Supertasters - Updating the Taste Test For the A & P Laboratory

Karen A. McMahon

Department of Biological Science
The University of Tulsa
Tulsa OK 74104

karen-mcmahon@utulsa.edu

Abstract: PROP testing recognizes supertasters, medium tasters, and nontasters. Supertasters have higher densities of fungiform papillae and avoid eating bitter vegetables and fruits ((Bartoshuk *et al*. 1994, Delwiche *et al*., 2001, Drewnowski *et al*. 1997, Dinehart *et al*.2006). Students investigated if reaction to unsweetened grapefruit juice predicted PROP status and papillae density. Blue dye was swabbed on the tongue to count nonstaining fungiform papillae. Most supertasters (77%) disliked grapefruit juice, but only 40% of medium tasters, and 0% of nontasters did. Mean papillae densities were significantly different (ANOVA, $p<0.001$) at 65.35/cm ^{2} (supertasters), 42.55/cm ^{2} (medium tasters), and 33.78/cm ^{2} (nontasters).

Introduction

Exercises about the sense of taste are either omitted or out-of-date in many human anatomy and physiology laboratory manuals. The most common exercise for testing taste is a map of the tongue. This exercise is discounted by current researchers because it is based on the misinterpretation of data from a study in the 1880s (Smith and Margolskee 2001). Another common exercise uses PTC (phenylthiocarbamide) taste papers to identify genetic variations (tasters or non-tasters) in the ability to discriminate bitterness. Fox (1931) discovered that phenylthiocarbamide (PTC) was extremely bitter to certain individuals but completely tasteless to others. Additional studies identified individuals in all age, gender, and ethnic groups as either tasters (approximately 75%) or nontasters (approximately 25%). It was hypothesized that the ability to taste PTC was due to the presence of at least one dominant allele and the pattern of inheritance followed Mendelian genetics. Later researchers (Bartoshuk *et al*.1994) observed that not all tasters were alike. Some tasters reacted more strongly and characterized PTC as very bitter. It was hypothesized that the homozygous dominant TT genotype characterized supertasters and the heterozygous Tt correlated with medium tasters. Many researchers have now abandoned PTC in taste experiments because it emits a detectable sulfurous odor and there were concerns about its toxicity. PROP (6-*n*-propythiouracil) is chemically similar to PTC and is now the standard for in research on the discrimination of bitter taste.

In 2003, Kim *et al.* located and sequenced the *TAS2R38* or *PTC* gene on chromosome 7 responsible for the PTC reaction. This gene encodes for one of the estimated 25 bitter–taste receptor proteins present in taste buds. Three common SNPS (single nucleotide polymorphisms) based on three amino acid substitutions have been identified in the *TAS2R38* gene and account for five different haplotypes found in human populations. The two most common are PAV (proline-alanine-valine) identified as the major taster haplotype and AVI (alanine-valine-isoleucine) as the major nontaster haplotype. Individuals with two copies of the AVI haplotype are largely nontasters whereas either one or two copies of the PAV haplotype were mostly tasters. PAV homozygotes are more sensitive to PTC/PROP than PAV/AVI heterozygotes (Kim and Drayna 2004, Minella *et al.* 2005, Reed *et al*. 2006).

Miller and Reedy (1990) developed a method using methylene blue solution to stain the anterior tongue. Filiform papillae which do not contain taste buds stained a deep blue whereas taste-bud containing fungiform papillae stained lightly and could be counted against the dark blue background of filiform papillae. They discovered that there were variations in both the number of fungiform papillae and the number of taste buds on the papillae among test subjects, and suggested that these differences might account for the observed variations in taste sensitivity among individuals. Several studies (Bartoshuk *et al.*1994, Delwiche *et al.* 2001) confirmed that the perceived bitterness of PROP tended to increase with the density of fungiform papillae.

In 1991, the National Cancer Institute launched the 5-A -Day-Program to encourage people to eat 5-9 servings of fruits and vegetables daily to promote consumption of phytochemicals as a dietary strategy for disease prevention. For most, taste is the main determinant in food selection and perceived bitterness in a food is often the primary reason for its rejection. Many phytochemicals, such as the flavonoid naringin in grapefruit juice and glucosinolates in cruciferous vegetables (broccoli, cabbage, kale, etc.) are bitter-tasting. Several studies reported that supertasters showed a tendency to avoid certain foods that they perceive as very bitter (Drewnowski *et al*.1997, Dinehart *et al*. 2006). The consequences of diet choice to health may be significant. A study of men over 65 who had been identified as supertasters had a significantly higher number of colon polyps, a finding which is associated with a higher risk of colon cancer (Milius, 2003). The supertasters reported that they avoided strong vegetable tastes. The diet of a supertaster appeared to be deficient in both protective phytochemicals and fiber which led to the higher formation of polyps, raising the risk of colon cancer.

In this study, students investigated if their taste reaction to unsweetened grapefruit juice can predict PROP taster status and the number of fungiform papillae.

Student Outline

Learning Objectives

1. Distinguish fungiform papillae (with taste buds) from filiform papillae (lacking taste buds) on the anterior human tongue.

2. Learn about the differential response of supertasters, medium tasters, and nontasters to the bitter compound PROP (*(6-n-propylthiouracil)*.

3. Understand how sensitivity to bitter taste may influence food choice and health.

Materials and Methods

Blue Food Coloring
Cotton-tipped Swab Applicators
Magnifying (5X) Hand Mirrors
Paper Cups, Small (3 ounce/88ml)
PROP Test Paper (6-*n*-propylthiouracil)
Reading Glass Magnifiers (optional)
Reinforcement Labels
Rulers
Digital Camera (optional)

1. Taste unsweetened grapefruit juice and record taste reaction as *dislike*, *neutral*, or *like*. Rinse mouth with water.

2. Take one PROP taste paper and place on tongue. Identify reaction as *strongly bitter*, *bitter*, or *no taste* which is indicative of a supertaster, medium taster, or nontaster respectively. Record your taster status. Rinse mouth with water.

3. Dab some blue food color on a cotton swab. Using a mirror for guidance, swab the tip of the tongue with blue food color. Fungiform papillae which contain taste buds will not stain and appear pink against the background of filiform papillae which do not contain taste buds and stain blue (Figure 1). If the color is too dark, rub the tongue on the roof of the mouth. If too light, dab on a little more blue dye.

Figure 1. Anterior tongue stained with blue food coloring. Fungiform papillae appear pink against the background of blue filiform papillae.

4. Place a reinforcement label (Figure 2) on the tongue tip (BBC 2003). Stick out your tongue to cover the lower lip. Gently close your mouth and use your teeth to hold the tongue in place. Shine a flashlight on the exposed tongue. With the 5X magnifying mirror, count the number of pink fungiform papillae in the center hole of the reinforcement label. Have your lab partner verify the count using a reading glass magnifier or by looking over you shoulder into the mirror. Record the number.

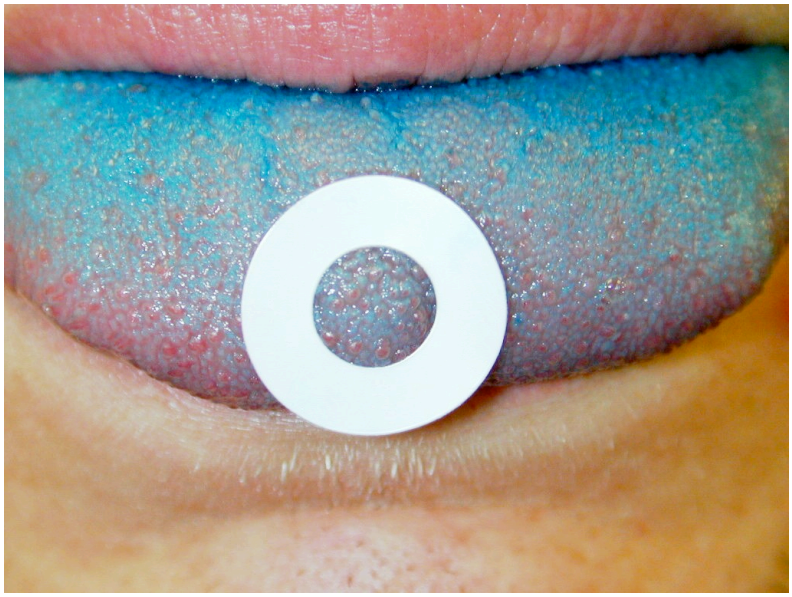

Figure 2. Adhesive reinforcement label placed on the tongue tip. Papillae are counted in the encircled area.

5. Calculate the density of the fungiform papillae per cm^2 in the area encircled. Recall that the area of a circle = radius ^{2} x π ($\pi = 3.14$). Calculate the density of the fungiform papillae per cm^2 in the area encircled.

6. Calculate the class mean for both the number and density of fungiform papillae for each PROP taster status: supertaster, medium taster, and nontaster.

7. How does PROP taster status correspond to the mean number and density of fungiform papillae? How does the taste reaction to grapefruit juice correspond to PROP taster status? Is the hypothesis, that a subject's taste response to grapefruit juice will predict both PROP status and the relative density of fungiform papillae, supported? Explain.

Results

Fifty individuals were tested during the spring and summer of 2007. Seventy-seven percent of PROP supertasters disliked the taste of unsweetened grapefruit juice, 18.5% were indifferent, and 4.5% liked unsweetened grapefruit juice. Medium tasters were divided in their response to the taste of unsweetened grapefruit juice. Nontasters either liked (54%) or were indifferent (46%) to the taste of

unsweetened grapefruit juice (Figure 3).

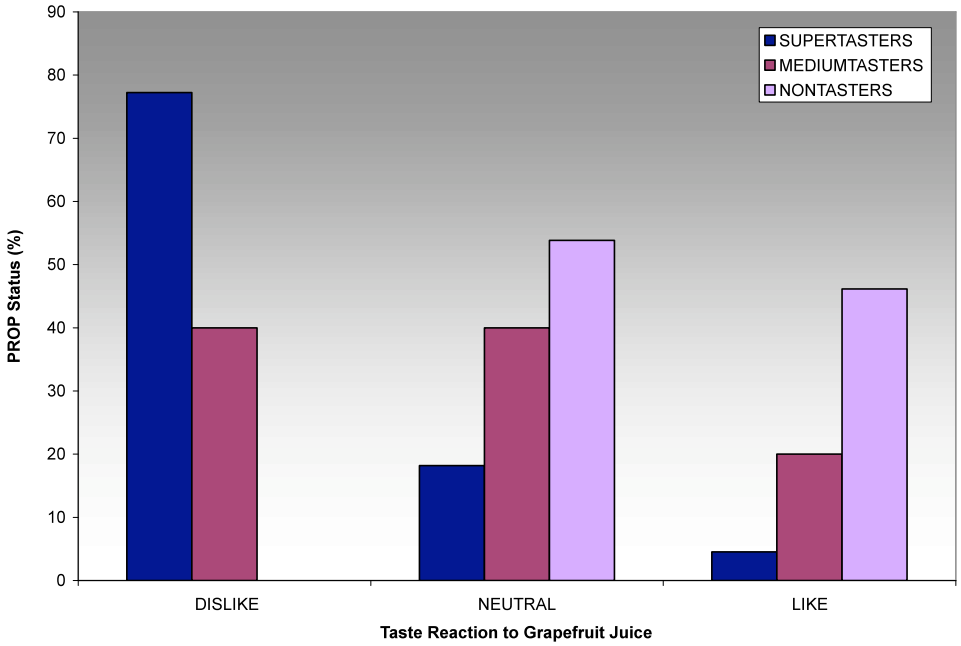

Figure 3. Taste reaction to unsweetened grapefruit juice according to PROP taster status.

Twenty-two individuals were identified by PROP testing as supertasters, 15 as medium tasters and 13 as nontasters. The highest count (36) and density of fungiform papillae (113.74/cm^2) were found in a PROP identified supertaster. Mean number and density of papillae varied according to PROP taster status (Table 1). Supertasters averaged 20.68 fungiform papillae in the sampled area and a mean density of 65.38 /cm^2; medium tasters had a mean of 13.47 papillae and a mean density of 42.55/cm^2; and nontasters averaged 10.69 papillae and a mean density of 33.78/cm^2. ANOVA Single Factor Analysis found the results to be significantly different at $p<0.001$. There were a few unexpected results. An individual who was identified as a supertaster based on PROP testing had both the lowest count (4) and density of fungiform papillae (18.96/cm^2). This individual was also the only supertaster who liked the taste of unsweetened grapefruit juice. Also, two medium tasters had papillae densities (107.42 /cm^2, 72.67/cm^2), which were greater than the mean density for supertasters. These individuals both recorded a dislike to grapefruit juice. One nontaster had a papillae density of 63.19/cm^2 approaching the mean for supertasters. This individual recorded a neutral reaction to the taste of grapefruit juice.

Discussion

Class data reflected the general trends seen in the published literature. Taste response to the bitter flavonoid naringin in unsweetened grapefruit juice is a fairly good predictor of PROP taste status. The majority of supertasters disliked the taste of unsweetened grapefruit juice. Medium tasters represented all reactions to the unsweetened grapefruit juice. Not a single nontaster disliked the taste of unsweetened grapefruit juice and the highest percentage of those who liked the taste of grapefruit juice was found in the nontaster group.

Table 1. Mean number of papillae and mean density of papillae/cm ^{2} according to PROP taster status. (N_{total} = 50, +/- standard deviation, area of sample = 0.3165 cm^2 , *ANOVA Single Factor, $p<0.001$)

Fungiform Papillae	Supertasters (n=22)	Medium Tasters (n=15)	Nontasters (n=13)
Mean	20.68 +/- 8.78*	13.47 +/- 7.01*	10.69 +/- 4.21*
Mean Density (/cm^2)	65.38 +/- 27.74*	42.55 +/- 22.14*	33.78 +/- 13.30*

Mean number and density of fungiform papillae were also generally reflected by PROP taster status with supertasters having the highest means and densities whereas nontasters had the lowest, but a few individual means and densities overlapped taster classes. One individual in the supertaster group had the lowest mean number and density of papillae of all taster classes. Three individuals identified as medium tasters had densities within the range reported for nontasters. Also, two medium tasters had papillae densities higher than the reported mean for supertasters.

PROP taster status, reaction to grapefruit juice, and number of fungiform papillae can also be used to investigate:

- Gender differences. More women are supertasters than men
- Smokers vs. nonsmokers. More smokers are nontasters than nonsmokers.
- Ethnic differences. More Africans and Asians are reportedly supertasters.
- Drinkers vs. non drinkers. Nontasters regularly consume more alcoholic beverages per year than supertasters.
- Stabilizing selection. The selection for heterozygotes and the adaptive differences of the taster (reject bitter substances that may be poisonous) and the nontaster (tolerate bitter substances that may have a medicinal or health value) phenotypes.

Instructor's Notes

After viewing digital photograph of tongues stained with blue food coloring, students quickly learned how to identify and count fungiform papillae. Fungiform papillae are found on the anterior of the tongue and are most abundant on the margins. It is probably important to sample the tongue in roughly the same region among individuals.

PROP test paper can be purchased from Ward's (PROP Test Paper - 14 W 4104 - VIAL OF 100, Ward's 800 962-660 or http://wardsci.com). Different brands of food color may vary somewhat in the intensity of the blue dye. I found McCormick and Adams Extract brands both worked.

All other supplies (magnifying mirrors, magnifying glasses, paper cups, food coloring, and reinforcement labels) are inexpensive and can be purchased locally at an office supply store, supermarket, or drug store.

Acknowledgments

I wish to thank the students and colleagues at The University of Tulsa who participated in the development of this exercise.

Literature Cited

BBC> Science & Nature > Human Body & Mind> The Body>The Nervous System> Test Your Tastebuds. 2003. http://www.bbc.co.uk/science/humanbody/body/articles/senses/tongue_experiment.shtml

Bartoshuk, Linda M., Valerie B. Duffy, and Inglis J.Miller. 1994. PTC/PROP tasting: anatomy, psychophysics, and sex effects. Physiology & Behavior, 56 (6): 1165 -1171.

Delwiche, Jeannine F. Zivjena Buletic, and Paul A. S. Breslin. 2001. Relationship of papillae number to bitter intensity of quinine and PROP within and between individuals. Physiology & Behavior, 74: 329 – 337.

Dinehart, M. E., J. E. Hayes, L. M. Bartoshuk, S. L. Lanier, and V. B. Duffy. 2006. Bitter taste markers explain variability in vegetable sweetness, bitterness, and intake. Physiology & Behavior, 87: 304 – 313.

Drewnowski, Adam, Susan Ahlstrom Henderson, and Amy B. Shore. 1997. Taste responses to naringin, a flavonoid, and the acceptance of grapefruit juice are related to genetic sensitivity to 6-*n*-propylthiouracil. The American Journal of Clinical Nutrition, 66: 391-397.

Fox, Arthur L. 1931. Six in ten *tasteblind* to bitter chemical. Science News Letter, 19: 249.

Kim, Un-kyung, Eric Jorgenson, Hilary Coon, Mark Leppert, Neil Risch, and Dennis Drayna. 2003. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science, 299: 1221 – 1225.

Kim, UK. and D. Drayna. 2004. Genetics of individual differences in bitter taste perception: lessons from the *PTC* gene. Clinical Genetics, 67:275 – 280.

Miller, Jr. Inglis J.and Frank E. Reedy, Jr. 1990. Variations in human taste bud density and taste

intensity perception. Physiology & Behavior, 47(6): 1213 – 1219.

Millius, Susan. 2003. Good taste in men linked to colon risks. Science News, 163 (9): 142.

Minnella, Julie, M. Yanina Pepino, and Danielle R. Reed. 2005. Genetic and environmental determinants of bitter perception and sweet preferences. Pediatrics, 115 (2): 216 – 222.

Reed, Danielle R., Toshiko Tanaka, Amanda H. McDaniel. 2006. Diverse tastes: genetics of sweet and bitter perception. Physiology & Behavior, 88: 215-226.

Smith, David V. and Robert F. Margolskee. 2001. Making sense of taste. Scientific American, 284 (3): 32 – 39.

About the Author

Karen A. McMahon earned her M. S. in botany from the University of Ohio and is currently an instructor at The University of Tulsa where she teaches laboratory courses in environmental biology, botany, organismal biology, and human anatomy and physiology. She has authored a textbook and a laboratory manual highlighting the varied uses of plants. This laboratory exercise was inspired by listening to a NPR interview of researcher Linda Bartoshuk of Yale University in March 2003. Karen is a nontaster who likes unsweetened grapefruit juice, broccoli, and many of the other fruits and vegetables that supertasters avoid.