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Relationships amongst phenylthio-carbamide sensitivity, body composition, coffee and tea consumption

Keywords: taste perception, single nucleotide polymorphism, electric impedance, Body Mass Index, food preferences

1. SUMMARY

Polymorphisms of TAS2R38 gene responsible for bitter taste perception elicit a bimodal receptor response in the population upon the detection of phenylthiocarbamide and 6-n-propylthiouracil, respectively. Genetic differences in sensitivity to phenylthiocarbamide and 6-n-propylthiouracil may affect body composition, food preferences, and frequency of consuming different food types. To date, no publication has been published in Hungary on the joint study of these factors.

The aim of the present research is to find correlations between phenylthiocarbamide taster status and body composition, and the frequency of consumption of different bitter-tasting foods.

In the study, a taster status survey of participants (n = 170), a bioimpedance-based body composition analysis (n = 96) and completed a food frequency questionnaire of bitter foods (n = 170) were conducted.

Descriptive statistical methods, cross-tabulation analysis, multiple correspondence analysis, and Mann-Whitney test were used for data analysis at 5% significance level.

The proportions of the taster and non-taster categories proved to be the same as reported by international literature (70%/30% respectively). There were no significant correlations among taster status and the other examined parameters, however, based on the multiple correspondence analysis, the observed trends are in accordance with the international literature. There were significant correlations among gender, body composition and some variables describing food preference.

Based on the literature data and our own results, there can be a relationship between genotype and body composition, and genotype and food choice. Further analyses with large-sample size and representative research are needed to substantiate these assumptions.

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Abbreviations:

PTC: phenylthiocarbamide; PROP: propylthiouracil; SNP: Single Nucleotide Polymorphism; GPCR: G Protein Coupled Receptor; PAV: Proline-Alanine-Valine; AVI: Alanine-Valine-Isoleucine; AAI: Alanine-Alanine-Isoleucine; PAI: Proline-Alanine-Isoleucine; PVI: Proline-Valine-Isoleucine; AAV: Alanine-Alanine-Valine; FFQ: Food Frequency Questionnaire; BIA: Bioelectrical Impedance Analysis; BMI: Body Mass Index; PBF: Percentage of Body Fat; VFA: Visceral Fat Area; MCA: Multiple Correspondence Analysis.

2. Introduction

Humans perceive their environment and its relation to them through their sense organs and senses. Five major senses are distinguished: sight, hearing, touch, olfaction, and gustation. There are further channels of sensations also known, e.g. balance, hunger, thirst, pain or discomfort [1]. Perception of taste and flavours are related to the oral and nasal area, including the sense of smell and the trigeminal sensation through the chemosensory system. It belongs to the chemical senses, and it focuses on the perception of the chemicals in our environment. Taste receptors detect the chemicals in the consumed food, which are generally called tastants. These are usually water-soluble molecules, which provide information on the quality and safety of food [2].

Taste perception is a direct contact process, which takes place in the oral cavity. The receptors can be found on the surface of the tongue, in the pharynx, on the palate and in the upper part of the oesophagus. Receptors are organized in the taste buds, which are located in the taste papillae. The sensory information is transferred through the VII; IX. and X. cranial nerves, then the brainstem's and the thalamus' nuclei and finally arrives to the frontal operculum and the gustatory cortex of the insula. These areas and the nuclei of tractus solitarius in the brainstem are linked with the hypothalamus and the amygdala, thus probably influencing hunger and satiety, homeostatic reactions to eating and any emotions linked to eating [2, 3].

Bitter taste often triggers a rejection, which is an innate human reaction. On one hand, this aversive reaction is due to the fact, that many bitter tasting compounds (secondary plant metabolites, e.g. alkaloids, some inorganic and synthetic compounds, and in case of food the rancid fat) are toxic, thus consuming them might be harmful, or life-threatening [4].

On the other hand, several bitter tasting compounds are known, which have beneficial effect from the pharmacological or nutritional point of view. These compounds are for example the glucosinolates and their decomposition products, the isocyanates, which are found in cabbage, broccoli or brussels sprouts (all belong to the *Brassicaceae* family). Coffee, tea, and cocoa contains methylated xanthine derivatives, like caffeine, theophylline, and theobromine; in beers we find the alpha-acids, which originate from the hop and are mainly responsible for the bitter taste. In case of the vegetable species, the bitter taste note might trigger rejection, in the case of the latter products; bitterness is an expected part of their sensory character [5, 6, 7].

In the field of taste perception, five basic tastes are distinguished: sweet, salty, sour, bitter and umami. This last one was accepted as a basic taste following the discovery of its specialized taste receptor in 2002 [8]. Among the five basic quality, the detection of bitter taste is the most complex; the TAS2R gene family, which consists of 25 functional genes, performs its regulation. These genes are coding the TAS2Rs receptors, which structurally bind to given bitter taste compounds (ligands), however in case of several receptors, their ligands is not identified yet [7].

Phenylthiocarbamide (PTC, also known as 1-phenylthiourea) and the 6-n-propylthiouracil (PROP) are colourless or white, crystalline, bitter tasting organic compounds: both have sulphur containing (SCN) functional group. Their use is different: phenylthiocarbamide is used as an industrial additive, colorant, while the propylthiouracil is applied as an antithyroid agent in case of hyperthyroidism [9, 10]. The structure of PTC and PROP shown in the **figure 1**.

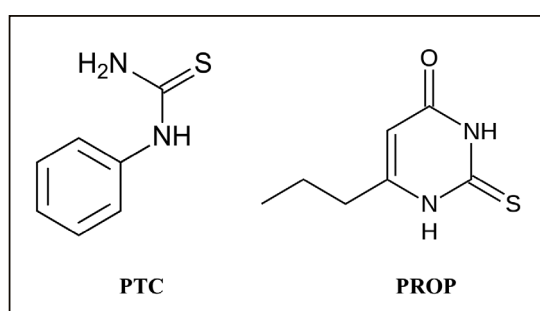


Figure 1. The chemical structure of phenyl-thiocarbamide (PTC) and 6-n-propylthiouracil

Peculiarity of these two compounds, that they trigger a bimodal reaction in humans: a part of the population is able to perceive their bitter taste, while others not. Its discovery is linked to the chemist Arthur Fox. In 1931, Fox working in a laboratory of the DuPont chemical company accidentally released some fine crystalline PTC to the atmosphere of the room. A colleague working nearby complained on perceiving bitter taste. Fox did not perceive any bitter taste, despite the fact that he directly contacted the fine dust. After this occasion, he tested his family and friends, and categorized the individuals as ‘taster’ or ‘non-taster’. Laurence Hasbrouck Synder geneticist, who identified that the inheritance of the non-taster status is a recessive phenomenon according to the Mendelian genetics [11], strengthened his results.

In the 1960’s the issue of changing of PTC to PROP has risen, because of the strong, sulphuric odour of PTC. In the 1980’s toxicological information also questioned the use of PTC, so researchers started to work with PROP after the comparison of the two compounds and measuring the threshold concentration of PROP [12].

Bartoshuk and co-workers discovered in 1991, that the non-taster group gives relatively homogenous responses, while the reaction of tasters were much more different, and one of their subgroups perceived the bitter taste of PROP much more intensively. Individuals, belonging to that subgroup were called *supertasters*. The supertaster status is not influenced by the genotype responsible for the taster status, but this discovery resulted in a third type of classification label, *medium taster* [13].

Taster status is defined by some variations of the genetic domain; in this case the single nucleotide polymorphisms (SNP). SNP’s are DNA sequence variations that affect one nucleotide, which are identified between the genetic domains of two individuals belonging to the same species. Each human genome has a unique SNP pattern, but these changes might be called SNP, if they show up at least in 1% of the total population. SNP’s are usually the results of errors during the DNA replication, or caused by DNA damage. They might be located in genes (both in coding and in non-coding sections), and between genes (intergenetically), thus might cause change in structure or in functions [14].

A database (dbSNP) is collecting these SNP’s, was created in 1999 by the American National Centre for Biotechnology Information and the National Human Genome Research Institute. The number of discovered SNP’s was dramatically increased by the Human Genome Project, which mapped the whole humane genome in 2003, thus resulting a total number of more than 650 million SNP’s in the database up to date (<https://www.ncbi.nlm.nih.gov/snp/>) [15].

In case of PTC or PROP sensitivity the SNP’s of TAS2R28 gene (responsible for bitter taste perception) define, whether the individual perceives bitter taste or not. This gene codes a heptahelical (including seven transmembrane domain), G-protein coupled bitter taste perceiving receptor, which binds to the N-C=S group of the compounds. In this case, the gene contains 1002 nucleotides, from which 3 are functionally missense-coding SNP’s, which cause a non-synonym changes, thus modifying the structure of the coded protein.

The amino acid sequence of this protein is shown in **Table 1**.

Table 1. Polymorphisms of TAS2R38 gene, and the amino acids of the coded protein based on [16, 17]

Position of the nucleotide	SNP allele	Position of the amino acid	The encoded amino acid
145	rs713598 – Cytosine	49	Proline
	rs713598 – Guanine		Alanine
785	rs1726866 – Cytosine	262	Alanine
	rs1726866 – Thymine		Valine
886	rs10246939 – Guanine	296	Valine
	rs10246939 – Adenine		Isoleucine

The two most frequent haplotype are the PAV and AVI. Individuals having dominant PAV/PAV, or PAV/AVI diplotype are usually belong to the taster group, while the recessive AVI/AVI diplotypes are non-tasters. With a much lower occurrence (1-5%), AAI, PAI, PVI and AAV haplotypes also occur in some ethnics and populations. In case of PVI and AAV the two status is usually balanced. Based on the studies it might can be concluded that the occurrence of the taster status varies between 55% and 85%, depending on the investigated population [16, 17, 18].

In Hungary, György Forray paediatrician and György Bánkövi mathematician performed a survey on children aged 7-15, in Budapest in 1967. During their study, they applied the Harris-Kalmus method with PTC solutions in order to measure the taste threshold of the children, and thus they concluded their taster status. According to their results 67.8% of the children belonged to the taster group, but they did not find a significant correlation between gender and taster status. They have published their research in the journal *Orvosi Hetilap* [19].

From the anatomic point of view the polymorphysm has a relationship with the number of taste buds: tasters have more fungiform papillae and more taste pores [12].

The study of PTC and PROP sensitivity's effect on other factors have started in the 1960's. The psychopharmacologist researcher Roland Fischer (born in Hungary) was the first, who assumed that there might be a relationship between taste perception and food preference [20]. Even until now several researchers study the relationship between taster status (and its haplo- and diplotypes) and body mass index [17, 21], food preference and frequencies of different food consumption (e.g. alcoholic drinks [22, 23], vegetables, especially the Brassicales [24, 25], coffee, tea [26], sweeteners [27]), and some diseases (e.g. Parkinson-disease, gastrointestinal tumours, chronic rhinosinusitis) and their symptoms [28, 29, 30].

3. Scope

The scope of the current research is to investigate correlations between taster status, body composition and the consumption frequency of bitter tasting foods. To achieve that we have performed PTC status survey, bioimpedance-based body composition measurement and used a food frequency questionnaire focused on bitter tasting foods.

4. Methods

Data collection took place in February and March of 2019, participants were volunteers from the Food Science Faculty of Szent István University, and Faculty of Health Sciences, Semmelweis University (students and staff), altogether 170 people. In the taster status survey 170 people participated, in the body composition study we had 96 participants, the food frequency questionnaire (FFQ) was filled out by 170 individuals. All data were recorded anonymously. To link the different type of data, all participants received an individual code. Participants were informed on the data handling according to the general GDPR guidelines (Regulation (EU) 2016/679).

Taster status was defined with PTC-impregnated paper strips (Precision Europe, Northampton, United Kingdom). PTC is present at 20 micrograms per strip. Individuals were assigned to the taster or non-taster category based on their responses after tasting the paper strips.

The body composition was measured with an InBody 770 (InBody USA, Cerritos, California) device, which works based on bioelectric impedance analysis (BIA). This method relies on the different levels of conductivity of the human body's tissues. The measurement is simple and non-invasive, which provides accurate data for several anthropometric parameters, e.g. percentage of body fat, and its distribution [31]. From the recorded data set we have used the body mass index (BMI, kg/m²), the body fat percentage (PBF, %) and the visceral fat area (VFA, cm²) for further analysis [32, 33, 34]. The FFQ questionnaire involves a list of specific foods or food types, and respondents have to indicate the consumption frequency of these items [35]. Our questionnaire was assembled including bitter tasting food types, consumption frequencies were measured with category scales. The final forms were implemented through the Google Forms platform, data recording was performed online. From the recorded data in this study we report the values concerning coffee and tea consumption, not only the frequency indices, but its type and flavorings also. In order to provide transparent data, the FFQ categories were merged into three major categories (see **Table 2**).

Table 2. Merging of the food frequency questionnaire categories

Categories	Merged categories used during the analyses
I consume it more than one time a day	Regularly
I consume it daily	
I consume it 1 to 3 times a week	
I consume it 1 to 3 times a month	Not typical
I consume it less often than a month	
I never consume it	Never

5. Statistical analyses

To analyse the recorded datasheet, we applied descriptive statistical methods (mean, standard deviation, percentages). Afterwards, data were transformed into category variables, thus suitable for contingency table analysis, multiple correspondence analysis (MCA) and Mann-Whitney test at 5% significance level [36]. XLStat 2020.1.3. and Microsoft® Office Excel® 2016 softwares were used for data analysis.

6. Results

6.1. Demographic parameters

55 males and 115 females participated in this study, so the ratio of genders are 32.5% male and 67.65% female. The youngest respondent was 19 years old, while the eldest was 40 years old, the average age was 23.85 ± 3.05 years. Based on their residence 44.70% lived in the capital of Hungary (Budapest), 55.30% lived in other locations. In the latter group 24.46% lived in Pest County (relating it to the total data that was 13.53%). There were only two Hungarian counties (Zala and Csongrád-Csanád) which were not indicated in any of the respondents.

6.1.1. Taster status

The distribution of taster status data (**Table 3.**) showed that 72.94% of the respondents were tasters, while 27.06% were non-tasters. The ratio of non-tasters among males was 23.63%, while in case of females it was 28.69%. Based on the results of the contingency table analysis there is no significant relationship between the gender and the taster status ($\chi^2(1, n=170)=0.483, p=0.48$).

Table 3. Results of the taster status survey according to genders and in total (number of individuals, $n = 170$)

Gender	Taster	Non-taster
Male	42	13
Female	82	33
Total	124	46

6.1.1.1. Results of investigation of body composition analysis and its relation to the taster status

Body composition analysis was performed in case of 23 males and 73 females, altogether on 96 individuals. The averaged data of these values are listed in **Table 4.**

Table 4. Averaged values of the body composition data (average \pm standard deviation, $n = 96$)

Gender	Height (cm)	Weight (kg)	Body Mass Index (BMI, kg/m ²)	Body Fat Percentage (PBF, %)	Visceral Fat Area (VFA, cm ²)
Male	$180,57 \pm 6,37$	$83,15 \pm 1,85$	$25,53 \pm 3,69$	$20,19 \pm 7,18$	$74,46 \pm 33,85$
Female	$166,34 \pm 6,58$	$61,75 \pm 10,62$	$22,29 \pm 3,50$	$27,91 \pm 6,75$	$76,63 \pm 37,95$

BMI data showed that among the males 11 individuals were obese (BMI from 25.0 to 29.9) and three individuals were overweight (BMI > 30.0). The percentage of body fat values showed obesity in case of 6 people (PBF > 27%), while the visceral fat area was higher than the upper limit of 100 cm² value in the case of 5 people.

Among the females the BMI showed undernourishment for 5 individuals (BMI < 18.5), 7 were obese, and 3 were overweight. The percentage of body fat data showed that 18 people was obese, and the visceral fat area was higher than 100 cm² for 15 participants.

Based on the statistical evaluations we did not find significant relationships in case of any of the obesity-indicating parameters and the taster status (BMI: $\chi^2(3, n=96)=0.42, p=0.93$; PBF: $\chi^2(1, n=96)=0.45, p=0.50$; VFA: $\chi^2(1, n=96)=0.01, p=0.90$). The multiple correspondence analysis results on **Figure 2** shows that the obesity indicating parameters have relationships with each other. The patterns show that non-tasters are positioned closer to the categories of normal body composition and body weight. Outcomes of the contingency table analysis showed that on the basis of BMI values the ratio of overweight individuals (compared to the normal weighted ones) were significantly higher among males, than among females ($\chi^2(3, n=96)=21.52, p<0.0001$).

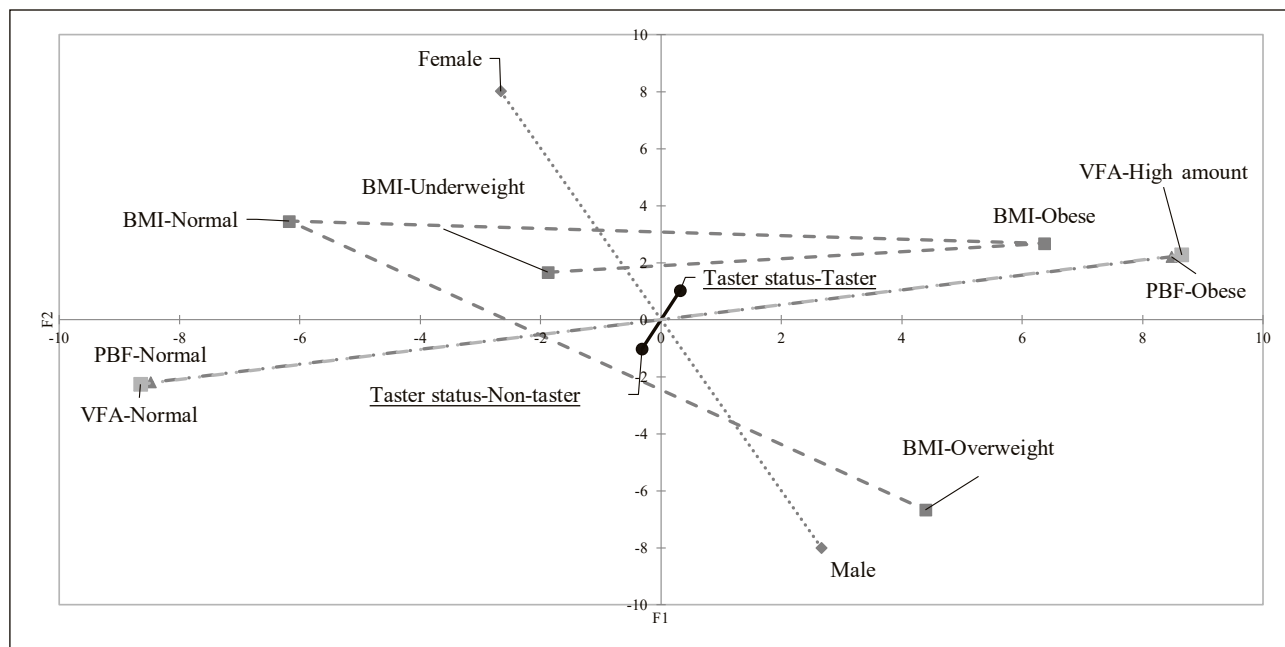


Figure 2. Results of multiple correspondence analysis for taster status, gender, and body composition parameters ($n = 96, p=0,05$).
Abbreviations: BMI = Body Mass Index; PBF = Percent Body Fat; VFA = Visceral Fat Area

6.1.1.2. Relationship of coffee consumption and taster status

Among the FFQ respondents, 27 individuals do not consume coffee, so their data was removed from the analysis. Flavouring categories were the following: 'with milk' (referring to the use of milk, dairy products, or milk substitutes) and 'with sweetener' (referring to the use of any sweeteners (sugar, natural or artificial sweeteners)). The 'mixed' coffee variety indicated the consumption of both Arabica and Robusta (individually or as a blend). From the 143 consumers 24 individuals drink their coffee black (without sweetener, milk, or milk substitute).

Based on the contingency table analysis there is no significant relationship among taster status and coffee consumption ($\chi^2(1, n=170)=0.02, p=0.88$), consumption frequency ($\chi^2(1, n=143)=2,57, p=0,10$) and the consumed type of coffee ($\chi^2(3, n=143)=4.21, p=0.24$). Similarly there was no significant relationship between the type of consumption, like black ($\chi^2(1, n=143)=0.60, p=0.43$), with milk ($\chi^2(1, n=143)=0.28, p=0.59$) or sweetened ($\chi^2(1, n=143)=0.17, p=0.67$) and the taster status.

The patterns of multiple correspondence analysis (**Figure 3**) shows that non-tasters consume coffee less frequently than the tasters, and they are unable to specify the type of coffee they consume. When the non-tasters consume coffee, they prefer the sweetened way. Tasters use Arabica type, and they usually do not add sweetener to it. Even if they add milk, it is not necessarily means the addition of sweetener. There is a clear distinction among genders: there are significantly more coffee consumers among women ($\chi^2(1, n=143)=3.65, p=0.05$), furthermore females have their coffee with milk and sweetener, while males prefer to drink it without milk (black). This is supported with the outcomes of contingency analysis (drinking coffee black: $\chi^2(1, n=143)=3.46, p=0.05$; with milk: $\chi^2(1, n=143)=6.51, p=0.01$).

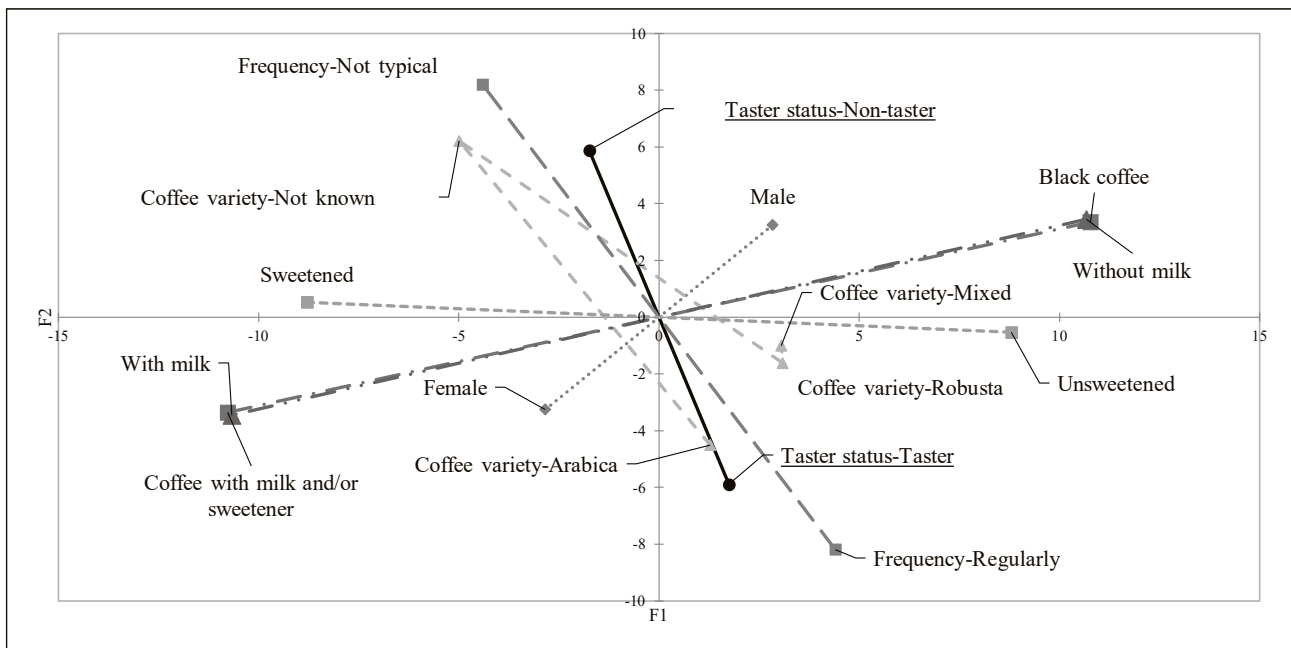


Figure 3. Relationships between coffee consumption and taster status and gender ($n = 143$, $p = 0.05$)
 Abbreviations: 'Milk' = flavored with milk, milk replacer, dairy product, 'Sweetened' = flavored with any sweetener (sugar, artificial and natural sweeteners),
 'Type of coffee' - Assorted: consumed alternately or as a blend (Arabica)

6.1.1.3. Relationship among taster status and tea consumption

Fourteen respondents reported that they do not consume tea, so their results were not analysed. The major categories were 'Several types including black tea' (consuming several tea types, including black tea); 'Several types, but no black tea' (consuming regularly other type of tea than black). The 'Sweetened' label refers to the use of any sweeteners (sugar, artificial and natural sweeteners) for tea consumption. The 'Flavouring – Variegated' category means the use of several ways of flavouring (sometimes with sugar, with lemon and sometimes without sugar), while the 'Flavouring – More items' refers to the use of sweetener and lemon. Among the 156 tea consumers 57 individuals drink their tea without flavouring (no sweetener, no lemon added).

During our analysis we did not find significant relationship among taster status and tea consumption ($\chi^2(1, n=170)=1.26$, $p=0.26$), its frequency ($\chi^2(1, n=156)=0.95$, $p=0.32$), the consumed tea types ($\chi^2(5, n=156)=2.57$, $p=0.76$) and the flavouring types of the tea ($\chi^2(4, n=156)=5.13$, $p=0.27$). There were also no significant differences among genders.

Pattern of the multiple correspondence analysis (**Figure 4**) shows that females and tasters consume tea more frequently, especially black teas and herbal infusions, both flavoured, or non-flavoured. Males and non-tasters consume tea less frequently, they prefer green tea, flavoured with lemon and sweetener, or only with lemon. It was not typical among the respondents that they might consume only fruit infusions.

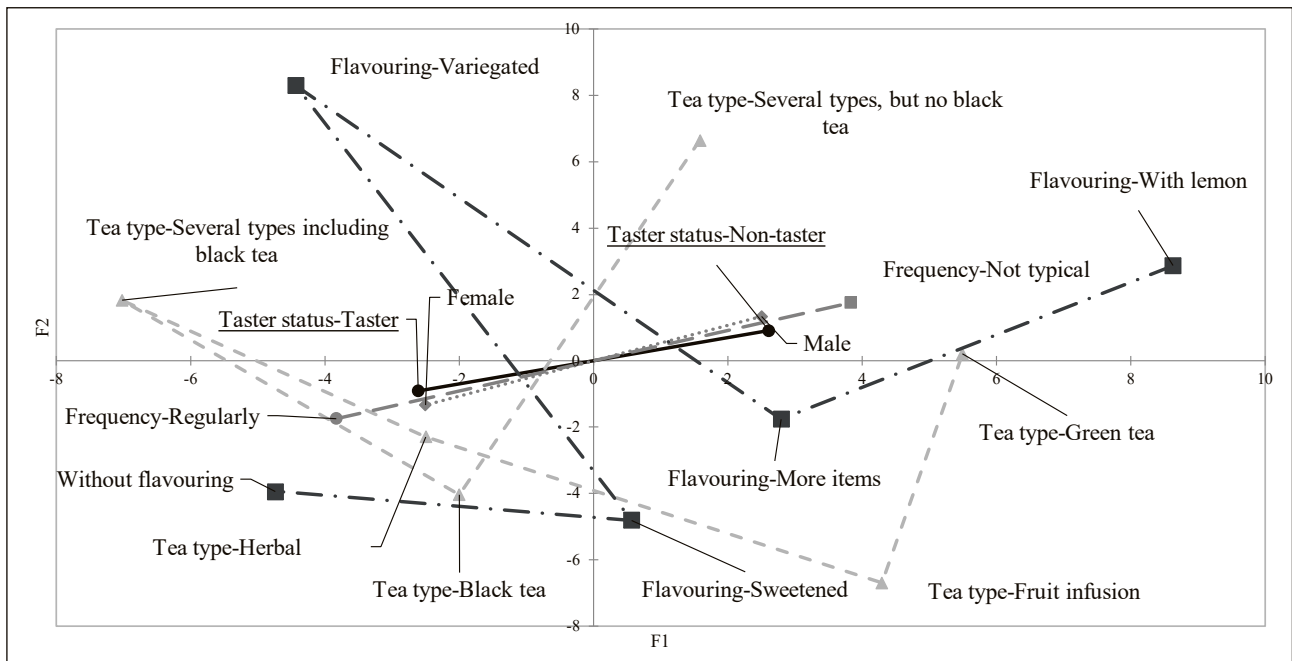


Figure 4. Correlations of tea consumption with taster status and gender ($n = 156, p=0,05$)
 Abbreviations: 'Tea type' – Several types including black tea: consumption of several tea types, including black tea; 'Tea type – Several types, but no black tea': consumption of several tea types, except black tea; 'Sweetened: with any sweeteners' (sugar, artificial and natural sweeteners); 'Flavouring – Variegated': occasionally different flavouring (sometimes sweetened and / or lemon, sometimes unflavoured); 'Flavouring – More items': flavouring with both sweetener and lemon.

7. Discussion

The ratio of tasters and non-tasters in our study is in accordance with those reported in the literature, namely 70% vs. 30% in the American and Caucasian population [6, 37]. We did not find relationship between taster status and BMI value, similarly to previous studies [17, 38]. Contrary to these results, some researchers were able to find significant correlations among these parameters [39]. Generally, the results on this field are controversial; there is no consensus among the researchers. Our new outcomes did not show relationships between taster status, body fat percentage and visceral fat area. However, our results showed significant differences between the genders in the overweight BMI category. The reason behind this is the muscle weight of the two genders: the BMI does not differentiate between fat tissue and non-fat tissue and does not take into consideration the distribution of body fat. Therefore, the BMI value's specificity is high, but its sensitivity is low [40]. In case of the male participants the skeletal muscle mass was significantly higher (Mann-Whitney $U=1664, n_1 = 23, n_2 = 73, p < 0.0001$, two-sided), so more of these individuals were put into the overweight category.

Although we did not find significant relationships in case of coffee consumption, we have observed trends, patterns according to the multiple correspondence analysis. Non-tasters consume coffee less frequently, and they are unable to specify its exact type. These two factors are probably related to each other, since those people who are less interested in coffee consumption, are also less interested in the exact type of coffee. When these individuals consume coffee, they usually add sweeteners, this is less typical in case of tasters, which is supported with literature data [41]. The difference among genders in flavouring or not flavouring the coffee might be related to a social expectation, that the espresso shot is more masculine, while the latte type drinks (e.g. milk espresso) is more feminine [42].

In case of tea consumption, we did not find significant relationships, but several trends were recognized, which are in accordance with the international literature, stating that tasters prefer green tea in a smaller extent [43, 44].

The limitation of our study, that it was not representative from the demographic point of view. During the tests, we have worked with commercially available paper strips, while using PTC or PROP solutions might lead to results that are more precise.

8. Conclusions

Both literature data and our own results show that there might be some level of relationships among genotype, body composition and food choice. It is very likely, that not the genotype, but the phenotype (taster – non-taster) will be the factor which indirectly, through the food choice and food preferences might contribute to obesity, and its related diseases. Since eating habits and food preferences are influenced by other factors (like sociodemographic or psychological ones), these effects might overwrite the expected consequences of the phenotype (preference or aversion toward bitter taste). Furthermore, representative studies with larger sample size are necessary to confirm these hypotheses.

9. Statements

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Conflicts of interest: The authors have no conflicts of interest.

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