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Determination of calcium salt solubility with changes in pH and P_{CO_2}, simulating varying gastrointestinal environments

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Abstract

The amount of calcium available for absorption is dependent, in part, on its sustained solubility in the gastrointestinal (GI) tract. Many calcium salts, which are the calcium sources in supplements and food, have pH-dependent solubility and may have limited availability in the small intestine, the major site of absorption. The equilibrium solubility of four calcium salts (calcium oxalate hydrate, calcium citrate tetrahydrate, calcium phosphate, calcium glycerophosphate) were determined at controlled pH values (7.5, 6.0, 4.5 and ≤3.0) and in distilled water. The solubility of calcium carbonate was also measured at pH 7.5, 6.0 and 4.5 with two CO_2 environments (0.3 and 152 mmHg) above the solution. The precipitation profile of $CaCO_3$ was calculated using in-vivo data for bicarbonate and pH from literature and equilibrium calculations. As pH increased, the solubility of each calcium salt increased. However, in distilled water each salt produced a different pH, affecting its solubility value. Although calcium citrate does have a higher solubility than $CaCO_3$ in water, there is little difference when the pH is controlled at pH 7.5. The partial pressure of CO_2 also played a role in calcium carbonate solubility, depressing the solubility at pH 7.5. The calculations of soluble calcium resulted in profiles of available calcium, which agreed with previously published in-vivo data on absorbed calcium. The experimental data illustrate the impact of pH and CO_2 on the solubility of many calcium salts in the presence of bicarbonate secretions in the intestine. Calculated profiles using in-vivo calcium and bicarbonate concentrations demonstrate that large calcium doses may not further increase intestinal calcium absorption once the calcium carbonate solubility product has been reached.

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Introduction

There are a variety of calcium supplements on the market, which contain different calcium salts. The advantages of one salt form over another in the extent of calcium absorption have been previously studied, often with comparisons made between calcium citrate and calcium carbonate. Two studies, one evaluating the true absorption of radiolabelled Ca^{2+} and one analysing the pharmacokinetic parameters after supplemental dosing, concluded that no difference in absorption existed between the two salts (Heaney et al 1999, 2001). On the other hand, several studies showed that calcium citrate has significantly greater absorption than calcium carbonate, as assessed by urinary Ca^{2+} excretion, faecal recovery of Ca^{2+}, and analysis of pharmacokinetic parameters (Nicar & Pak 1985; Harvey et al 1988; Heller et al 1999). The above data are contradictory and could be owing to the presence of food and the method of study.

When calcium citrate has shown greater absorption, it has been attributed to the salt's superior solubility over that of calcium carbonate. On the other hand, large increases in calcium salt solubility do not necessarily result in comparable increases in absorption, as reported in studies by Heaney et al (1990a) and Sheikh et al (1988). In these studies, the solubility was determined in water, and the pH of the solution was not reported. The pH of the gastrointestinal (GI) tract changes with time and region, from the very acidic environment of the stomach to the less acidic, nearly neutral environment throughout the intestine. The difference in pH could have a significant impact on the amount of dissolved calcium, thereby affecting the absorption of calcium. The effect of gastric pH was shown by Recker

(1985) when achlorhydric (i.e. those that do not secrete hydrochloric acid) subjects absorbed significantly less calcium from a calcium carbonate tablet than healthy subjects, indicating that hydrochloric acid is needed to dissolve the calcium salt. Whether calcium is absorbed by active transporters, located in the duodenum, or passively throughout the jejunum and ileum, it is assumed that calcium must be in its soluble form (Favus 1996; Bronner 2003).

Determining calcium solubility at different pH values can mimic the salt's behaviour in the various GI environments, but the analysis can be complex and difficult to achieve. There are studies in which the effect of pH on a measure of calcium salt solubility was determined (Roth-Bassell & Clydesdale 1992; Cayon & Roquer 1997; Assoumani 1998). While each study recognized the potential impact of pH, the various methods utilized were not the standard method of determining equilibrium solubility, defined as the equilibrium concentration of calcium salt that is in solution in the presence of excess solid. Once solubility of a calcium salt is known, the value can be widely used, since it is an intrinsic value of the salt at a specific pH and independent of the amount of calcium salt, although it is often somewhat dependent on ionic strength (Bromley 1973). In the case of large amounts of calcium, such as supplement doses, it is reasonable to expect incomplete solution in the pH environments near absorption sites, such that the solubility becomes an important determinant of calcium absorption.

In addition to regional and temporal differences in pH along the GI tract, the partial pressure of carbon dioxide (P_{CO_2}) may affect the solubility of calcium and other minerals. The concentration of dissolved CO_2 changes with the addition of bicarbonate (HCO_3^-), primarily from pancreatic secretions, into the small intestine to neutralize stomach contents, such that the partial pressure of CO_2 can reach approximately 300 mmHg (Rune 1972), as opposed to 0.3 mmHg, the atmospheric P_{CO_2} in which most solubility experiments are performed. Of particular importance is the solubility of calcium (Ca^{2+}) in the presence of carbonate (CO_3^{2-}), which will be affected by the CO_2 in the system. Carbonate, from the dissolved $CaCO_3$, and bicarbonate (HCO_3^-), secreted intestinally to increase pH, have an equilibrium relationship with dissolved CO_2 (Figure 1) (Butler 1991). When this equilibrium is taken into account, determining $CaCO_3$ solubility could prove difficult, since addition of carbonate (CO_3^{2-}) from dissolving $CaCO_3$ shifts the reaction toward CO_2, thereby generating CO_2 that evolves from the solution, possibly overestimating the solubility.

In this study, we determined the equilibrium solubility of five calcium salts at four pH values to ascertain how the solubility of each salt may change throughout the GI tract. The solubility of each calcium salt was determined in water for comparison with published values. Calcium oxalate and calcium glycerophosphate were chosen based on their extreme solubility (low and high, respectively) at the pH range of the GI tract, and three common salts, calcium citrate, phosphate and carbonate, with reported solubility values between the extremes were also studied. The effect of the CO_2 environment was also evaluated by determining $CaCO_3$ solubility at partial pressures of 0.3 mmHg CO_2, comparable with atmospheric intestinal environment, and 152 mmHg CO_2, an average based on elevated intestinal bicarbonate concentrations (McNamara et al 2003). Using in-vivo values of calcium and

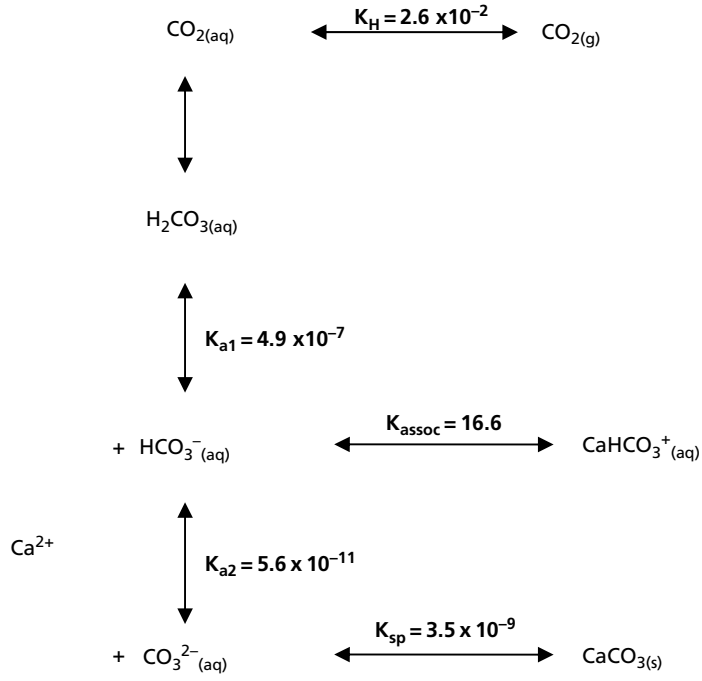

Figure 1 Equilibrium relationship between carbon dioxide (CO_2) and calcium carbonate ($CaCO_3$); g, gaseous; aq, aqueous; s, solid.

HCO_3^-, as well as the equilibrium relationship between the different species, the precipitation profile of $CaCO_3$ in the small intestine was determined for a range of doses.

Materials and Methods

Materials

Calcium oxalate hydrate [$CaC_2O_4 \cdot H_2O$] (Lot 11812EU), calcium citrate tetrahydrate [$Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$] (Lot 12202TA), calcium phosphate [$CaHPO_4$] (Lot 11K0165) and calcium carbonate [$CaCO_3$] (Lots 50K0205 and 17406EB) were purchased from Sigma-Aldrich (St Louis, MO, USA). Calcium glycerophosphate [$Ca(C_3H_7O_6P)$] (Lot C102034) was purchased from PCCA (Professional Compounding Centers of America; Houston, TX, USA). Hydrochloric acid (Lot 419060), sodium hydroxide (Lot 035207), and lanthanum solution (Lot 3346-11) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Calcium glycerophosphate was USP grade and all other chemicals were reagent grade. Carbon dioxide (20% in air) was obtained from Airgas, Inc. (Waterbury, CT, USA).

Effect of pH

Excess solid of each salt (10–100 g) was added to 100 mL distilled water (n = 3). Each sample was adjusted to the desired pH: 7.5, 6.0, 4.5 and 2.0 (Thermo Orion Model 710A pH meter; Thermo Orion, Beverly, MA, USA). Calcium carbonate solubility could not be determined at pH values less than 4.5 because a stable pH could not be maintained due to the release of CO_2. Calcium glycerophosphate solubility could not be measured at pH values less than 3.0 due to the high viscosity of the solution, creating difficulties with pH measurement and sample withdrawal. After adjustment with HCl or NaOH, each solution was continually stirred at room temperature. The concentration of the acid and base used for pH adjustment ranged from 10 M to 0.1 M, depending on the extent of adjustment needed. Periodically, each solution was analysed for pH and readjusted if necessary. Once the target pH (±0.1) was maintained for at least 4 h, samples (5–10 mL) were withdrawn and filtered (0.2 μm). When there was less than a 5% difference in calcium solubility between consecutive time points, equilibrium was assumed. In a separate study, 10 g of each salt was added to 100 mL doubly distilled water and continually stirred for 24 h (n = 3). Samples were withdrawn to determine calcium salt solubility and the pH was measured.

Filtered samples were diluted in 0.1 M HCl for analysis by flame atomic absorption (AA) spectrometry (Varian Spectra AA 200; Varian, Walnut Creek, CA, USA) with air/acetylene flame (13.02:2.00 L min^{-1}) and Ca/Mg lamp wavelength of 422.7 nm and current of 10 mA. Lanthanum solution (5%) was added to each sample to minimize chemical interference common in AA analysis. A calibration curve was generated prior to each analysis.

The composition of the excess solid remaining, after equilibrium solubility had been reached, was analysed by X-ray powder diffraction (XRPD) to ensure that there was no conversion of the solid to another form, such as a hydrate, during the solubility study. The XRPD scans compared peaks of the solid sample after solubility determination with peaks of the original solid. Analysis of the solid remaining at equilibrium solubility confirmed that there was no conversion of the solid, except the calcium citrate tetrahydrate at pH 2.0, which showed emergence of new peaks in the XRPD scan. The excess solid could not be identified by a standard XRPD library (Adama); the solid may have been either an unidentified hydrate or mixture of hydrates. The diffractometer (Model D5005; Bruker AXS Inc., Madison, WI, USA) used Cu$K\alpha$ radiation, a voltage of 40 kV and a current of 40 mA. The scanning rate was 5° min^{-1} over a 2θ range of 10–60°, with a sampling interval of 0.02°.

Effect of CO_2

Calcium carbonate solubility was determined in a beaker open to the atmosphere (0.3 mmHg CO_2) and in a closed container in an atmosphere of 152 mmHg CO_2 to simulate an increased intestinal CO_2 environment. The 'open' experiments were conducted with the same method as other salts, that is constant exposure to the atmosphere during pH adjustment and stirring. The 'closed' experiments were performed by flowing air with a partial pressure of 152 mmHg CO_2 above the solution during the pH adjustment and stirring each solution in a sealed container maintained at a partial pressure of 152 mmHg CO_2. In the 'closed' experiments, a CO_2 monitor (YSI Biovision 8500; YSI, Dayton, OH, USA) was used to verify the mmHg CO_2 dissolved in the solution at each pH adjustment.

The same methodology for sample dilution, AA sample analysis and XPRD solid analysis as the previous solubility determinations was used.

Statistical analysis

A two-way rank analysis of variance test was used to determine if there were significant differences in solubility between calcium citrate and calcium carbonate at pH 4.5, 6 and 7.5. These two salts were chosen because they are the most common calcium supplements on the market, and solubility versus absorption is often compared. The same statistical test determined differences in solubility of calcium carbonate at P_{CO_2} of 0.3 and 152 mmHg and at pH 4.5, 6 and 7.5. The level of significance was $P = 0.05$ with Bonferroni adjustment.

Calculation of the intestinal level of soluble calcium

Once the concentrations of calcium and carbonate exceed the solubility product (K_{sp}) in a solution, calcium carbonate will precipitate:

$$Ksp = [Ca^{2+}][CO_3^{2-}] \quad (1)$$

It is possible to estimate the concentration of calcium remaining in solution, and thereby available for systemic absorption, in the various pH environments of the intestine. All calculations are the first approximation, neglecting contributing effects such as other counter-ions or association of calcium

with other components. Although these effects may be significant in-vivo, the possible extent of precipitation of calcium by carbonate species was the focus of this investigation. The mass balance of calcium is as follows:

$$[Ca_{total}] = [Ca^{2+}] + [CaHCO_3^+] + [CaCO_3] \quad (2)$$

where total calcium, Ca_{total}, is the sum of soluble calcium ($Ca^{2+} + CaHCO_3^+$) and insoluble calcium ($CaCO_3$). When the intestinal bicarbonate concentration is known, the concentration of soluble calcium bicarbonate can be determined from:

$$[CaHCO_3^+] = K_{asn}[Ca^{2+}][HCO_3^-] \quad (3)$$

where K_{asn} is 16.6, the association constant of calcium and bicarbonate (Butler 1991), and $[Ca^{2+}]$ is the intestinal free ionized calcium concentration, which is dependent on the supplemental dose. Carbonate concentration can be determined at a specific pH using the following equation and shown in the equilibrium relationship in Figure 1:

$$[CO_3^{2-}] = K_a[HCO_3^-]/[H^+] \quad (4)$$

where K_a is 5.62×10^{-11} (Butler 1991), the equilibrium constant of carbonate and bicarbonate. Once the concentration of carbonate is known, the amount of calcium carbonate that exceeds K_{sp} (10^{-9}) can be calculated. Thus, the solubility of calcium ($Ca^{2+} + CaHCO_3^+$) in any set of conditions, pH and bicarbonate concentration, can be determined.

Results

Effect of pH

The solubility in terms of elemental calcium of five calcium salts commonly found in supplements and food was determined at various pH values to mimic the different environments each salt may encounter in the GI tract. The oxalate salt had the lowest solubility and calcium glycerophosphate had the greatest solubility at each pH measured (Table 1).

With the exception of calcium oxalate and calcium glycerophosphate, the rank order of calcium solubility changed depending on pH (Figure 2). At pH 7.5, the order was: phosphate < carbonate < citrate. Calcium carbonate solubility increased as pH decreased (note that Figure 2 is a logarithmic scale), which is expected according to the equilibrium described in Figure 1, since CO_2 was allowed to escape into the atmosphere, leaving calcium chloride (a very soluble salt) in solution. Calcium citrate solubility remained constant until the pH dropped below 4, where the citrate and phosphate salts had similar solubilities, while calcium carbonate had significantly greater solubility than calcium citrate. The solubility of each salt at the adjusted pH values is reported in Table 1. Also reported are the solubilities in water with no pH adjustment and values from the literature in order to compare previously reported values with no pH adjustment with the experimental values at specific pH values. Data are plotted as the median solubility versus pH due to the values below the limit of quantification. The graphs are superimposable with

Table 1 Observed solubility ($mg\,mL^{-1}$) of calcium at different pH values compared with previously reported solubility values ($mg\,mL^{-1}$)

Salt	Reported aqueous solubility	pH	Observed solubility[a]
Calicum oxalate		2.0	0.011 (0.002)
		4.5	<0.005[b]
		6.0	<0.005[b]
		7.5	<0.005[b]
	0.0061 (Hodgman 2004)	8.5 (water)	<0.005[b]
Calcium phosphate		2.0	21 (1)
		4.5	0.63 (0.04)
		6.0	0.079 (0.001)
	0.0012 (Hodgman 2004)	6.3 (water)	0.037 (0.010)
		7.5	0.019 (0.001)
Calcium citrate		2.0	17 (1)
		4.5	0.33 (0.03)
	0.20 (Linke 1958)	5.6 (water)	0.23 (0.00)
		6.0	0.20 (0.00)
		7.5	0.23 (0.01)
Calcium glycerophophate		3.0	76 (19)
		4.5	36 (1)
		6.0	10 (1)
		7.5	5.7 (0.4)
	No previously reported value	8.8 (water)	7.2 (0.3)
Calcium carbonate		4.5	46 (7)
		6.0	3.8 (0.4)
		7.5	0.13 (0.01)
	0.0066 (Hodgman 2004)	8.5 (water)	<0.005[b]

[a]Values in parentheses are the standard deviation of the three replicates. [b]Below the limit of quantification.

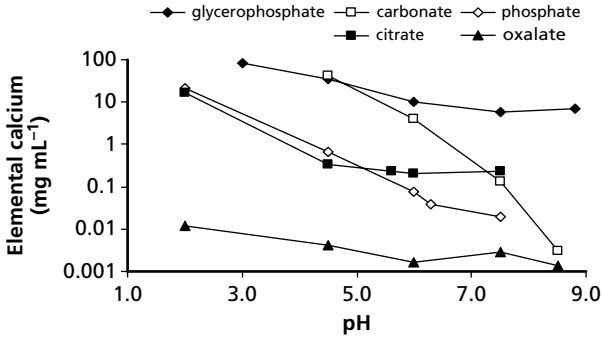

Figure 2 Log solubility plot of calcium salts (median value) in aqueous solutions open to the atmosphere.

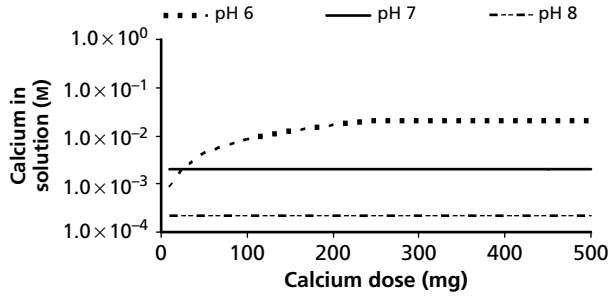

Figure 4 Concentration of calcium remaining in solution with increasing calcium dose. Assumptions: 10 mEq L^{-1} intestinal bicarbonate concentration, calcium dose in a volume of 250 mL.

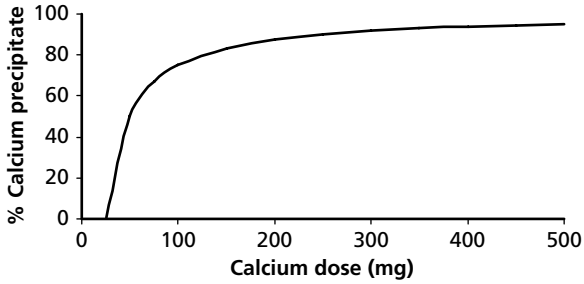

Figure 5 Percentage of calcium precipitated with increasing calcium dose. Assumptions: pH 7, 10 mEq L^{-1} intestinal bicarbonate concentration, calcium dose in a volume of 250 mL.

average solubility versus pH plots, indicating normal distribution of the replicate samples.

Effect of CO_2

Due to the higher partial pressure of CO_2 in the GI tract, the solubility of calcium carbonate was evaluated at two CO_2 partial pressures (0.3 and 152 mmHg). At pH 7.5, the solubility in the presence of 0.3 mmHg CO_2 was an order of magnitude greater than the solubility in the presence of 152 mmHg CO_2 (Figure 3). At pH 4.5, the calcium carbonate solubility at 152 mmHg CO_2 was significantly greater than the solubility at 0.3 mmHg CO_2.

Calculation of the intestinal level of soluble calcium

The precipitation profile of $CaCO_3$ in intestinal environments was determined based on the assumption that there is no counter-ion effect, such as the buffering and association effect of citrate, and the assumption that each calcium dose is administered with a volume of 250 mL. The endogenous calcium concentration is only approximately 1% of the calcium concentration after supplement administration (Lindahl et al 1997), so it is negligible in the total calcium concentration. A duodenal bicarbonate concentration of 10 mEq L^{-1} (Johnson 2001), which represents a low value over the typical range of HCO_3^- concentrations, at pH 7 and 8, corresponds to P_{CO_2} values of 60 mmHg and 6 mmHg, respectively. Under these two conditions, the maximum amount of calcium in solution was independent of calcium dose greater than 25 mg (Figure 4), because the K_{sp} was exceeded at 25 mg Ca^{2+}. At pH 6, where the carbonate concentration is 0.01 mEq L^{-1}, the soluble calcium concentration increased with calcium dose until the K_{sp} was reached at a dose of 250 mg, after which any further calcium added to the solution precipitated as calcium carbonate. The profile of calcium remaining in solution at pH 7 (Figure 4) can also be expressed as the percentage of calcium that precipitates out of solution with increasing calcium dose (Figure 5), which exceeded 75% at calcium doses approaching 100 mg. The graph agreed with published clinical data comparing calcium dose with fractional absorption (Heaney et al 1990b). The reported decrease in calcium absorption with increasing calcium dose had the same curve shape as the increase in calcium precipitation in Figure 5, with a transition to a plateau occurring at a calcium dose over the range of 25–100 mg.

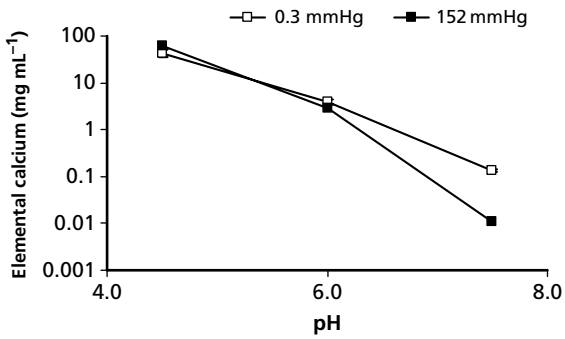

Figure 3 Log solubility plot of $CaCO_3$ (median values) under different CO_2 environments.

Discussion

The purpose of this study was to determine how the solubility of calcium from calcium salts changed in the different pH

environments and CO_2 environments of the GI tract, emphasizing the differences between the common supplements, calcium citrate and calcium carbonate. As pH increased to 7.5, the solubility of each calcium salt, with the exception of oxalate, decreased. Therefore, at higher doses, greater concentrations of ionized calcium dissolved by acid in the stomach may precipitate in the more neutral intestine before absorption occurs.

The reported aqueous solubility values of calcium salts from the literature were similar to the solubilities in water with no pH adjustment (Table 1), resulting in large differences in the final pH of each solution. Previous reported values of solubilities in water led to the conclusion that calcium citrate solubility is at least one order of magnitude greater than that of calcium carbonate (Linke 1958; Hodgman 2004). According to the results in Table 1, the order of magnitude difference exists only when the pH of the calcium carbonate solution exceeds 7.5. Calcium citrate solubility in water results in a final pH comparable with the proximal small intestine (pH 5.6), while at its solubility point in unbuffered water, calcium carbonate has a higher pH than in-vivo (pH 8.5). When different calcium salts are dissolved in water, the pH is determined by the counterion. However, in-vivo, it is expected that although the physiological buffer capacity is low, the local pH is governed by secretion of hydrochloric acid in the stomach and sodium bicarbonate in the intestines. The solubility values determined at known acidities corresponding to those in-vivo are more applicable to understanding the effect of solubility on calcium available for absorption. The data presented here suggest that simple aqueous solubility is not a key factor in the fractional absorption differences previously seen between calcium citrate and carbonate (Nicar & Pak 1985; Harvey et al 1988; Heller et al 1999), since over the small intestinal pH range (5.5 to 7.7; Fallingborg 1999) the salt solubilities are in a crossover region. That is, at pH 6, calcium carbonate solubility ($3.6\ mg\ mL^{-1}$) is greater than calcium citrate solubility ($0.20\ mg\ mL^{-1}$) and, at pH 7.5, calcium carbonate solubility ($0.12\ mg\ mL^{-1}$) is lower than calcium citrate solubility ($0.24\ mg\ mL^{-1}$) where values are reported in terms of elemental calcium.

Each calcium salt studied showed a decrease in solubility when the pH was increased from the more acidic pH of the stomach to the more neutral pH of the intestine. Therefore, while most calcium salts, with the exception of calcium oxalate, are expected to dissolve in the low pH of a healthy stomach, calcium may not remain in solution when introduced to the less acidic environment of the small intestine, the major site of absorption.

The Biopharmaceutics Classification System categorizes drugs based on the solubility and permeability in order to aid in determining a drug's potential for development (Amidon et al 1995), with Class I representing drugs high in both categories and Class IV representing drugs relatively low in both categories. The same concept can be applied to calcium supplements to obtain a clearer picture of the intrinsic barriers to calcium absorption. The permeability of the calcium ion is considered low due to its apparent permeability of $10^{-6}\ cm\ s^{-1}$ (Surendran et al 1995) across Caco-2 cell monolayers, a system previously correlated with in-vivo data (Giuliano & Wood 1991). The solubility component of the Biopharmaceutics Classification System is determined by solubility (highest dose in 250 mL) over the pH range 1–7.5. At higher pH values, which are comparable with intestinal pH values, all calcium salts, except for calcium glycerophosphate, would be considered 'low' solubility compounds. Therefore, the calcium supplements generally administered would be considered Class IV compounds.

The partial pressure of CO_2 in the intestine further affects solubility values. The differences in calcium carbonate solubility in the 152 mmHg CO_2 environment when compared with the 0.3 mmHg CO_2 environment can be explained by the equilibrium of carbonate described in Figure 1. The CO_2-rich environment in the solution at 152 mmHg CO_2 increases all the carbonate species, particularly the concentration of carbonate (CO_3^{2-}) at pH 7.5, thereby causing more precipitation of the calcium carbonate ($CaCO_3$), as seen in Figure 3. At pH 4.5, bicarbonate (HCO_3^-) is much more prevalent than carbonate (CO_3^{2-}) as compared with pH 7.5, and the 152 mmHg CO_2 environment creates an abundance of HCO_3^- to associate with calcium to form calcium bicarbonate ($CaHCO_3^+$), a soluble ion.

The concentration of CO_2 in the intestine may have a significant impact on the amount of calcium available for absorption in the small intestine. The effect can be seen in this study on the solubility of calcium carbonate, but it may be a factor in-vivo regardless of the calcium salt administered orally. In the duodenum, the average pH is 6.0 (with a range of 5.7–6.2) and gradually increases throughout the small intestine to pH 7.5 (with a range of 7.3–7.7) (Fallingborg 1999). Under these conditions, our results indicate that the solubility of $CaCO_3$ is approximately $3.5\ mg\ mL^{-1}$. The calcium source, whether calcium citrate, carbonate, phosphate or glycerophosphate, will dissolve in the acidic conditions of the stomach and be subject to the same CO_2 environment in the intestine, due to the secretion of HCO_3^- (a source of CO_3^{2-}) to neutralize stomach contents. Therefore, for salts that dissolve to the same extent in the stomach, the solubility of the salt itself may have a limited impact, since much of the free calcium ion in the intestine may precipitate out of solution as $CaCO_3$, regardless of its source salt. This may explain why calcium salts have similar fractional absorptions (Sheikh et al 1987) despite the water solubilities ranging over four orders of magnitude.

It should be noted that other factors in the GI tract, besides pH and CO_2, may affect calcium solubility. The presence of bile salts may enhance the solubility and therefore more calcium may be available for absorption. Phosphate ions in the GI tract may also associate with calcium, in a similar manner to CO_3^{2-}, to form $CaPO_4$ and precipitate out of solution. There are also dietary components, such as protein, amino acids and fibre, which could bind with calcium, thereby altering absorption (Favus 1996). Calcium absorption will also be dependent on the sojourn time in each intestinal segment, particularly the duodenum, where highly efficient active transporters are present

(Bronner & Pansu 1999). The extent of each of these effects needs further study.

A first approximation estimate of soluble calcium can be made by focusing on the major factors, pH and P_{CO_2}. Using the in-vivo concentration of calcium and bicarbonate, the precipitation of calcium carbonate was estimated using ionic equilibrium calculations (Figure 4), and the profile agreed with previously published in-vivo data (Heaney et al 1990b). By considering the interaction of each carbonate species with calcium, the concentration of calcium in solution, and therefore the concentration of soluble calcium available for absorption, can be evaluated. The precipitation of calcium carbonate in the intestine, as illustrated by Figure 5, suggests that high calcium doses offer no advantage in increasing calcium absorption.

At pH 6, the concentration of calcium in solution increases with increasing dose until a plateau is reached (Figure 4). Once the pH is increased above pH 6 in the profile, there is no difference in the concentration of calcium, regardless of increasing dose. At doses greater than 200 mg, soluble calcium concentration is limited by solubility at each pH value and deceases by an order of magnitude with each increasing pH unit. The higher pH values are more relevant in the jejunum and ileum regions of the small intestine, where the majority of large supplemental doses are absorbed after the active transporters in the duodenum are quickly saturated (Bronner 2003). The precipitation profile at pH 7 (Figure 5) indicates that once the calcium dose is greater than 100 mg, administered with a 250 mL glass of water, the majority of calcium exists as the insoluble calcium carbonate salt, with only 10% of the calcium in solution at doses greater than 200 mg. The possibility of this physicochemical mechanism explaining, in part, the poor absorption of calcium from supplements is supported by previous in-vivo data (Heaney et al 1990a, b) that suggest that lower calcium doses are more efficiently absorbed.

Conclusion

The different pH environments throughout the GI tract may have a significant impact on the concentration of calcium absorbed, assuming calcium must be in its ionized form for absorption. Controlling pH during solubility determination is essential for accurate values. For example, calcium citrate is often considered more soluble than calcium carbonate, which is true for the solubility in water, where pH depends on the salt. However, at pH values below 7, the solubility of carbonate is actually greater than citrate. As solubility decreases with increasing pH, the amount of soluble calcium in the intestinal fluid diminishes, thereby likely diminishing the amount of absorbed calcium for a given sojourn time.

Using equilibrium relationships between specific intestinal species and in-vivo concentrations of these species, it is possible to calculate the precipitation of calcium carbonate. The percent of precipitated calcium increases as the dose increases. A plateau is approached at 100 mg, suggesting that a larger calcium dose does not result in a comparable increase in soluble calcium, because soluble calcium concentration is limited by solubility. The data agree with the absorption profile calculated from in-vivo data, demonstrating a possible correlation between intestinal precipitation and intestinal absorption of calcium (Heaney et al 1990b).

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