Effect of Caseinphosphopeptide on Absorbability of Co-ingested Calcium in Normal Postmenopausal Women

Robert P. Heaney¹, Yasuhiro Saito², Hajime Orimo³
¹Creighton University Omaha, Nebraska, USA
²Meiji Seika Kaisha, Ltd., Saitama, Japan
³University of Tokyo, Tokyo, Japan

Abstract:
Caseinphosphopeptide in a dose of 87.5 mg was administered to 35 normal postmenopausal women as a part of a standard test meal containing a calcium load of 250 mg. Absorption of calcium was tested both with and without caseinphosphopeptide, using an intrinsic ⁴Ca label in the calcium source. The mean quotient of absorption with/without caseinphosphopeptide was greater than 1.0, but nonsignificantly so. However, when analysis was confined to women with low absorption values, caseinphosphopeptide administration was associated with significantly better absorption of co-ingested calcium. Those findings suggest that caseinphosphopeptide supplementation is particularly useful for persons with low basal absorptive performance.

Key words: calcium, calcium absorption, caseinphosphopeptide, bioavailability

Introduction
Caseinphosphopeptide (CPP) has been reported to enhance calcium phosphate absorbability in rats (1-3), but a review by Kitts and Yuan (4) of studies involving CPP indicated that there was still uncertainty as to the effect of CPP on calcium bioavailability. An adequate calcium intake is essential for bone health as well as for protection from several other chronic diseases (5-8). Thus food factors that enhance calcium availability may help offset the health impact of the frequently low calcium intakes that are typical of the diets of industrialized nations. Because of differences in digestive physiology between rats and humans, it seemed desirable to test CPP in humans. This report describes the results of such a human test.

Methods
Investigational Design
The design was a randomized cross-over involving the addition of either CPP (CPP+) or nothing (CPP-) to a standard test meal. CPP was ingested at a dose of 87.5 mg. For 29 women the calcium source was calcium carbonate, and for six, it was calcium phosphate (CaHPO₄). The ingested calcium load for both sources was 250 mg. This regimen provided a CPP:Ca ratio of 0.35:1 (mg : mg). Minor amounts of calcium were contributed by other components of the test meal (including the CPP itself). The total calcium load, from all ingested sources, was 260 mg. Each subject was tested twice at an interval of three to four weeks.

Please address all reprint requests to: Y. Saito, Director of R & D Management Division Bio-Science Laboratories Meiji Seika Kaisha, Ltd. 5-3-1, Chiyoda, Sakado-shi, Saitama, 350-02, Japan
Doses of both the calcium source and CPP were delivered in individually weighed, tared, gelatin capsules (Lilly). Both were consumed midway through a light breakfast consisting of low-calcium white bread, toasted, with butter or margarine, and tea or coffee (with artificial sweetener, if desired). The time of ingestion was carefully noted. A single blood sample was taken at exactly 5 hours after ingestion of the labeled CaCO₃ or CaHPO₄. All tests were performed in the morning after an overnight fast of 10-12 hours’ duration.

Subjects
Thirty-five postmenopausal women were recruited through the efforts of a local survey research firm. All subjects gave informed consent, and the project was approved by the Creighton University Institutional Review Board. The mean age (±S.D.) of the subjects was 59.1 (±3.8). The subjects were screened to exclude major medical illnesses, as well as extremes of weight for height.

Caseinphosphopeptide used in this study was a phosphopeptide-enriched commercial product (Meiji Seika Kaisha, Tokyo). This product was made from the tryptic hydrolysate of whole bovine casein by purification of the phosphopeptide fraction using calcium chloride and ethanol (9) and spray dried. Phosphopeptide content of the test material was 86.6 w/w % (m.f.b.) by HPLC analysis (9).

Source Labeling
The labeled calcium sources were prepared in the laboratories of the Center for Hard Tissue Research, Creighton University. ⁴⁵CaCl₂, high specific activity was added to solutions of the reactants prior to precipitating the final products. Sufficient ⁴⁵Ca was added to produce a tracer level of 0.05±Ci/dose. CaHPO₄ was prepared using the method of Jensen and Rathlev (10). Calcium carbonate, was prepared by precipitating CaCl₂ with Na₂CO₃. The final precipitate was washed with 2 percent NH₄(OH) to remove traces of NaCl. Molar ratios and recoveries for both products were very close to theoretical values.

Analyses
Fractional absorption was measured from blood samples taken at five hours after oral ingestion using published algorithms (11, 12). Briefly, in women, absorption fraction is given by:

\[ F x Abs = 0.3537 \times (SA_5^{0.3187}) \times (Ht^{0.1847}) \times (Wt^{0.2721}) \]

in which \( FxAbs \) equals absorption fraction; \( SA_5 \) equals 5-hour serum calcium specific radioactivity (fraction of oral dose per gram calcium); \( Ht \) equals height (meters); and \( Wt \) equals weight (kilograms).

Because ⁴⁵Ca is an isotope with a relatively long half life (165 days), it is necessary to correct the serum radioactivity values obtained at later tests for residual radioactivity remaining from earlier tests. Values for such residual radioactivity in our own laboratory experience were pooled with the data of Table 28 from Marshall (13) for this purpose. At three weeks, residual radioactivity averages 4.68 percent of the counts present in the five-hour sample at the prior test, and at four weeks, 2.5 percent. For tests performed at intervals between these times, residual counts were estimated by logarithmic interpolation. Then residual net counts were subtracted from current net counts obtained at the second test before computing serum specific activity related to the second dose. The correction is small, but if it were not made, second tests would slightly over-estimate absorption.

⁴⁵Ca was analyzed by liquid scintillation counting on a Packard Model No 1900CA instrument (Packard Instrument Corporation, Meriden, CT). Stable calcium in serum and calcium sources was analyzed by atomic absorption spectrophotometry (model 2380, Perkin-Elmer, Norwalk, CT).

Statistical Analysis
The design yielded both a set of differences (between CPP+ and CPP−), and a set of quotients (in which one value is expressed as a fraction of the other). The means of these values were tested against zero difference (or a quotient of 1.0) by ordinary t-tests. Pooling of data for CaCO₃ and CaHPO₄ was accomplished by first normalizing the values for each source (mean 0, SD=1) and then working with these standardized values. Standard least squares regression analysis was performed on the relationship between variables. Standard power calculations revealed that, for a sample size of 35 (N for the aggregate dataset), \( (1-\beta) = 0.90 \) for a difference in absorption fraction of 0.06 or greater.

Results
Table 1 presents the results of two experiments in postmenopausal women. The mean difference between

<table>
<thead>
<tr>
<th>Ca Source</th>
<th>CPP</th>
<th>N</th>
<th>Mean Absorption (%)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>CaCO₃</td>
<td>−</td>
<td>29</td>
<td>38.7±12.2</td>
<td>NS</td>
</tr>
<tr>
<td>CaCO₃</td>
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<td>36.9±10.5</td>
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<tr>
<td>CaHPO₄</td>
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<td>6</td>
<td>28.6±9.0</td>
<td>NS</td>
</tr>
<tr>
<td>CaHPO₄</td>
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<td>6</td>
<td>33.0±12.4</td>
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