Biofortified and bioavailable: The gold standard for plant-based diets

Article *in* Proceedings of the National Academy of Sciences · March 2008

DOI: 10.1073/pnas.0712330105 · Source: PubMed

Jeeyon Jeong
Amherst College

Biofortified and bioavailable: The gold standard for plant-based diets

Jeeyon Jeong and Mary Lou Guerinot*

Department of Biological Sciences, Dartmouth College, Hanover, NH 03755

Much of the world's population relies on a few staple foods (rice, maize, wheat, and cassava) that are poor sources of essential nutrients. Biofortification, the process of enriching the nutrient content of crops as they grow, provides a sustainable solution to malnutrition worldwide, because other methods, such as diversifying people's diets or providing dietary supplements, have proved impractical, especially in developing countries (1). One of the first biofortified crops was golden rice, which was engineered to produce beta-carotene or provitamin A in the edible portion of the grain (2). Since then, there have been similar successes with other crops, giving us a variety of carotenoid-enriched foods (1) as well as crops enriched with other micronutrients such as vitamin E (3) and folate (4). However, in each of these cases, assumptions about whether the nutrients are bioavailable—i.e., whether the nutrients can be readily absorbed by humans—remain untested. In a recent issue of PNAS, Morris *et al.* (5), using feeding studies with both mice and humans, report that carrots genetically engineered to accumulate twice as much calcium as control carrots are indeed a good source of this essential nutrient, resulting in a $\approx$50% increase in calcium absorption.

Calcium is a critical mineral nutrient for bone health, and it is the most abundant mineral in the human body. Because the skeleton functions as a calcium reserve, calcium deficiency results in low bone mass, which is a major cause of osteoporosis (6). Studies have shown that adequate intake of calcium reduces the risk of osteoporotic fractures, as well as other diseases (6). According to the Institute of Medicine, the recommended adequate intake for calcium is 1,000–1,300 mg/d for adults and 1,300 mg/d for children above 9 years old (7). However, a significant percentage of both children and adults consume less than the recommended amount of calcium (6). Dairy foods are good sources of calcium (8), but the amount of bioavailable calcium in fruits and vegetables is generally low (9). Although many vegetables contain high levels of calcium, plants also have oxalic acid and phytate, which inhibit calcium absorption (9).

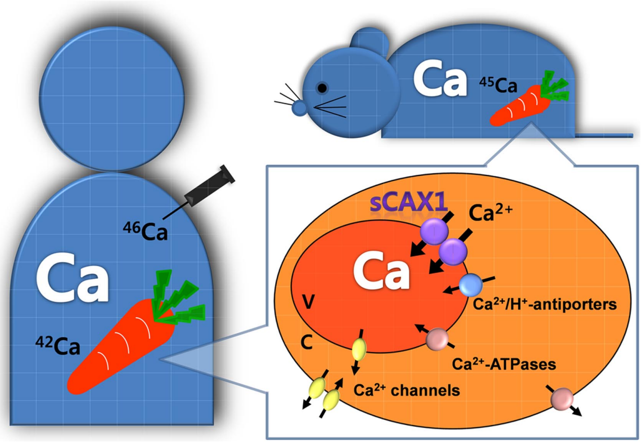

Fig. 1. Schematic diagram of *sCAX1*-expressing carrots showing enhanced calcium bioavailability in mice and humans. Stable isotopes, ^{42}Ca and ^{46}Ca, were used to trace calcium absorption in human subjects, whereas the radioactive isotope ^{45}Ca was used for mice. sCAX1 constitutively transports calcium into the vacuole, increasing the content of bioavailable calcium in the engineered carrots. Although not drawn in this figure, the ER is another important calcium store in the cell (23). C, cytoplasm; V, vacuole.

Calcium, like other mineral nutrients, is taken up by plants from the environment. Mineral enhancement strategies, therefore, focus on increasing uptake, transport, and/or accumulation in edible portions of the plant. The first successful biofortification effort for a mineral nutrient was iron-fortified rice; in this crop, the iron storage protein ferritin was engineered to be overexpressed in the grain (10). The strategy used by Morris *et al.* (5) also relies on increasing storage. In the case of calcium, the plant vacuole is an important storage location. The vacuolar calcium pool is maintained by Ca^{2+}-ATPases and Ca^{2+}/H$^+$ antiporters located on the vacuolar membrane (Fig. 1). The *Arabidopsis* CAX1 (cation exchanger 1) was the first Ca^{2+}/H$^+$ antiporter identified in plants, on the basis of its ability to complement a yeast mutant defective in vacuolar calcium transport (11). Expression of *CAX1* is highly induced in response to exogenous calcium (12). At the posttranslational level, the N-terminal region of CAX1 undergoes an intramolecular interaction and inactivates itself via a conformational change (13). *In planta*, the N terminus is not cleaved to activate CAX1, but when it is artificially truncated, a shorter version of CAX1 or sCAX1 is translated and results in constitutive CAX1 activity (13).

Previously, the Hirschi group (12) showed that tobacco plants expressing the *Arabidopsis* full-length *CAX1* contained significantly increased levels of calcium. In fact, these tobacco plants accumulated more calcium in their roots than they did in the shoots (12). This finding led to the idea that an engineered version of CAX1 could be used for biofortification, by increasing calcium levels of edible roots, such as carrots. It is generally more efficient to construct biofortified crops that accumulate a nutrient of interest in their roots, because the nutrient does not have to be translocated to the aerial parts. The attempt to increase calcium content in carrots was successful, with the sCAX1-expressing carrots accumulating 1.4- to 1.6-fold more calcium in the edible part compared with calcium levels in control plants, without perturbing growth, development, or fertility, under the laboratory conditions tested (14).

Feeding trials by Morris *et al.* (5) demonstrated that calcium absorption was significantly increased in both mice and humans with diets containing sCAX1-expressing carrots. The authors first validated that either radioactive or

---

Author contributions: J.J. and M.L.G. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 1431 in issue 5 of volume 105.

*To whom correspondence should be addressed. E-mail: guerinot@dartmouth.edu.

stable calcium isotopes could be used for the feeding studies (5). Using stable isotopes for human subjects is a safe method that meets ethical standards even for children, and it provides technical advantages, because long-distance collaborations combining field studies and feeding or clinical studies can be easily developed (15). Meanwhile, it is noteworthy that the authors conducted both extrinsic and intrinsic labeling, and they obtained consistent results. Because the bioavailability of calcium depends on the exchangeability of multiple calcium species in the food, extrinsic labeling, or adding the tracer as calcium salts, can be informative (15, 16). By using intrinsic labeling, the tracer is uniformly distributed among the calcium ions. Thus, this method of labeling is useful if the food contains antinutrients that inhibit the absorption of a nutrient (17), which is the case for calcium in vegetables containing oxalic acid.

It should be stressed that increased levels of nutrients are not necessarily correlated with enhanced bioavailability. In fact, the calcium absorption efficiency from *sCAX1*-expressing carrots was lower than that from control carrots, probably because not all of the extra calcium in the vacuole was bioavailable due to the antinutrients within the carrots (5). The sCAX1 carrot is still a better source of calcium, because total calcium absorbed from sCAX1 carrots was higher than that from the same amount of control carrots (5). However, this result might not be the case for other modified crops. Meanwhile, this finding also suggests that, in addition to increasing nutritional content, eliminating or reducing antinutrients that interfere with bioavailability could be another strategy for biofortification (1), as the example of Medicago *cod5* (calcium oxalate deficient 5) mutant demonstrates (18). *cod5* plants have significantly less oxalic acid but have similar levels of calcium compared with wild-type Medicago plants, and they showed increased calcium bioavailability in mice (18). Another strategy to improve the nutritional qualities of plants is to use promoters or enhancers for absorption, such as inulin for calcium or ascorbate for iron and zinc (1, 19).

Increased levels of nutrients are not necessarily correlated with enhanced bioavailability.

As the authors themselves point out (5), *CAX1*-expressing crops might have increased bioavailability of other metals, because calcium is not the only substrate of CAX1 (20, 21). Under the hydroponic growth conditions used for the authors' labeling studies, no increase in the content of other minerals was seen, with both control and sCAX1 carrots having values similar to the U.S. Department of Agriculture food composition guide. However, if plants were to be grown in soils contaminated with toxic heavy metals, such as cadmium, there is the potential for unwanted accumulation. By using site-directed mutagenesis, it was shown that substrate specificity of sCAX1 could be manipulated (22). This finding suggests that CAX1 can potentially be used to fortify other nutrients or engineered to exclude toxic metals. Meanwhile, because calcium acts as a signaling molecule involved in various metabolic processes (23), overexpressing *CAX1* or altering cellular calcium pools may produce undesirable metabolites.

Other concerns regarding sCAX1 carrots, or biofortified crops in general, include social and economic issues, such as cost-effectiveness and social acceptance. There is no question that the nutritional qualities of plants can be enhanced through conventional breeding by taking advantage of natural variation (1, 19). However, breeding alone is often not an adequate solution, because of the characteristics of the plant species itself or the nutrient of interest (1, 19). The best biofortifcation strategies will probably involve breeding where practical and genetic modification when necessary.

Despite the questions remaining, the work by Morris *et al.* (5) provides a well executed and timely example of what needs to be done for all biofortified crops. Seven years after the introduction of golden rice, scientists are still questioning its nutritional benefits, and to date, no feeding studies have been reported (24). It is no longer sufficient to simply show that a particular food has increased levels of a nutrient. We must ensure that such foods are "fed" into a pipeline to test bioavailability. Such studies are critical for public acceptance of biofortified foods.

1. Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) *Trends Plant Sci* 12:548–555.
2. Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) *Science* 287:303–305.
3. Van Eenennaam AL, Lincoln K, Durrett TP, Valentin HE, Shewmaker CK, Thorne GM, Jiang J, Baszis SR, Levering CK, Aasen ED, *et al.* (2003) *Plant Cell* 15:3007–3019.
4. Bekaert S, Storozhenko S, Mehrshahi P, Bennett MJ, Lambert W, Gregory JF, III, Schubert K, Hugenholtz J, Van Der Straeten D, Hanson AD (2008) *Trends Plants Sci* 13:28–35.
5. Morris J, Hawthorne KM, Hotze T, Abrams SA, Hirschi KD (2008) *Proc Natl Acad Sci USA* 105:1431–1435.
6. Office of Dietary Supplements (2004) *Dietary Supplement Fact Sheet: Calcium* (Office of Dietary Supplements, National Institutes of Health Clinical Center, National Institutes of Health, Bethesda). Available at http://ods.od.nih.gov/factsheets/calcium.asp.
7. Yates AA, Schlicker SA, Suitor CW (1998) *J Am Diet Assoc* 98:699–706.
8. Cotton PA, Subar AF, Friday JE, Cook A (2004) *J Am Diet Assoc* 104:921–930.
9. Weaver CM, Proulx WR, Heaney R (1999) *Am J Clin Nutr* 70:543S–548S.
10. Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) *Nat Biotechnol* 17:282–286.
11. Hirschi KD, Zhen RG, Cunningham KW, Rea PA, Fink GR (1996) *Proc Natl Acad Sci USA* 93:8782–8786.
12. Hirschi KD (1999) *Plant Cell* 11:2113–2122.
13. Pittman JK, Shigaki T, Cheng NH, Hirschi KD (2002) *J Biol Chem* 277:26452–26459.
14. Park S, Kim C, Pike L, Smith R, Hirschi K (2004) *Mol Breeding* 14:275–282.
15. Abrams SA (2003) *Forum Nutr* 56:312–313.
16. Avalos Mishaan AM, Zavaleta N, Griffin IJ, Hilmers DC, Hawthorne KM, Abrams SA (2004) *J Pediatr* 145:26–31.
17. Heaney RP, Weaver CM, Recker RR (1988) *Am J Clin Nutr* 47:707–709.
18. Morris J, Nakata PA, McConn M, Brock A, Hirschi KD (2007) *Plant Mol Biol* 64:613–618.
19. White PJ, Broadley MR (2005) *Trends Plants Sci* 10:586–593.
20. Shigaki T, Pittman JK, Hirschi KD (2003) *J Biol Chem* 278:6610–6617.
21. Mei H, Zhao J, Pittman JK, Lachmansingh J, Park S, Hirschi KD (2007) *J Exp Bot* 58:3419–3427.
22. Shigaki T, Barkla BJ, Miranda-Vergara MC, Zhao J, Pantoja O, Hirschi KD (2005) *J Biol Chem* 280:30136–30142.
23. Pittman JK, Hirschi KD (2003) *Curr Opin Plant Biol* 6:257–262.
24. Krawinkel MB (2007) *Nat Biotechnol* 25:623 (lett), 624 (author reply).