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Biotechnologically-modified Carrots: Calcium Absorption Relative to Milk

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Abstract

Background: Biotechnology to increase the nutrient content of fruits and vegetables is an innovative strategy to address insufficient mineral intakes. A novel biotechnologically modified carrot which has higher levels of calcium than control carrots has been developed.

Objective: For dietary guidance, it is necessary to understand the relative servings of any specific product that would be needed to provide calcium compared to a standard source, such as milk.

Methods: In a crossover study we used stable isotopes to measure calcium absorption from milk in 30 young adults and compared it to calcium absorption from both biotechnologically modified (MOD) and control (CON) carrots.

Results: Using a total meal calcium of 300 mg of which 35-40 mg of the calcium is derived from the test product, fractional calcium absorption from milk was slightly higher than from the MOD carrot (50.1 ± 3.0% vs. 42.6 ± 2.8%, Mean ± SEM, $p<0.05$) but was similar to that from the CON carrot (50.1 ± 3.0% vs. 52.8 ± 3.3%; $p=0.7$).

Conclusions: Biotechnologically-modified carrots have calcium bioavailability levels only slightly below that of milk. Serving sizes of enhanced carrots remain too large to be considered full substitutions for usual sources such as milk, but can supplement these sources effectively. Further biotechnological enhancements of a range of vegetable sources may lead to substantial benefits in nutritional status for minerals such as calcium with significant population-deficient intakes.

Keywords: Calcium; Absorption; Bioavailability; Biotechnology; Stable isotope; Carrot

Abbreviations: MOD: Modified; CON: Control

Introduction

The biotechnological modification of plants to increase their nutritional benefits in the food supply is a rapidly expanding field of nutritional investigation (Freese and Schubert, 2004; DellaPenna, 2007; Sevenier et al., 2002; Mackey, 2002). The terms "biotechnology" and "genetically modified/enhanced" have been used to describe various strategies which implement some form of plant biochemistry modification. Modern biotechnology has been utilized in the United States food supply since the early 1990s (White and Broadley, 2005). Currently, the majority of manufactured foods marketed in the United States contain modified soybean or corn ingredients (Institute of Food

Technologists Expert Panel, 2000). Most crops are modified primarily for insect resistance or to improve tolerance to herbicides. However, increasingly, crops are being modified to enhance the nutritional profile of the plant in an effort to decrease nutritional deficiencies, promote health and well being, and to enhance taste. Modified plants have been analyzed for changes in plant metabolism and nutrient composition, however the functional outcomes related to their use has rarely been evaluated.

In the United States, milk and other dairy products account for the majority of dietary calcium intake (Huang, 1997). However, persistent inadequacies of calcium intake throughout the life cycle has led to the recognition that alternative approaches are required to provide adequate calcium to the population. These approaches include increasing the bioavailability of calcium (Miller et al., 2001; Wienk et al., 1999; Van Dokkum, 1992) so that more calcium is absorbed and is available for metabolic purposes.

It has recently been demonstrated that through biotechnological modification the amount of bioavailable calcium in plants such as potatoes, tomatoes, and carrots can be increased (Park et al., 2004; Park et al., 2005). Carrots are a commonly consumed vegetable in the United States; however, like many vegetables they are a poor source of dietary calcium. A single medium-sized carrot (about 60 g) can only provide about 5% of the calcium of a single 240 mL serving of milk (US Department of Agriculture, 2007).

We have recently developed a modified carrot with elevated levels of calcium. In a group of young adults we reported that the total amount of calcium absorbed from the carrot was significantly greater than from a control carrot (Morris et al., 2008). In brief, the calcium levels in the carrots were engineered through high-level expression of a deregulated *Arabidopsis* calcium transporter called Cation Exchanger 1.

However, in order to make specific nutritional recommendations, and to evaluate the level of progress made and future progress needed in this area, it was necessary to compare the total amount of absorbed calcium from such a product with an accepted dietary source of the nutrient. In this report, we describe such a comparison using additional data from the absorption study previously reported (Morris et al., 2008). In this case we evaluated the absorption of calcium from milk and compared it to the absorption of calcium from both modified and control carrots. We hypothesized that one modified carrot (about 60 g) would provide equivalent absorbed calcium as that of an ounce of milk.

Materials and Methods

Subject Enrollment

We enrolled healthy young adults, 21.0-29.9 years of age, whose body mass index (BMI) was < 95th percentile for age and gender. To qualify for the study, subjects had to have an average calcium intake of 600-1200 mg/d based on an assessment made of three nonconsecutive days of 24 hr dietary recalls. Subjects could not regularly be taking any prescription medication other than oral contraceptives. The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals approved this protocol and written informed consent was obtained from all subjects.

Calcium Absorption Study

Subjects arrived after an overnight fast to Texas Children's Hospital (National Institutes of Health, M01-RR00188, General Clinical Research Center) for two outpatient study visits that occurred two weeks apart. Subjects were randomized to receive either the genetically modified (MOD) carrot or the control (CON) carrot at the first visit, receiving the other type of carrot at the second visit. Details of the production and characterization of these carrots have been reported (Morris et al., 2008). Carrots were given with a test meal that consisted of either 65 g MOD carrot or 120 g CON carrot. These carrot weights were chosen so that each serving provided approximately 38 mg calcium, the amount present in 30 g milk. This determination of the relative total amount of calcium in the carrots was based on analysis of previous batches of carrots and we confirmed our test meals with individual analysis of each carrot used in the study. With this meal, we also gave 30 mL of low-fat milk. In addition, the test meal included 170 g of calcium-fortified orange juice (Minute Maid, Coca-Cola Co., Atlanta, GA) to insure the test meal provided approximately 300 mg calcium, such as would be found in a typical breakfast. Both types of carrots were intrinsically labeled with ^{42}Ca stable isotope and milk was labeled with ^{48}Ca stable isotope. Labeling of the milk was done via the addition of 1 mg ^{48}Ca as the chloride salt approximately 24 hr prior to its being fed. Extrinsic labeling has been shown to be accurate as a measure of milk absorption after allowing adequate time for tracer equilibration (Nickel et al., 1996).

After the test meal, subjects received 15 µg ^{46}Ca stable isotope intravenously and then completed a 24 hr urine collection. This urine collection was done at home using timed collection containers.

Diets on the study day were designed so that the subjects received approximately 900 mg calcium per day, with one-third of the total daily calcium being provided during the test meal. The remaining intake was divided between lunch and dinner, with one optional snack. Lunch and the snack were provided containing approximately 300 mg calcium total. For dinner, subjects were instructed by the study dietitian about consuming a meal with 300 mg calcium based on a preselected food list. Subjects reported back the following day as to what they consumed. This process was repeated at the second visit when the subject received the reciprocal carrot test meal. Subjects were contacted between the two visits for 24 hr dietary recalls on two nonconsecutive days to insure that their calcium intake was maintained relatively constant during the study.

Calculation of Mineral Absorption

Serum and urine samples were analyzed for stable isotope enrichment using inductively coupled plasma mass spectrometry to measure the ^{42}Ca and ^{48}Ca enrichment and thermal ionization mass spectrometry to measure the ^{46}Ca enrichment. Sample preparation and analysis were identical to those we have reported previously (Chen et al., 2003; Griffin et al., 2003; Abrams et al., 2007) and have been validated (Abrams et al., 1994). Calcium absorption was calculated as the relative recovery in the urine of the oral isotope divided by the recovery of the intravenous isotope during the 24 hr after isotope administration (from time of the first oral dose until 24 hr after the last oral dose). For the second visit, the proportional recovery of ^{46}Ca from the first visit was used as it was unlikely that a substantial change in distribution kinetics had occurred in otherwise healthy adults over a short time period.

Data Analysis and Interpretation

Comparison of calcium absorption was done between milk and each of the types of carrots by paired t-tests.

Results

We enrolled and completed studies in 15 males and 15 females (Table 1). Baseline dietary calcium intake determined from the 24 hr recall data was 865 ± 229 mg/d. Subjects consumed 796 ± 97 mg calcium on the first study day and 797 ± 103 mg calcium on the second study day, two weeks later ($p > 0.5$ between visits). Between the two study days, subjects consumed 1166 ± 461 mg calcium, maintaining calcium intakes within the inclusion criteria. Fractional absorption results for the test meal days are provided in Table 2.

Characteristics	Values
Male/Female	15/15
Age (yr)	24.9 ± 2.4
Weight (kg)	71.5 ± 13.1
Height (cm)	174.6 ± 10.2
BMI (kg/m^2)	23.4 ± 3.2
Ethnicity	22W/4H/3A/1ME*
Calcium intake (mg/d)	865 ± 229

All data are Mean ± SD
*W, White; H, Hispanic; A, Asian; ME, Multiethnic

Table 1: Characteristics of Subjects at Baseline.

We further calculated the number of servings of each type of carrot that would be needed to achieve the total amount of calcium absorbed as a typical 240 mL (8 ounce) glass of milk. This was done by calculating the total amount of calcium absorbed from 240 mL of milk and then determining the weight of carrots that needed to be consumed to provide the same amount of absorbed calcium. Typical weights of medium sized carrots (65 g) (US Department of Agriculture, 2007) were then used to indicate the number of carrots this would represent. These results are shown in Table 2 and indicate that about 10 MOD or 15 CON carrots would be needed to equal the absorbed calcium from 240 mL of milk.

Discussion

We evaluated a novel strategy to increase the bioavailability of calcium in the diet by using modified carrots to provide more calcium in each serving of the vegetable. We found that each of these carrots would provide a level of absorbed calcium just below that of about one ounce of milk. While this amount is inadequate to resolve calcium intake deficiencies in the population, it is an important step in enhancing the use of vegetables as sources of minerals deficient in the usual diet, such as calcium. Furthermore, such vegetables would reflect an intrinsic value-

	Milk	MOD carrot	CON carrot
Intake (mg calcium)	38	40	35
Serving volume	30 mL	65 g	120 g
Fractional absorption*	50.1 ± 3.0%	42.6 ± 2.8%	52.8 ± 3.3%
Total abs/100 g	64 mg	26 mg	15 mg
Relative servings**	240 mL	10 carrots (650 g)	15 carrots (1000 g)

All data are Mean ± SEM
MOD=biotechnologically modified; CON=control
*Milk vs MOD, $p < 0.05$, Milk vs CON, $p = 0.7$.
**This serving size of milk provides 300 mg of calcium.

Table 2: Fractional Absorption of Calcium for Milk, MOD carrot, and CON carrot.

added property to both producer and consumer.

The current "Fruits and Veggies Matter" campaign by the National Cancer Institute and Centers for Disease Control and Prevention is designed to promote increased fruit and vegetable consumption (Centers for Disease Control and Prevention, 2008). This campaign defines a standard serving as 1 cup of raw vegetables (e.g., two medium carrots or 12 baby carrots) and promotes consumption based on age, gender and physical activity. However, goal intakes are not widely achieved (Guenther et al., 2006). Therefore, strategies to promote modified vegetables should include focus on overall education to promote vegetable intake (Heaney and Barger-Lux, 1994; Fulgoni et al., 2007).

Heffernan and Hillers found that approximately 50% of consumers surveyed would purchase modified foods if fewer pesticides were used in their production compared to natural foods; however 80% reported the importance of considering the environmental impact of such agricultural advances (Heffernan and Hillers, 2002). When consumers were provided with information that the modified food had an enhanced nutritional profile when compared to the control food, acceptability of the modified product increased (Brown and Ping, 2003). Aside from nutritional benefits to increase calcium levels, the use of food biotechnology to increase the calcium content of vegetables could also improve plant productivity, maintain crop firmness during transport, and extend product shelf life (Raz and Fluhr, 1992; Dris and Niskanen, 1999), thus meeting consumer demands for environmental consideration with biotechnology.

The World Health Organization (WHO) has reported on the availability of modified vegetables with increased levels of beta-carotene, iron, protein, isoflavones and antioxidants (WHO, 2005). This emerging science may be a step towards diminishing nutritional deficiencies worldwide. Enhanced nutritional benefits of modified foods are likely to increase acceptability as personal risk perception declines. Increased calcium bioavailability could influence people to choose these plants over traditional plants in order to avoid potential calcium deficiency or osteoporosis.

Conclusions

Our results allow our earlier report about the relative bioavailability of modified and control carrots to be interpreted in terms of a usual calcium source, milk. We found that the modified carrot came close to the fractional absorption of milk. Further research is needed to enhance the calcium content or bioavailability of modified carrots to decrease the number of carrots needed to provide equivalent total calcium compared to a serving of milk.

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