

In Vitro Gastrointestinal Calcium Release from an Organic Calcium–CPP Granule Matrix and Its Translational Relevance to Bioavailability

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ABSTRACT

Objective: This study evaluated an NVTIA organic calcium–casein phosphopeptide (CPP) composite tablet built from calcium citrate malate, calcium L-lactate, CPP, inulin, resistant dextrin and microencapsulated vitamin D3. **Method:** The optimized granule system achieved 84.7% calcium release after 30 min in gastric-phase medium and 61.5% soluble calcium retention after 60 min in intestinal-phase medium. Corresponding values were lower in a CPP/inulin-free comparator (76.2% and 50.8%), a wet-granulated vitamin D3 comparator (78.5% and 52.1%), and a direct-blending comparator (70.4% and 46.7%). **Results:** Human studies of calcium citrate malate and inulin-type fructans provide clinically relevant context for these physicochemical findings and a useful framework for future direct quantification of absorption with the complete formulation. **Novelty:** Taken together, the evidence supports a formulation-level strategy in which organic calcium dissolution, CPP-associated maintenance of calcium solubility, and fermentable-fiber effects are considered jointly, with single-ingredient studies serving as complementary mechanistic context.

INTRODUCTION

Calcium nutriture is frequently discussed in terms of total intake, yet the physiological value of a calcium-containing preparation also depends on the fraction that enters solution, remains chemically available across changing luminal conditions, and is ultimately accessible for intestinal transport. This distinction is especially relevant for solid dosage forms, in which tablet architecture, particle-level contact, disintegration and the chemical environment generated during dissolution can alter the amount of soluble calcium presented to the intestinal surface.

Calcium citrate malate (CCM) has been tested directly in human absorption studies. In a randomized crossover study of 25 healthy premenopausal women, a 500-mg calcium load supplied as CCM in fortified orange juice produced a 0–9 h serum-calcium response corresponding to an estimated absorbed amount of 148 ± 9.0 mg, compared with 100 ± 8.9 mg from a tricalcium phosphate/calcium lactate fortification system [1]. Other controlled data show that citrate- and malate-containing fortified matrices can both provide substantial fractional calcium absorption [2], while comparisons with other calcium salts demonstrate that matrix and feeding conditions materially influence apparent source differences [3]. These findings highlight the value of considering formulation matrix and absorption behavior alongside labeled elemental calcium content when evaluating delivery quality.

The present formulation combines CCM with calcium L-lactate, CPP, inulin and resistant dextrin in a wet-granulated matrix, while microencapsulated vitamin D3 is

protected from the wet process and introduced through a light-shielded stepwise premix. The scientific question addressed here is whether this granule-level organization produces a gastrointestinal release profile that is directionally consistent with improved calcium availability, and how far such physicochemical results can be interpreted in light of published human evidence on organic calcium and prebiotic fibers.

MATERIALS AND METHODS

Formulation design

The optimized tablet contained 50.000% calcium citrate malate, 15.000% calcium L-lactate, 2.500% CPP, 5.000% inulin, 2.000% resistant dextrin, 1.000% hydroxypropyl methylcellulose, 12.150% microcrystalline cellulose, 9.000% mannitol, 1.800% croscarmellose sodium, 0.200% microencapsulated vitamin D3 powder, 0.550% silicon dioxide and 0.800% magnesium stearate by final tablet mass. The microencapsulated vitamin D3 powder had a specified potency range of 90,000–110,000 IU/g. The study therefore reports formulation percentage and potency range; per-tablet IU exposure can be specified in dose-defined work once tablet mass and exact lot potency are established.

Granulation and final blending

Before granulation, calcium citrate malate, calcium L-lactate, CPP, inulin, resistant dextrin and hydroxypropyl methylcellulose were screened through a 60-mesh sieve. The screened powders were blended for 5 min, after which purified water was incorporated at 10% of the dry blend mass. The wet mass was passed through a 20-mesh screen. Drying was carried out at 48°C until the granules reached 2.2% moisture, and the dried material was then sized through a 24-mesh screen. Under light-shielded conditions, microencapsulated vitamin D3 was premixed by stepwise equal-increment dilution with an amount of microcrystalline cellulose corresponding to 10% of the total microcrystalline cellulose. For final blending, the granules and vitamin D3 premix were combined with the remaining microcrystalline cellulose, mannitol and croscarmellose sodium at 20 rpm for 10 min. Silicon dioxide and magnesium stearate were incorporated last, with mixing continued for a further 3 min before rotary tableting.

Gastrointestinal-phase evaluation

Tablets were first exposed to artificial gastric juice at pH 1.2 and 37°C for 30 min. Calcium released during this phase was determined by EDTA titration or atomic absorption spectroscopy and expressed as a percentage of total sample calcium. The gastric-treated material was subsequently transferred to artificial intestinal juice at pH 6.8 and 37°C for 60 min. Calcium remaining soluble in the filtrate was measured relative to total sample calcium. Testing covered four formulation conditions: the optimized granule formulation, a CPP/inulin-free comparator, a comparator in which vitamin D3 entered wet granulation, and a direct-blending comparator without prefabricated granules.

Translational evidence synthesis

A targeted search of PubMed-indexed human literature through August 2026 was used to identify controlled studies relevant to calcium citrate malate or related

citrate/malate systems, inulin-type fructans and CPP. Randomized crossover trials, randomized controlled trials and stable-isotope calcium-absorption studies were prioritized. Published human evidence was integrated to contextualize the translational relevance of the formulation findings and to complement future complete-formulation human evaluation. Given the available experimental record, formulation outcomes were summarized descriptively without reconstruction of effect sizes or P values.

Table 1. Composition of the optimized NVTIA composite calcium tablet (% of final tablet mass).

Component	wt%
Calcium citrate malate	50.000
Calcium L-lactate	15.000
Casein phosphopeptide (CPP)	2.500
Inulin	5.000
Resistant dextrin	2.000
Hydroxypropyl methylcellulose	1.000
Microcrystalline cellulose	12.150
Mannitol	9.000
Croscarmellose sodium	1.800
Microencapsulated vitamin D3 powder	0.200
Silicon dioxide	0.550
Magnesium stearate	0.800

RESULTS

The optimized system generated the highest gastric calcium release and intestinal soluble-calcium retention among the four evaluated formulation conditions. At 30 min in the gastric phase, calcium release was 84.7%, versus 76.2% for the CPP/inulin-free comparator, 78.5% for the wet-granulated vitamin D3 comparator, and 70.4% for direct blending. After transfer to the intestinal phase, soluble calcium retention at 60 min was 61.5%, compared with 50.8%, 52.1% and 46.7%, respectively. Relative to the CPP/inulin-free condition, the optimized matrix therefore showed absolute improvements of 8.5 percentage points in gastric release and 10.7 percentage points in intestinal soluble-calcium retention. These observed differences are presented descriptively; replicate-level variance data in future work would enable inferential statistical characterization.

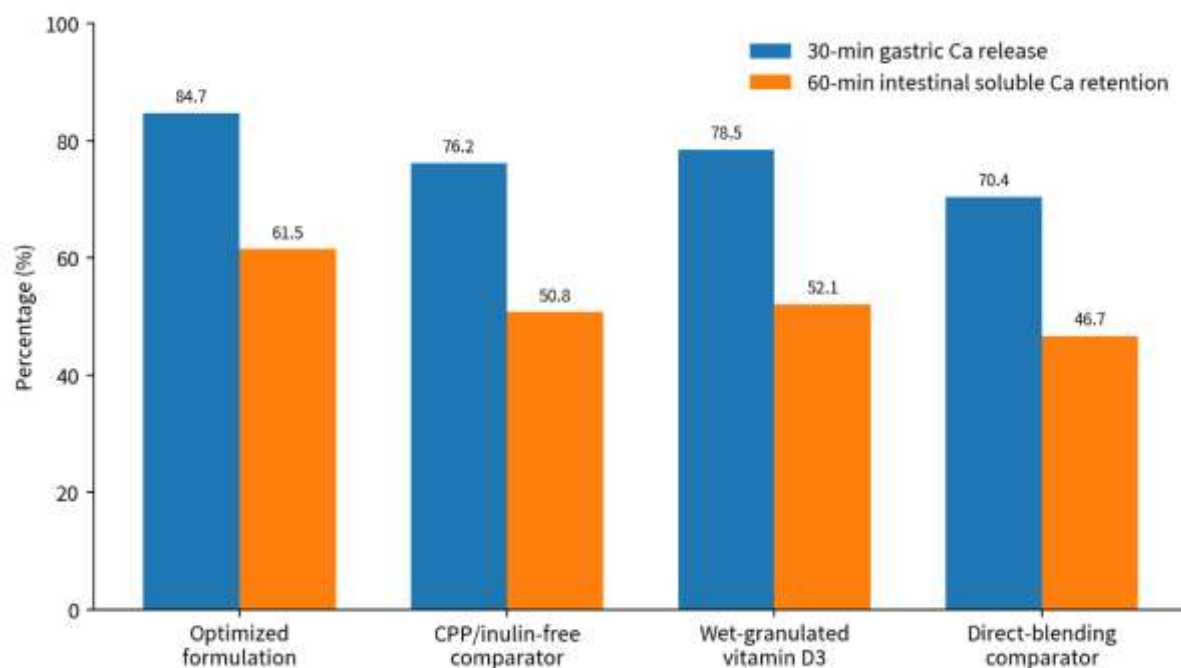


Figure 1. Descriptive gastrointestinal calcium performance across four formulation conditions. Observed values are presented descriptively; replicate-level variance estimates can support error bars and inferential analysis in future evaluation.

The optimized tablet had a hardness of 82 N, friability of 0.42%, and a disintegration time of 18.6 min. For the direct-blending comparator, the corresponding measurements were 69 N, 0.86%, and 27.8 min. The release comparison therefore reflects differences in dosage-form structure in addition to differences in the listed formulation.

The human literature is informative for individual formulation components, but the findings are mixed. Heaney and colleagues observed greater absorbed calcium from a beverage supplying 500-mg calcium as CCM than from a tricalcium phosphate/calcium lactate system [1]. Pak et al., however, reported similar fractional absorption (~40%) from citrate- and malate-enriched calcium-fortified orange juices [2]. Results for inulin-type fructans are also variable. In adolescent trials, 8 g/d mixed inulin-type fructans increased calcium absorption after 8 weeks and one year and was accompanied by greater one-year gains in whole-body bone mineral measures [4]. Among responders, kinetic analysis placed most of the fructan-associated increase in absorption within a period consistent with colonic uptake [5]. A separate trial using 9 g/d oligofructose-enriched inulin found no significant absorption or retention advantage in girls who already had high calcium absorption efficiency [6]. In a small crossover study of postmenopausal women, 1 g CPP did not acutely alter calcium-metabolism markers [7].

Table 2. Human studies relevant to calcium-source bioavailability and prebiotic-associated calcium absorption.

Study	Population/design	Intervention	Key result	Interpretive relevance
Heaney 2005 [1]	25 healthy premenopausal women; randomized crossover	500 mg Ca as CCM vs tricalcium phosphate/calcium lactate	CCM: estimated absorbed Ca 148 ± 9.0 mg vs 100 ± 8.9 mg; $P < 0.001$	Supports matrix-dependent CCM bioavailability
Pak 1994 [2]	16 healthy adults; crossover absorption study	300 mg Ca in citrate- vs malate-enriched fortified juice	Fractional absorption $40.1 \pm 8.3\%$ vs $40.6 \pm 8.6\%$; $P = 0.81$	Shows both citrate/malate systems can be bioavailable
Abrams 2005 [4]	Pubertal adolescents; randomized trial	8 g/d mixed inulin-type fructans vs maltodextrin	Absorption difference $+8.5 \pm 1.6\%$ at 8 wk and $+5.9 \pm 2.8\%$ at 1 y	Supports a human prebiotic-calcium interaction
Abrams 2007 [5]	13 young adults; kinetic analysis of 8 responders	8 g/d mixed inulin-type fructans	Responders: $22.7 \pm 11.3\%$ to $31.0 \pm 15.3\%$; most increment after 7 h	Suggests colonic contribution in responders
Martin 2010 [6]	14 girls; randomized double-blind crossover	9 g/d oligofructose-enriched inulin	No significant absorption or retention difference	Demonstrates response heterogeneity
Narva 2003 [7]	9 postmenopausal women; double-blind crossover	1 g CPP in milk matrices	No significant acute calcium-metabolism effect	Suggests context dependence in CPP-related clinical translation

Discussion

Soluble calcium after the transition to intestinal pH is useful for interpreting the release profile because gastric release does not show whether calcium remains in solution when the medium becomes weakly alkaline. In the optimized system, 61.5% of total sample calcium remained soluble at the end of the intestinal phase. This value describes what happened to calcium after the pH shift, rather than only how much was released under gastric conditions.

The optimized matrix brings CCM, calcium L-lactate and CPP together during wet granulation, so the observed retention is best interpreted as a matrix-level response rather than a response to calcium salt identity alone. One possible contribution of CPP is its

interaction with dissolved calcium through phosphoserine-rich regions, which could limit local precipitation as the medium shifts toward intestinal pH. This mechanism is consistent with the observed in vitro retention profile and provides a useful basis for considering the role of CPP within the complete matrix. In the crossover trial by Narva et al. [7], CPP produced no acute change in calcium-metabolism markers. Any contribution of CPP in vivo may therefore depend on the calcium source, dose, study population and the endpoint being measured.

Inulin needs to be considered separately from the events measured during gastric dissolution. Some human stable-isotope studies reported higher calcium absorption with inulin-type fructans [4], [5], [8]. In the kinetic analysis by Abrams et al., much of the additional absorption among responders occurred more than seven hours after dosing [5], which is consistent with a contribution from colonic uptake. The dosing formats differ between those studies and the present formulation: inulin represents 5% of tablet mass here, whereas the cited interventions used 8–9 g/d. The human literature therefore provides mechanistic and translational context for the formulation, while a dose-defined study of the complete tablet could clarify how formulation exposure relates to the reported absorption responses. Additional human evidence showed that oligofructose increased fractional calcium absorption in male adolescents [9], while oligofructose-enriched inulin has also been evaluated in postmenopausal women for effects on calcium and magnesium absorption and bone-turnover markers [10]. Mechanistic literature describes intestinal calcium uptake as the combined result of active transcellular and passive paracellular transport [11], with vitamin D serving as a major regulator of active intestinal calcium absorption [12]. Broader reviews further emphasize that calcium bioavailability depends on dose, chemical form, food or supplement matrix, and host factors [13]. Pharmacokinetic comparisons of calcium supplements and methodological analyses of bioavailability likewise show that formulation, meal conditions, and the measurement approach can materially influence apparent absorption differences [14], [15].

Human comparisons also show that calcium salt form should not be considered in isolation. Heaney et al. reported a marked difference between two fortification systems [1], whereas another study found comparable absorption of calcium carbonate and citrate when they were consumed with food [3]. Interpretation depends on more than the source alone: food or dosage-form matrix, timing, dose, baseline calcium status and host physiology can all affect the measured response. The present in vitro experiment contributes formulation-level evidence on the maintenance of calcium in soluble form through the intestinal phase. This favorable profile provides a practical basis for subsequent pharmacokinetic or stable-isotope evaluation of complete-formulation bioavailability.

Study Considerations and Future Perspectives

The experiments reported here were designed around physicochemical gastrointestinal behavior; published human studies were used to place those observations in translational context. A subsequent study could compare the complete

tablet with a composition-matched direct-blend control in a stable-isotope crossover design, with serial calcium, parathyroid hormone and urinary-calcium measurements collected alongside the absorption endpoint. This would test whether the observed 10.7-percentage-point advantage in intestinal soluble-calcium retention is accompanied by a measurable increment in human absorption. Defining the tablet dose would also permit the contributions of inulin and CPP to be examined apart from effects associated with granule architecture.

CONCLUSION

Fundamental Finding : Under the in vitro conditions used here, the prefabricated organic calcium–CPP granule formulation yielded 84.7% calcium release in the gastric phase and 61.5% soluble-calcium retention after the intestinal phase; both were the highest values among the four formulations tested. The current experiment showed better release and maintenance of soluble calcium with the NVTIA matrix under the test conditions. **Implication:** Together with the published human evidence, this formulation-level profile supports the translational relevance of the NVTIA matrix and provides a strong basis for complete-formulation bioavailability evaluation. **Limitation:** Under the in vitro conditions used here, the prefabricated organic calcium–CPP granule formulation yielded 84.7% calcium release in the gastric phase and 61.5% soluble-calcium retention after the intestinal phase; both were the highest values among the four formulations tested. **Future Research:** Human studies of CCM and inulin-type fructans show that calcium absorption can vary with source, matrix, dose and population, and the available CPP evidence also depends on study context.

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