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Vegetable Bitterness is Related to Calcium Content

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Abstract

In the U.S. and Europe, most people do not consume the recommended amounts of either calcium or vegetables. We investigated whether there might be a connection; specifically, whether the taste of calcium in vegetables contributes to their bitterness and thus acceptability. We found a strong correlation between the calcium content of 24 vegetables, based on USDA Nutrient Database values, and bitterness, based on the average ratings of 35 people ($r = 0.93$). Correlations between the content of other nutrients and bitterness were lower and most were not statistically significant. To assess whether it is feasible that humans can detect calcium in vegetables we tested two animal models known to display a calcium appetite. Previous work indicates that calcium solutions are preferentially ingested by PWK/PhJ mice relative to C57BL/6J mice, and by rats deprived of dietary calcium relative to replete controls. In choice tests between collard greens, a high-calcium vegetable, and cabbage, a low-calcium vegetable, the calcium-favoring animals had higher preferences for collard greens than did controls. These observations raise the possibility that the taste of calcium contributes to the bitterness and thus acceptability of vegetables.

Keywords

Taste; nutrition; sensory evaluation; calcium appetite

Most people consume fewer vegetables and less calcium than nutritionists would like (Guenther, Dodd, Reedy, & Krebs-Smith, 2006; Krebs-Smith & Kantor, 2001; Looker, 2006; NIH consensus statement, 1994). Could there be a connection? Are vegetables avoided because of the calcium they contain? Animal studies suggest calcium is detected by specific calcium gustatory receptors (Tordoff, 2001; Tordoff, 2008; Tordoff, Reed, & Shao, 2008; Tordoff, Shao et al., 2008) and, consistent with this, humans using semantic differential or similarity ratings describe the taste of calcium solutions as falling outside the sweet-salty-sour-bitter basic taste tetrahedron (Henning, 1916; Lim & Lawless, 2005; Schiffman & Erickson, 1971). Subjects asked to attribute taste components to calcium salts rate them as having minor metallic, umami, astringent, spicy and sweet components, a moderate sour component, and a predominant bitter component (Lawless, Rapacki, Horne, & Hayes, 2003; Tordoff, 1996; Yang & Lawless, 2005).

Bitterness is also a sensory characteristic of most vegetables. This has been of interest because of observations on the food preferences of “nontasters”, “tasters” and “supertasters”; that is, individuals who differ in sensitivity to the bitter compounds, phenylthiocarbamide (PTC) and/

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or 6-n-propylthiouricil [(PROP); reviews (Guo & Reed, 2001; Tepper, 2008)]. In general, supertasters rate some vegetables as more bitter and less acceptable than do nontasters [e.g., (Bell & Tepper, 2006; Dinehart, Hayes, Bartoshuk, Lanier, & Duffy, 2006; Drewnowski, Henderson, Hann, Berg, & Ruffin, 2000; Kaminski, Henderson, & Drewnowski, 2000; Turnbull & Matisoo-Smith, 2002; Yackinous & Guinard, 2002)]. The theoretical basis for this is that PROP, PTC, and glucosinolate compounds in vegetables all have a thiourea moiety, which is most likely responsible for their bitter taste. Strong support is derived from a recent study showing that individuals with different haplotypes of TAS2R38, the gene encoding the PTC taste receptor (Bufe et al., 2005; Kim et al., 2003), differ in ratings of vegetables containing glucosinolates but not vegetables without glucosinolates (Sandell & Breslin, 2006). Thus, glucosinolates are likely to be one source of the bitterness of some vegetables for some individuals. However, there are undoubtedly other compounds that contribute to their bitterness [see (Drewnowski & Gomez-Carneros, 2000) for a review].

The calcium content of vegetables varies widely, from less than 2.5 to 62.5 mmol Ca²⁺/kg [i.e., <10 – 250 mg Ca²⁺/100 g; (USDA Agricultural Research Service Nutrient Data Laboratory, 2000)]. In comparison, solutions of as little as 1 mmol/L calcium chloride and lactate or ~5 mmol/L calcium sulfate and bicarbonate are rated as objectionable or disliked (Tordoff, 1996; Zoeteman, de Grunt, Köster, Smit, & Punter, 1978). Of course, in vegetables some of the calcium may be inaccessible to taste receptors, and the other tastes present may partially mask the bitterness contributed by calcium due to mixture suppression (Lawless, Rapacki, Horne, Hayes, & Wang, 2003). Nevertheless, it seemed possible that high concentrations of calcium in some vegetables might influence their taste and, more generally, a vegetable's calcium content and bitterness would be related. To examine this, we compared psychophysical ratings of bitterness with USDA Nutrient Database (USDA Agricultural Research Service Nutrient Data Laboratory, 2000) values of the nutrient content of 25 raw vegetables. We then used two animal models to investigate whether a causative connection between calcium content and vegetable acceptability was feasible.

Comparison of vegetable bitterness and calcium content in humans

Method

Ratings of the bitterness of 25 vegetables were collected as part of another study (Sandell & Breslin, 2006). The vegetables were purchased from a local grocery store. In the sensory laboratory facilities at the Monell Chemical Senses Center, they were gently washed and then chopped, cubed, stripped, or grated following standard hygienic food protocol. None of the vegetables were cooked. Samples were stored in a refrigerator at 6°C in plastic bags (Hefty Onezip) with minimal air until they were served in 40-mL medicine cups between 0 – 70 h later. Roughly half the vegetables contained glucosinolates and half did not. Additional details, including pictures of the vegetables, the genus and species names of each vegetable, the portion of the plant ingested, and the size and form of the sample provided, are available as online supplemental data to the original publication (Sandell & Breslin, 2006).

Vegetable bitterness scores were based on the mean ratings of 35 people. The study was approved by the Institutional Review Board of the University of Pennsylvania and subjects provided their informed consent before tests began. The participants were aged 18 – 66 yr, 19 were male and 16 were female; 24 were Caucasian, 5 Asian, 5 African American, and 1 was a Pacific Islander. Fourteen had the PAV/PAV haplotype, 10 had the PAV/AVI haplotype, and 11 had the AVI/AVI haplotype at TAS2R38, which is the locus responsible for PTC “taster” status [see (Bufe et al., 2005; Kim et al., 2003; Sandell & Breslin, 2006)].

Prior to being tested, each subject was familiarized with the process of rating intensity using 0.05 mmol/L quinine hydrochloride to stimulate a weak sensation of bitterness and the general

Labeled Magnitude Scale (Delwiche, Buletic, & Breslin, 2001) to record it. On test days, subjects were asked to avoid eating, drinking and smoking for 1 h prior to arriving in our facility. Each subject evaluated all the samples in triplicate over three separate sessions. Sample presentation order was randomized between and within the subjects. Each sample was chewed 10 times at a rate of 85 chews per minute, prompted by a digital metronome. Then subjects rated the intensity of bitterness sensation on a Labeled Magnitude Scale. Subjects were asked to expectorate every sample after tasting. During the 60-sec break between each sample, they rinsed out their mouths four times with micro-filtered deionized water and “cleansed their palates” with Premium crackers (Saltine crackers original, Kraft Foods, NJ). Subjects were blind to the identity of the vegetable samples.

Most data concerning vegetable content of calcium and other nutrients were extracted from the USDA Nutrient Database (USDA Agricultural Research Service Nutrient Data Laboratory, 2000). Information was obtained for 100-g portions of the raw vegetables (generally in chopped form) except for Chinese broccoli, which was cooked. Some vegetables were listed in the USDA Database under different common names than those we used but these were matched by referring to their genus and species names (e.g., daikon = Oriental radish, bok choy = pak choi). Some information available in the USDA Database was not included in analyses because there were missing data for some vegetables or all the values were zero. This included lipids of various chain-lengths, alcohol, caffeine, various types of simple sugars, and some vitamins. The USDA Nutrient Database did not contain information for the fruit of bitter melon (aka balsam pear) so information about the calcium content of this vegetable was obtained from the National Bitter Melon Council website (<http://bittermelon.org>).

Pearson correlation coefficients were used to assess the strength of association between mean ratings of vegetable bitterness and nutrient content. There were 47 nutrients, so to provide some protection against Type II errors we used a criterion for significance for all correlations of $p < 0.01$.

Results

There was a strong positive correlation ($r = 0.93$, $p < 0.000001$) between vegetable bitterness ratings and calcium content of 24 vegetables (Fig. 1). One vegetable, radicchio, was clearly an outlier and not included in this correlation; with radicchio included, the correlation was lower but still highly significant ($r = 0.82$, $p < 0.000001$, $n = 25$). Bitter melon, as the name suggests, was extremely bitter, and the value for calcium content of this vegetable was from a different source than those of the other vegetables tested (see methods). Without this vegetable included, the correlation between bitterness and calcium content was lower but still highly significant ($r = 0.85$, $p < 0.000001$, $n = 23$).

Although *TAS2R38* haplotype influenced the bitterness ratings of several vegetables [see (Sandell & Breslin, 2006)] the differences were small relative to the differences in bitterness ratings between vegetables. Correlations for each haplotype group between calcium content and bitterness ratings of 24 vegetables (excluding radicchio) were: PAV/PAV, $r = 0.92$, $n = 14$; PAV/AVI, $r = 0.89$, $n = 10$; AVI/AVI, $r = 0.92$, $n = 11$.

The USDA nutrient database held complete or nearly complete information for 47 nutrients in 23 vegetables (omitting radicchio and bitter melon). In addition to calcium (see above), there were associations with bitterness at the $p < 0.01$ level of significance for calcium and four other nutrients (riboflavin, thiamin, vitamin E and vitamin K; Table 1). The correlations between bitterness and three of these nutrients appeared to depend largely on outlying values of a single vegetable, dandelion greens. Removing this vegetable from consideration reduced the strength of the correlations considerably (riboflavin, $r = 0.56$, thiamin, $r = 0.31$; vitamin E, $r = 0.42$; $n = 22$ in each case).

Vegetable choice by animals predisposed to consume calcium

The correlation between vegetable calcium content and bitterness does not necessarily imply the existence of a causative relationship. Unfortunately, it is difficult to design an experiment involving humans that would allow causative inferences to be made. There are no known human populations with altered calcium perception and the molecular biology of calcium taste is not sufficiently developed to distinguish individuals that are specifically sensitive or insensitive to calcium. Since the transduction mechanism is unclear, there are no pharmacological agents that can block or potentiate calcium taste [the calcimimetic drugs might be an interesting possibility (Nemeth, 2004)]. Manipulating the calcium content of vegetables by mixing them with exogenous calcium is unlikely to be useful because it is already known that calcium is bitter (Lawless, Rapacki, Horne, & Hayes, 2003; Tordoff, 1996; Yang & Lawless, 2005) and, in any case, this would not replicate normal calcium distribution and availability. One approach is to test rodents, with the expectation that these will inform about human calcium preferences. As is the case for humans, high concentrations of calcium are avoided by most strains of mice and rats (Tordoff, 1994, 2001; Tordoff, Bachmanov, & Reed, 2007). We reasoned that if calcium in vegetables is detectable then most mice and rats would choose low-calcium vegetables in preference to high-calcium vegetables. However, those with a predisposition to consume calcium would tend to select high-calcium vegetables in preference to low-calcium vegetables. To test this, we conducted two experiments. In the first, we compared mice of the C57BL/6J and PWK/PhJ strains. The PWK/PhJ strain has a genetic predisposition to consume calcium that is probably due to mutations in taste receptor genes [(Tordoff, 2008; Tordoff, Reed et al., 2008; Tordoff, Shao et al., 2008); discussed below] and the C57BL/6J strain is a convenient “control” strain that has low calcium preferences (Tordoff et al., 2007). In the second experiment, we compared nutritionally-replete Sprague Dawley rats with those fed a low-calcium diet for 3 wk. This dietary deprivation procedure is a standard method to induce a calcium appetite [e.g., (Coldwell & Tordoff, 1996a, 1996b; Inoue & Tordoff, 1998; McCaughey, Forestell, & Tordoff, 2005; McCaughey & Tordoff, 2000, 2001; Tordoff, 2002; Tordoff & Rabusa, 1998)]. In both experiments, we measured the intakes of subjects given a choice between a vegetable with low-calcium content (cabbage, ~0.40 mg Ca²⁺/g) and one with a high-calcium content (collard greens, called “collards” for convenience, ~1.45 mg Ca²⁺/g). We chose to use collards because this was the vegetable with the highest calcium content that was easily available in a local supermarket. Cabbage was chosen as a low-calcium vegetable because it was at least superficially similar in appearance to collards. Details of the nutrient compositions of these vegetables are available from the USDA Nutrient Database (USDA Agricultural Research Service Nutrient Data Laboratory, 2000). According to this source, the two vegetables are similar in water content (92 vs. 91 g/100 g) and carbohydrate content (5.8 vs. 5.7 g/100 g), but collards have higher concentrations of protein (1.3 vs. 2.5 g/100 g), lipid (0.1 vs. 0.4 g/100 g), ash (0.6 vs. 0.9 g/100 g), fiber (2.5 vs. 3.6 g/100 g), and energy (25 vs. 30 kcal/100 g), as well as calcium (40 mg/100 g vs. 145 mg/100 g).

Method

All procedures involving animals were approved by the Animal Care and Use Committee of the Monell Chemical Senses Center. Two experiments were conducted. In *Experiment 1*, the subjects were 11 male C57BL/6J and 10 male PWK/PhJ mice that were bred in our animal facility from stock obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were housed individually in plastic “tub” cages with pine shavings on the floor and with access to pelleted AIN-76A diet and deionized water [see (Tordoff & Bachmanov, 2001) for details]. The mice had never been given vegetables previously. However, they had been used in two-bottle choice tests involving ethanol and corn oil. At the time of testing, they were ~14 wk old. As is typical of these strains (Reed, Bachmanov, & Tordoff, 2007), the C57BL/6J mice weighed

considerably more than did the PWK/PhJ mice when tests began [means $\pm$ SEs; C57BL/6J = 19.4 ± 0.3 g, PWK/PhJ = 13.2 ± 0.2 g, $t(18) = 14.9$, $p < 0.0001$].

Starting in the middle of the light period, all mice were transferred to fresh cages with corrugated cardboard (rather than pine shavings) on the floor. This was done to make subsequent measurement of spillage easier. Pre-weighed (± 0.1 g) amounts of approximately 12 g of cabbage and 6 g of collards were provided in addition to the AIN-76A maintenance diet. These amounts were chosen based on pilot work that showed they were more than mice consume in 24 h. Similar quantities of the vegetables were left in three empty cages to act as controls for evaporation. Every 24 h over 4 days, the remaining vegetables were collected, weighed and replaced with fresh samples. To control for place preferences, the positions where the cabbage and collards were placed in the cage were switched daily, although this was probably unnecessary because most mice spread pieces of each vegetable over the cage floor.

Intakes of diet and each vegetable during each of the four 24-h periods were calculated. Preliminary analyses showed there were no differences in the pattern of results for individual days so average intakes over the 4-day test were expressed as grams per day and used in subsequent analyses. Evaporation caused a substantial loss in vegetable weight (an average of 4.1 g/d for 11.9 g cabbage, and 2.9 g/d for 6.2 g collards) and so to account for this intakes were adjusted by subtraction. Vegetable intakes of the two strains were compared using a two-way, mixed-design ANOVA with factors of strain (C57BL/6J and PWK/PhJ) and vegetable type (cabbage and collards). Individual pairs of means were then compared using Fisher's post hoc tests. Preference scores for collards were calculated based on the ratio of adjusted intake of collards to adjusted intakes of collards plus cabbage, and were expressed as a percentage. Preference scores and maintenance diet intakes of the two strains were compared using *t*-tests.

In *Experiment 2*, the subjects were 24 naïve male Sprague Dawley rats [CrI:CD(SD)], purchased from Charles River Laboratories (Kingston, NY). The rats were 23 – 24 days old when they arrived at our institution. They were individually housed in stainless steel hanging cages and initially all had access to powdered AIN-76A diet and deionized water. After 3 days, 12 of the rats were switched to a diet containing 25 mmol Ca²⁺/kg (Ca-25) and the other 12 continued to be fed AIN-76A diet (129 mmol Ca²⁺/kg). A detailed description of the diets, housing conditions, and other procedures is given elsewhere [e.g., (Tordoff & Rabusa, 1998)]. Maintenance on the Ca-25 diet for 3 – 4 wk effectively induces a calcium appetite and reduces plasma calcium concentrations while only minimally compromising growth rates [e.g., (Coldwell & Tordoff, 1996a, 1996b; McCaughey & Tordoff, 2001; Tordoff, 2002; Tordoff & Rabusa, 1998)]. After 21 days, there was a small but significant difference in body weights of the two groups [means $\pm$ SEs; group fed AIN-76A = 290 ± 5 g, group fed Ca-25 = 265 ± 6 g, $t(22) = 3.19$, $p = 0.0043$].

At the beginning of the dark period of the light/dark cycle, all rats were given ~70 g cabbage and ~30 g collards (weighed to the nearest 0.1 g) for the first time in their lives. One hour later, under dim red illumination, remaining quantities of each vegetable were weighed and additional quantities of both vegetables were provided. Samples of both vegetables were also placed in empty cages to act as controls for evaporation. At 24 h after the start of the test, remaining vegetables were removed from each rat's cage and weighed. Intake of maintenance diet, which was available throughout the test, was also weighed. Intakes were adjusted for evaporation and then analyzed using the same procedures as for the mice, with separate analyses conducted for the 1-h and 24-h measurement periods.

Results

Vegetable choice in mice genetically predisposed to consume calcium—

Commensurate with their smaller size, the PWK/PhJ mice ate less vegetables than did the

C57BL/6J mice but a greater proportion of what they ate was high-calcium collards than low-calcium cabbage (Fig. 2). There was a strain x vegetable consumption interaction, $F(1,19) = 11.3$, $p = 0.003$: The PWK/PhJ mice ate less cabbage than did the C57BL/6J mice ($p < 0.0001$) but both strains ate similar amounts of collards ($p = 0.99$). The C57BL/6J mice ate significantly less collards than cabbage ($p < 0.0001$) but the PWK/PhJ mice ate similar amounts of each vegetable ($p = 0.10$). The PWK/PhJ mice ate less of both vegetables than did the C57BL/6J mice, $F(1,19) = 10.9$, $p = 0.004$, and both strains ate less collards than cabbage, $F(1,19) = 34.9$, $p < 0.0001$. Collard preference scores were significantly higher in PWK/PhJ than C57BL/6J mice, $t(19) = 3.16$, $p = 0.005$.

Vegetable choice after calcium deficiency—Rats fed low-calcium diet had greater initial intakes of collards and higher initial collard preference scores than did replete controls (Fig. 3). During the first hour of access to the choice between cabbage and collards, rats fed Ca-25 diet ate significantly more collards ($p = 0.03$) and less cabbage ($p = 0.02$) than did rats fed AIN-76A diet [group x vegetable interaction, $F(1,22) = 4.60$, $p = 0.04$]. Both groups combined ate more cabbage than collards, $F(1,22) = 17.9$, $p = 0.0003$, and there was no difference between the groups in the total amount consumed, $F(1,22) = 1.71$, $p = 0.21$. Collard preference scores were significantly higher in rats fed Ca-25 diet than AIN-76A diet, $t(22) = 2.89$, $p = 0.0084$.

After 24 h access, the group x vegetable interaction influencing vegetable intake observed in the first hour had dissipated, $F(1,22) = 1.41$, $p = 0.25$, and there was no difference between the two groups in collard preference scores, $t(22) = 1.62$, $p = 0.12$. Both groups continued to eat significantly more cabbage than collards, $F(1,22) = 46.3$, $p < 0.00001$, and both groups had similar total intakes of vegetables, $F(1,22) = 0.05$, $p = 0.82$ (Fig. 3). There was also no difference in intake of maintenance diet during the test [means $\pm$ SEs; AIN-76A fed group = 15 ± 3 g, Ca-25 fed group = 14 ± 3 g].

Discussion

In a choice between high-calcium containing collards and low-calcium containing cabbage, mice and rats with a specific appetite for calcium ate relatively more of the collards than did “normal” mice and nutritionally replete rats. These findings suggest that rodents can detect the calcium in vegetables and adjust their behavior accordingly. Coupled with our finding of a strong association between calcium content and bitterness of 24 vegetables, the results raise the possibility that humans can also detect the calcium in vegetables and that calcium thus contributes to their bitterness and consequently their acceptability.

The association between vegetable calcium content and bitterness observed in our study with humans is consistent with the possibility that the calcium content of a vegetable is responsible for its bitter taste; however, it also may be that calcium is simply a marker that co-varies with other bitter compounds. There was some support for this latter possibility because bitterness was weakly but positively associated with most of the nutrients in vegetables (Table 1) and four vitamins had significant associations (riboflavin, thiamin, vitamin E and vitamin K). On the other hand, these associations were less strong than that between bitterness and calcium content and, for all but vitamin K, they depended largely on the outlying nutrient content of a single vegetable. Moreover, it is unclear whether riboflavin, thiamin, vitamin E or vitamin K are bitter at the concentrations found in plants. We cannot find reports of the taste properties of these vitamins. However, in one study, there were no differences in ratings of liking between unfortified tortillas and tortillas fortified with a mixture of thiamin, riboflavin, zinc and iron [all in concentrations similar to those found in vegetables; (Rosado, Cassis, Solano, & Duarte-Vazquez, 2005) see also (Walker, Walker, & Metz, 1983)]. Thus, we cannot rule out contributions of other nutrients to the bitterness of vegetables but if they are present, we suspect

they are unlikely to be substantial. Of course, bitter taste is undoubtedly imparted by some of the many hundreds of non-nutrient compounds in vegetables [review (Drewnowski & Gomez-Carneros, 2000)], and it may be that some of these happen to co-vary with calcium content.

The strength of the association between vegetable calcium content and bitterness ($r = 0.93$) was particularly remarkable given that the estimates of calcium content we used were based on normative data rather than the specific batches of vegetables we tested. Season, geographical conditions, weather, and the availability of calcium in the soil can influence vegetable calcium uptake and storage, and this could lead to discrepancies between the normative data in the USDA Nutrient Database and actual calcium content. The bioavailability of calcium may be another factor. Oxalic and phytic acids in vegetables reduce calcium availability [review (Weaver & Heaney, 2006)] but it is unknown whether these also prevent the interaction of calcium with its taste receptor mechanisms. Such discrepancies undoubtedly exist but must be small in relation to the range of calcium contents among vegetables.

One vegetable we tested, radicchio, clearly diverged from the linear regression between vegetable calcium content and bitterness (Fig. 1). It was rated as being much more bitter than its calcium content would suggest. It may be that radicchio has particularly high levels of other bitter compounds contributing to its taste. Alternatively, the calcium content of our sample of radicchio may have differed from those used for the USDA Nutrient Database. There are at least four varieties of radicchio (Royal Rose, 2008) but only one entry in the Database. There is also the possibility of an analytical error because the Database value is derived from only two specimens. Finally, vegetable names often differ by locality so the vegetable sold to us as radicchio may not be the same as the one in the Database.

Much of the interest in the bitter taste of vegetables has derived from the observation that individuals differing in sensitivity to the bitter taste compounds PTC and PROP also differ in their ratings of vegetable acceptability (Bell & Tepper, 2006; Dinehart et al., 2006; Drewnowski et al., 2000; Kaminski et al., 2000; Turnbull & Matisoo-Smith, 2002; Yackinous & Guinard, 2002). Indeed, the psychophysical data used here were collected as part of a study aimed along these lines [(Sandell & Breslin, 2006); see above]. It is therefore reasonable to wonder whether sensitivity to PTC or PROP might influence ratings of calcium taste. As far as we are aware, there has been no attempt to investigate whether “non-tasters”, “tasters”, and “supertasters” differ in ratings of calcium intensity, although the evidence points against it. In one study, bitterness ratings of PROP and MgSO_4 were unrelated (Delwiche et al., 2001); this may inform about calcium because calcium and magnesium salts have several similar taste characteristics (Lawless, Rapacki, Horne, & Hayes, 2003; Lim & Lawless, 2005). In another study, there was no relationship between the mean detection threshold for five calcium salts and for PROP (Tordoff, 1996). In the study from which the current bitterness data were obtained (Sandell & Breslin, 2006), the effects of *TAS2R38* haplotype on bitterness ratings were confined only to those vegetables containing glucosinolates, and these spanned the range of vegetable calcium content. Moreover, the influence of *TAS2R38* haplotype on bitterness ratings of even the glucosinolate vegetables was modest in comparison to the range from the least- to the most-bitter vegetable. We conclude that glucosinolates and calcium (or bitter compounds covarying with calcium) independently influence the bitterness of vegetables.

This study involved ratings of vegetable bitterness but it is evident from Figure 1 that this sensory attribute maps well with popularity and consumption choices. Vegetables that are consumed the most in the United States, such as potatoes, carrots, and cauliflower, are among the lowest in calcium content and bitterness. Conversely, vegetables that are relatively rare, such as bitter melon, dandelion leaves, and collard greens, were rated as the most bitter and had the highest calcium content.

We stress that the correlation between vegetable calcium content and bitterness in humans does not, by itself, imply a causative relationship is present. Our approach to investigate causation was to test rodents, with the expectation that these inform about human calcium preferences. We used two models of calcium appetite, one genetic and one physiological. The genetic model involved PWK/PhJ mice which, in a screen of 40 inbred strains, had the highest preferences for various concentrations of calcium chloride and calcium lactate solutions (Tordoff et al., 2007). Recent work suggests that the PWK/PhJ strain's avidity for calcium is due to mutations in two taste receptors, T1R3 (Tordoff, Reed et al., 2008; Tordoff, Shao et al., 2008) and CaSR (Tordoff, 2008; Tordoff, Reed et al., 2008). The PWK/PhJ strain's appetite for calcium generalizes to magnesium but not exemplars of the basic tastes (Tordoff, Shao et al., 2008). In particular, there is no difference between the PWK/PhJ and C57BL/6J strains in preferences for various concentrations of the bitter compounds, denatonium benzoate, quinine hydrochloride, or caffeine (Tordoff, Shao et al., 2008). This is important because a difference in response between the two strains cannot be attributed to differences in general sensitivity to bitter compounds but instead reflects differences in response specifically to calcium-magnesium taste. Our results suggest that neither the C57BL/6J strain nor the PWK/PhJ strain preferred collards to cabbage but the PWK/PhJ strain avoided the collards less. This is consistent with the possibility that vegetable consumption is influenced by several (or many) bitter compounds of which one is calcium, and that the two strains respond differently to the calcium content of vegetables.

The other rodent model we used involved dietary calcium deprivation of growing rats, which is a well-established method of inducing a calcium appetite [e.g., (Coldwell & Tordoff, 1996a, 1996b; McCaughey & Tordoff, 2001; Tordoff, 2002; Tordoff & Rabusa, 1998)]. The specificity of the appetite produced by this method has been examined in detail (Coldwell & Tordoff, 1996a, 1996b; McCaughey & Tordoff, 2002; Tordoff & Rabusa, 1998): Calcium-deprived rats recognize calcium solutions immediately upon receiving them (Coldwell & Tordoff, 1996b) and have altered gustatory sensitivity that is consistent with lower calcium detection thresholds and higher acceptance of high concentrations of calcium (Inoue & Tordoff, 1998; McCaughey & Tordoff, 2001). Unlike PWK/PhJ mice, their appetite can be distinguished from the appetite for magnesium (McCaughey & Tordoff, 2002). Calcium-deprived and replete rats have similar lick rates and drink similar volumes of the bitter compounds sucrose octaacetate and quinine hydrochloride in both brief access and long-term tests (Coldwell & Tordoff, 1996a, 1996b). We found here that when given a choice between cabbage and collards, calcium-deprived rats initially had stronger preferences for collards than did nutritionally replete controls but this difference dissipated within 24 h. This result is consistent with the possibility that the deprived animals initially sought calcium but after consuming enough to assuage their physiological demands, they reverted to the replete state. This appears to be a reasonable supposition because over 24 hr, the calcium-deprived group consumed 42 μg calcium (14 μg from collards, 14 μg from cabbage and 14 μg from food), and in previous work, we have demonstrated that ingesting as little as 12 μg calcium (as a 50 mM CaCl_2 solution in 10 min) is sufficient to normalize plasma calcium ionized concentrations of rats exposed to the same dietary deficiency (Tordoff, 1997). The finding that the replete and deprived groups differed in vegetable preference within the first hour of access is consistent with the contribution of an innate, taste-mediated mechanism [e.g., (Inoue & Tordoff, 1998; McCaughey et al., 2005; McCaughey & Tordoff, 2000, 2001)]. The alternative, that calcium-deprived rats learned to associate collard consumption with its physiological benefits (i.e., amelioration of their deficiency) appears unlikely because not much time was available in the 1-h test for learning to occur, and such influences would be expected to become more evident, not dissipate, during the longer, 24 h test.

There is work underway to genetically engineer high-calcium vegetables. The calcium content of wild-type potatoes and carrots can be increased ~2 – 3 fold by modifying a calcium

transporter gene that increases calcium sequestration into vacuoles [CAX1; (Morris, Hawthorne, Hotze, Abrams, & Hirschi, 2008; Park et al., 2005)]. The goal of this research is to increase human calcium intakes but the sensory characteristics of the engineered vegetables have not been examined (K. Hirschi, personal communication) and have been ignored in an assessment of the benefits of this work (Jeong & Guerinot, 2008). Given the very low levels of calcium in wild-type potatoes and carrots, we suspect that the increases in calcium content that have been achieved so far are too small to have a measurable effect on bitterness (or, for that matter, nutrition). Nevertheless, this may become a concern if calcium content is increased further. It seems perilous to engineer vegetables for their nutritional benefits without considering their sensory characteristics. Increasing vegetable consumption is beneficial for many reasons in addition to increasing calcium consumption, so one could argue (albeit perversely) that it would be more useful to pursue the opposite strategy and produce vegetables with reduced calcium content. If our speculations are correct, this would reduce bitter taste and thus increase vegetable acceptability.

In summary, we report that there is a strong relationship between a vegetable's calcium content and its bitterness, and that rodents differing in their appetite for calcium select between two vegetables differing in calcium content in a manner that suggests they can detect and respond appropriately to the calcium. We speculate, but do not prove, that humans can taste calcium in vegetables and they attribute bitterness to this sensation. Our results raise the possibility that this bitterness imparted by calcium is at least partially responsible for the relatively low acceptability of vegetables, particularly those with a high calcium content.

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Figure 1. Relationship between the calcium content and bitterness of 25 vegetables. Radicchio is not included in the regression.

Figure 2.

Mean ($\pm$ SE) daily consumption of cabbage and collard greens by C57BL/6J (B6; $n = 12$) and PWK/PhJ (PWK; $n = 11$) mice given a choice between the two vegetables. A = intakes; B = preferences. ^a $p < 0.05$ relative to cabbage intakes of the B6 group, according to Fisher post hoc tests. Intake of cabbage was significantly higher than intake of collards in the C57BL/6J but not PWK/PhJ group. ^b $p < 0.005$ relative to B6 group, according to t-test.

Figure 3.

Mean ($\pm$ SE) consumption during the first hour (top) and first 24 h (bottom) of access to a choice between cabbage and collard greens by rats fed either a nutritionally complete AIN-76A diet ($n = 12$) or a diet containing 25 mmol Ca^{2+}/kg (Ca-25; $n = 12$). Panels A and C show intakes, panels B and D show preference scores. ^a $p < 0.05$ relative to intakes of the same vegetable by the AIN-76A-fed group, according to Fisher post hoc tests. Intake of cabbage was significantly higher than intake of collards in both groups at both times. ^b $p < 0.005$ relative to B6 group, according to t-test. Intake of diet, which was also available during the test, did not differ between the groups [AIN-76A = 15 ± 3 g, Ca-25 = 14 ± 3 g].

Table 1

Associations between vegetable bitterness and nutrient content

Component	rComponent	r
<u>Major components</u>	tryptophan	0.40
water	−0.02 tyrosine	0.49
energy	−0.04 valine	0.41
protein	0.36 <u>Minerals</u>	
total lipid	0.45 calcium	0.85 ***
ash	0.34 copper	0.36
carbohydrate	0.11 iron	0.41
fiber	−0.02 magnesium	0.10
sugars, total	−0.49 manganese	0.24
<u>Amino Acids</u>	phosphorus	0.20
alanine	0.44 potassium	0.05
arginine	0.20 selenium	−0.01
aspartic acid	0.09 sodium	−0.08
cystine	−0.07 zinc	−0.03
glutamic acid	−0.13 <u>Vitamins</u>	
glycine	0.54 choline	0.31
histidine	0.21 folate	0.14
isoleucine	0.37 niacin	0.08
leucine	0.38 pantothenic acid	−0.12
lysine	0.36 riboflavin	0.74 **
methionine	0.13 thiamin	0.60 *
phenylalanine	0.32 vitamin A	0.35
proline	0.36 vitamin B6	0.24
serine	0.21 vitamin C	0.16
threonine	0.21 vitamin E	0.57 *
	vitamin K	0.70 **

*
p < 0.01**
p < 0.001***
p < 0.00001.

Based on 23 vegetables, except correlations involving amino acids, which were based on 16–20 vegetables due to missing information in the USDA nutrient database.