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Title: Facial affective reactions to bitter-tasting foods and body mass index in adults

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Abstract

Differences in food consumption among body-weight statuses (e.g., higher fruit intake linked with lower body mass index (BMI) and energy-dense products with higher BMI) has raised the question of why people who are overweight or are at risk of becoming overweight eat differently from thinner people. One explanation, in terms of sensitivity to affective properties of food, suggests that palatability-driven consumption is likely to be an important contributor to food intake, and therefore body weight. Extending this approach to unpalatable tastes, we examined the relationship between aversive reactions to foods and BMI. We hypothesized that people who have a high BMI will show more negative affective reactions to bitter-tasting stimuli, even after controlling for sensory perception differences. Given that hedonic reactions may influence consumption even without conscious feelings of pleasure/displeasure, the facial expressions were included in order to provide more direct access to affective systems than subjective reports. Forty adults (28 females, 12 males) participated voluntarily. Their ages ranged from 18 to 46 years (M=24.2, SD=5.8). On the basis of BMI, participants were classified as low BMI (BMI<20; n=20) and high BMI (BMI>23; n=20). The mean BMI was 19.1 for low BMI (SD=0.7) and 25.2 for high BMI participants (SD=1.8). Each subject tasted 5 ml of a grapefruit juice drink and a bitter chocolate drink. Subjects rated the drinks' hedonic and incentive value, familiarity and bitter intensity immediately after each stimulus presentation. The results indicated that high BMI participants reacted to bitter stimuli showing more profound changes from baseline in neutral and disgust facial expressions compared with low BMI. No differences between groups were detected for the subjective pleasantness and familiarity. The research here is the first to examine how affective facial reactions to bitter food, apart from taste responsiveness, can predict differences in BMI.

Key words: Affective facial reaction; bitter food; body mass index; overweight; taste responsiveness

Introduction

Research on obesity has revealed that overweight/obese people display different eating behaviours to lean people (for review, cf. French, Epstein, Jeffery, Blundell &Wardle, 2012; Mesas, Muñoz-Pareja, López-García & Rodríguez-Artalejo, 2012). Several studies with both children and adults agree that individuals with a higher body mass index (BMI, kg/m^2) not only consume larger amount of, e.g., energy-dense snacks (Berteus Forslund, Torgerson, Sjostrom & Lindroos, 2005; Nicklas, Yang, Baranowski, Zakeri & Berenson, 2003), soda/sweetened beverages (Blum, Jacobsen & Donnelly, 2005; Malik, Schulze & Hu, 2006; Nicklas et al., 2003) and fast food (Bowman &Vinyard, 2004; Schröder, Fito & Covas, 2007) compared to those with a lower BMI; but also less fruit and vegetables (Alinia, Hels & Tetens, 2009; Kahn et al, 1997; Lin & Morrison, 2002; Mohindra, Nicklas, O'Neil, Yang & Berenson, 2009). Dietary patterns or changes in patterns of food choice over time have also been linked to BMI status (e.g., Maskarinec, Novotny & Tasaki, 2000; Pachucki, 2012). Pachucki using cluster analysis with dietary data showed that transitions to lower diet quality clusters (e.g., from fruits and legumes to low/high-fat meat and soda) were associated with a higher BMI. Since excessive fat versus inadequate vegetable and fruit intake have been identified as risk factors for developing obesity and major diseases (e.g., Bray & Popkinand, 1998; Boeing et al., 2012), there is an urgent need to understand why people at risk of obesity choose and eat differently from thinner people.

Among the determinants of food preferences related to weight status, some studies have considered whether a predisposition to overeating might be related to hedonic processes (cf. Blundell & Finlayson, 2004; Mela, 2001). In terms of taste preference (Drewnowski, 1997), these studies are based on the assumption that differences in the perceived pleasantness of foods (hedonic sensitivity), and not only in sensory perception (taste responsiveness), should explain the individual variability in BMI. Given that pleasure “comprises the positive dimension of the more general category of hedonic processing [...], which also includes other negative and unpleasant dimensions” (Berridge & Kringelbach, 2008), an attractive possibility is to extend this hedonic eating-based approach of overweight people to aversive tastes. Thus, subjects with a higher sensitivity to the affective value of food might be likely to have a stronger drive to eat pleasurable food as well as a higher avoidance of aversive tastes, promoting the overconsumption of palatable energy-dense products and the rejection of unpalatable healthy bitter substances. Although the results are mixed, the view that BMI is increased by a heightened liking for highly palatable foods has received support from several sources of evidence, including data from longitudinal (e.g., with the obesity-prone Pima Indian population; Salbe, DelParigi, Pratley, Drewnowski & Tataranni, 2004) and cross-sectional studies (e.g., with the distribution of BMIs among the high-fat phenotypes; Blundell et al., 2005). Particularly interesting are the studies on the relationship between sensitivity to food reward and BMI (e.g., Davis & Fox, 2008; Franken & Muris, 2005). For instance, Davis, Strachan and Berkson (2004) pointed out that overweight women were significantly more sensitive to the hedonic reward of food, when comparing the self-reported scores on the *Physical Anhedonia Scale* with those of their normal weight counterparts. Franken and Muris (2005) also found that reward sensitivity, as indexed by *Sensitivity to Punishment and Sensitivity to Reward Questionnaire*, was positively associated with BMI in young women.

Regarding the assumption that body mass is affected by variations in reactivity to unpleasant tastes, to date no study has specifically investigated the relation between negative affective (aversive) responses to bitter-tasting foods and body weight. The available studies which have explored weight differences as a function of taste are based on sensory (e.g., threshold or intensity; for review, cf. Donaldson, Bennett, Baic & Melichar, 2009) but not affective variations. Although this line of evidence does not directly address our question, results seem to point out that the perception of taste intensity of bitter compounds (e.g., 6-n-propylthiouracil [PROP]) could ultimately impact body weight (e.g., Lumeng, Cardinal, Sitto & Kannan, 2008; Tepper & Ullrich, 2002). These studies suggest that greater responsiveness to the bitter taste of PROP is positively associated with a higher BMI in children, but this relationship is negative in adults. Unfortunately, it is not obvious from the gustatory perception data how the individual differences in aversive reactions may influence the body weight status, especially when sensory versus affective aspects of taste stimuli have been separated via physiological, psychological and pharmacological manipulations in animals and humans (Berridge, 2000; e.g., keeping the sensory properties of a taste unchanged, while altering its pleasantness). Therefore, the purpose of the present experiment was to compare the aversive responses to bitter-tasting stimuli, measured by subjective ratings and behavioural observations in a taste reactivity paradigm, between two healthy adult groups of varying BMI. Taking into account the evidence for a non-linear relationship between sensitivity to reward and BMI, indicating a positive relationship only in the normal and overweight range of BMI (Davis & Fox, 2008), the present study was limited to the BMI range of 17.7-29.9. We anticipated that individuals at risk of becoming overweight (BMI $\approx$ 25) would be more responsive to the unpleasant properties of food than those with a low body weight (BMI $\approx$ 19). That is, high BMI should show lower scores on hedonic ratings and higher intensity of disgust facial expressions compared to low BMI, even after controlling the differences in taste responsiveness.

In view of the importance of providing a relatively pure indication of affect (isolated from the sensory and motivational properties of tastes), facial expressions were used here (cf.

Berridge, 2000). This way of assessing responses to food, beyond self-report measures alone, was hoped to obtain a more exact evaluation of the relationship between the aversive reactions and BMI, given that objective measures of liking reactions may sometimes provide more direct access to hedonic systems than subjective reports (Berridge, Robinson & Aldridge, 2009). In addition, it should be noted that many studies investigating taste preferences have found no hedonic differences as a function of body weight (for review, cf. Bartoshuk, Duffy, Hayes, Moskowitz & Snyder, 2006), the methods used to compare sensory and affective experiences across groups being one possible explanation for these conflicting results. Concretely, psychophysical errors derived from subjective measures (e.g., visual analogue or category scales) have been suggested as a factor masking the relationship between orohedonic response and obesity (Bartoshuk et al., 2006). Moreover, unlike facial patterns, self-ratings might not represent accurate measures of pleasure/displeasure, because they may often conflate affective and motivational (i.e., desire to eat) components of food and be too overlaid with cognitions to pick up underlying core differences in food liking (Mela, 2001). In this sense, the present study additionally sought to extend prior findings (e.g., Danner, Sidorkina, Joechl & Duerrschmid, 2013) on the contribution of facial expressions to sensory evaluation and affective testing of bitter food; as well as explore the validity of hedonic self-report measures as assessment instruments of the affective experience when they are employed with bitter tastes.

Materials and Method

Participants

Forty healthy adults (28 females, 12 males) from the Faculty of Agrarian Sciences (Pontificia Catholic University of Argentina, Argentina) were selected from a pool of people.

Their ages ranged from 18 to 46 years (M = 24.2, SD = 5.8). Participants were asked to report their height and weight. On the basis of their BMI, two groups were formed: low BMI, consisting of lean subjects (BMI < 20; n = 20); and high BMI, encompassing participants that were at risk of becoming overweight and overweight (BMI > 23; n = 20). The BMI values of 20 and 23 corresponded to percentile 40 and 60 respectively of the reference sample and were deliberately selected in these ranges in order to establish a clear separation between BMI groups. The mean BMI was 19.1 for low BMI (SD = 0.7) and 25.2 for high BMI participants (SD = 1.8), being statistically different ($p < .05$). Exclusion criteria were aversions, smoking (more than 5 cigarettes per week; Sato, Endo & Tomita, 2002), illnesses, a history of eating disorders, diabetes and allergy for the foods offered. Specially, participants who described themselves as being on weight-loss diets or actively losing weight were excluded; this factor might be associated to bias in reporting of sensory and affective perceptions of stimuli or influence the relationship between bitter responsiveness and body weight (Tepper & Ullrich, 2002). Subjects were contacted by e-mail and asked to participate in a research study investigating preferences for bitter foods. The experiment was approved by the Ethics Committee of the Pontificia Catholic University of Argentina. Participants were informed about the purpose of the study and that the experimental procedure would be video recorded. All subjects gave their written consent, including agreement to be recorded on video, and participated voluntarily.

Food solutions

Subjects received solutions of liquor chocolate (Natural Cocoa Liquor Refined NA760, Cargill Agricola S.A., Brazil) and grapefruit juice, which were selected by their different bitter compounds. The energy density was 28.9 and 0.4 kcal/g for the chocolate and the grapefruit, respectively. The chocolate drink presented a high aromatic intensity at tasting temperature (55ºC; data not shown), a strong bitter taste and a high viscosity (viscosity > 1000). The grapefruit juice exhibited a more neutral sensory profile (aroma and bitter taste; viscosity < 10) at tasting temperature (20ºC), which was prepared from natural pink grapefruit obtained from a local store using an electric citrus juicer. Viscosity was measured by means of a rotational viscometer (Brookfield DV-LVT; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) using the UL/Y adapter with S-00 spindle (chocolate) and S-38 spindle (grapefruit). The sample chamber was placed in a water jacket connected to a bath (TC-502 Brookfield) to perform the determinations at tasting temperature. PH values were 6.0 for the chocolate and 3.1 for the grapefruit. The pH was measured using a pH-meter (HANNA-pH 210, Germany), except for the chocolate (determined by method IOCCC, 9/1972, in 10% solution; Gerkens Cacao, Brazil). No sugar or sweeteners were added to the solutions.

Dependent variables

Eating behaviour questionnaires and caloric intake assessment

Preference and consumption of bitter substances were measured with a food preference questionnaire (FPQ; with Cronbach's alpha (α) of .88), a food frequency questionnaire (FFQ; α = .51) and a reduced version of the Spanish translation of Diet History Questionnaire (DHQ, National Cancer Institute; α = .83). Although these instruments might not include all possible dietary sources of bitter substances, they were meant to cover most bitter items in the Argentinian diet. Factors that are thought to influence people's dietary choices were examined with a version of the Food Choice Questionnaire in Spanish population (FCQ-SP; Jáuregui-Lobera & Bolaños Ríos, 2011; α = .88). The size and the nature of the last meal before each testing session were measured with a food record to obtain the amount of calories consumed. Caloric intake was calculated by consultation with the USDA National Nutrient Database for Standard Reference, Release 25 (December, 2011).

Bitter taste responsiveness and time-intensity measurements

To determine how responsive the subjects were to the taste of PROP (Sigma Chemical Company, St Louis, USA), three concentrations were used: 0.010, 0.032 and 0.600 mmol/L (belonging to the regular PROP series for taste detection thresholds; e.g., Drewnowski, Henderson & Shore, 1997a). All solutions were prepared in distilled water ≥ 1 day before testing. The perceived bitter sensations of the PROP solutions over time (recorded every 0.35 s) were characterized using a computerized time-intensity (T-I) software program by moving a cursor along a 500-pixel line that represented a 20 cm unstructured line scale anchored at both extremes 0 - 100 on the monitor (cf. Galmarini, Zamora & Chirife, 2009), after receiving verbal instructions: 0 = not at all bitter and 100 = extremely bitter. The software provided the T-I curve as well as the parameters that described it: maximum intensity reached (Imax; 0-100), time elapsed to maximum intensity (Tmax; in seconds), area under curve (AUC; representing the overall bitterness perception of the whole stimuli perceived over the total time of recording) and rate of increase of bitter (Rinc). The question asked was as follows: “How bitter do you find this solution now in your mouth?” The subjects also rated the bitterness of the two food solution. The rating method, question and software were the same as those for the PROP solutions.

Self-report measures of food attributes

Hedonic value (i.e., subjective pleasure) was rated on a 9-point hedonic scale with opposing extremes of liking from 1 (dislike extremely) to 9 (like extremely), and with a neutral point at 5 (neither like nor dislike), by answering the following question: "How pleasant is this food now in your mouth?" In addition, given the importance of incentive value (i.e., desire to eat) and familiarity (i.e., knowledge of and experience with the taste of stimuli) to the people's daily food and beverage choices, these attributes were examined as well. To account for this, subjects rated the incentive value and familiarity of each food stimulus using 9-point category scales, where 1 was "not at all" and 9 was "extremely". The questions were as follows: ''How much do you want to eat this food?'' and "How familiar are you with this food?" respectively.

Facial expressions to foods

A behavioural measure of taste-elicited affective reactions was provided by the analysis of the facial patterns. Facial reactions were videotaped with a digital video camera (JVC GZ-MS150SU), which was located in a hole of the booth wall, directly above the computer screen and in front of the subject at a distance of 1.5 m. The illumination of the participant's face was optimized by using daylight lamps (6500 k), in addition to the ceiling lights. The participants sat on a wooden school chair and were kept from turning their head by answering the questions and rating the bitterness of the food solution on a computer screen. The cups used were transparent so that they did not interfere with the recording. In addition, the camera had face detection technology which identified people's faces following their movements and made adjustments to achieve the optimum focus, exposure and white balance. The experimenter followed the facial expressions in real time watching the camera screen without being seen by the subjects. The video files were run through the FaceReader 4 software (Noldus Information Technology, Wageningen, The Netherlands) and processed frame-by-frame at 50 Hz, scaling the facial expressions from 0 (not present at all) to 1 (maximum intensity of the fitted model). Approximately 85% of the video frames were analyzable by the software. This software distinguished between seven facial reaction patterns or expressions (happy, sad, angry, surprised, scared, disgusted and neutral) using the Active Appearance Modelling (cf. van Kuilenburg, Wiering & den Uyl, 2005). In order to standardize the measurements and to compare the facial expressions (of different duration and latency), the ten seconds before and after tasting the food stimuli were taken for analysis. The facial analysis before tasting served as baseline. The intensity of each facial expression was calculated by subtracting the average intensity of the baseline period from the average intensity after tasting.

Procedure

Before starting the experimental session, participants completed the questionnaires and were also presented with the PROP solutions in 10-ml plastic cups and asked to rate the bitter intensity, rinsing between samples. PROP solutions were presented from lower to higher intensity in order that the receptors were not saturated. The experimental session took place in an individual booth kept at 22 ± 2 °C. The booth was equipped with a computer (Samsung NP300E4AH) and software for the presentation of the instructions and recording subjects' responses. The session lasted about 25 minutes and was subdivided into (1) a record of food eaten for the evaluation of caloric intake; (2) presentation of neutral pictures (from the Geneva Affective Picture Database; Dan-Glauser & Scherer, 2011) for a total time of 10 min in order to minimize differences in motivational state; (3) delivery of food samples and tasting; and (4) rating of hedonic, incentive, familiarity and bitter dimensions of each food sample. During the tasting stage, subjects received 5 ml of a grapefruit juice drink and a bitter chocolate drink in 10-ml plastic cups in counterbalanced order. There was a period of 120 seconds between the presentation of one sample and the presentation of the next sample. They took each sample into the mouth and tasted it using whole mouth tasting, but were instructed not to swallow the solutions (sip-and-spit technique). Subjects were told to rinse with mineral water (presented in 120-ml thermal cups) before each food sample. PROP solutions and mineral water were offered at room temperature. The experimenter was not visible to the subjects.

Data Analysis

Comparisons between BMI conditions for the eating behaviour questionnaires (FPQ, FFQ, DHQ, FCQ-SP) and caloric intake were tested using independent samples t-tests. T-I curves were first analyzed visually in order to remove the irrelevant points on the graph caused by the use of the mouse. These points corresponded to small regions of the curves with abrupt changes to very low or very high value, and were replaced by an average of the preceding and following points (Lallemand, Giboreau, Rytz & Colas, 1999; Le Berrre, Boucon, Knoop & Dijksterhuis, 2013). The data for the T-I curve for each solution was separately averaged by low and high BMI. Differences in T-I parameters for PROP and food solutions (Imax, Tmax, AUC, Rinc), self-ratings of food attributes (hedonic, incentive, familiarity) and intensity of facial expressions (angry, disgusted, happy, neutral, sad, scared, surprised) were analyzed using a two-way repeated measures ANOVAs. Independent variables included BMI (Low vs. High) and Food (Chocolate vs. Grapefruit) or PROP (0.010 vs. 0.032 vs. 0.600 mmol/L). Greenhouse-Geisser correction was used in case of violation of the assumption of Sphericity. All pairwise comparisons of individual means for effects found to be significant in the ANOVA were carried out by using Tukey's multiple comparison tests to control for Type I error. Pearson’s or Spearman’s correlations were used, when appropriated, to assess associations among taste responsiveness, hedonic ratings or facial expressions and BMI status and between hedonic ratings and facial expressions. Regression models were calculated to predict BMI using intensity of the disgust facial expression and to predict the facial expression intensity using hedonic self-report.

Results

Eating behaviour questionnaires and caloric intake

The mean values of the eating behaviour questionnaires and the caloric intake are shown in Table 1. The total scores from the FPQ, FFQ, DHQ and total caloric consumption did not differ between the BMI conditions (largest t [38] = 1.24, p = .22). Regarding FCQ-SP, analyses indicated significant differences on one of the factors (t [38] = 2.33, p < .05). Specifically, the low BMI rated sensory appeal (e.g., taste, smell or appearance) as more important in their food choices than did the high BMI group.

(Table 1 about here)

Bitter taste responsiveness and time-intensity measurements

The sum of bitter ratings for the three PROP solutions was used to assess the PROP taster status (Kaminski, Henderson & Drewnowski, 2000; Ly & Drewnowski, 2001). The participants whose summed responses were 59 or less (i.e., 10th percentile or less) were classified as non-tasters, while those with summed ratings in excess of 59 were classified as tasters. Only four participants were PROP non-taster, two with high BMI and two with low BMI. As can be seen, the average bitterness T-I curves of the three PROP solutions for the two BMI conditions over the time course of 20 sec are shown in Figure 1. The PROP concentrations were differently perceived by the subjects according to Imax ($F [2, 68] = 36.83$, $p < .001$, $\eta^2 = .52$), Tmax ($F [1.5, 52.7] = 6.67$, $p < .01$, $\eta^2 = .16$) and Rinc ($F [1.7, 57.7] = 19.25$, $p < .001$, $\eta^2 = .36$). There were no significant main effects of BMI or interactions between BMI and PROP concentration (largest $F [1.5, 52.7] = 3.33$, $p = .085$). Post hoc comparisons showed that the 0.600 mmol/L presented lower Tmax and higher Imax and Rinc values compared to 0.010 mmol/L (ps < .05); and higher Imax and Rinc than 0.032 mmol/L (ps < .05). On the other hand, the 0.032 mmol/L showed lower Tmax and higher Rinc values than 0.010 mmol/L (ps < .05). In contrast, a significant interaction between BMI and PROP concentration on AUC was found ($F [1.8, 59.9] = 6.91$, $p < .001$, $\eta^2 = .17$). This interaction revealed that only the lowest PROP concentration was perceived differently by the BMI conditions, for which the subjects with high BMI perceived 0.010 mmol/L to be bitterer than those with low BMI ($t [28.66] = -2.78$, $p < .01$). On the other hand, both BMI groups showed differences in the AUC among PROP stimuli (smallest $F [2, 34] = 5.20$, $p < .05$, $\eta^2 = .23$), with higher values in the highest PROP compared with the intermediate PROP concentration ($p < .05$).

Regarding food solutions, the average bitterness T-I curves of the chocolate and grapefruit for the two BMI conditions over the time course of 20 sec are shown in Figure 2. A visual inspection of curves showed that the solutions were perceived differently according to BMI. Concretely, all subjects with low BMI started the curves with zero or very close to zero values for the grapefruit and chocolate, whereas approximately 32% of high BMI subjects presented values higher than 30 for the chocolate. However, the statistical analyses revealed no effect of BMI or their interaction with Food (largest $F [1, 37] = 3.23$, $p = .085$) on Imax, Tmax, AUC and Rinc. There was a Food effect on Imax ($F [1, 37] = 114.06$, $p < .001$, $\eta2 =.75$), Tmax

(F [1, 37] = 156.47, $p < .001$, $\eta 2 = .81$), AUC (F [1, 37] = 77.23, $p < .001$, $\eta 2 = .68$) and Rinc (F [1, 37] = 12.25, $p < .01$, $\eta 2 = .25$), showing higher values on Imax, AUC and Rinc for chocolate compared with grapefruit. Tmax showed a lower value for chocolate compared with grapefruit.

Additionally, the question of whether BMI could be related to bitter taste responsiveness was examined. Results of the analysis showed that BMI was not correlated with the bitter taste perception of PROP concentrations or food solutions determined by the Imax, Tmax, AUC and Rinc parameters ($ps \geq .13$).

(Figure 1 about here)

(Figure 2 about here)

Self-report measures of food attributes

Ratings on the hedonic value varied between the food solutions (F [1, 38] = 69.7, $p < .001$, $\eta^2 = .65$), reflecting higher pleasure ratings for the grapefruit (rating = 6.3) that for the chocolate (rating = 2.8; which perceived as strongly unpleasant). Although foods' hedonic scores of high BMI were smaller than those of low BMI (4.1 vs. 4.9) and inspection of data revealed that 70% of the low BMI compared with scarcely 40% of the high BMI subjects evaluated grapefruit with values 7-9 on the hedonic scale or 65% of the low BMI compared with 80% of the high BMI participants evaluated chocolate with values 1-3, there was no significant effect of BMI or their interaction with Food (largest F [1, 38] = 3.00, $p = .91$). Additionally, the question of whether BMI could be related to hedonic ratings was examined. Results of the analysis showed that BMI was not associated with hedonic scores for chocolate ($r = -.077$, $p = .64$) or grapefruit ($r = -.157$, $p = .33$).

Incentive ratings varied significantly between foods (F [1, 38] = 72.59, $p < .001$, $\eta^2 = .66$) and BMI groups (F [1, 38] = 7.83, $p < 0.01$, $\eta^2 = .17$), but there was no a significant BMI x Food interaction (F [1, 38] = 1.96, $p = .17$). These effects revealed that low BMI wanted to drink more bitter foods (rating = 4.9) than high BMI (rating = 3.5), and that the desire to eat was higher for the grapefruit (rating = 5.9) than chocolate (rating = 2.5). Familiarity varied between the food solutions (F [1, 38] = 11.78, $p = .001$, $\eta^2 = .24$). There were no significant main effect of BMI or their interaction with Food (largest F [1, 38] = 2.56, $p = .12$), indicating that the grapefruit solution was rated as more familiar that the chocolate. Familiarity ratings for the both food solutions were in the moderate-to-high range (ratings > 7.5).

Facial expressions to foods

On average, the times to reach the maximum intensity of negative emotions after tasting the food stimuli were 1.67 seconds for ‘disgusted’, 2.43 seconds for ‘sad’ and 3.28 seconds for ‘angry’. The mean changes from baseline in intensity of facial expressions for BMI conditions and food solutions are shown in Figure 3 and Figure 4, respectively. Analyses on taste-elicited facial patterns revealed a main effect of Food concerning the facial expression “disgusted” (F [1, 38] = 14.47, $p < .01$, $\eta^2 = .28$), “angry” (F [1, 38] = 5.30, $p < .05$, $\eta^2 = .12$) and “ neutral” (F [1, 38] = 6.24, $p < .05$, $\eta^2 = .14$); and a main effect of BMI concerning the expressions “disgusted” (F [1, 38] = 4.90, $p = .053$, $\eta^2 = .11$) and “neutral” (F [1, 38] = 4.03, $p = .052$, $\eta^2 = .10$). No significant BMI x Food interactions were observed (largest F [1, 38] = 3.15, $p = .09$). Regarding the main effect of Food, the chocolate produced an increase in expressions “disgusted” and “angry”, and a decrease in “neutral” compared to the grapefruit.

Regarding the BMI effect, the results showed that the bitter foods elicited significantly more intense reactions of "disgusted" and strongest reduction of "neutral" in high BMI than in low BMI. As an additional check for the possibility that sensory but not affective responsiveness to bitter taste might have contributed to the observed between-group differences on facial expressions, two-way analyses of covariance (BMI x Food) were performed on both "disgusted" and "neutral" expressions using as covariate the overall bitterness perception (AUC) for the 0.010 mmol/L PROP concentration (that was significantly different across BMI groups in previous analyses). The results showed that the ANCOVA and ANOVA produced similar conclusions –with a significant effect of BMI on the expressions "disgusted" ($F [1, 35] = 3.41$, $p = .07$, $\eta^2 = .09$) and "neutral" ($F [1, 35] = 4.54$, $p < .05$, $\eta^2 = .11$. Additionally, the relationship between BMI and disgust facial expression was examined, showing a significant positive correlation for chocolate ($r = .304$, $p < .05$), though the percent of variance explained was very low, with a coefficient of determination (R^2) of .092 ($F [1, 39] = 3.87$, $p = .056$). There was no significant linear correlation between BMI and "disgusted" for grapefruit or between BMI and the neutral facial expression (for chocolate or grapefruit) and BMI ($ps > .2$).

(Figure 3 about here)

(Figure 4 about here)

Association between self-reported hedonic value and facial expressions

Correlations of hedonic ratings with the different facial expressions indicated that the hedonic scores were negatively associated with the intensity of disgust facial expression ($r = -.463$, $p < .01$) for chocolate. Results for grapefruit showed a negative relationship between the hedonic scores and the disgust facial expression ($r = -.323$, $p < .05$) and a positively correlation related to the neutral facial reactions ($r = .405$, $p < .01$). To further examine the significant associations, linear regressions analyses were performed (on primary data, but means were plotted on the graphs for each point of the hedonic scale for clarity; Figure 5). The results showed low R^2 values; $R^2 = .133$ for "disgusted" to chocolate ($F [1, 39] = 5.83$, $p < .05$); $R^2 = .055$ for "disgusted" to grapefruit ($F [1, 39] = 2.22$, $p = .14$); and $R^2 = .164$ for "neutral" to grapefruit ($F [1, 39] = 7.43$, $p < .05$). It should be noted that the point 5 of the hedonic scale, which is the neutral value to pass from liking to disliking, matched with the inflection point changing positive to negative facial expression values.

(Figure 5 about here)

Discussion

According to recent hedonic eating theories of obesity, we hypothesized that overweight individuals would be more reactive to unpleasant tasting food than lean people. Facial expression results were consistent with our hypothesis, showing that bitter-tasting stimuli (grapefruit and chocolate) elicited significantly more intense disgust reactions and less neutral state reactions in the high BMI than in the low BMI condition. Furthermore, the disgust intensity response to strong bitter (chocolate) was positively related to BMI, though the percent of variance explained was very low (≈ 10%). To our knowledge, this is the first study which has revealed a link between aversive patterns of taste reactivity and weight status. Partial support was also obtained by hedonic ratings, which showed a trend toward lower preference scores for bitter foods in high BMI; but failed to provide significant differences between BMI groups. Although the reasons for this difference are unclear, some possibilities may be suggested (see below).

One interpretation is that these different aversive reactions were related to an enhanced perception of bitter intensity in the high BMI compared with the low BMI participants. If we consider that overweight individuals had a heightened acuity for bitterness, it should not be surprising that they reflected increased dislikes for bitter-tasting foods (Drewnowski, Henderson & Shore, 1997b) and therefore a higher facial reactivity compared with their normal weight counterparts. It is well established that functional or structural differences (e.g., number of taste buds and density of taste buds per papilla) in the gustatory system may affect taste preferences and, ultimately, body weight (cf. Donaldson, Bennett, Baic & Melichar, 2009). For instance, higher BMI and higher propensity to be overweight was found in individuals with a genetically mediated ability to taste PROP (tasters) compared with nontasters (Fischer, Griffin, England & Garn, 1961; Lumeng, Cardinal, Sitto & Kannan, 2008; but see Keller, Steinmann & Nurse, 2002). This interpretation cannot be completely ruled out given the complexity of taste perception; however, it seems unlikely in view of our sensory evaluation data using time-intensity methodology. In fact, no effect of BMI status on sensory response to bitter food samples was detected when the comparison was done in terms of Imax, Tmax, AUC and Rinc. This lack of sensory difference for bitter compounds between overweight and normal weight subjects is not new, and it has been reported in adults and children (e.g., Drewnowski, Henderson & Cockroft, 2007; Goldstein, Daun & Tepper, 2007; Nasser, 2001). Responsiveness to PROP concentrations also did not differ between the BMI groups, except for a slight variation in the AUC of the low PROP concentration (0.010 mmol/L) which was higher in high BMI. Even so, after treating this parameter as a confounding factor and covariant, the observed differences in disgust and neutral facial reactions between the groups remained at least marginally significant. Therefore, the greater reactivity to affective component of taste in high BMI could not be attributed to differences in bitterness intensity alone.

That the high BMI participants expressed an enhanced sensitivity to the affective properties of taste compared with the low BMI participants is an alternative interpretation of the current data. Thus, pleasantness of taste could be considered a mediator variable of the relationship among the bitter taste perception and food selection, dietary patterns, and ultimately body weight. As pointed by Tepper, White, Koelliker, Lanzara, d'Adamo & Gasparini (2009), variations in bitterness perception may not be sufficient to alter food acceptability, since bitterness represents only one facet of the complex sensory profile of a food. It is also important to consider the role of other factors, such as hedonic processes. Our findings supported this explanation: overweight participants experienced a similar bitterness perception to those of lean participants; further, the overweight people disliked bitterness more. A number of observations seem to indicate a heightened affective response to bitter compounds in overweight individuals. For instance, Bartoshuk et al. (2006) found that the maximum disliking for the food/beverages (including dark chocolate and grapefruit juice) rose with BMI. Interestingly, a stronger hedonic response to palatable food has recently been implicated as a factor for weight gain (e.g., Salbe et al., 2004). Since reward and aversion might be mediated by overlapping neural systems and constitute an affective continuum (Umberg & Pothos, 2011), it is possible that liking of sweetness and disliking of bitterness express the same psychobiological trait in the risk for weight gain and overeating. Confirmation of this possibility might have considerable implications for nutritional, health and weight status.

The tendency to avoid bitter vegetables and fruits, which contain water, dietary fibre (Howarth, Saltzman & Roberts, 2001), human health-bioactive compounds (Drewnowski & Gomez-Carneros, 2000) and have a low fat content, could reduce satiety and increase energy (palatable) intake, body weight (cf. Rolls, Ello-Martin & Tohill, 2004) and the risk of some of the diet-related chronic diseases (Slavin & Lloyd, 2012). This tendency to avoid bitter foods was confirmed by our results; ratings of desire to eat revealed that high BMI participants wanted less to drink bitter stimuli compared with the low BMI group. Comparing the responses given with regards to the motivational factors that underlie the food choices (FCQ-SP scales), the sensory appeal was rated as more important by the low BMI than high BMI group. No differences related to mood, health and natural content, weight control, convenience, familiarity and price were observed. In contrast, reported total dietary intake of vegetables and fruits from food frequency and diet history questionnaires did not support a reduced consumption of these substances among overweight subjects. Bitter foods included grapefruit juice, spinach, kale, coleslaw, broccoli, cauliflower, brussels sprouts, as well as beer, wine, tea and coffee. A more accurate assessment of the relationship between body weight and bitter food consumption would require a study of the effects of particular fruit/vegetable intake and explore how these foods are eaten by separating them by preparation: fresh, baked, or fried; in mixtures; or with other accompaniments (Lin & Morrison, 2002).

The use of taste reactivity also provided insights of interest for the sensory and consumer evaluation. Although facial reactivity has been used in infants and adults to study the hedonic function of taste (e.g., de Wijk, Kooijman, Verhoeven, Holthuyzen & de Graaf, 2012; Steiner, Glaser, Hawilo & Berridge, 2001), it has not previously been applied to overweight adults. Similar to other studies (e.g., Danner et al., 2013), FaceReader technology was a sufficiently suitable and accurate method, in our case for differentiating between the two bitter foods: stimulus perceived as more bitter (i.e., chocolate, Imax = 69.4 vs. grapefruit, Imax = 34.3) was more strongly disliked (on the basis of the intensity of the elicited disgust and angry expressions) in both BMI groups. Moreover, although the study was not addressed to the question of whether the relationship between aversive taste sensitivity and BMI could be mediated by the energy density of foods, it should be noted that the patterns of aversive reactions were not affected by the energy content (chocolate = 28.89 vs. grapefruit = 0.39 kcal/g). As noted in Epstein, Truesdale, Wojcik, Paluch and Raynor (2003), hedonics and the reinforcing value of high-calorie foods measured by subjective ratings and behavioural observations in a taste reactivity paradigm seem to be separate processes in humans. Still, the influence of energy density on aversive taste processing currently remains unexplored to the best of our knowledge.

Regarding the validity of hedonic self-reports, we explored whether these reports reflected a genuine affective response to aversive value of foods, rather than a cognitive or motivationally determined response. As an affective measure, hedonic self-reports should be highly related to facial patterns (a well-established measure of the hedonic evaluation of taste stimuli; Berridge, 2000). Investigations have demonstrated the reliability and the validity of the nine-point hedonic scale in assessing product likes and dislikes (cf. Stone & Sidel, 2004). However, moderate associations (≈ -.40) of subjects' facial expressions of disgust to bitter foods with their hedonic ratings of these same solutions were found. This is a level generally considered acceptable, though we cannot rule out the fact that the nine-point hedonic scale measured other aspects, not only pleasure/displeasure, but also intensity of sensation, social desirability or cognitions regarding bitter foods. It can be seen that this potential bias might have blunted the differences on hedonic ratings between high and low BMI. Some other explanations of the failure of hedonic self-reports to provide significant BMI group differences can be suggested. For example that the relatively small sample size limited the ability to detect an effect; that the 9-point hedonic scales provided invalid group comparisons for bitterness because of psychophysical errors (assuming erroneously that intensity perception is the same for subjects in different BMI groups; as pointed out by Bartoshuk et al., 2006, for sweet taste in the obese vs. non-obese). Further studies are needed to confirm these possibilities. In addition, it is worth considering that these potential sources of error were not sufficient to make BMI differences in incentive motivation disappear, reporting that low BMI participants showed a stronger desire to eat the bitter stimuli than high BMI participants. It would seem that the question “How much do you want to eat this food?” is more sensitive than “How pleasant is this food now in your mouth?” for measuring differences between conditions.

Several limitations of this study should also be discussed. First, our study tested bitter perception with time-intensity methodology, a tool for fundamental research on bitterness (cf. Cliff & Heyman, 1993). Because of the complexity of the measurements, participants should be trained (Dijksterhuis & Piggott, 2000). However, the participants only had a relatively short training in order to learn how to move the mouse on the scale on the screen. It might be asked, would we have found more differences (and in which direction?) if both BMI groups had been trained? Another detail to note was the beginning of the time-intensity curves, especially for the chocolate, in which all low BMI subjects started the curves with zero values and, approximately, 32% of high BMI subjects started with values higher than 30. Would it be possible to consider that high BMI subjects can perceive bitter tastes faster? This question requires further investigation and another approach such as reaction time methodology could be used (Bonnet, Zamora, Buratti & Guirao, 1999; Guirao & Zamora, 2000). Finally, as pointed by Danner et al., (2013), it is also important to recognize that motor artefacts caused by drinking could be misinterpreted by the FaceReader as expression. In order to minimize artefacts, liquid samples which need less processing in the mouth were used.

In summary, although BMI is a complex variable for which aversive reactions explain only a small portion, hedonic (appetitive or aversive) over-responding may be one factor contributing to the susceptibility to weight gain also through avoidance of health-promoting food. Additional research is therefore needed to examine affective mechanisms that control dietary selection and food consumption, given the increasing incidence of obesity.

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Table 1. Scores of the eating behaviour questionnaires and caloric intake for the BMI groups.

Characteristic	**Low BMI**	**High BMI**
FPQ	108.3 ± 6.7	101.1 ± 6.4
FFQ	2.9 ± 0.5	3.8 ± 0.6
FCQ-SP: Health	16.4 ± 0.9	16.1 ± 1.7
FCQ-SP: Mood	11.7 ± 0.8	12.8 ± 0.9
FCQ-SP: Convenience	12.4 ± 0.8	10.8 ± 1.0
FCQ-SP: Sensory appeal	14.3 ± 0.3	12.8 ± 0.6*
FCQ-SP: Natural content	5.6 ± 0.4	6.3 ± 0.5
FCQ-SP: Price	7.0 ± 0.4	7.2 ± 0.5
FCQ-SP: Weight control	6.5 ± 0.5	6.3 ± 0.5
FCQ-SP: Familiarity	6.3 ± 0.4	5.7 ± 0.5
DHQ	48.0 ± 4.1	46.3 ± 4.8
Intake before testing session (Kcal)	470.4 ± 42.1	445.1 ± 57.9

Note. Values are means (± SEM). FPQ: food preference questionnaire; FFQ: food frequency questionnaire; FCQ-SP: Food Choice Questionnaire in Spanish population; DHQ of Diet History Questionnaire (Spanish translation). Kcal: kilocalories. *$p < .05$, significant differences for comparisons between low and high BMI conditions.

Figure Captions

Figure 1. Time-intensity curves for average bitterness obtained of the three PROP solutions (0.010, 0.032 and 0.60 mmol/L) for the two BMI conditions (low and high).

Figure 2. Time-intensity curves for average bitterness obtained of the chocolate and grapefruit solutions for the two BMI conditions (low and high).

Figure 3. Changes from baseline in intensity of facial expressions for BMI conditions (low and high). Bars express the mean changes from baseline (±SEM).

Figure 4. Changes from baseline in intensity of facial expressions for food solutions (chocolate and grapefruit). Bars express the mean changes from baseline (±SEM).

Figure 5. Regression of facial expression intensity of disgusted (A: chocolate; B: grapefruit) or neutral (C: grapefruit) against self-reported hedonic rating (1-dislike extremely to 9- like extremely).

Figure 1

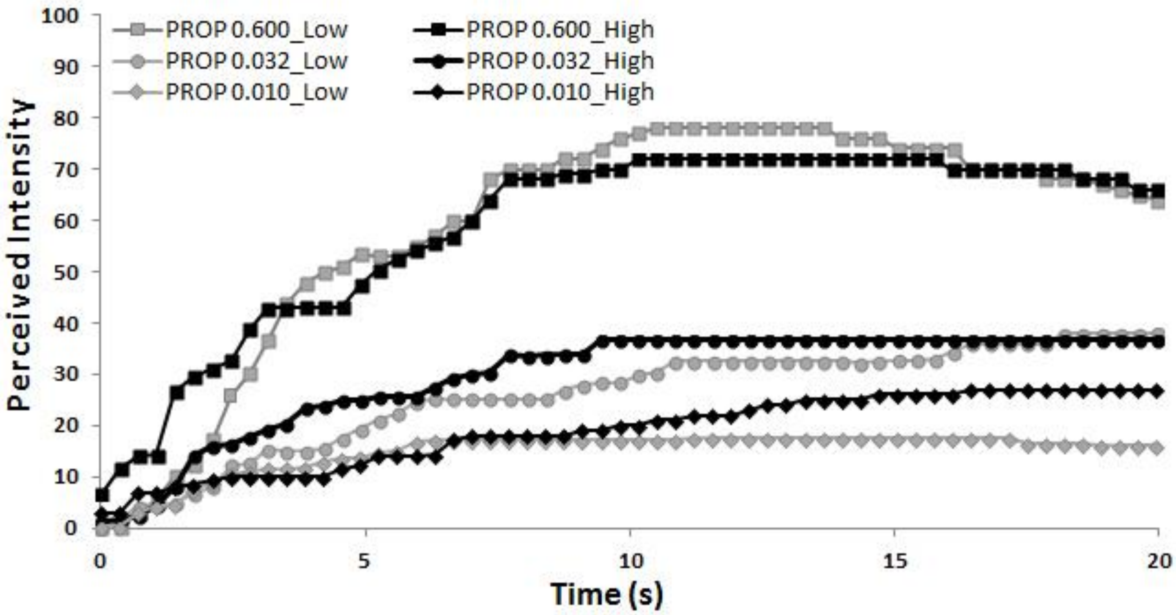

PROP 0.600_Low
PROP 0.032_Low
PROP 0.010_Low
PROP 0.600_High
PROP 0.032_High
PROP 0.010_High
Perceived Intensity
Time (s)
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20

Figure 2

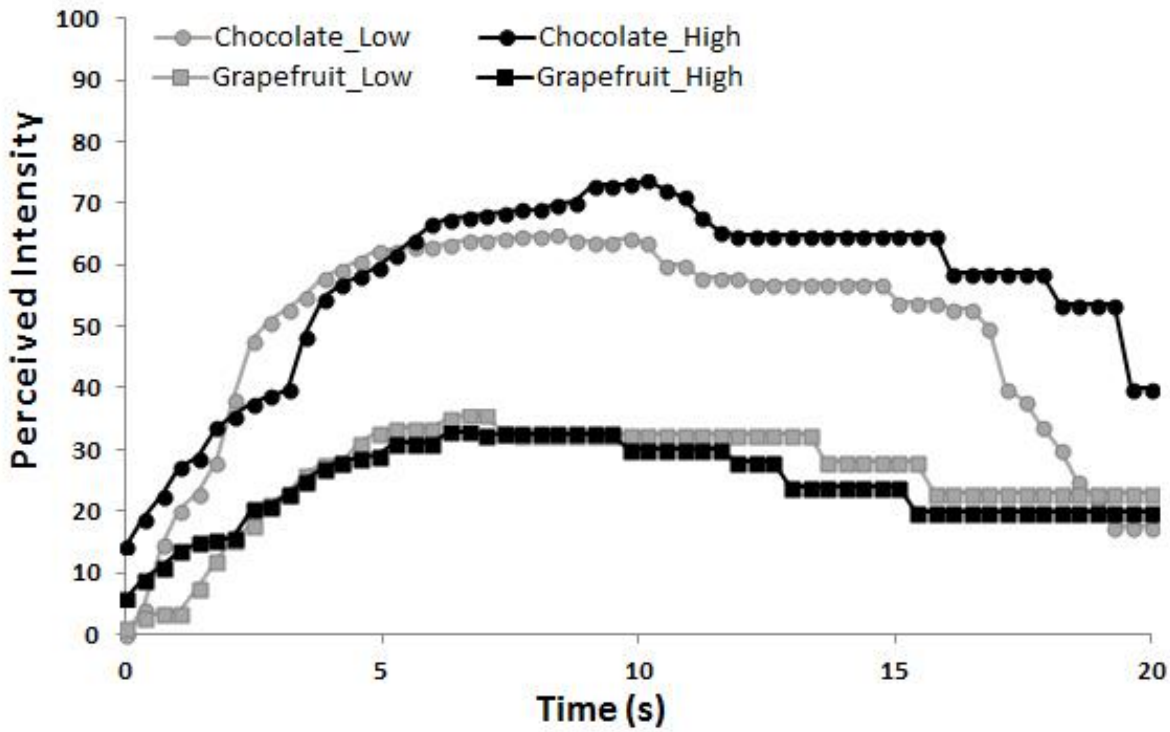
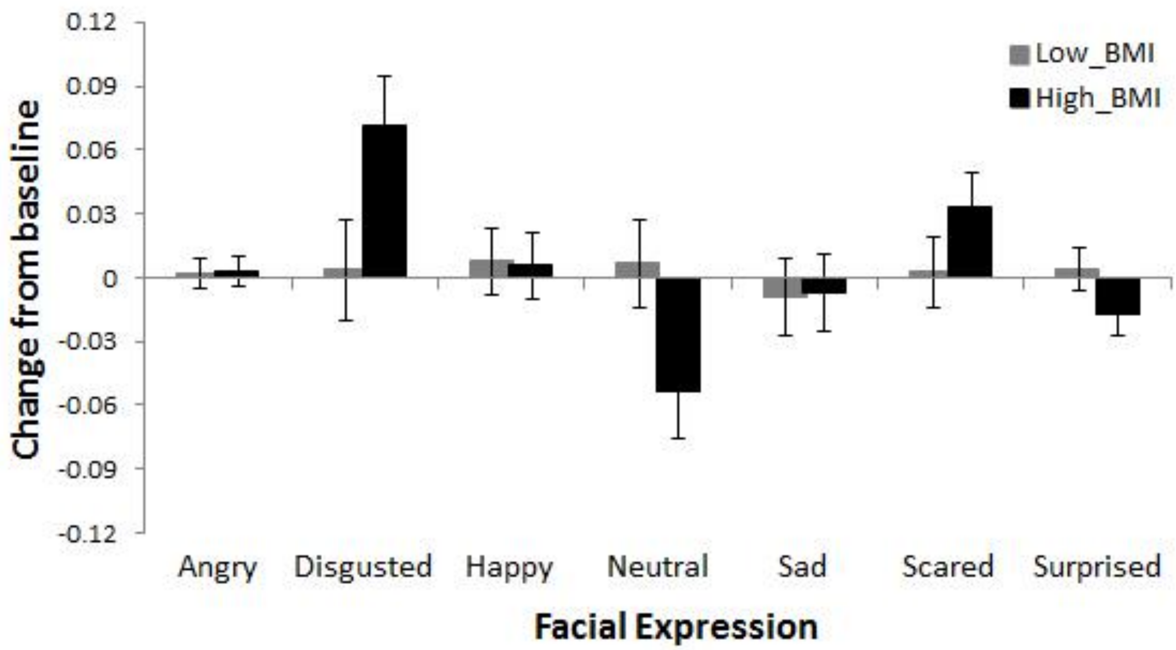

Chocolate_Low
Chocolate_High
Grapefruit_Low
Grapefruit_High
Perceived Intensity
Time (s)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20

Figure 3



0.12
0.09
0.06
0.03
0
-0.03
-0.06
-0.09
-0.12
Change from baseline
Low_BMI
High_BMI
Angry
Disgusted
Happy
Neutral
Sad
Scared
Surprised
Facial Expression

Figure 4

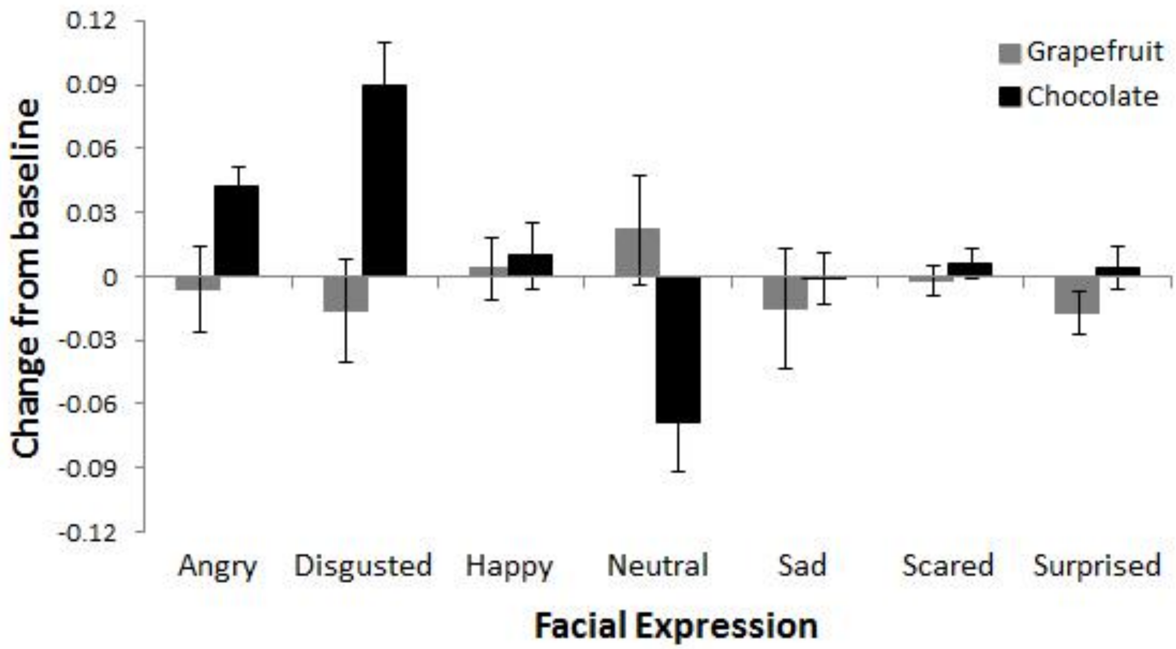
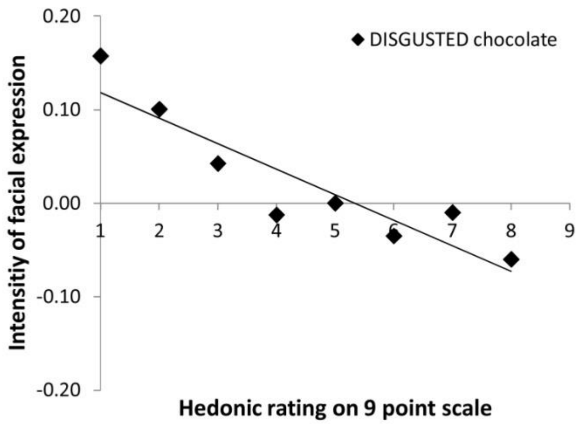

0.12
0.09
0.06
0.03
0
-0.03
-0.06
-0.09
-0.12
Change from baseline
Grapefruit
Chocolate
Angry
Disgusted
Happy
Neutral
Sad
Scared
Surprised
Facial Expression

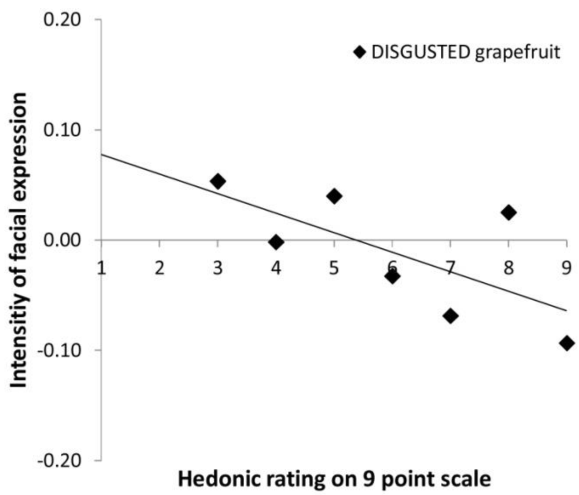
Figure 5

A)



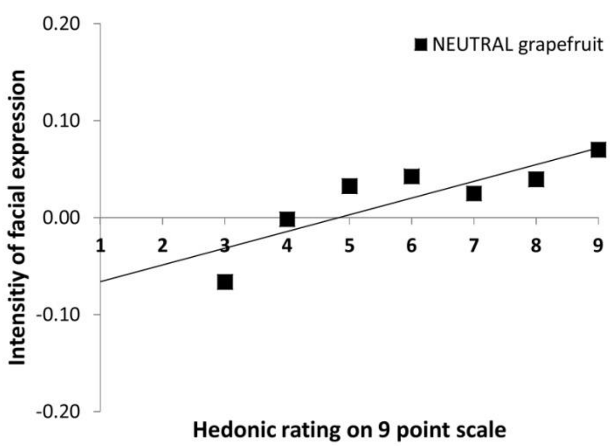
0.20
0.10
0.00
-0.10
-0.20
1
2
3
4
5
6
7
8
9
DISGUSTED chocolate
Intensitiy of facial expression
Hedonic rating on 9 point scale

B)



0.20
0.10
0.00
-0.10
-0.20
1
2
3
4
5
6
7
8
9
DISGUSTED grapefruit
Intensitiy of facial expression
Hedonic rating on 9 point scale

C)



0.20
0.10
0.00
-0.10
-0.20
1
2
3
4
5
6
7
8
9
NEUTRAL grapefruit
Intensitiy of facial expression
Hedonic rating on 9 point scale