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Original Paper

Effect of ageing process on fungiform papillae density

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Ageing is accompanied by physiological changes in the human oral cavity. These include potential reduction of the fungiform papillae (FP) density which has been associated with impaired taste acuity. Because the studies have demonstrated either contrary or non-significant evidences, we decided to verify the hypothesis about FP reduction and ageing. Total of 150 human living subjects aged 19–87 years participated in the study. The tongues were stained with blue food dye, quantification area with 10 mm diameter was defined, and images of the tongues were captured with digital camera. The number of FP has been scored on each photo by five individual scorers according to a decision tree. The scoring was accomplished in ImageJ open source program using a cell counter plugin. The mean FP density value within all subjects was 18.02 ±15.95 FP per cm^{-2}, the lowest and the highest values were 0.25 ±0.57 FP per cm^{-2} and 65.00 ±1.66 FP per cm^{-2} of the tongue tip, respectively. Based on age, the subjects were divided into categories with 6.0 increasing step, in order access effect of ageing on FP densities. Age categories were significantly different in mean FP densities as determined by Welch's F (8, 22.04) = 28.81, P <0.01). The Games-Howell *post hoc* test revealed that participants aged 18–24 years had significantly higher FP densities than subjects aged more than 48 years. High degree of intra-groups variance was observed, which could reflect in non-significant differences between the other age groups. Nevertheless, the results were sufficient to support the hypothesis that FP quantity diminish with age.

Keywords: fungiform papillae, density, tongue, ageing

1 Introduction

The dorsal anterior tongue in humans is rich in fungiform papillae (FP), which play an important role in taste functioning and oral sensation. Most of the FP contains at least one taste bud comprising of the taste cells (Zhang et al., 2008; Fischer et al., 2013). Each taste bud has single apical pore through which the taste cells project microvilli into the oral cavity. A cell membrane of each microvillus possesses multiple taste receptors providing a surface for the reception of taste stimuli and transduction of chemical into electrical signals (Chandrashekar et al., 2006; Cvijanovic et al., 2015).

The FP density commonly varies across the anterior tongue. In general, the highest values for healthy participants were recorded near the tip. Since 99% of papillae in humans contain at least 1 taste bud, papillae density is related to taste sensitivity. The subjects who have higher numbers of papillae are being more sensitive to taste stimuli (Correa et al., 2013; Fischer et al., 2013; Zhang et al., 2008; Just et al., 2006).

Up to now, it is not clear what factors are associated with the FP number or with possible changes in FP density over time (Fischer et al., 2013). In the past, it was suggested that higher density of FP may explain biological background of PROP super-tasting ability (Shahbake et al., 2005). However, the newest evaluating models revealed that predictors of taste sensitivity to PROP are age, sex, and haplotype rather than FP density (Garneau et al., 2014).

By contrast, some conditions such as degeneration or loss of taste following neural damage are accompanied by reduced density of FP (Zhang et al., 2008). The patients with transected chorda tympani nerve, during middle ear surgery because of cholesteatoma, exhibited a significant decrease of taste function and FP density on the respective side of the tongue (Just et al., 2006).

Keratinisation of FP, decreased capillary vessels and distortion of filiform papillae has been reported for heavy smokers, but there was no significant difference in taste sensitivity and FP density, compared to non-smokers (Konstantinidis et al., 2010). Moreover, the FP density is related to alcohol consuming. Heavy alcohol drinkers exhibit lower densities, compared to non-heavy ones Fischer et al., 2013).

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Finally, a relationship between FP reduction and ageing process has also been examined. Some studies have not reported either significant association or gender differences (Zuniga et al. 1993; Just et al., 2006; Zhang et al. 2008), but others have done (Segovia et al. 2002; Pavlidis et al. 2012; Correa et al. 2013; Fischer et al. 2013). The mechanisms that cause age-associated declines in the peripheral taste structures remain mostly unclear (Feng, Huang and Wang, 2013). This issue is the serious one accounting for taste disorders in elderly, which may alter appetite, lead to malnutrition and be concerned with morbidity and mortality of elderly persons (Imoscopi et al., 2012; Ikeda et al., 2007).

Since contrary evidences have been published, we decided to examine the hypothesis whether ageing is associated with reduction of FP density.

2 Material and methods

2.1 Subjects

The number of participants was 150 (44 men and 106 women), aged from 19 to 87 years (mean age: 50.27 ±22.40 years). Totally, 21 participants were smokers. Besides, 80 of them were being treated on some diseases (the most frequently on cardiovascular diseases and diabetes) when the study was conducted. Written consent was obtained from each participant once he had read an information sheet describing the purpose of the study.

2.2 Quantification of fungiform papillae

Acquisition of FP data was done following a protocol of Nuessle et al. (2015). Using a macro setting, the photos were captured with digital camera Canon EOS 550D attached to a tripod. The number of FP has been scored on each photo by five individual scorers according to a decision tree. The scoring was accomplished in ImageJ open source program using a cell counter plugin. Thus, 5 different values for each subject were obtained, each of which presented the number of FP per 10 mm diameter stained section of the tongue tip. The values were recalculated in order that final mean FP density value corresponded to 1 cm^2 area of the tongue tip.

2.3 Statistical analysis

Descriptive statistics was applied on individual mean FP density values and results were presented graphically in box-and-whisker plot. Based on FP densities (dependent variable), the participants were divided into 12 age categories (independent variable) with the 6.00 increasing step. Totally, 2 categories (C: 30–36, D: 36–42) were discarded because there was lack of the participants. A Shapiro-Wilk test was used to verify normal distribution of dependent variable for each category of independent variable. Extreme outliers were detected by Grubbs' test and then removed. Subsequently, Levene's test was performed to compare equality of variances. Because the variance and the size of depended variable for each category of independent variable were not equal, a Welch ANOVA was performed in order to determine whether age-categorised participants differ significantly each other by FP densities. Finally, a Games-Howell *post hoc* test was conducted to identify any significantly different pairs of groups. A significance level in all tests was set to 0.05. The statistical analysis, including graphical presentations, was performed using the XLSTAT (Addinsoft, 2016) package program.

3 Results and discussion

Concerning the morphology, a majority of FP had mushroom-like appearance. They were elevated, light-stained structures with either oval or round shape. In addition, some too small and either amorphous or recessed FP were also recognised across all tongues. The mean FP density and SD value within all subjects was 18.02 ±15.95 FP per cm^{-2}, indicating high inter-individual variability. The lowest and the highest values were 0.25 ±0.57 FP per cm^{-2} and 65.20 ±1.66 FP per cm^{-2}. The most of the subjects (i.e. 48%) was aged 18–24 years (age category A), with mean FP density value of 35.90 ±13.08 FP per cm^{-2} of the tongue tip. By contrast, the lowest frequencies (bellow 5 participants) were observed for B, E and F age intervals. Interestingly, the oldest participants (aged 85 and more years) had above 9-times lower mean FP density than subjects aged 18–24 years. It was suggested mean FP density values tend to show descending order across age categories (Figure 1, Table 1).

There was statistically significant difference between the means of groups as determined by Welch's F (8, 22.04) = 28.81, P <0.01). The Games-Howell *post hoc* test revealed that participants aged 18–24 years had significantly higher FP densities than subjects aged 48 years and more. These are the only significant differences observed. However, some p-values suggest there is almost provable significant difference between particular categories (Table 2). It has been assumed either low number of subjects or high inter-individual variability may have been the causation of inability to demonstrate the statistically significant differences between other age categories. Similarly, Shahbake et al. (2005) and Zhang et al. (2008) quantified FP in young adults. The mean age of participants in these studies was about equal whereas the number of them was different. The first study reported for 30 adults (aged 20–24 years) an average of 156 ±5.80 FP per cm^{-2}. On the contrary, 182 subjects (aged 18–23 years) had an average of 96.96

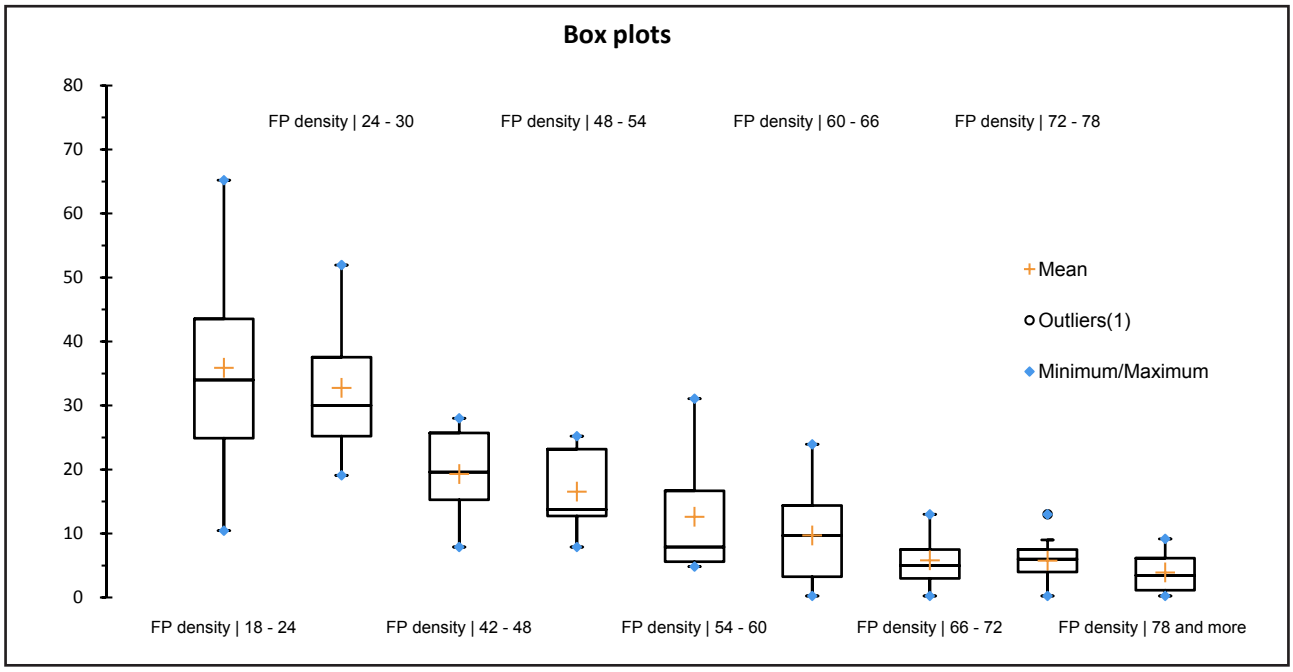

Figure 1 Center lines show the median, red crosses show mean, box limits indicate the 1st and 3rd quartiles, whiskers (terminated by blue rhombuses) extend 1.5 times the interquartile range from the 1st and 3rd quartilesn

±3.06 FP per cm^{-2} in the second study. Thus, quantity of subjects and inter-individual variability seems to be relevant factors.

Several studies have investigated the age- and lifestyle-related changes in FP density and consequent impact on taste acuity. For instance, Segovia et al. (2002) found out children have significantly higher FP densities, much higher taste pore densities and rounder FP than adults, despite of the fact they have smaller FP and taste pore diameters.

In addition, Correa et al. (2013) conducted study with 30 adults and 85 children (7–12 year olds). Statistical analysis revealed significant differences in the total number of FP in the anterior tongue among age groups, but not between sex ones. Post hoc tests have shown that only 7–8 year old children had significantly more papillae than adults. Because there were no significant differences between other groups, the finding suggested that number of FP stabilised by 11–12 years of age, which is very close to the age that cessation of growth of the anterior tongue occurs.

Moreover, in the study with 156 non-smokers (74 males, 82 females), aged 10–80 years, the density of FP at the tip of the tongue decreased significantly in men aged >50 years and in women aged >60 years compared with younger individuals. The mean FP density value reported for elderly individuals was similar to that one in our study. Moreover, ageing was also significantly associated

Table1 An overview of age categories – mean and SD of age and FP values

Age category	Endpoints	Frequency of subjects	Mean age	Mean FP density
A	18–24	48	20.88 ± 0.82	35.90 ± 13.08
B	24–30	4	25.25 ± 0.96	32.76 ± 13.97
E	42–48	5	46.00 ± 0.71	19.30 ± 8.12
F	48–54	5	50.60 ± 1.82	16.55 ± 7.35
G	54–60	7	57.43 ± 1.27	12.62 ± 9.67
H	60–66	32	63.03 ± 1.69	9.70 ± 6.81
I	66–72	23	68.43 ± 1.53	5.82 ± 3.28
J	72–78	15	74.00 ± 1.48	5.77 ± 3.39
K	78 and more	7	82.86 ± 2.79	3.92 ± 3.42

Table 2 *p*-values of pairwise differences. Values in bold indicate significant difference

	18–24	24–30	42–48	48–54	54–60	60–66	66–72	72–78	78 and more
18–24	1.00								
24–30	1.00	1.00							
42–48	0.07	0.73	1.00						
48–54	0.02	0.56	1.00	1.00					
54–60	<0.01	0.38	0.91	0.99	1.00				
60–66	<0.01	0.26	0.39	0.61	1.00	1.00			
66–72	<0.01	0.19	0.16	0.23	0.67	0.13	1.00		
72–78	<0.01	0.18	0.15	0.22	0.67	0.20	1.00	1.00	
78 and more	<0.01	0.16	0.10	0.14	0.46	0.08	0.90	0.95	1.00

with higher electrogustomery thresholds. Both sexes aged >60 years have also shown significantly decreased vascular density and worsened morphology of vessels. Influencing the taste function in elderly subjects, all these factors were suggested to be relevant (Pavlidis et al., 2012).

Finally, Fischer et al. (2013) in a comprehensive study with 2371 participants (females = 1,263, males = 1,108), aged 21–84 years, found out significantly inverse linear relationship between FP density and age. Females had a significantly greater FP density than men. Papillary density was found to be significantly lower in former and current smokers compared with never smokers. A significant dose-response relationship between level of consumed alcohol and papillary density was observed among those who consumed alcohol in the past year. Both smoking and alcohol consumption were termed as modifiable factors contributing to overall taste quality.

4 Conclusions

The results from this study supported the hypothesis FP densities decrease during ageing. Due to the fact that individual FP densities extremely varied in particular age categories, it was not possible to demonstrate statistically significant difference between such groups. Nevertheless, the data were sufficient for the purpose of the study. In addition to high intra-groups variance, there was also lack of participants in some age groups. All these factors should be considered in designs of future studies.

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