A Research Note

In Vitro Solubility Characteristics of Six Calcium Salts

HELEN A. ROTH-BASSELL* and FERGUS M. CLYDESDALE

Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, Massachusetts 01003

*Present address: Ocean Spray Cranberries, Inc., One Ocean Spray Drive, Lakeville, MA 02349

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ABSTRACT

Six calcium salts (lactate, carbonate, citrate, gluconate, phosphate, and citrate malate) were evaluated for ionic and total soluble calcium content utilizing a sequential pH treatment to simulate gastrointestinal pH conditions. At pH 2.0 (gastric pH), 80-90% of all soluble calcium present was in the ionic form. However, when the pH was brought to 7.0 (intestinal pH), a unique solubility pattern for each calcium source was evident. Both calcium citrate and calcium citrate malate formed significantly higher levels of a soluble complex. By contrast, calcium phosphate precipitated extensively under the same conditions. These results indicate the importance of pH adjustment during in vitro testing of calcium bioavailability.

An increasing public awareness of the importance of adequate dietary calcium has led to an overwhelming demand for supplements and calcium fortification. Calcium’s solubility is believed to be an important component to its absorption. Furthermore, it is dependent on digestive processes as well as food composition, preparation, processing, and storage. There have been a few conflicting in vivo studies which have attempted to determine the role of gastric acid secretion on net calcium absorption. Recker (7) found that the absorption of calcium from calcium carbonate by fasting achlorhydric patients was significantly lower than for normal subjects and also lower than the absorption of calcium from calcium citrate in both the normal and achlorhydric subjects. However, when calcium carbonate was given as part of a breakfast, normal calcium absorption was seen in the achlorhydric patients. Bo-Linn et al. (1) found gastric acid secretion and gastric acidity did not play a role in absorption of dietary calcium from milk, calcium carbonate, or calcium citrate in normal patients. However, this study has been criticized (7), due to the lavage technique used to measure calcium absorption which could have dissolving some of the calcium carbonate. In addition, the pH of the food itself may have contributed to the solubilization of the calcium salt in both studies. Heaney et al. (3) have studied the effects of meal ingestion on calcium absorption from calcium carbonate, milk, and calcium citrate malate using rat and human studies. The results showed an enhancement of calcium absorption with a meal, and the authors proposed this effect was due to greater gastric acid secretion and slower stomach emptying, thus allowing better dispersal and dissolution of the calcium as well as permitting complexation of the relatively insoluble material with starch and protein molecules of the food (3).

As illustrated by these studies, there are many factors which contribute to calcium solubility. A basic understanding of the potential solubility problems associated with the calcium salts themselves will allow both the fortification of foods and the preparation of supplements using the most bioavailable forms. Therefore, this investigation was performed to provide a complete in vitro solubility profile of a variety of calcium salts following a simulated gastrointestinal pH treatment.

MATERIALS AND METHODS

Mineral stock solutions were prepared with the following salt sources and double distilled deionized water: calcium lactate (EM Science, Cherry Hill, NJ), calcium phosphate, monobasic (Sigma Chemical Co., St. Louis, MO), calcium citrate (EM Science), calcium gluconate (J. T. Baker, Inc., Phillipsburg, NJ), calcium carbonate (Fisher Scientific Co., Medford, MA), and calcium citrate malate (provided, compliments of the Proctor and Gamble Co., Cincinnati, OH).

At a calcium concentration of 10 mM, each calcium source underwent a sequential pH treatment to simulate gastrointestinal pH changes using a modification of the methods described previously by Lee and Clydesdale (4) and Platt and Clydesdale (6). The mixture was stirred at a continuous rate while the pH was adjusted to 2.0 by dropwise addition of 1.0 N HCl to simulate the gastric acid secretion of the stomach. The solution was left for 30 min while stirring was continued. To simulate the neutral environment of the intestine, the pH of the solution was then raised to 7.0 using 1.0 N NaOH left for 30 min. All solutions were analyzed (4.6) at pH 2.0 and at pH 7.0 for total calcium, total soluble calcium, and ionic calcium as described below.

Total calcium

Duplicate 10-ml aliquots of the sample to be analyzed were placed into two 100-ml digestion flasks with 20 ml of concen-
trated HCl and three boiling beads. The solution was digested for 30 min, cooled, and filtered through ashless filter paper (Whatman No. 40) into 100-ml volumetric flasks. In order to prevent any interference by phosphate ions, lanthanum chloride (0.5%) was added to all samples. The samples were then made up to 100 ml with double distilled deionized water, and the samples were analyzed using an atomic absorption spectrophotometer (Perkin Elmer Lambda 3, Wellesley, MA).

Total soluble calcium
Duplicate 40-ml aliquots of the sample to be analyzed were centrifuged at 3,335 x g for 20 min. The digestion procedure, as described for total calcium determination, was then performed on the supernatant and the calcium concentration determined by atomic absorption spectrophotometric analysis.

Ionic calcium
The ionic calcium concentration was determined using an Orion calcium electrode (Orion Research, Inc., Cambridge, MA). To each calcium sample to be analyzed, 2 ml of ionic strength adjust (Orion Research, Inc.) was added. The ionic calcium concentration was then read directly from the standardized meter, while stirring was maintained at a constant rate.

Soluble calcium complex
The soluble complex, referred to in these studies, was based on the following relationship:

\[(TSC) - (IC) = (SCC)\]

where: 
- TSC = total soluble calcium
- IC = ionic calcium
- SCC = soluble calcium complex

RESULTS AND DISCUSSION

In order for calcium to be absorbed at the brush border, it must be in a soluble form. Fig. 1 graphically illustrates the effects of pH treatment on the amount of soluble calcium present in the ionic form for each calcium salt. At pH 2.0 (gastric pH), approximately 80-90% of all calcium present was in the ionic form. However, as the pH was increased to 7.0, to simulate the neutral environment of the intestine, a unique solubility pattern for each calcium source was evident. Both calcium citrate and calcium citrate malate exhibited higher amounts of soluble calcium complex formation (Fig. 1). This high degree of soluble complexation may be the reason for enhanced absorption in humans of calcium citrate as compared to calcium carbonate (2) and calcium citrate malate was in the ionic form. However, as the pH was raised to 7.0, to simulate the neutral environment of the intestine, a unique solubility pattern for each calcium source was evident. Both calcium citrate and calcium citrate malate exhibited higher amounts of soluble calcium complex formation (Fig. 1). This high degree of soluble complexation may be the reason for enhanced absorption in humans of calcium citrate as compared to calcium carbonate (2) and calcium citrate malate (8). Calcium citrate malate has also been shown to be more bioavailable than calcium carbonate (5). In this study, the calcium phosphate and calcium gluconate salts showed a small decrease in the total amount of ionic calcium present (Fig. 1). However, all of the soluble calcium from calcium lactate and calcium carbonate was in the ionic form. In the case of the latter, the carbonate was probably liberated as carbon dioxide during the pH adjustment and thus may not participate in complex formation. It is important to note that the high level of ionic calcium present at pH 7.0 would be quite reactive with intestinal food components and insoluble complexes may be formed.

Figure 2 illustrates the effect of pH treatment on the total soluble calcium. At pH 2.0, there was little difference in soluble calcium levels among all the calcium sources tested. When the pH was raised to 7.0, almost all the calcium phosphate present precipitated. This was in contrast to the other tested calcium forms which showed little change in total solubility with the exception of calcium lactate in which approximately 20% of the total soluble calcium became insoluble.

This investigation, utilizing a sequential pH treatment designed to simulate gastrointestinal pH changes, compared the reactivity of calcium from a variety of different sources. The unique changes in solubility of each of these salts illustrated the importance of pH adjustment during in vitro testing of bioavailability. These studies extend our understanding of calcium solubility under conditions that may be encountered during ingestion and provide information which could be applied to the improvement of food fortification practices.

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EFFECT OF pH TREATMENT ON IONIC CALCIUM

Figure 1. Percentage of total soluble calcium in the ionic form from calcium carbonate, calcium citrate, calcium citrate malate (CCM), calcium lactate, calcium phosphate and calcium gluconate at a pH of 2.0 (gastric pH) and after adjustment to a pH of 7.0 (intestinal pH). Values shown represent the mean of duplicate samples. All standard deviations were within 5% of the mean. The final concentration of each source measured was 10 mM.

EFFECT OF pH TREATMENT ON TOTAL SOLUBLE CALCIUM

Figure 2. Percentage of total calcium in a soluble form, from calcium carbonate, calcium citrate, calcium citrate malate (CCM), calcium lactate, calcium phosphate and calcium gluconate at a pH of 2.0 (gastric pH) and after adjustment to a pH of 7.0 (intestinal pH). Values shown represent the mean of duplicate samples. All standard deviations were within 5% of the mean. The final concentration of each source measured was 10 mM.
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REFERENCES


