

Investigating perception and liking of non-nutritive sweeteners in individuals representing different taste receptor genotypes

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Abstract

This study investigates whether variations in taste receptor genotypes account for differences in perception and liking of the non-nutritive sweeteners sucralose and Rebaudioside A (RebA). Single nucleotide polymorphisms (SNPs) of sweet taste receptor subunits *TAS1R2* and *TAS1R3* (8 SNPs), bitter taste receptors *TAS2R4* and *TAS2R14* (2 SNPs), and carbonic anhydrase 6 (*CA6*, *GUSTIN*) were studied. Consumer liking and perception of apple beverages varying in sucralose or RebA concentration were measured. Of the sweet receptor SNPs, *TAS1R2 rs12137730* had a significant effect on sweet perception of sucralose beverages. No sweet taste receptor SNPs had any significant effect on liking. The bitter taste receptor SNP *TAS2R4 rs2234001*, however, significantly affected bitter perception of stevia beverages; the more bitter sensitive consumers, homozygous for the GG allele, liked the RebA-sweetened drinks substantially but not significantly less than the homozygous CC group.

Introduction

Individual differences exist in liking and perception of sucrose sweetness [1]. Consumers can be classified into ‘sweet likers’ and ‘sweet dislikers’ (SLs, SDs) [2]. SDs like sweet taste at relatively high levels; their liking of sucrose solutions decreased at around 12 % (w/v), whereas SLs continued to like sucrose at 36 % (w/v) [2]. Whether hedonic phenotypes for non-nutritive sweeteners correlate with distinct genotypes is less clear. G-protein coupled receptors responding to sweet stimuli, are T1R2 and T1R3. Several SNPs in *TAS1R2* and *TAS1R3* genes have been investigated, focusing on either sweet perception or carbohydrate intake. One study of *TAS1R2* SNPs found *rs12033832* was significantly associated with sucrose taste thresholds and sugar intake, yet confounded by the body mass index (BMI) [3]. A study of *TAS1R3* found correlations between sucrose sensitivity and two SNPs, *rs307355* and *rs35744813*, where in both cases individuals carrying the T allele were less sensitive to sucrose [4]. *TAS1R3 rs35744813* has been reported to impact on a preference for sucrose concentrations [5]. Regarding diet, *TAS1R2 rs35874116* has been shown to influence carbohydrate intake [6]. Two dental studies found that *TAS1R2 rs3935570*, *rs35874116*, and *rs307355* are related to dental caries risk [7, 8]. Finally, the *CA6* gene is linked to taste cell proliferation; SNP *rs2274333* A allele carriers have been shown to have produced more taste cells [9]. There is a lack of research into genotype/phenotype associations and non-nutritive sweeteners. Sucralose is a widely used artificial sweetener, whereas steviol glycosides (SGs), such as RebA, are natural non-nutritive sweeteners obtained from the leaves of the *Stevia rebaudiana* shrub. However, SGs are also bitter due to their affinity for *TAS2R4* and *TAS2R14* receptors. SNPs *rs2234001* and *rs3741843*, of *TAS2R4* and *TAS2R14*, respectively, have been proposed to account for individual differences in bitter perception from SGs [10, 11]. This study investigates associations between receptor genotype and differences in individual liking and perception of the non-nutritive sweeteners, sucralose and stevia (RebA).

Experimental

Subjects, study design and stimuli

Participants (n=62; 11 male, 51 female, ages 18-62, non-smoking) were recruited (study number 34/16). Each participant attended two 30 min visits. In visit 1 they rated liking of beverages, had buccal samples collected and answered demographic questions. On visit 2 they rated perception of the same beverages. An Apple cordial beverage was developed containing an apple flavouring (0.017% w/v, International Flavours and Fragrances), malic acid (0.2% w/v, Sigma-Aldrich), potassium sorbate (0.02% w/v, Young's Group), sucrose (2% w/v, Silver Spoon), water (Harrogate Spa), plus the non-nutritive sweetener (sucralose, Tate and Lyle; RebA, Cargill) at varying levels (Table 1). To calculate equivalent sweetness (ES), it was estimated that sucralose and RebA were 600 and 250 times sweeter than sucrose, respectively.

Table 1: Concentration of sweetener added to apple cordial beverage models

<i>Equivalent sweetness to sucrose (% w/v)</i>	<i>Equivalent sweetness to sucrose required from sweetener</i>	<i>Sucralose (g/L)</i>	<i>Equivalent sweetness to sucrose (% w/v)</i>	<i>Equivalent sweetness to sucrose required from sweetener</i>	<i>Reb A (g/L)</i>
3	1	0.017	4	2	0.08
11	9	0.15	6	4	0.16
20	18	0.30	8	6	0.24
28	26	0.43	16	14	0.56
36	34	0.57	32	30	1.2

Sensory methods

The liking of samples was rated on a 9-point hedonic scale. The five sucralose-sweetened samples were presented first (monadic sequential presentation, balanced order, random code labelling and allocation), with a 30 s time delay to cleanse the palate (water, crackers) between samples. Following a 5 min break, the five RebA-sweetened samples were presented in the same manner. Perceived sweetness (all samples) and bitterness (RebA samples) were rated using the general Labelled Magnitude Scales (gLMS). Prior to sample rating, a gLMS practice session was performed where four food items ("salty crisps", "black coffee", "lemon", and "honey") were rated for their respective tastes (by recall). Testing was carried out in individual booths with artificial daylight at 23°C. Data were collected using Compusense at-hand software (Canada).

Genotyping

Two replicate buccal swab samples were collected per participant by rubbing a sterile swab along the inside of the cheek for 1 min. Swab heads were placed into individual tubes with Isohelix Dri-capsules and stored in a dry place at ambient temperature. Samples were sent to iDNA Genetics Ltd (Norwich, UK) for genotyping.

Statistical analysis

Analysis was carried out within the individual sweetener sample set. To avoid scale bias, sweet and bitter perception data were normalised using the gLMS practice data. ANOVA was used to investigate liking and taste perception depending on the sweetener concentration. Agglomerative Hierarchical Clustering (AHC) of liking data used dissimilarity (Euclidean distance) and agglomeration by Ward's method. A chi-squared test of independence determined associations between receptor genotypes. Due to the high number of significant associations, subsequent analysis by ANOVA was performed

for each SNP independently. Liking and taste perception were reanalysed fitting sample, genotype and interaction. Using Bonferroni correction for multiple testing, a significance of the main effect was assumed at $p < 0.001$. Trends at $p < 0.05$ were discussed due to the small sample size. Multiple pairwise comparisons used Tukey HSD ($p < 0.05$). XLSTAT software (Paris, France) was used for all statistical data analysis. Error bars on all figures represent standard error of the mean.

Results and discussion

Population genotype

Genotypes for the receptor SNPs examined are given in Table 2. Proportion of participants with the minor allele types was similar to those reported in the literature, except for CA6 *rs2274333*, where the proportion of the GG genotype was much lower (5%) as compared to previous literature (21%) [9].

Table 2: Distribution of receptor genotypes within the study population

Cat.	Receptor Gene	SNP	Allele Frequency	Homozy. wild type n (%)	Heterozy. type n (%)	Homozy. polymorphic type n (%)
s	TAS1R2	<i>rs35874116</i>	T > C	36 (58)	23 (37)	3 (5)
s	TAS1R2	<i>rs12033832</i>	G > A	27 (43)	29 (47)	6 (10)
s	TAS1R2	<i>rs12137730</i>	A > C	30 (48)	25 (40)	7 (12)
s	TAS1R2	<i>rs4920566</i>	G > A	16 (26)	32 (52)	14 (22)
s	TAS1R2	<i>rs3935570</i>	G > T	31 (50)	30 (48)	1 (2)
s	TAS1R2	<i>rs4920564</i>	G > T	32 (52)	24 (39)	6 (9)
s	TAS1R3	<i>rs307355</i>	C > T	42 (68)	14 (23)	6 (9)
s	TAS1R3	<i>rs35744813</i>	C > T	46 (74)	10 (16)	6 (10)
g	CA6	<i>rs2274333</i>	A > G	33 (53)	26 (42)	3 (5)
b	TAS2R4	<i>rs2234001</i>	C > G	23 (37)	25 (40)	14 (23)
b	TAS2R14	<i>rs3741843</i>	A > G	45 (72)	11 (18)	6 (10)

*Cat. = category; s = sweet, g = gustin, b = bitter

Influence of sweet stimuli and genotype on the sweet perception

Sweetness increased with increasing sweetener concentration as expected. Fig. 1a demonstrates psychophysical relationship between perceived sweetness against stimuli concentration (log-log plot). As samples contained two different types of sweetener, and in all cases 2 % sucrose was included, the stimulus concentration is represented as ES. In the case of sucralose, the relationship for sweetness approximated a decelerating relationship (exponent 0.7), whereas for stevia the relationship is close to proportional (exponent 0.9). There was no effect of CA6 *rs2274333* on sweetness perception (data not shown). Of the 8 type-1 receptor SNPs investigated, there was only one significant association between sweetness perception of sucralose which was for TAS1R2 SNP *rs12137730* ($p = 0.0001$) (Fig. 1b), with a tendency for an effect of *rs35874116* ($p = 0.011$) (data not shown). Of these two SNPs, TAS1R2 SNP *rs12137730* also had a tendency for association with sweetness perception of stevia ($p = 0.005$) (Fig. 1c). However, there was no clear link to the wild or minor allele (Fig 1b-c); consumers with the AC genotype rated sweetness higher than either homozygous group for both sweetener types. This result should be treated with caution as the CC group size was small ($n = 7$). In the case of

TAS1R2 rs35874116, there was a tendency for the TT homozygotes to rate sweetness from sucralose higher than the CC homozygotes, but this effect was not replicated for stevia, and the CC group was extremely small (n=3) (data not shown). Neither of these two SNPs influenced liking.

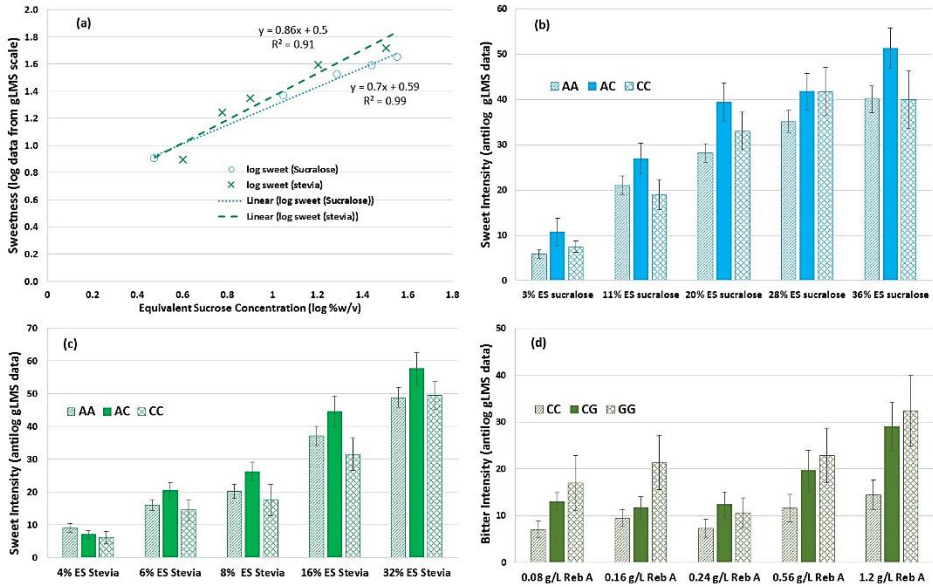


Figure 1: (a) Psychophysical relationship between perceived sweetness (log gLMS data) and equivalent sucrose concentration (log %w/v), (b) Sweet perception of sucralose sweetness according to *TAS1R2 r12137730* genotype, (c) Sweet perception of stevia sweetness according to *TAS1R2 r12137730* genotype, (d) Bitter perception of stevia beverages according to *TAS2R4 rs2234001* genotype. (ES = equivalent sweetness)

Influence on genotype on bitter perception of Stevia

In addition to sweet taste, RebA imparts bitter taste and liquorice flavour [10]. Previous studies have shown that bitterness becomes noticeable above 1000 μ M [10], which is equivalent to 0.97g/L, between samples 4 and 5 in the present study. The relationship between bitterness and Reb A concentration was far less than proportional (exponent 0.28 on log-log plot, data not shown), and indeed the bitterness perceived was very low until 0.56 g/L. In the present study, there was no relationship between *TAS2R14 rs3741843* and RebA bitter perception; however the influence of *TAS2R4 rs2234001* was significant ($p < 0.0001$), where the homozygous GG group (n=14) rated bitterness significantly higher than the CC group ($p < 0.0001$) (Fig. 1d), as expected from previous literature [10]. In addition, the CA6 SNP *rs2274333* demonstrated a relationship that was close to significance ($p = 0.003$; data not shown); the homozygous GG group tended to rate bitterness lower than the other two groups, however, there were extremely few GG consumers (n=3). Although *TAS1R3 rs307355* and *rs35744813* did not influence sweet perception, there was a trend for an effect on bitter perception ($p = 0.004$ and $p = 0.003$, respectively; data not shown). For both SNPs the homozygous polymorphic type (TT) rated bitterness lower than the wild type CC groups ($p = 0.005$; data not shown) however, there were only 6 TT participants for each of these SNPs. These SNPs were not associated with the type 2 bitter receptor genotypes tested, therefore, there is no clear hypothesis for this trend.

Influence on sweet stimuli and genotype on liking of Apple Cordial Samples

Table 3 demonstrates liking of the apple beverages across the study population. With both sweetener types the mean liking increased from the first to second concentration, plateaued from the second to fourth sample, and decreased at the highest sweetener concentration. The sweetness varied from an ES of 3 to 36 % (w/v) sucrose.

Table 3: Mean liking of apple beverages sweetened with varying levels of sucralose or rebaudioside A (with 2% sucrose w/v). (S1 to S5 = samples 1 to 5). ^{abc}Values without the same letter significantly different ($p < 0.05$)

	S1	S2	S3	S4	S5	
Sucralose (g/L)	0.017	0.15	0.3	0.43	0.57	
Reb A (g/L)	0.08	0.16	0.24	0.56	1.2	Significance (p)
Sucralose	4.3 ^a	6.3 ^c	6.2 ^c	6.1 ^{bc}	5.3 ^b	<0.0001
RebA	4.4 ^{ab}	5.6 ^c	5.6 ^c	5.0 ^{bc}	3.7 ^a	<0.0001

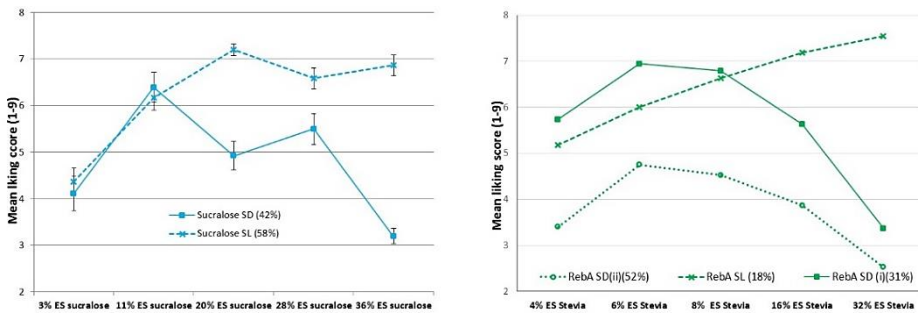


Figure 2: (a) Liking of sucralose beverages by consumers clustered into two distinct liking groups, (b) Liking of RebA beverages by consumers clustered into three distinct liking groups. (ES = equivalent sweetness, SD = sweet liker, SL = sweet liker)

AHC revealed two liking clusters for sucralose beverages (Fig. 2a): for the larger cluster (58%), sucralose SLs, liking reached a maximum at an ES of 19.5%, which was then maintained. The sucralose SDs reached maximum liking at 11% ES, above which liking decreased. For RebA there were 3 clusters (Fig. 2b): there was an outright RebA SL group (18%), where liking increased with increasing RebA, however, there were two SD groups. The first SDs(i) (31%) showed a similar pattern of liking to the SLs up to an ES of 6-8%, above which their liking ratings decreased. The second SD(ii) group (52%) rated their liking at all levels of RebA lower than the other 2 groups, and again their liking for the RebA sweetened beverages decreased when ES above 8%. Considering the sweet perception of the sweet liking groups, there was no significant difference between the sucralose sweet perception between the 2 clusters ($p=0.07$). However, there was a significant difference in sweet perception for the stevia sweet liking clusters ($p=0.006$) where the SLs had lower sweet perception than both the SD(i) and SD(ii) groups ($p=0.002$ and 0.006 , respectively). In addition, there was a difference in bitterness perception between these groups, where the participants that particularly disliked RebA beverages (SD(ii)) had significantly higher bitter ratings than the SD(i) group, that only disliked the higher RebA levels ($p=0.008$).

None of the sweet receptor SNPs, nor the CA6 SNP, had any significant effect on liking of either sucralose or RebA sweetened beverages at $p < 0.001$. However, for RebA there were trends for two *TAS1R2* SNPs (*rs4920566* $p=0.01$; *rs12033832* $p=0.04$), the 2

TAS1R3 SNPs (*rs307355* $p=0.01$, *rs35744813* $p=0.008$) and the *TAS2R4* *rs2234001* ($p=0.004$). The more bitter sensitive *TAS2R4* *rs2234001* GG group liked the ReBA sweetened drinks substantially less than the homozygous CC group ($p=0.003$). However, for *TAS1R3* *rs307355* and *rs35744813*, the CC groups which rated bitterness higher had a tendency to give higher mean liking scores which cannot readily be explained. For *TAS1R2* *rs4920566* the trend in liking was attributed clearly to the minor allele, as the heterozygotes rated liking higher than either homozygous group. For *TAS1R2* *rs12033832* the homozygotes with the minor allele (AA) rated liking higher for stevia beverages. Although they did not differ here in sweet perception, a previous study [3] found the AA group of normal BMI to have higher taste thresholds for sucrose.

In conclusion, consumers varied in their liking for sweetness of sucralose and ReBA, as previously shown for sucrose. Such differences in liking were not associated with differences in their perception of sucralose. However, for stevia-sweetened beverages our study revealed that those participants with a higher liking had a lower sweet perception, and those that particularly disliked these beverages found them to be more bitter. There were a number of trends for the receptor genotypes tested to influence perception and liking of the apple beverages, however, there were only two significant differences at $p<0.001$: *TAS1R2* *rs12137730* had a significant effect on the sweet perception of the sucralose beverages, and *TAS2R4* *rs2234001* had a significant effect on the bitter perception of the stevia beverages. To reduce free sugar intake, beverage manufacturers are replacing sugar with non-nutritive sweeteners. The findings of this study may help to explain why consumers differ in their sensorial appreciation of non-nutritive sweeteners. 23% of our study sample were of the *TAS2R4* *rs2234001* GG genotype, suggesting that a substantial proportion of the population may find ReBA to be too bitter, which may influence their beverage choice.

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