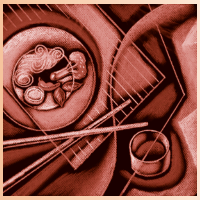

Not All Calcium-fortified Beverages Are Equal

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The physical state of calcium fortification of 14 calcium-fortified beverages available to consumers was evaluated and compared with unfortified, fat-free milk. Fortification was evaluated by extrinsic labeling of each beverage with a calcium isotope, followed by equilibration in the refrigerator for 17 hours, and then by centrifugation and separation of the calcium into solid and soluble moieties. Exchangeability of the 2 physical components was evaluated by measuring how well the isotope partitioned between the calcium in the 2 phases.The cow milk referent had 11% of its total calcium separable by centrifugation, but that calcium had achieved 91% of the tracer level predicted for its calcium content, indicating a high degree of exchangeability. All of the soy and rice beverages had most of their calcium in a separable, particulate form, in amounts ranging from 82% to 89% of the total calcium of the beverage. The orange juices had lesser amounts of their calcium separable by centrifugation (range: 8.1%-50.4%). Tracer equilibration of the particulate calcium ranged from a low of 17% of predicted to a high of 85% for the orange juices, and from 25% to 79% for the soy and rice beverages. Two of the orange juices had profiles comparable to cow milk, but most of the remainder fell between the extremes of cow milk and the milk substitute soy beverages. An earlier study had shown that poorly exchangeable, particulate calcium in a fortified beverage exhibited reduced bioavailability in human tests. Many of the beverages tested in this study exhibited similar physical characteristics, suggesting that their bioavailability would be compromised as well. We conclude that the quality of calcium fortification in currently available beverages is uneven at best, with the result that consumers are likely to be misled with respect to the calcium benefit the beverage is presumed to confer. The beverage industry should establish standards that would ensure a uniform, high quality of calcium fortification.

With the growing recognition that typical calcium intakes in North America fall far short of current recommendations, there has been a rapid increase in the numbers and varieties of calcium-fortified foods appearing on the shelves of US grocery stores. Some products have had the quality of their fortification tested by explicit measurement of calcium bioavailability in humans. Most, however, have little or no evidence as to how well the added calcium may be assimilable by the body. We have previously shown that one calcium-fortified soy beverage available in Midwestern markets delivered into the blood stream only 75% of the calcium predicted for its measured content.[1]

The purpose of the investigation reported here was to expand on these earlier observations, and to examine certain features of the many calcium-fortified beverages that have appeared in the market since our earlier study. In this report we confine our attention to orange juices and cow milk substitutes.

Orange juices and cow milk substitutes were tested

Methods

The various beverages tested are listed in Table 1, along with expiration dates, lot numbers tested, and the

Table 1. Products Tested

	Product ID	Product	Lot Numbers	Expiry Date	Fortification System
Cow milk					
	1	Roberts Fat-free Milk	25-162 09:00	28 Jul, 2003	NA
Soy and rice beverages					
	2	Rice Dream	L0312602 14:49; L0312602 11:37	6 May, 2004	Tricalcium phosphate
	3	Soy Dream	0308002 11:36; 0308002 15:19	21 Mar, 2004	Tricalcium phosphate
	4	Vita Soy	F1 02017 14:46; F1 02017 14:47	4 Mar, 2004	Tricalcium phosphate
	5	Silk	NA 17:05B; NA 17:06B	21 Mar, 2004	Calcium carbonate
Orange juices					
	6	Wells Blue Bunny	22 19113	19 Jul, 2004	Tricalcium phosphate; malic acid, citric acid
	7	Albertsons	P 5-2	19 Aug, 2003	Calcium phosphate, calcium lactate
	8	Florida's Natural	20:56 16 8	15 Aug, 2003	Tricalcium citrate
	9	Old Orchard	K 41	30 Apr, 2005	Calcium lactate, tricalcium phosphate
	10	Kroger	05P	10 Jun, 2003	Calcium phosphate, calcium lactate
	11	Shurfine	3 P 22 D 13	NA	Tricalcium phosphate calcium lactate, calcium hydroxide, phosphoric acid
	12	Tropicana	48FM0429	10 Jul, 2003	CCM (calcium-citrate-malate)
	13	Tropicana with vitamin D	48JH1921	4 Aug, 2003	CCM (calcium hydroxide, malic acid, citric acid)
	14	Minute Maid	AD41758	Feb 14, 2004	Tricalcium phosphate calcium lactate
	15	Minute Maid with vitamin D	PWE 10:03 CT349LM	14 Jul, 2003	Tricalcium phosphate calcium lactate

fortification system used for each. All were tested prior to labeled expiration or "best if sold before" dates.

For each beverage, 5 μCi ^{45}Ca (Amersham, Arlington Heights, Ill) was added to a 100 g aliquot. The mixture was capped, agitated, and stored overnight in a refrigerator, as described elsewhere.[2,3] Next morning the sample was separated by centrifugation at 8,740 g for 20 minutes. Each component, as well as the unfractionated beverage, was analyzed for its total calcium and ^{45}Ca contents.

Each fraction was ashed at 600° C and the ash analyzed by atomic absorption spectrophotometry (AAnalyst 100, Perkin-Elmer, Norwalk, Conn) and by liquid scintillation spectrometry (Packard 1900TR, Perkin Elmer Life Sciences, Downers Grove, Ill), using suitable standards. Specific activity of the total beverage, as well as of its separable components, was computed as the quotient of radioactivity (in dpm) divided by calcium content. Two main variables were computed: (1) the proportion of the total calcium in the beverage that was separable by centrifugation; and (2) the proportion of the ^{45}Ca found in the precipitate, relative to what would be predicted from its carrier calcium content. The latter serves as a measure of the extent to which undissolved, suspended calcium had exchanged with calcium in solution. The presumption is that particles too large to exchange adequately would also be too large to be absorbed efficiently. This is a presumption that was validated in our prior study of calcium-fortified soy beverage.[1]

Finally, a beverage fortification score was confected in such a way as to produce values that rise as both dissolved calcium and the specific activity of precipitate calcium rise. Because the latter was judged more critical, the formula gives predominant weight to the extent of tracer equilibration, as follows:

$$\text{Beverage score} = \left[\begin{matrix}\textit{\% of Ca in}\\ \textit{supernate}\end{matrix}\right] + \left[\begin{matrix}\textit{\% of Ca in}\\ \textit{pellet}\end{matrix}\right] \times \sqrt{\textit{pellet tracer (fraction of predicted)}}$$

Results

Table 2 sets forth the partition data for both stable and tracer calcium for the 15 beverages analyzed. Measured total content per serving ranged from 97% to 139% of the labeled contents, and the proportion of that calcium which was separable by centrifugation spanned a full order of magnitude ranging from 8.1% to 88.8%. Tracer content in the calcium of the centrifuged pellet ranged from a low of 17% of that predicted (had full isotopic equilibration between the solid and soluble moieties occurred) to a high of 91%. The beverage fortification score ranged from a low of 57.5 to a high of 99.4, with the milk referent and several of the orange juices in the top tier (≥95%), and all of the soy beverages plus 2 or 3 of the orange juices, in the bottom tier.

In general, for the orange juices, there was a strong inverse relationship between the fraction of the beverage calcium separable by precipitation, and the degree of equilibration of the suspended particulate calcium (Figure 1). This was not the case for the cow milk substitutes.

The best of the orange juices (the 2 Tropicana products) used the Procter & Gamble–patented CCM process,[4] while the poorest of the orange juices used calcium citrate. Other than the contrast between these 2 extremes, which is largely as might have been expected from the relative solubilities of the respective fortification systems, it is not possible to draw any general conclusions from these data about the calcium salt or system used for fortification. For example, beverages 2, 3, and 4 each used tricalcium phosphate, and while all had more than 85% of their calcium in a form separable by centrifugation, the degree of exchangeability of the solid phase ranged from a low of 25% for one of them, to a high of nearly 79% for another.

Discussion

While simple physical separation by centrifugation is at best a very crude test, it sufficed in this case to show that some portion of the calcium in these several fortified products was in a form that may not exhibit the same absorbability characteristics as the calcium in solution (and may not even be ingested). In fact, the physical state of the fortificant in some of the soy beverages was so poor that, in initial analyses, the total calcium content of the beverage seemed to fall far short of the labeled content. On inspection of the container, a dense sludge was found in the bottom of the cartons, which required vigorous, prolonged agitation to suspend. While the cow milk substitutes carried a "shake well before opening" instruction, in our hands casual shaking would not have sufficed to suspend most of the sediment.

By contrast, the orange juices, as a group, were much more uniformly suspended. Even so, as the Table shows, substantial quantities of calcium were separable by centrifugation from several of them (particularly Product 8). Additionally, the more the calcium in the pellet, the less good the equilibration between the pellet calcium and the supernatant liquid (see Figure 1). This probably is a function of particle size, as large particles would be more likely to settle out of suspension, and, because exchange is a surface phenomenon, large particles are less likely to equilibrate with solution-phase calcium.

The beverage scores calculated for these products are presented very tentatively. In our previous study of a soy beverage, in addition to using centrifugation and tracer equilibration, absorption itself was measured. That product would have produced a score of 57.4 had the current formula been applied, and its absorbability was shown to be ~25% below that of cow milk. With this sole exception, the beverage fortification scores have not been validated against absorbability. Nevertheless, they are not unreasonable on their face. A score of 100 arbitrarily designates a source that would be predicted to achieve the maximum absorbability possible for the calcium load size concerned. As developed, the formula gives full weight to

Table 2. Partition of Stable and Tracer Calcium in Milk and in Calcium-fortified Beverages

	Ca Per Serving, mg		Ca in Pellet		
Product	**Labeled**	**Measured**	**% of Total**	**Tracer Calcium as % of Predicted**	**Beverage Score***
Cow milk referent					
1	300	292	11.1	91.0	99.5
Soy and rice beverages					
2	300	382	88.8	78.9	90.1
3	300	367	85.3	25.2	57.5
4	300	345	88.2	44.5	70.6
5	300	385	82.0	34.3	66.0
Orange juices					
6	300	418	28.1	28.1	86.8
7	350	371	12.3	55.7	96.9
8	350	398	50.4	17.0	70.4
9	300	312	11.5	59.8	97.4
10	350	377	15.1	55.4	96.1
11	300	376	14.0	59.5	96.8
12	350	388	8.1	82.8	99.3
13	350	385	9.7	85.4	99.3
14	350	373	17.5	47.7	94.6
15	350	421	14.8	36.5	94.1

*See text.

calcium in solution, and discounts pellet calcium only to the extent that it equilibrates poorly. Thus, a source with a large fraction of its calcium separable by centrifugation could nevertheless be fully "redeemed" if its pellet calcium had the tracer content predicted for its calcium content, ie, if the pellet calcium and the supernatant calcium had the same specific activity. We propose the score mainly as an interim way to compare products and as a tool for fortified-beverage manufacturers to assess their formulations economically, without immediately incurring the expense of bioavailability tests in humans.

Suspended particulate calcium, while not perhaps exactly what the consumer might have expected, is nevertheless not, by itself, evidence of poor absorbability, at least so long as it is sufficiently suspended so as to be consumed. (It may, actually, differ little from taking a Tums-EX™ tablet at the time one drinks a glass of unfortified juice.) That is why our beverage score was constructed to allow exchangeability to redeem physical separation. But poor exchangeability of the particulate, despite 17 hours of incubation in mildly acidic orange juice (as in beverages 6 and 8), raises more serious concerns.

The projected shortfall in bioavailability does not, by itself, mean that the beverages concerned were nutritionally poor. Any extra calcium at all improves the nutritional value of the products concerned. But where the level of fortification, and in some cases the marketing, of these products explicitly positions them against cow milk, then the potential for misinformation arises.

These beverages do provide more calcium than do unfortified versions, but more work needs to be done to see what their actual absorption is

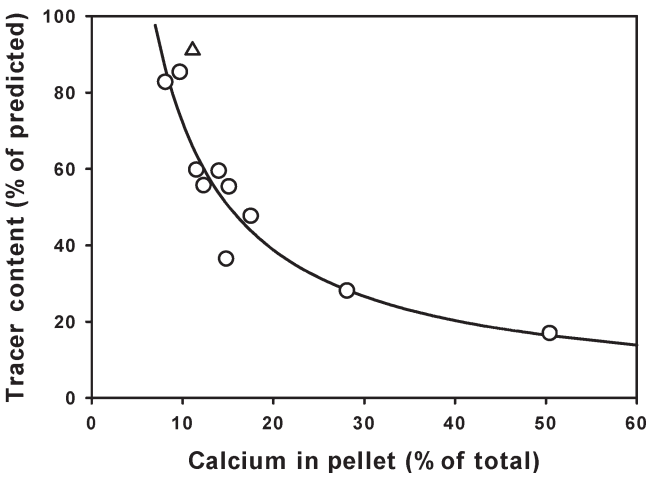

Figure 1. Plot of the inverse relationship between pellet tracer content and proportion of total beverage calcium found in the pellet, for the 10 orange juices. The regression line is a 2-parameter hyperbola plus a constant. The no-fat milk value is shown as a triangle (but its value was not included in calculating the regression line). (Copyright Robert P. Heaney, 2003. Used with permission.)

Consumers would naturally believe they were absorbing from the labeled serving content of calcium about what they would have absorbed from one serving of cow milk, when in fact something less would be the case. Only in the instance of the 2 Tropicana products tested could it be predicted that the calcium delivered would be fully comparable to that of cow milk. This is shown, for example, by the very similar beverage fortification profiles for cow milk and the 2 juices concerned.

The precise chemical or physical nature of the calcium in the pellets cannot be determined from these analyses. Given the complex nature of the matrices in which the fortificants have been inserted, one would predict that the calcium in the pellets would consist of some combination of 1) calcium complexed with fixed anionic groups that are a part of physically dense particulates in the native product, 2) solution calcium entrained in the precipitate, and 3) fortificant calcium particles. The fact that a large fraction of the calcium should be in the form of suspended particles in several of these beverages is hardly surprising, on solubility grounds alone. Considered only as an aqueous solution, a serving of orange juice would be expected to dissolve only ~43 mg calcium as the citrate salt, in contrast with the 398 mg actually found per serving in Product 8. With the exception of CCM (Products 12 and 13), similar solubility considerations apply for most of the other fortificants.

Because details of fortification methods constitute proprietary information, it is not possible to draw many conclusions about any one fortification *system* versus another. But as far as the beverages tested are concerned, the CCM-fortified products clearly exhibited the best physical features. That is probably because the entire fortificant is completely or nearly completely solubilized at some stage of the process. The other fortificants must be present to some extent (or perhaps mainly) as suspensions, as suggested by the separation produced either on the supermarket shelf or by centrifugation. As noted, beverages 2, 3, and 4 all used tricalcium phosphate as the fortificant, but obviously something more than the chemical identity of the salt was involved. Beverage 2 achieved nearly 80% of the theoretical maximum for its pellet tracer content, while beverage 2 achieved only 25%; yet both consisted of tricalcium phosphate.

What seems clear from this very simple study is that the state of calcium fortification in various beverages is at best quite uneven, and would likely result in less calcium delivery into the body than the calcium content of the beverage itself would suggest. This is particularly true for beverages in which the fortificant settled out into the bottom of the container on the supermarket shelf, altogether apart from whatever may have been the intrinsic absorbability of the sediment. But it is likely to be true, also, for those products with more stable suspensions which exhibited isotopic labeling that fell substantially short of predicted values.

In brief, more work needs to be done by food producers choosing to fortify their products so as to ensure realization of the ostensible benefit of the added calcium.

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Soy May Boost IVF Success

High doses of soy-derived estrogens can improve pregnancy rates in women undergoing in vitro fertilization and embryo transfer, Italian researchers have found.

Women who took 1500 milligrams of soy isoflavones per day, along with progesterone injections, had significantly greater rates of implantation, ongoing pregnancy, and delivery than women given progesterone injections with placebo, Dr Vittorio Unfer of the Obstetrics and Gynecology Center in Rome and colleagues report.

Progesterone injections after egg retrieval are standard practice, Unfer and his team write in the medical journal *Fertility and Sterility*, but the role of estrogen supplementation has been controversial.

They conducted the current study to determine if high doses of plant estrogens—previously shown by the researchers to have estrogen-like effects on the uterine lining of postmenopausal women—could help support implantation of the early embryo.

The researchers randomly assigned 213 women to take soy supplements or inactive placebo supplements after egg retrieval. The women continued to take the daily supplement until a pregnancy was confirmed or ruled out.

Among women given the supplements, 25% of the embryos transferred achieved implantation, compared to 20% among women given placebo.

Pregnancy occured in 39% of the women on the soy estrogens and 21% of those on placebo. The rate of successful delivery per pregnancy was 30% among women who took the active supplement compared with 16% for women given the placebo.

Source: *Fertil Steril*. 82(1)