Calcium Transport from Mineral Waters Across Caco-2 Cells

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In the present study the absorption of calcium from 13 different mineral waters has been examined. For this purpose the mineral waters were first digested in vitro by simulating gastric and intestinal digestion. Afterward, the absolute and fractional transport rates of calcium from these digested solutions across human colon adenocarcinoma (Caco-2) cell monolayers grown on bicameral filters were measured. Results showed that the fractional transport rates for calcium lie between 1.65 and 6.72% after 90 min of incubation time. The absolute transport values varied between 90.4 and 624.7 pmol/(min·cm^2). The transport values [pmol/(min·cm^2)] for calcium from the mineral waters were in general not concentration dependent, showing neither signs of saturation kinetics nor unsaturable uptake mechanisms. In addition, the fractional transport rates from mineral waters with similar calcium concentrations were greatly different in some cases. On the basis of these results, it can be concluded that calcium biovailability from mineral waters could vary dependent on probably several factors.

Keywords: *Calcium; transport; mineral waters; Caco-2 cells*

INTRODUCTION

Bioavailability could be defined as the fraction of the ingested nutrient that is utilized from the body for the maintenance of normal physiological functions or storage. One of the main pillars of bioavailability is without doubt the digestion and resorption of nutrients in the gut, which could be influenced by a large number of factors. This could be, for example, the different ingredients of foods that could mutually impair their bioavailability. In the ideal case, nutrient bioavailability should be studied in human subjects, but because of the inherent heterogeneity in humans, a large interindividual variability is seen in many bioavailability studies. Additionally, human studies are often time-consuming and costly to perform. The possible hazardous effect of radioisotopes should also be considered in these studies.

Therefore, attempts have been made to simplify and standardize bioavailability investigations. Primarily, rats have been used as a model for man, but studies that use rodents have the disadvantage that prevailing differences in the metabolism between man and rat could lead to inaccurate data. This was recently described by Reddy and Cook (1991), who reported that rat studies cannot be used to assess the quantitative dietary factors in human iron nutrition. In the 1980s, models based on in vitro digestion accompanied by equilibrium dialysis of dialyzable components from foods were successfully used to study the bioavailability of micronutrients (Miller et al., 1981; Hazell and Johnson, 1987). This method was improved by using continuous removal of the dialyzed component (Wolters et al., 1993). The main disadvantage of these in vitro methods is the fact that the complex, rate-limiting transport processes across the intestinal epithelial monolayer could not be studied. To fill this gap, the human colon carcinoma cell line, Caco-2, was used recently to study food iron bioavailability (Glahn et al., 1996; Garcia et al., 1996). Caco-2 cells, when grown on collagen-coated polycarbonate filters, form a monolayer of well-polarized cells that show many functional and morphologic properties of mature human enterocytes, such as having apical tight junctions and microvilli or producing high levels of brush border associated enzymes. Therefore, these cells represent an excellent tool for transport (Quaroni and Hochman, 1996), biochemical (Ekmekcioglu et al., 1996), and also bioavailability studies.

The working groups of Garcia et al. (1996) and Glahn et al. (1996) investigated the biovailability of iron from foods by using Caco-2 cells in different test models. For this purpose they primarily digested the foods in vitro and afterward measured the transepithelial or intraepithelial transport of ^{59}Fe added to the digested food mixture at the beginning of the assays. These studies combined in vitro digestion with transepithelial transport of a micronutrient and were, therefore, superior to investigations studying only one of these biovailability components. In addition, it was shown that the Caco-2 cell system represents a rapid, inexpensive method for performing bioavailability studies.

Previously it has also been described that culturing Caco-2 cells for 21 days in the presence of biliary/pancreatic fluid with fetal calf serum did not impair the barrier function of the monolayer and therefore probably had no cytotoxic effect on the cells (Finley et al., 1994).

In the present study we used Caco-2 cells grown on bicameral filters to examine the transport of calcium from different mineral waters that were subjected to in vitro digestion. Such investigations have not been

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published before, to our knowledge. This mineral was chosen because it exerts important physiological and biochemical effects in vivo. In addition, it can be found in mineral waters at relatively high concentrations, leading to an important dietary mineral source.

We measured the luminal to serosal transepithelial transport of nonlabeled, naturally occurring calcium, which was present in mineral waters at different concentrations. The fractional and absolute transport rates were determined and compared with each other. Additionally, we examined possible cytotoxic effects of the digested mineral waters on the cells and verified the accuracy of the nonradioactive analytical method used.

MATERIALS AND METHODS

Cell Culture. Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD) and were used between passages 23 and 40. The cells were maintained in 175 cm^2 flasks in Dulbecco's minimal essential medium (DMEM) containing 10% fetal calf serum, 20 mmol/L HEPES, 2 mmol/L L-glutamine, 1% nonessential amino acids, 100 U/mL penicillin G, and 100 μg/mL streptomycin. The cells were harvested by using trypsin/EDTA solution. Culture medium was changed every 2 days, and monolayers reached confluence usually after 7−8 days.

For transport experiments the cells were seeded onto collagen-coated polycarbonate filter cell culture chamber inserts (area = 4.72 cm^2; pore diameter = 0.4 μm; Transwell Costar, Vienna, Austria) at a density of 3×10^5 cells per filter. The Transwell filters were placed into six well plates dividing an apical or a donor-like compartment from a basal or acceptor compartment. The apical to basal transport of calcium from the digested mineral waters was measured. Experiments were performed with differentiated cells at 15 days after seeding.

Digestion and Preparation of the Mineral Waters. Peptic and intestinal digestions of the mineral waters were carried out at 37 °C in a shaking water bath. The following reagents (from Sigma, Vienna, Austria) were used for this purpose: (1) pepsin [2 g of pepsin (EC 3.4.23.1) powder from porcine stomach mucosa was dissolved in 50 mL of 0.1 mol/L HCl]; (2) pancreatin−bile extract (0.3 g of pancreatin from porcine pancreas and 1.5 g of bile extract were suspended in 125 mL of 0.1 mol/L $NaHCO_3^-$).

The digestion was started by adjusting the pH of 50 mL of mineral water to 2.0 ± 0.2. Afterward, 1 mL of the pepsin solution was added to the mixture that was then incubated for 2 h, and pH deviations were corrected every 30 min with 6 mol of HCl/L. Subsequently, the pH of the solution was gradually raised to 5 with 1 mol of HCO_3^-/L, and 5 mL of the pancreatin−bile extract mixture was added to start the intestinal digestion. The pH was adjusted to 7.0, and the mixture was incubated for an additional 2 h. Afterward, the digested mixture was centrifuged at 250*g* for 5 min to remove nondissolved particles from the pancreatin−bile extract. Subsequently, 1% v/v fetal calf serum and 10 mmol/L HEPES were added to the supernatant to inhibit the proteases of the digestive supplements and stabilize the pH for the transport studies, respectively. Contamination with calcium (0.032 mmol/L Ca^{2+}) from the fetal calf serum was minimal. Finally, the osmolality of the treated mineral waters was adjusted to 290 ± 5 mOsm/kg H_2O with NaCl Suprapur from Merck (Vienna, Austria). The proteases were inhibited with fetal calf serum and not by heat inactivation because the latter method would have been unphysiological. The completed test solutions for the assays were stored at −20 °C and used within 2 weeks.

Monolayer Integrity. Shortly before and immediately after the transport studies, the integrity of the cell monolayer was examined by determining the transepithelial passage of phenol-red, a pH indicator (molecular mass = 354 Da), which is transported only paracellularly. For this purpose, 1.5 mL of complete culture medium, containing ~42 μM phenol-red, was added to the apical compartment and 2.6 mL of phenol-red-free medium to the basal compartment. Afterward, the apical to basal transport of phenol-red was measured by spectrophotometry at 479 nm (isosbestic point) in 1 mL of the acceptor solution. A transport rate of <0.25%/h compared to the transport without cells indicated to us that the monolayer was intact.

Transport Experiments. The filter inserts were carefully washed three times with phosphate-buffered saline$^-$ and transferred to fresh six well plates containing 2.6 mL of the prewarmed acceptor solution. This was composed as follows (in mmol/L): 135 NaCl, 5 KCl, 5 glucose, 10 HEPES, pH 7.4. The contamination of the solution with calcium was negligible. To start the experiments, 1.5 mL of the pretreated mineral water was pipetted into the apical compartment, and the incubation was conducted for 90 min in a shaking water bath at 37 °C. Preliminary experiments showed that longer incubation times resulted in an impairment of the monolayer.

At the end of the incubation period filter inserts were carefully removed, and the basal solution was pipetted off and transferred to plastic tubes for the determination of the amount of calcium transported across the monolayer. In addition, the volume of both compartments was measured at the end of the assays to exclude possible dilution or concentration effects due to either water movement between the compartments or evaporation. This would have influenced the results of the study. The sample volume in both compartments remained constant throughout the assays. Analysis of calcium in all samples was accomplished by using a sequential atomic emission spectrometer ARL 3520 ICP equipped with a Paschen-Runge spectrometer and a ARL MDSN nebulizer. Samples were used for analysis without pretreatment. Calcium concentrations in the respective samples were obtained after three replicate measurement in a definite volume of sample and expressed as the mean value of these determinations. The intra-assay variability of the analytical method was 2.76 ± 2.35%. All reagents used were of analytical grade (Merck Suprapur). All standard solutions were prepared from stock solutions containing 1 mg/mL of each element (Merck Titrosol, Merck, Darmstadt, Germany). Transport data were presented either as fractional transport in percent of apical mineral concentration at the beginning of the assay or as absolute transport in pmol/(min·cm^2). Absolute transport was evaluated by calculating the calcium amount (in pmol) in 2.6 mL of the basal solution and dividing this value by (90 min × 4.72 cm^2).

Fractional transport (FT) was determined according to the equation

$$FT\ (\%) = (Ca_B/Ca_A) \times 100$$

where Ca_B is the amount of calcium after 90 min in the basal solution and Ca_A is the amount of calcium in the apical solution at the beginning of the assay (baseline value at zero time).

In separate experiments, we determined the concentration-dependent transport of calcium (1−10 mmol/L) from a nondigested HEPES-buffered solution across the monolayer to compare these transport values with those from the digested mineral waters. Kinetic constants for the calcium concentration-dependent assays were calculated using nonlinear regression with the method of least squares and then fitting the data into a modified Michaelis−Menten equation (Guiliano et al., 1991) including a saturable and a nonsaturable component.

Transepithelial ^{45}Ca Transport Studies. The transepithelial transport of the ^{45}Ca isotope in comparison to the transport of non-radiolabeled calcium was measured under the same conditions to verify the accuracy of the analytical method used in this study.

For this purpose, Caco-2 cells were grown in the filter inserts for 15 days. On the day of the experiment, they were washed with phosphate-buffered saline$^-$ and the filters were transferred to fresh six well clusters containing a 2.6 mL of calcium-free acceptor buffer that contained (in mmol/L) 135 NaCl, 5 KCl, 5 glucose, and 10 HEPES, pH 7.4. The experiment was started by adding 1.5 mL of transport buffer (composed of 130

Table 1. Composition of the Mineral Waters

mineral water	Na^+ (mmol/L)	Ca^{2+} (mmol/L)	Mg^{2+} (mmol/L)	HCO_3^- (mmol/L)	Cl^- (mmol/L)	SO_4^{2-} (mmol/L)	others (mg/L)
MW-1	0.18	5.19	1.66	4.00	0.07	5.70	47.0
MW-2	0.65	0.46	0.01	1.09	0.20	0.35	29.5
MW-3	12.70	2.72	1.12	17.20	3.57	0.06	73.1
MW-4	14.37	5.95	2.17	29.58	1.70	0.70	123.7
MW-5	5.22	6.52	6.99	36.25	0.85	0.08	50.1
MW-6	1.91	2.50	1.52	7.62	0.46	1.14	12.6
MW-7	0.99	2.08	1.37	4.32	0.58	3.01	57.7
MW-8	23.13	3.91	1.68	29.31	5.87	0.23	1.1
MW-9	22.35	4.50	1.62	37.16	2.17	0.34	289.4
MW-10	0.58	3.76	2.70	7.01	0.12	3.11	40.1
MW-11	0.30	3.14	2.30	4.49	0.19	0.96	5.5
MW-12	10.49	.99	0.29	11.55	0.60	0.48	53.1
MW-13	0.93	2.62	1.63	4.37	0.60	0.01	100.9

mmol/L NaCL, 5 mmol/L KCl, 5 mmol/L glucose, and 10 mM HEPES, pH 7.4) to the apical compartment, which contained either 5 mmol/L $CaCl_2$ or 5 mmol/L $CaCl_2$ plus 5 μCi (^{45}Ca)/mL. The cluster dishes were incubated for 90 min in a shaking water bath at 37 °C. At the end of the incubation period an aliquot of the acceptor solution was pipetted off. ^{45}Ca transported across the monolayer was measured with a liquid scintillation counter and the nonlabeled calcium with ICP-AES. Finally, the transport rates in pmol/(min·cm^2) were calculated and compared with each other.

Determination of the Sucrase and Alkaline Phosphatase Activities. Sucrase (EC 3.2.1.48) and alkaline phosphatase (EC 3.1.3.1) activities were measured after the transport studies to examine whether the digested solution in the apical compartment might have a cytotoxic effect on the monolayer. These brush border enzymes were chosen because they are in direct contact with the treated mineral waters and therefore may be impaired in the case of toxic effects during the incubation period. For enzyme studies, cells were scraped off the filters with a rubber policeman and the cell pellet was suspended at 4 °C in HEPES/mannitol buffer (20 mmol HEPES/L, 280 mmol mannitol/L), pH 7.2, followed by disruption through ultrasonication. Sucrase activity was measured in the cell homogenate according to the method of Messer and Dahlquist (1966) and alkaline phosphatase activity according to the method of Garen and Levinthal (1960). The enzyme activities of cells exposed to digested mineral waters were compared with those of control cells that were incubated in the presence of culture medium.

Statistical Analysis. Results are presented as mean ± SD. Each set of experiments was done at least two times with $n = 8$, and data presented here are from representative experiments. Statistical significance of differences between mean values was assessed with ANOVA using the Scheffé procedure as a post-hoc test. Test values that resulted in $p < 0.05$ were considered to be significant.

RESULTS

Mineral Waters. Thirteen commercial available natural mineral waters from Austria with different mineral compositions were used for this study. The calcium concentration in the mineral waters varied between 0.46 and 6.52 mmol/L (Table 1).

^{45}Ca Assays. Before we conducted the bioavailability assays, the accuracy of the nonradioactive analytical method used was verified by comparing the transport rates between labeled ^{45}Ca with the stable isotope. The results from these assays revealed no significant difference between both transport rates, with 540 ± 110 pmol/(min·cm^2) for nonlabeled Ca and 630 ± 65 pmol/(min·cm^2) for ^{45}Ca. This indicates that the measurement of nonlabeled calcium across Caco-2 monolayers is justifiable.

Enzyme Assays. The sucrase and alkaline phosphatase activities of Caco-2 cells, exposed for 90 min to four empirically chosen digested mineral waters, were not significantly different from those of control cells that were incubated with standard culture medium (Table 2).

Table 2. Sucrase and Alkaline Phosphatase Activities in Caco-2 Cells Exposed to Digested Mineral Waters versus Culture Medium (Control)[a]

variable	sucrase (% of control)	alkaline phosphatase (% of control)
control	100 ± 6.7	100 ± 8.3
MW-1	97.9 ± 7.6	103.3 ± 3.6
MW-2	91.3 ± 14.4	89.8 ± 8.2
MW-3	99.5 ± 3.0	99.5 ± 4.4
MW-4	106.5 ± 4.6	90.7 ± 9.0

[a] Values are presented as percent (mean ± SD) of control. Control cells were incubated with standard culture medium. Statistical differences between mineral waters and control were assessed with ANOVA ($n = 8$, values not different from control). These results indicated that the digested solutions were probably not cytotoxic to the Caco-2 cells.

Table 3. Fractional and Absolute Transport Rates of Calcium from Digested Mineral Waters[a]

	Ca^{2+}	
mineral water	fractional transport (%)	absolute transport [pmol/(min·cm^2)]
MW-1	1.65 ± 1.10	228.5 ± 152.4
MW-2	6.05 ± 2.73	90.4 ± 40.8
MW-3	4.17 ± 0.60	357.8 ± 51.5
MW-4	3.24 ± 0.52	624.7 ± 100.3
MW-5	2.68 ± 0.47	444.2 ± 77.9
MW-6	6.26 ± 1.64	488.0 ± 127.8
MW-7	5.13 ± 0.91	344.1 ± 61.0
MW-8	3.43 ± 0.66	320.2 ± 61.6
MW-9	3.29 ± 1.31	420.9 ± 167.6
MW-10	3.89 ± 0.95	391.6 ± 95.2
MW-11	3.15 ± 1.41	303.8 ± 136.0
MW-12	6.72 ± 1.38	204.6 ± 42.0
MW-13	4.17 ± 1.22	308.3 ± 90.2

[a] Fractional transport rates were presented as percent of apical concentration at the beginning of the assay (baseline value at zero time). Values are presented as mean ± SD.

Transport Experiments. The fractional transport values for calcium from the different mineral waters varied between 1.65 and 6.72% after 90 min of incubation time (Table 3).

The fractional transport rates between mineral waters with similar calcium concentrations were greatly different in some cases (Tables 1 and 3).

The absolute transport values were between 90.4 and 624.7 pmol/(min·cm^2) (Table 3). The absolute transport rates showed in general no homogeneous increase, like in unsaturable uptake, or characteristics of saturation kinetics at rising mineral concentrations that could be

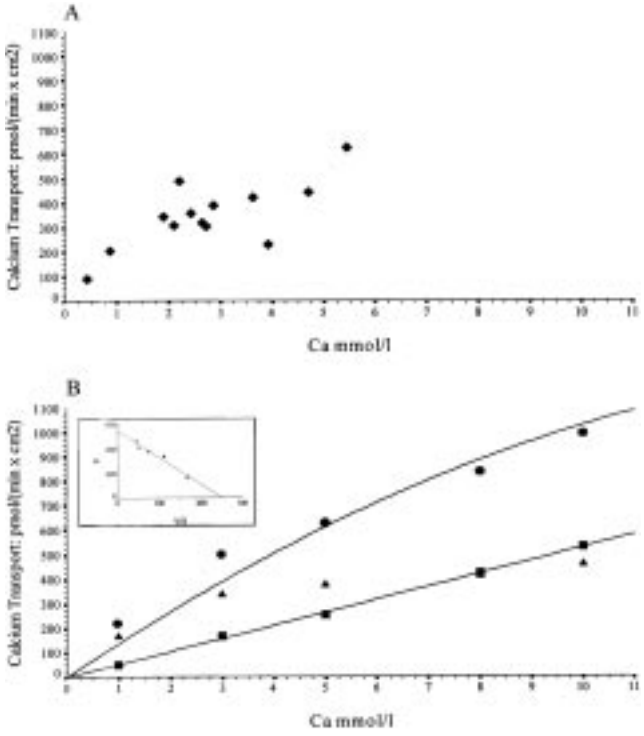

Figure 1. (A) Calcium transport from different digested mineral waters across the Caco-2 cell monolayer. Each symbol (◆) represents the mean transport in pmol/(min·cm^2) from one digested mineral water. (**B**) Calcium transport from a nondigested HEPES-buffered solution across the Caco-2 cell monolayer. The upper curve represents the total concentration-dependent transport (●) in pmol/(min·cm^2). The data were fitted into a modified Michaelis−Menten equation to derive parameters for saturable (▲) and nonsaturable (■) transport. The kinetic analysis for the saturable component was performed by using an Eadie−Hofstee plot (inset) and revealed K_m = 2.17 mmol/L and V_{max} = 545.37 pmol/(min·cm^2). Transport values for the nonsaturable, linear component can be calculated using 53.1 pmol·(cm^2)$^{-1}$·min^{-1}·(mmol/L)$^{-1}$·mmol/L calcium. The mean values (n = 8) without SD for each variable were presented because of graphic quality.

normally expected under standardized conditions (Figure 1A). This was verified by studying the concentration-dependent calcium transport from a nondigested HEPES-buffered solution showing a curvilinear course (Figure 1B) including a saturable and nonsaturable component. Considering these results, it can be concluded that the calcium transport rates between the investigated mineral waters varies dependent on probably many causes.

DISCUSSION

In the present study we investigated the bioavailability, that is, transport, of Ca^{2+} from 13 different mineral waters across the Caco-2 monolayer. The reason for our choice to take a liquid rather than a solid was the fact that there are only a few studies available that have compared the bioavailability of nutrients from liquids (Couzy et al., 1995; Halpern et al., 1991; Loennerdal et al., 1993; Van Dokkum et al., 1996; Wynckel et al., 1997).

Before carrying out the transport studies we digested the mineral waters in vitro. The reason for digesting the liquids was the following consideration: First, it is known that there is a basal secretion of bile and pancreatic fluid (including enzymes) to the upper intestine in the interdigestive phase (Keane et al., 1981; Regan et al., 1980; Solomon, 1994; Svenberg et al., 1982) that is stimulated in the cephalic phase or especially after ingestion of foods, that is, in the gastric and intestinal phases. Therefore, it is obvious that the mineral waters will be mixed thoroughly to a varying extent with pancreatic bile fluid dependent on the momentary digestive phase. Second, we wanted to investigate the effect of digested foods on the Caco-2 cells.

Our results indicated that the Caco-2 cells tolerated the addition of digested solutions prepared under the test conditions described without showing signs of monolayer impairment or brush border enzyme toxicity. Therefore, we believe that this model is valuable for conducting bioavailability studies.

In addition, we showed that the transport rate of calcium could vary among the mineral waters. One reason for the different bioavailabilities could be, for instance, the presence of anions such as HCO_3^- or SO_4^{2-} that are dissolved in the mineral waters at different concentrations and probably affect, in some cases, the transport rates of these two minerals. It might also be possible that magnesium ions in the mineral waters inhibited calcium transport to some degree. Previously it was shown that magnesium inhibited dose-dependent mucosa-to-serosa calcium flux in rat ileum (Karbach and Rummel, 1990). In contrast to this observation, an inhibiting effect of calcium on magnesium transport in rodents and man has been published by Behar (1975) and Norman et al. (1981). Nevertheless, these considerations remain hypothetical unless specific investigations are performed, which could be realized, for example, by adjusting the calcium concentration in the mineral waters to an identical value. Afterward, correlations could be performed between the transport rates and the different anion concentrations to identify nutrients responsible for the impairment in calcium bioavailability. However, this kind of pretreatment would be unphysiological and, in addition, it was beyond the scope of this paper to explore this problem.

Another explanation for the differences in the calcium absorption rates among the mineral waters could be a modulation of the paracellular transport pathway. It is known that calcium absorption in the gut is controlled by transcellular and paracellular transport routes. The transcellular pathway is a saturable, presumably carrier-mediated process that is stimulated by vitamin D_3. In contrast, the paracellular permeation route is traditionally defined as a vitamin D_3-independent passive pathway for calcium transport. Passive intestinal calcium absorption was demonstrated not only in Caco-2 cells (Guiliano et al., 1991; Blais et al., 1997; the present study) but also in animals (Nellans, 1990) and in humans (Sheikh et al., 1990). There is growing evidence that different substances could modify the tightness of the paracellular barrier. Some of these factors implicated in this regard are, for example, bile salts (Webling and Holdsworth, 1966), second messengers (Stein and Kottra, 1997), or also polyvalent cations such as Ca^{2+} (Powell, 1981; Palant et al., 1983). Therefore, it may be plausible that some of the electrolytes in the mineral waters, possibly calcium itself, could have modulated passive calcium transport by affecting the intercellular absorption route.

In separate experiments, we have additionally shown that the concentration-dependent transport of calcium from a nondigested HEPES-buffered solution is curvi-

linear (Figure 1B), thus indicating saturable and passive uptake mechanisms. These outcomes are in agreement with previous investigations studying vitamin D-regulated calcium transport in Caco-2 cells (Giuliano and Wood, 1991).

One question that needs further clarification is, "Are the results from this study directly applicable to human conditions?". It is obvious that the best bioavailability studies could be carried out with human subjects. However, these investigations are often difficult to perform and may be very expensive. To overcome such kinds of problems, different in vitro methods were established through the years and were used worldwide to study nutrient bioavailability. These various methods differ in their complexity and functionalism. However, each of them has advantages and also limitations. The principal goal of all of these in vitro methods is to achieve results that are comparable with those of in vivo conditions. At present, several laboratories regard the Caco-2 cell model as one of the best in vitro methods to investigate intestinal nutrient bioavailability (Glahn et al., 1996; Garcia et al., 1996). Referring to previous publications in this area, it seems likely that results from nutrient transport studies across Caco-2 cell monolayers could be transferred in general to in vivo conditions. For example, it was previously demonstrated that Caco-2 cell iron uptake from meat and casein digests is in agreement with results from human trials (Glahn et al., 1996). Furthermore, Ma et al. (1994) found that biotin uptake by Caco-2 monolayers is very similar to in vivo conditions. Vectorial calcium transport across Caco-2 cells was also described to correspond to the mammalian small intestine regarding the transport kinetics and the stimulatory effect of vitamin D_3 (Guiliano et al., 1991). Considering these previous investigations, we believe that the results from this study could be applicable, with certain restrictions, to human conditions. However, it must be remarked that this cellular in vitro system differs is some points from in vivo conditions. The transport area of the filter system, for example, is only a minor fraction from that of the small intestine. Furthermore, the morphological and physiological situations in vivo vary in some points as in vitro, for example, the different populations of cells in the gut such as Goblet, Paneth, and Crypt cells, which are less organized and therefore leakier. There is also a lack in the regulatory control by neuroendocrine cells and through the blood. Other handicaps of this model are the static transport conditions and the fact that the incubation times cannot be set up very long like the sojourn time in vivo without risking an impairment of the monolayer. Therefore, it is not fully justified to compare results such as fractional transport values from this kind of an in vitro study with that of a human trial performed under equal preconditions. We found, for instance, that the fractional absorption rates for calcium from mineral waters in human studies tend to be distinctly higher than in our study (Couzy et al., 1995; Van Dokkum et al., 1996). However, we were especially interested in the relative fractional transport rates among the mineral waters rather than in comparing the absorption values directly with in vivo results. The main purpose of our comparative study was to show that intestinal calcium transport could differ among a similar group of liquids. Consequently, we can suggest that it is likely that these mineral waters will have similar calcium bioavailabilities in the human intestine. The differences in the calcium absorption rates among the mineral waters may probably be not important in daily life, when calcium intake is mainly accomplished through other dietary sources. However, the results of our paper could be relevant in cases when mineral waters are used solely to supplement dietary calcium, especially in patients suffering, for example, from osteoporosis, who require large quantities of this mineral.

In conclusion, we have demonstrated that the Caco-2 cell system could be used successfully for bioavailability studies from digested liquids. Although this cell model obviously has limitations like other in vitro systems, it also possesses advantages as compared to human trials. One major advantage is that this system is standardized and yields reproducible results. These properties are very important, especially in comparative studies such as this one. We presented evidence that intestinal calcium transport among mineral waters could vary. Reasons for this heterogeneity in the transport rates are only speculative and should be further investigated in the future.

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