetter & Allen (1989) estimated the effect of an additional 50 g of protein intake to be an increase of 60 mg urinary Ca, whereas the effect of an additional intake of Na of 2–3 g is in the order of a 20–25 mg increase in urinary Ca (McParland *et al.* 1989; Zarkadas *et al.* 1989). So the amounts of protein and Na in the spaghetti meal explain only a small part (25%) of the effect. Since we observed a significant correlation (r 0·61; $P < 0·025$) between Ca absorption and urinary Ca excretion (see Fig. 1), it is likely that the increase in urinary Ca associated with the ingestion of the spaghetti meal merely reflects a direct stimulating effect of the meal on Ca absorption. This observation may be of significance for optimal Ca absorption: Ca intake with food is preferred over Ca ingestion by tablets. Our measurements on urinary Ca excretion do not allow us to draw any conclusions about effects on Ca retention, since we have not studied Ca absorption from other foods consumed over the study period and since we have not analysed the Ca intake via these foods. The positive effect of the spaghetti meal on Ca absorption, expressed as fractional Ca absorption, was a 24% increase. If expressed in mg of Ca, the positive effect on absorption would have been even higher, since the total Ca intake with the spaghetti meal combined with the mineral water was estimated at 181 mg and equilibration of the isotopic Ca label with the Ca from the spaghetti meal will have occurred at least in part after ingestion of the meal.

Our results also demonstrate that Ca-rich fresh cheese does not interfere with the absorption of added Fe. This is an important observation considering the attention that is currently focused on Fe–Ca interactions at the intestinal level.

Since fractional Ca absorption decreases with Ca intake (Heaney *et al.* 1990) we decided to test the different cheeses at amounts which provided similar quantities of Ca (i.e. 150 mg). Since standard fresh cheese contains 720 mg Ca/kg, while the new fresh cheese has a Ca content of 1600 mg/kg, 208 and 99 g of these respective products were provided. We did not find a significant difference in fractional Ca absorption between these products, which means that for an equal amount of fresh cheese (say a portion of 50 g) Ca absorption in absolute terms will be higher from the new cheese, because of its much higher Ca content.

We also found that the Ca bioavailability of the enteral product Enterogil was comparable with that of conventional foods.

Habitual Ca intake was not found to be correlated significantly with Ca absorption as measured in the present study. This indicates that the results are valid over at least a wide range of habitual Ca intake levels.

New methodologies, such as stable-isotope research, are useful tools for the food industry to assess the nutritional value of foods processed with new technology (fresh cheese), natural food (e.g. mineral water), or entirely formulated foods. From the results of the present study we conclude that true fractional Ca absorption from the newly developed fresh cheese, from the enteral food and from the mineral water is not significantly different from that of commercially available fresh cheese. Thus a new food process, aimed at recovering Ca loss occurring with the traditional process, would have a

positive nutritional effect in relation to Ca intake. As far as Ca is concerned, the nutritional value of an entirely synthetic food product, intended to cover the nutritional needs of seriously affected patients is comparable to that of fresh cheese. Ca-carbonated mineral water, particularly if combined with a meal, is a useful additional Ca source, especially for populations who do not cover their daily Ca requirements, such as young women on low-energy diets or aged people.

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