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Genetic Taste Responses to 6-*n*-Propylthiouracil Among Adults: a Screening Tool for Epidemiological Studies

Adam Drewnowski[1,2], Alan Kristal[1,2] and Jennifer Cohen[2]

[1]Nutritional Sciences Program and the Department of Epidemiology, University of Washington School of Public Health and Community Medicine, Seattle, WA 98195 and [2]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Correspondence to be sent to: Adam Drewnowski, Nutritional Sciences Program, 305 Raitt Hall, Box 353410, University of Washington, Seattle, WA 98195-3410, USA. e-mail: adamdrew@u.washington.edu

Abstract

Genetically mediated taste responsiveness to 6-*n*-propylthiouracil (PROP) has been linked to reduced acceptance of some bitter foods. In this community-based study male ($n = 364$) and female ($n = 378$) adults enrolled in a self-help dietary intervention trial were screened for PROP taster status. Respondents, aged 18–70 years, were mailed filter papers impregnated with PROP or with aspartame solutions. They received instructions to rate taste intensity and hedonic preference using nine point category scales. Women rated PROP as more bitter than did men. Both sweetness and bitterness ratings were lower for older adults. Taste responsiveness to PROP was unrelated to body mass index in women or men. Higher bitterness ratings for PROP were weakly associated with higher sweetness ratings for aspartame, but were unrelated to sweet taste preferences. Successful administration of PROP filter papers by mail suggests new avenues for the screening of taste phenotypes in epidemiological studies.

Introduction

Taste responsiveness to phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP) is a heritable trait (Fox, 1931; Hartman, 1939; Kalmus, 1958; Reddy and Rao, 1989). While the phenotypic taste responses to PTC and PROP are well described in the literature, the gene responsible for this trait has not been described and its exact location is unknown (Reed, 2000). Genetic linkage studies have linked the ability to taste PROP with a locus at 5p15, with a modifier locus on human chromosome 7 (Reed *et al*., 1999).

The ability to taste PROP has been linked with a rejection of other bitter compounds and with reduced acceptance of some bitter foods (Fischer *et al*., 1961; Drewnowski *et al*., 1997b, 2000; Kaminski *et al*., 2000). The most frequently cited example of bitter food rejection is the reported avoidance by PTC tasters of bitter compounds found in raw cruciferous vegetables: cabbage, broccoli and Brussels sprouts (Niewind *et al*., 1988; Jerzsa-Latta *et al*., 1990). In recent studies PROP tasting was further associated with reduced preferences for grapefruit juice, Japanese green tea, Brussels sprouts and selected soy products (Akella *et al*., 1997; Kaminski *et al*., 2000). These foods are among the major dietary sources of bioactive phytochemicals, some of which are thought to reduce cancer risk (Drewnowski and Rock, 1995). Tasters and supertasters of PROP may have reduced dietary exposure to bitter but beneficial phytonutrients found in vegetables and fruit (Drewnowski *et al*., 2000).

Screening for PROP taster status may therefore have a public health importance. Increasing the consumption of vegetables and fruit to at least 5 servings/day is the key dietary strategy for prevention of cancer and other chronic disease (Steinmetz and Potter, 1996). However, the current laboratory-based methods for assessing PROP sensitivity are not suited for use in large-scale population studies.

Whereas early studies on taste genetics used PTC crystals or PTC-impregnated filter papers (Fox, 1931; Blakeslee and Fox, 1932), most later studies relied on taste detection thresholds established using a series of solutions (Harris and Kalmus, 1949). The bimodal distribution of PTC or PROP detection thresholds served to distinguish between tasters and non-tasters (Kalmus, 1958, 1971). Noting that the taster group showed much variability in detection thresholds, Bartoshuk proposed that a separate sub-population of 'supertasters' might also exist (Bartoshuk, 1993). Supertasters were distinguished from regular tasters of PROP by their elevated ratio of perceived bitterness of above-threshold PROP solutions relative to the perceived saltiness of NaCl solutions (Bartoshuk, 1993).

The PROP paper test provides a crude but rapid way to test supra-threshold responses to PROP in large groups

(Bartoshuk *et al*., 1994). Blakeslee and Salmon were the first to note that females were more sensitive than males to the bitter taste of PTC (Blakeslee and Salmon, 1931). Bartoshuk *et al*. confirmed the sex effect using PROP-impregnated filter paper (Bartoshuk *et al*., 1994). Although the early PTC literature had shown both race and age effects, Bartoshuk *et al*. reported that the current data, including their own, did not have adequate numbers to provide compelling evidence on either point (Bartoshuk *et al*., 1994). At least one study showed that PROP detection thresholds increased with age and that older respondents rated concentrated PROP solutions as less bitter than did younger persons (Whissell-Buechy, 1990; Drewnowski *et al*., 1999).

The proportion of PTC/PROP tasters in the US population has been estimated at 70% (Bartoshuk *et al*., 1994). Anthropological studies, mostly conducted using the filter paper method, found an even higher proportion of tasters among Africans and Asians, as opposed to Caucasians (Parr, 1934). While taste data on minority groups in the US are scarce, recent studies on PROP tasting in Africa, India and China seem to support that general conclusion (Hladik *et al*., 1986; Guo *et al*., 1998).

One question of interest is the relationship between PROP tasting and body mass index (BMI, kg/m^2) or some other measure of body fatness. Observing an inverse relationship between PROP tasting and body mass indices, some researchers have even suggested that PROP tasting may protect against obesity (Tepper and Nurse, 1997; Tepper, 1998, 1999). While such a finding would have major public health implications, the studies were for the most part limited to very small samples of college students (Tepper and Nurse, 1997; Tepper 1998, 1999). While of great potential interest, the association between PROP tasting and body weight remains unproven.

Our goal was to determine PROP taster status of adult men and women enrolled in a randomized community trial of dietary change. The question was whether taste tests can be a part of standard evaluation procedures in large-scale population-based studies. Laboratory techniques, such as the determination of PROP detection thresholds, are singularly unsuited for that purpose. PROP- and aspartame-impregnated filter papers were delivered by mail to a subset of study participants. We hypothesized that the broad effects of sex, age and race, some of which had been observed using PROP solutions, would be confirmed using the PROP paper tests.

Materials and methods

Respondents

Respondents were a random sample of adults enrolled in the Group Health Cooperative of Puget Sound, a large health maintenance organization in Washington State. They were taking part in the Puget Sound Eating Patterns (PEP) Study, a randomized trial of a tailored, self-help intervention to promote lower fat and higher fruit and vegetable intakes. Details on the design and results of the PEP study have been provided elsewhere (Kristal *et al*., 2000). For the purposes of recruitment and randomization participants were stratified evenly in six groups, defined by sex and age category (ages 18–34, 35–54 and 55–69 years). All 1269 PEP participants who completed the 12 month final follow-up survey received materials for the taste sensitivity study by mail. Responses were obtained from 364 men and 378 women. The response rate to the taste sensitivity questionnaire was 59.2% overall and did not differ by intervention or control group assignment. For the study reported here demographic information was collected at baseline and weight (self-reported) and fruit and vegetable intakes in estimated servings per day were assessed at the time of the 12 month survey. More detailed dietary intake data were collected from 326 intervention group participants who completed food frequency questionnaires.

Taste stimuli

PROP-impregnated filter paper

To prepare PROP-impregnated filter papers sheets of Whatman no. 1 filter paper were dipped into a hot supersaturated solution of PROP. The solution was heated close to the boiling point. The papers were dried and cut into 1 inch squares that were then stored in glassine envelopes (Kaminski *et al*., 2000).

Aspartame-impregnated filter paper

To prepare aspartame-impregnated filter papers sheets of Whatman no. 1 filter paper were dipped in warm aspartame solution containing 0.2 g/l. The papers were dried and cut into 1 inch squares that were then stored in glassine envelopes.

Scaling procedures

Respondents were given written instructions to place the PROP-impregnated filter paper on the tongue, allow it to moisten and rate its bitterness using a nine point category scale. The printed scale was anchored at each end with '1 = not at all bitter' and '9 = extremely bitter'. Overall acceptability ratings were obtained using the standard nine point hedonic preference scale. This fully anchored nine point category scale ranges from 1 ('dislike extremely') to 9 ('like extremely'), with a neutral point at 5 ('neither like nor dislike') (Drewnowski *et al*., 1997a,b; Kaminski *et al*., 2000). The same procedures have been used in other studies. Respondents were then classified into three taster groups: those who rated bitterness intensity as 1 or 2; those who rated bitterness intensity at between 3 and 7; those who gave ratings of 8 or 9. Bartoshuk *et al*. used an identical procedure to assign subjects tested at a lecture into three categories (Bartoshuk *et al*., 1994). In some early studies (Kalmus, 1958) subjects who indicated that a filter paper impregnated with PTC was very or extremely bitter were

also classified as tasters, whereas non-tasters rated filter papers as 'not at all' or 'slightly' bitter. Ratings of the aspartame-impregnated filter papers followed the same procedure. The sweetness intensity scale ranged from '1 = not at all sweet' to '9 = extremely sweet'. The nine point hedonic preference scale was the same as described above.

Questionnaire instruments

Respondents completed a variety of health and demographic questionnaires. Self-report data were obtained on respondent sex, age, height, weight, ethnicity, marital status and tobacco use. BMI values (kg/m^2) were calculated for each respondent.

Statistical analyses

Statistical tests were conducted using SPSS for Windows 8.0 (SPSS Inc., Chicago, IL). Assignments of PROP taster status were based on bitterness intensity ratings of PROP filter papers. Following Bartoshuk *et al*. (Bartoshuk *et al*., 1994), the three groups were defined as low (rating 1 or 2), medium (3–7) and high responders (8 or 9). Respondents were also divided into four categories by age: 18–29, 30–44, 45–59 and 60–70 years. Comparisons by sex and age category were based on cross-tab analyses followed by χ^2 tests. Regression analyses were used to explore the relationship between PROP tasting and BMI and between PROP tasting and sensory response to sweetness.

Results

Respondent characteristics

The final sample was based on 364 men and 387 women. Mean ages were 48.0 years for men and 48.2 years for women (range 18–70 years). Mean BMI values were 26.9 for men and 26.2 for women. The sample was 93% Caucasian. These data are summarized in Table 1.

Table 1 Subject characteristics

	Women (n = 378)	Men (n = 364)
Age (years)	48.2 ± 14.2	48.0 ± 15.1
Height (cm)	164.9 ± 0.3	178.9 ± 7.1
Weight (kg)	71.2 ± 15.9	86.1 ± 14.6
BMI (kg/m^2)	26.2 ± 5.8	26.9 ± 4.2
Race (%)		
White	89.8	92.0
Black/African	2.4	2.2
American Indian	0.0	0.3
Asian/Pacific	4.5	3.6
Other	3.2	1.9

Data are means ± SD (except for race).

PROP tasting by sex

Bitterness intensity ratings for PROP filter papers followed a bimodal distribution, as shown in Figure 1 (top left). The proportion of non-tasters (i.e. low PROP responders) was 15% for women and 24% for men. Women rated PROP filter papers as more bitter than did men, as confirmed by Pearson χ^2 analysis of the nine bitterness categories by sex [χ^2 (df 8) = 44.5, $P < 0.01$]. Although everyone disliked the bitter PROP papers, women tended to dislike them more strongly than did men [χ^2 (df 8) = 45.9, $P < 0.01$] (Figure 1, top right). As expected, greater perceived bitterness was associated with increased dislike of PROP filter papers (r = –0.69, $P < 0.01$).

Sweetness intensity ratings for aspartame filter papers showed a normal, as opposed to a bimodal, distribution. The two bottom panels of Figure 1 show that sweetness intensity and hedonic ratings for aspartame were the same for both men and women. Both sweetness intensity and hedonic ratings followed a unimodal distribution. Respondents expressed a mild liking for aspartame-impregnated filter papers.

PROP tasting by age category

The distribution of PROP taster status by age category is shown in Figure 2, separately for men and women. For women the percentage of low responders increased with age category, whereas the percentage of high responders decreased with age category [χ^2 (df 6) = 12.7, $P < 0.05$]. The same effect of age was observed for men [χ^2 (df 6) = 18.6, $P < 0.01$].

Race/ethnicity

Early studies suggested that the ability to taste PROP was more common among Asians and Africans than among Caucasians (Parr, 1934). Pearson χ^2 test for the distribution of taster categories by ethnic status (Caucasian/non-Caucasian) confirmed that this was indeed the case [χ^2 (df 2) = 8.4, $P < 0.05$].

Body mass index

A multiple linear regression model was used to determine the relationship between PROP tasting and BMI, controlling for such confounding variables as age, ethnicity and tobacco use. Separate regression analyses with BMI as the dependent variable were conducted for men and women. No significant relationship between PROP intensity ratings and BMI or PROP hedonic ratings and BMI was observed. Whereas BMI was influenced by age and ethnicity for men, it was not influenced by PROP taster status, either for men or women.

To explore this issue further an additional measure of taste response to PROP was constructed by combining bitterness intensity and hedonic ratings. A scatter plot of this composite measure against BMI is shown in Figure 3. Regression analyses showed no influence of PROP taste response on BMI values in men or women. There was no

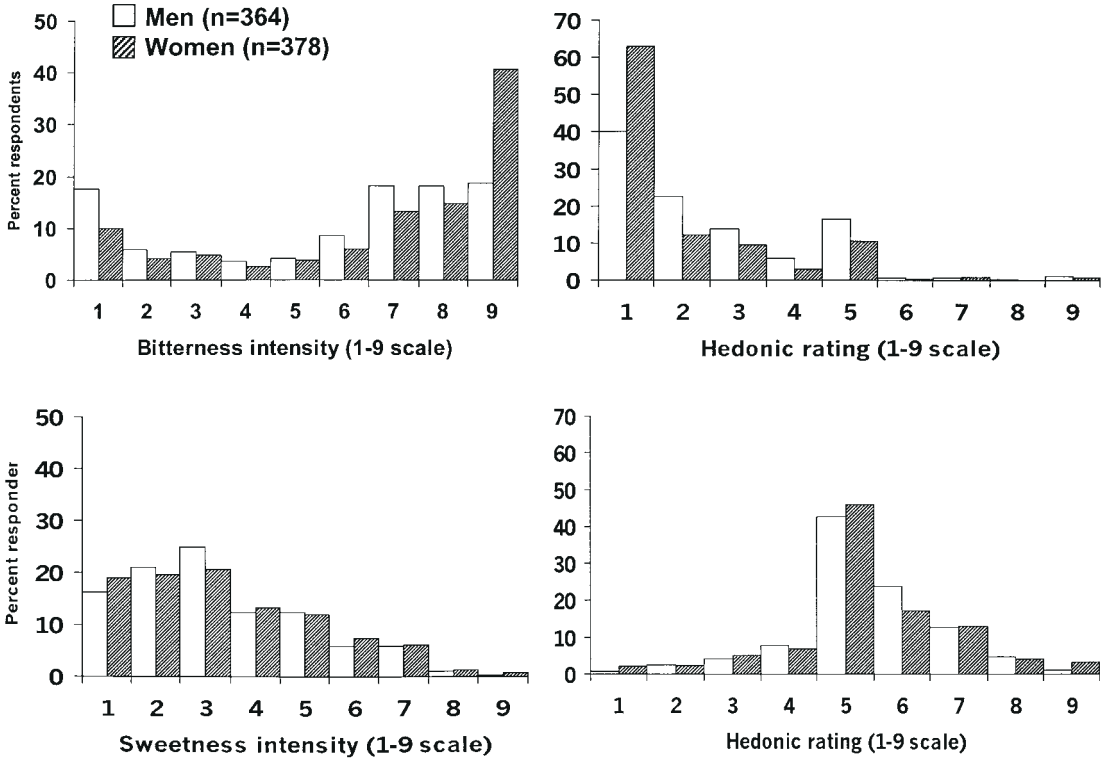

Figure 1 (Top) The distribution of bitterness intensity (left) and hedonic ratings (right) for PROP filter papers by sex. (Bottom) The distribution of sweetness intensity (left) and hedonic ratings (right) for aspartame filter papers by sex.

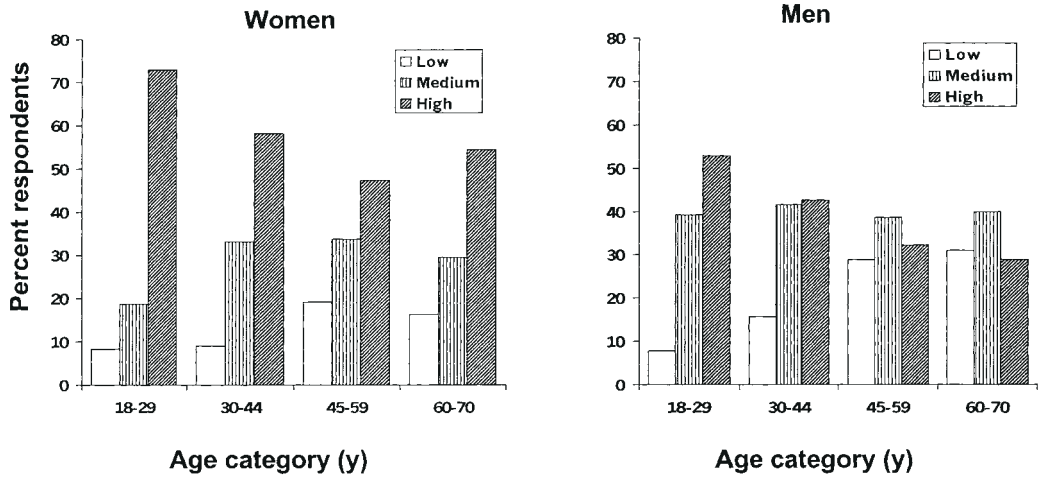

Figure 2 Distribution of PROP responses (low, medium and high) by age category for women (left) and men (right).

evidence whatever to link PROP tasting to BMI in this sample of adult men and women.

PROP tasting and response to sweet

Stepwise linear regression with sweetness intensity ratings as dependent variable showed that age, intensity ratings for PROP and ethnic status predicted intensity ratings for sweet ($P < 0.01$). On the other hand, hedonic ratings for sweetness were weakly predicted by age ($P < 0.10$) but were unrelated to sex, ethnic background or bitterness ratings for PROP.

PROP tasting and dietary habits

The consumption of total fruit and vegetables, total vegetables, salads or cooked vegetables in terms of servings per week was assessed using dietary behavior questionnaires, as opposed to food records. In multiple regression analyses controlling for sex, age and race there were no

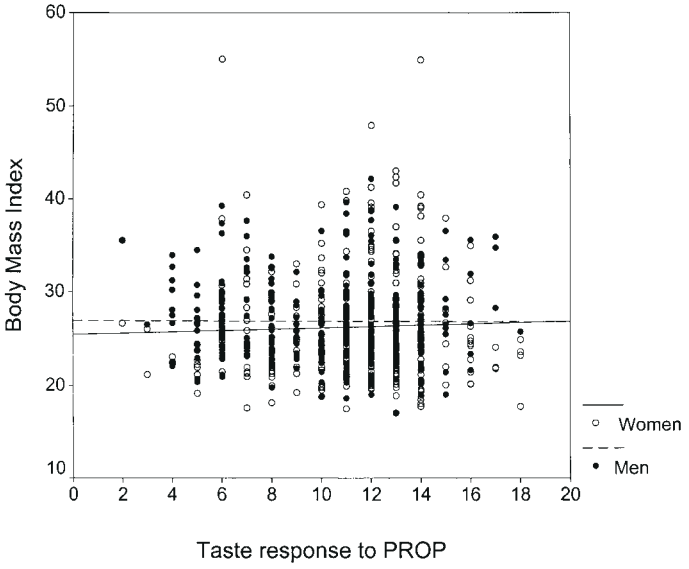

Figure 3 Absence of a relationship between PROP responsiveness and BMI (kg/m^2). PROP response is a composite score of intensity and hedonic ratings.

associations of PROP responsiveness with these measures of consumption. For a smaller subgroup of respondents (n = 230) for whom more detailed dietary data were available PROP tasting (taster versus non-taster) was associated with significantly lower mean consumption of coffee (4.6 versus 5.4 servings/week, $P < 0.05$), consistent with past reports. Although PROP tasting was also associated with reduced consumption of cruciferous vegetables, the observed difference was small and did not reach statistical significance (2.7 versus 2.9 servings/week).

Discussion

The use of taste tests in large-scale epidemiological studies poses a number of challenges for the investigator. Methods based on detection thresholds or intensity ratings of taste solutions are often unsuitable for use outside the laboratory. Studies on PROP taster status in particular were often limited to college students, most often women, and to relatively small subject samples of convenience (Looy and Weingarten, 1992; Tepper and Nurse, 1997; Kaminski *et al.*, 2000). In other cases PROP filter papers were passed to subjects tested at a lecture (Bartoshuk *et al.*, 1994).

In this study PROP filter papers were delivered by mail to respondents enrolled in a population-based study of diet and health. Generally the data showed the same patterns as laboratory results obtained using PROP detection thresholds and intensity scaling of PROP solutions (Bartoshuk, 1993; Bartoshuk *et al.*, 1994; Drewnowski *et al.*, 1997a,b). Using classical PTC threshold detection procedures, Whissell-Buechy placed the proportion of non-tasters among adult Berkeley men at 16% and among women at 17% (Whissell-Buechy, 1990). Using PROP detection thresholds, Bartoshuk *et al.* identified 35 of 220 subjects as non-tasters (16%) (Bartoshuk *et al.*, 1994). The data of Bartoshuk *et al.*, based on PROP filter papers for 487 subjects, showed that the proportion of non-tasters was ~22% for women and 26% for men (Bartoshuk *et al.*, 1994). The present filter paper method allowed us to identify 15% of women and 24% of men as non-tasters of PROP.

The proportion of high responders among females in our sample was 55%, higher than the ~30% observed in the data of Bartoshuk *et al.* (Bartoshuk *et al.*, 1994), also based on PROP filter papers and an analogous nine point scale. Whether or not these high responders were in fact supertasters is another issue. Technically supertasters can only be identified by the ratio of their response to PROP relative to salt solutions and not by their response to PROP alone. Leaving this issue aside, women generally rated PROP as more bitter than did men. No bimodal distribution or sex effects were observed for aspartame filter papers.

The present data also showed a sharp decline in PROP ratings with age. These age effects are consistent with data from laboratory studies. Some past studies have observed a modest increase in PROP detection thresholds with age (Whissell-Buechy, 1990; Drewnowski *et al.*, 1999). Others have found a modality-specific decline in intensity ratings for some bitter compounds, again using the method of solutions (Weiffenbach, 1984). A shift from high to low responders by age category was observed for both women and men.

There was no relationship between PROP tasting and BMI for women or for men. Published abstracts that purported to show a relationship between PROP tasting and BMI have been based on very small respondent samples. One study by Dabrila *et al.* correlated responses to PROP filter paper with BMI values, but for only 44 women (Dabrila *et al.*, 1995). Another report (Tepper, 1999) was based on only 30 male respondents and the relationship was not significant in any case. In a recent study with PROP filter papers (Duffy *et al.*, 1999) the relationship between perceived PROP bitterness and BMI was inconsistent. PROP bitterness showed a negative relationship with BMI, but only for normal weight persons. In contrast, a positive relationship was observed for overweight and obese persons.

Perhaps most important, none of the above studies had sufficient power or took measures to control for confounding variables. BMI is influenced by age, sex, ethnicity, education, income and tobacco use. In order to establish an independent relationship between PROP tasting and body weight as many of these variables as possible must be accounted for. The present regression analyses showed no relationship between measures of PROP tasting and BMI, at least for this sample of adult men and women.

Another question is whether the filter paper method can serve as a screening tool in epidemiological studies.

Laboratory studies have shown high correlations between bitterness intensity ratings of PROP filter papers and those for PROP solutions (Drewnowski *et al*., 1997a; Kaminski *et al*., 2000). Mean perceived bitterness of PROP solutions was correlated, in turn, with PROP detection thresholds (Drewnowski *et al*., 1997a). Whereas threshold detection methods remain the gold standard of taste psychophysics, they are too labor intensive for use in the field. Successful testing of PROP filter papers delivered by mail to community respondents may be the better screening tool in epidemiological studies of taste phenotypes. Of course, the use of PROP filter papers also has important limitations. The ability to perceive the bitterness of filter paper is critically dependent on adequate wetting, contact with the tongue and the time of exposure. Although subjects were asked to keep the filter paper on the tongue for a specific period of time, we have no way of verifying that these procedures were followed.

Identifying factors that influence food selection and may promote or prevent dietary change is a key concern of public health nutrition. Inherited taste factors influence taste responses to some foods and may have an influence on food choice (Drewnowski and Rock, 1995; Drewnowski, 1997; Drewnowski *et al*., 1999). Screening for PROP taster status may bear on current efforts to promote the consumption of healthful diets, notably those rich in bitter constituents of plant foods.

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