



Article

Basal Levels of Salivary Alpha-Amylase Are Associated with Preference for Foods High in Sugar and Anthropometric Markers of Cardiovascular Risk

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Abstract: Salivary alpha-amylase (sAA) influences the perception of taste and texture, features both relevant in acquiring food liking and, with time, food preference. However, no studies have yet investigated the relationship between basal activity levels of sAA and food preference. We collected saliva from 57 volunteers (63% women) who we assessed in terms of their preference for different food items. These items were grouped into four categories according to their nutritional properties: high in starch, high in sugar, high glycaemic index, and high glycaemic load. Anthropometric markers of cardiovascular risk were also calculated. Our findings suggest that sAA influences food preference and body composition in women. Regression analysis showed that basal sAA activity is inversely associated with subjective but not self-reported behavioural preference for foods high in sugar. Additionally, sAA and subjective preference are associated with anthropometric markers of cardiovascular risk. We believe that this pilot study points to this enzyme as an interesting candidate to consider among the physiological factors that modulate eating behaviour.

Keywords: salivary alpha-amylase; food preference; eating behaviour; body composition; glycaemic index; carbohydrates

1. Introduction

Salivary alpha-amylase (sAA) hydrolases starch into smaller oligosaccharides as the first step of carbohydrate digestion. In addition, this enzyme is involved in the perception of taste [1,2] and texture [3]. This property is notably relevant in relation to eating, as physicochemical attributes determine the orosensory response (i.e., palatability) to food [4,5] and can therefore play a key role in modulating motivational (approach, consumption) and affective (hedonic response) constituents of eating behaviour [6]. Hence, together with psychological and cultural factors, physiology can be particularly important in establishing food preferences. Whether early physiological components of food processing, like salivary amylase activity, promote preference towards foods with relevant psychological salience is a query yet to be answered.

Recently, a low copy number of the salivary amylase gene (*AMY1*) has been linked to a greater risk of developing obesity [7,8]. However, given that obesity is a multifactorial condition [9–11], how this genetic variation leads to an obese phenotype is still unclear. Foods rich in refined starch and sugar have been pointed out as major contributors to obesity and overeating [12–15]. Therefore, as the number of copies of *AMY1* is directly correlated with the amount of sAA [7], a reasonable question

would be whether this enzyme influences the behavioural outcome related to weight gain, like the preference for foods with fattening properties.

In the last decade, a trend pointing at higher carbohydrate intake as a determinant factor for obesity gained momentum. However, research has shown that it is not the carbohydrate content per se that is related to weight gain, but how processed these carbohydrates are and how fast they can increase blood glucose [16]. Based on this feature, foods are attributed a glycaemic index (GI), with a higher GI meaning a faster increasing glucose concentration in blood. There is evidence showing a clear relationship between GI and weight gain [17]. However, many researchers point to the glycaemic load (GL) instead of the GI as a more relevant measure, since GL takes into account the portion size and the carbohydrate content per portion. Regardless, both GI and GL have proved useful when exploring the relationship between obesity and carbohydrates [18,19]. Given the influence of sAA on orosensory properties, and how it affects later absorption of carbohydrates, it is reasonable to ponder how the GI and GL of different foods affect food preference.

In this pilot study, we explored two hypotheses to address this matter. First, given the relationship between *AMY1*, sAA, and obesity, we hypothesised that high levels of sAA would be associated with a low preference for refined carbohydrates. Here, we define "preference" as a construct with two dimensions, one subjective, and other self-reported behaviour. We believe this distinction is appropriate given that both constituents should be distinguished in the context of eating behaviour, since the desire to eat certain foods, or groups of food (subjective component), is not necessarily followed by actual consumption (behavioural component) [20]. Second, given the digestive properties of sAA, we expected lower levels of this enzyme to be associated with self-reported behavioural preference better than with subjective preference for these foods. In addition, because of the association between the intake of high GI foods and overweight, we also explored the relationship between body composition and basal levels of sAA. Finally, given the reported differences between men and women with regards to food preferences [21], we also tested sex differences.

2. Material and Methods

2.1. Participants and Procedure

Sixty participants (21 males and 39 females, from age 18 to 41) were recruited from the University of Trier via e-mail digest to fill in the preliminary version of the Food Craving Inventory in German (FCI-DE). Volunteers were invited to come to the laboratory facilities where the study would take place. Smoking, pregnancy, and previous or current history of psychiatric and cardiovascular disorders were criteria for exclusion. After a brief introduction, body measures from the participants were taken with a measurement tape ranging from 0 to 180 cm. Together with height and body weight, neck, forearm, wrist, waist, and hip circumferences were registered. These measures would be later used to estimate body composition features. After this, the FCI-DE first, and the German version of the Three-Factor Eating Questionnaire second, were handed to each participant. The order of the questionnaires was selected to avoid highlighting that restrained eating practices could affect responses to food craving measures. The total time to complete both questionnaires was 10 to 15 min. Upon completion, questionnaires were given back, and unstimulated saliva was collected. After this, participants received either 5 euros or a credit exchange sticker of a 30 min value. Informed consent was obtained from all individual participants included in the study. All procedures complied with the principles from the University Ethics Committee for Experimental Procedures with human subjects.

2.2. Body Composition

Height and waist circumference were registered in cm and weight was measured in kilograms (kg). Body Max Index (BMI) and Waist-to-Height Ratio (WHR) were calculated from body measures. Body Fat percentage (BF%) and Body Adiposity Index (BAI) were estimated using the standardised formulas developed by Deuremberg [22] and Bergman [23], respectively.

2.3. Evaluation of Food Preference

Taking the FCI as a starting point, we created a list of food items to evaluate both subjective and self-reported behaviour dimensions of food preference. The first part of the instrument (subjective scale) asked the participant how often, from 1 "never" to 5 "always/almost always", he or she had felt cravings for a given food in the last week (Appendix A). The second part of the instrument (self-reported behaviour scale), asked, on the same item list, how often the person gave in to this appetence (Appendix B). The sum of the different scores of each scale conformed the Total Inventory Score (TIS), a measure used to evaluate the overall preference. A nutritional breakdown of the foods was performed, and the total carbohydrate, sugar, and starch content of each food was determined according to the tables of food composition [24] and the food database from the German government. The GI of the products was obtained from the University of Sydney database and the publication by Foster-Powell and collaborators [25]. The GL of each product was calculated as the result of the GI and total carbohydrate content product, divided by 100 [GL = (GI \times total carbohydrate)/100)]. Items with $GI \ge 55$ and $GL \ge 10$ were considered "high" in the respective categories, according to generalised consensus. Mean values were considered to categorise foods as high/low in starch and high/low in sugar. Subjective and self-reported behaviour scores of each participant were calculated as the mean of all food items. Scale scores for the food categories (High/Low starch, sugar, GI, and GL) were calculated as the mean of the foods included in each category.

2.4. Salivary Alpha-Amylase Determination

Unstimulated saliva was collected between 8:30 a.m. and 3:30 p.m. Participants were instructed to come to the laboratory fasted (minimum 3 h). Salivette (Sarstedt, Nümbrecht, Germany) tubes were used to collect the saliva, following the manufacturer's protocol. Briefly, the swab was removed from the Salivette and placed inside the mouth. After one minute of chewing, the swab was placed back inside its tube. Samples were stored until further analysis at 4 °C. For sAA determination, Salivettes were centrifuged at 4 °C, 10,000 rpm for 10 min. Alpha-amylase activity was determined by the standard ELISA procedure (Sigma-Aldrich Chemie Gmbh, Munich, Germany).

2.5. Data Analysis

From the 60 volunteers, three (one man and two women) had to be excluded from the analysis because of extreme amylase levels and BMI values (women), as well as failure to complete the questionnaires (man). Normality of the data was checked with the skewness value and the Shapiro-Wilk test of normality. A t-test was performed to analyse differences between groups. Correlation and regression analyses were performed to explore the relationship between the variables. Cohen's d and Cohen's f^2 were calculated for the effect size of group comparison and regression analysis, respectively [26]. Confident intervals (CI) are also reported. Participants were included in the healthy or overweight groups, depending on their WHR [27]. Given the ultradian variability of salivary amylase, the time at which the saliva samples were taken was also analysed. Data values are shown as mean \pm S.E.M. Standard statistical significance was established at an alpha below 0.05. In those analysis where multiple comparisons were performed (t-test), a false discovery rate was applied following the Benjamini-Hochberg procedure. Amylase activity is shown as the log transformation of unit saliva per ml (U/mL). All the statistical procedures were conducted using the Statistical Software Package SPSS (version 23; IBM SPSS, Chicago, IL, USA).

3. Results

3.1. Participant Characteristics

Demographic and anthropometric data from the participants are presented in Table 1. Men presented a slight although non-significant overweight value (25.34 vs. overweight \geq 25) in terms of BMI. In terms of BAI, WHR, and BF %, both sexes fell into the normal healthy range [23,28,29].

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Basal levels of sAA were similar in both sexes. Men and women differ significantly in their BMI (t = -5.21, p < 0.001), BF% (t = 4.01, p < 0.001), WHR (t = -5.13, p < 0.001), and BAI (t = -5.14, p < 0.001).

	Men	Women		
	(n = 21)	(n = 37)		
	Mean \pm s.d., Asymmetry \pm d.e.			
Weight	82.62 ± 12.69 , 0.95 ± 0.501	62.58 ± 13.16 , 0.13 ± 0.388		
Body Mass Index	25.34 ± 3.62 , 1.52 ± 0.501	$21.94 \pm 4.73, 0.49 \pm 0.388$		
Body fat %	$20.03 \pm 5.40, 0.93 \pm 0.501$	25.23 ± 5.13 , 1.01 ± 0.388		
Body Adiposity Index	21.43 ± 3.18 , 1.12 ± 0.501	$18.07 \pm 2.27, 1.05 \pm 0.388$		
Waist to Hip Ratio	0.52 ± 0.07 , 1.06 ± 0.501	0.44 ± 0.05 , 1.04 ± 0.388		
sAA	85.06 ± 11.80 , 1.01 ± 0.501	$95.85 \pm 12.50, 1.09 \pm 0.388$		

Table 1. Anthropometric measures and sAA in men and women.

sAA = salivary alpha-amylase, s.d. = standard deviation, d.e. = deviation error.

3.2. Food Preference

The correlation between subjective and self-reported behavioural measures was strong and highly significant for TIS (r = 0.720, p < 0.001), foods high in starch (r = 0.669, p = 0.001), foods high in sugar (r = 0.630, p = 0.003), foods with high GI (r = 0.534, p = 0.015), and foods with high GL (r = 0.553, p = 0.011).

The *t*-test showed higher scores for subjective preference in comparison with self-reported behavioural preference in TIS (p < 0.001, d = 0.49, CI = 3.07 to 9.39), the high sugar category (p < 0.001, d = 0.488, CI = 1.53 to 5), the high GI category (p < 0.001, d = 0.528, CI = 2.03 to 6.57), and the high GL category (p < 0.001, d = 0.466, CI = 2.14 to 8.02) (Figure 1). We found no differences between men and women in terms of subjective and self-reported behavioural preference.

3.3. Food Preference and Anthropometric Markers of Health Risk

A significant, positive correlation was found in women between BMI and self-reported behavioural preference TIS (r = 0.287, p = 0.038), foods high in sugar (r = 0.421, p = 0.004), with high GI (r = 0.305, p = 0.03), and with high GL (r = 0.295, p = 0.034). Furthermore, in women, BF% correlated with self-reported behavioural preference for foods high in sugar (r = 0.400, p = 0.006), with high GI (r = 0.286, p = 0.039), and with high GL (r = 0.280, p = 0.042). In men, self-reported behavioural preference for foods high in sugar correlated negatively with BMI (r = -0.421, p = 0.032) and BF% (r = -0.386, p = 0.046). No correlation was found between subjective preference and body measurements in men or in women.

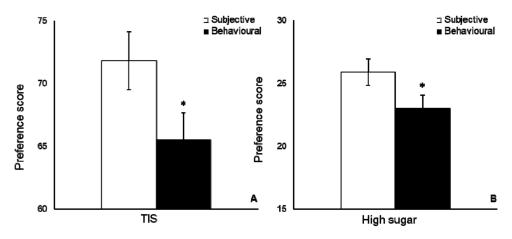


Figure 1. Cont.

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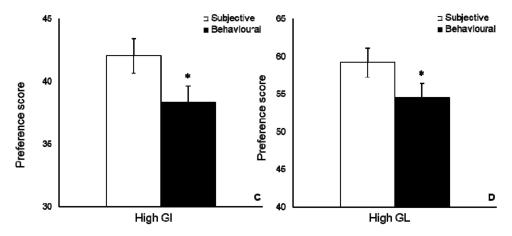


Figure 1. Subjective and self-reported behavioural preference. Preference score for Total Score Inventory (TIS) (**A**), foods high in sugar (**B**), foods with high GI (**C**), and foods with high GL (**D**). Behavioural = Self-reported behavioural. Data is presented as mean \pm s.e.m. * Significant p < 0.01.

The regression analysis showed a significant effect of subjective preference on WHR ($R^2 = 0.239$, p = 0.011, $f^2 = 0.377$) and BAI ($R^2 = 0.236$, p = 0.012, $f^2 = 0.372$) when men and women were analysed together. Self-reported behavioural preference had no impact on body measurements (Figure 2). When the analysis was performed on men and women separately, neither subjective nor self-reported behavioural preference seemed to be significantly associated with body measurements.

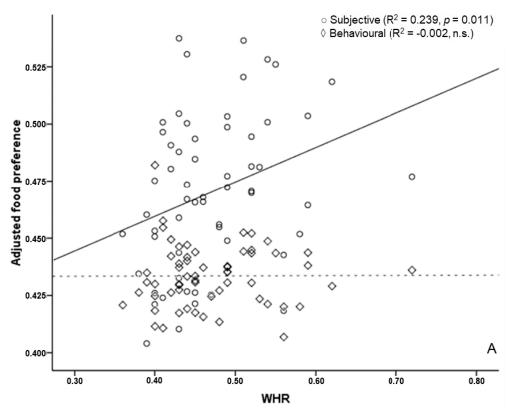


Figure 2. Cont.

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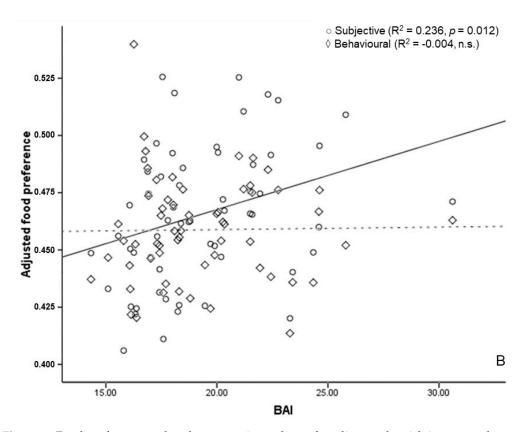


Figure 2. Food preference and anthropometric markers of cardiovascular risk in men and women. Adjusted predicted value of food preference for Waist-to-Hip Ratio (**A**) and Body Adiposity Index (**B**) in total sample. The straight line represents the line of best fit of the subjective category. The dotted line represents the line of best fit of the behavioural category.

3.4. Effect of Time in sAA Levels

We performed a regression analysis to examine if the saliva samples were influenced by sAA levels. When the total sample was analysed, no effect was found. This was consistent when we analysed the samples by sex. Given the lack of influence of the moment in which the saliva was collected, further analysis was performed, omitting this variable.

3.5. sAA and Anthropometric Markers of Health Risk

A significant, positive correlation was found between sAA and WHR (r = 0.229, p = 0.039) and BAI (r = 0.231, p = 0.038) when data from both sexes were analysed together. When analysed separately, this correlation was only observed in women. No significant relationship was found in men (Table 2).

Table 2. Correlation between salivary alpha-amylase and anthropometric measurements.

		Log_{10} sAA
	BMI	0.154
147	WHR	0.323 *
Women	BAI	0.327 *
	BF %	0.209
	BMI	0.119
Men	WHR	0.149
Men	BAI	0.14
	BF %	0.173

BMI = Body Mass Index, WHR = Waist-to-Hip Ratio, BAI = Body Adiposity Index, BF = Body Fat. * Significant, p < 0.05.

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When the regression analysis was performed on men and women together, no association between sAA and body measurements was found. When split by sex, however, sAA was associated with WHR ($R^2 = 0.111$, p = 0.038, $f^2 = 0.142$) and BAI ($R^2 = 0.114$, p = 0.035, $f^2 = 0.128$) in women (Figure 3). No association was found in men.

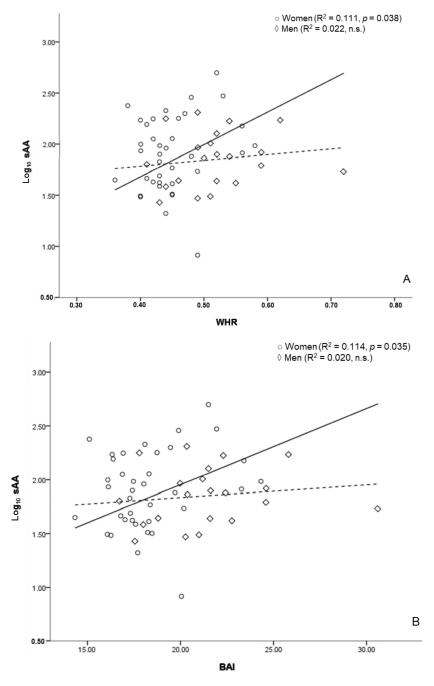


Figure 3. Salivary alpha-amylase and anthropometric markers of cardiovascular risk. Association of the log_{10} transformed sAA values of Waist-to-Hip Ratio (**A**) and Body Adiposity Index (**B**). The straight line represents the line of best fit of the subjective category. The dotted line represents the line of best fit of the behavioural category.

3.6. sAA and Food Preference

A significant, negative correlation was found between sAA and subjective preference for foods high in sugar (r = -0.244, p = 0.034) when data from men and women were analysed together. When split by sex, the correlation analysis showed a significant, negative relationship between sAA

and preference for food high in sugar, both in subjective (r = -0.323, p = 0.026) and self-reported behavioural measures (r = -0.283, p = 0.045). No correlation was found in men (Table 3).

Table 3.	Correlation	between	salivary	alpha-am	vlase and	d food	categories.

Sex	Preference	Category	Log ₁₀ sAA
		TIS	-0.254
		Starch	-0.115
	Subjective	Sugar	-0.323 *
		ĞĪ	-0.244
Women		GL	-0.258
Violiteit		TIS	-0.206
		Starch	-0.081
	Self-reported behaviour	Sugar	-0.283 *
		GI	-0.154
		GL	-0.173
		TIS	-0.072
		Starch	0.105
	Subjective	Sugar	-0.138
		GI	0.046
Men		GL	-0.044
Wich		TIS	0.044
		Starch	0.241
	Self-reported behaviour	Sugar	-0.011
		ĞĪ	0.209
		GL	0.125

TIS = Total Inventory Score, GI = Glycaemic Index, GL = Glycaemic Load. * Significant, p < 0.05.

The regression analysis indicated no relation between sAA and food preference in the overall sample. A second analysis was performed, including WHR and BAI in the model. When these anthropometric markers of health risk were accounted for, sAA was inversely associated with subjective, but not self-reported behavioural preference for foods high in sugar ($R^2 = 0.282$, p = 0.037, $f^2 = 0.095$). Although not as significant, this result was consistent when the analysis was performed for women only ($R^2 = 0.104$, p = 0.051, $f^2 = 0.126$) (Figure 4). No effect was observed for men.

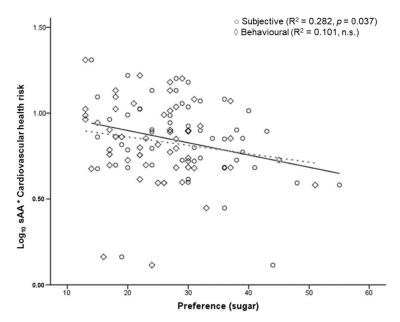


Figure 4. Salivary alpha-amylase and food preference. Association between the log₁₀ transformed sAA values and WHR in terms of subjective preference for foods high in sugar (in women). The straight line represents the line of best fit of the subjective category. The dotted line represents the line of best fit of the behavioural category.

3.7. SAA, Body Composition, and Eating Behaviour

A correlation analysis was performed to explore the relationship between the three dimensions of the TFEQ and sAA first, and body composition, secondly. No correlation was found between the restrained eating scale of the TFEQ and sAA, BMI, WHR, BAI, or BF%.

4. Discussion

We put together a list of foods to assess in two dimensions, according to personal preference. Subjective and self-reported behavioural preference scales reflected how often the person had the desire to eat a specific food, and how often they would give into that appetence, respectively. Eating is not only a matter of re-stabilising physiological homoeostasis. Therefore, distinguishing these two domains of preference may be relevant. In this work, we explored the hypothesis of basal levels of sAA being associated with food preference and various body measurements associated with health status, coherent with the evidence presented in recent research [7,30,31]. In addition, we explored whether food preference, measured as subjective and behavioural dimensions (self-reported), would affect body composition in different ways. Our intention was to provide preliminary evidence of the possible association between these factors. Nevertheless, given the reported differences between men and women in food preference, we also looked at this in our study.

Several conclusions can be drawn from our data. First, food preference can be effectively differentiated into separate dimensions; one subjective, characterised by volition without the need of an action towards consuming food; and another in which this will is followed by consummatory behaviour. Further, our data indicate that, although being highly correlated, each of these dimensions are differentially associated with (1) basal levels of sAA, (2) various food categories, (3) body composition measurements, and (4) sex. Such results are consistent with other research reporting differences between subjective and self-reported behavioural evaluation of the same foods [32,33].

Second, the subjective, but not the behavioural, dimension seems to effectively predict body composition; specifically, WHR and BAI. Interestingly, both subjective and self-reported behavioural preference correlate with body composition. We found that this relationship is different in men and women, up to a point where self-related behavioural preference is negatively associated with these variables in men. Concretely, our data show a negative association between BMI and BF% and self-reported behavioural preference for foods high in sugar (in men). This sex-related discrepancy could be explained by previously reported differences describing how men tend to prefer more savoury rather than sweet foods. Assuming that foods with a greater sugar content are associated with a greater measure of obesity, it is expected that lowering their intake would correlate with better body composition values.

Finally, we demonstrate that sAA is associated with body composition in women only and that this enzyme affects subjective, but not self-reported behavioural, food preference. Concretely, we show that basal levels of fasting sAA inversely predict subjective preference for foods high in sugar, but not other food groups or behavioural measures.

Understanding the relationship between sAA and the two preference dimensions becomes pertinent after considering the evidence showing that lower levels of sAA represent a greater risk of developing insulin tolerance and type 2 diabetes [31]. Societies and individuals with a lower copy number of the amylase gene present a greater risk for developing obesity, regardless of environmental and other biological factors [7]. Interestingly, the amount of amylase found in saliva correlates directly with the number of copies of the *AMY1* gene [7]. Again, this is consistent with our findings of lower sAA, reflecting fewer *AMY1* copies, associated with a greater preference for foods that have been similarly linked to weight gain and demonstrated fattening properties [34,35]. Coherently, our results indicate that lower levels of sAA correlate positively with greater preference, mostly for foods high in sugar. This suggests that lower levels of this enzyme could be an important aspect of the physiological environment by which individuals are influenced when making dietary choices. We think this is

particularly relevant, given that it could contribute to partially explain the obesity phenotype in those individuals carrying low copy numbers of the amylase gene.

The second hypothesis of this study was that sAA could also be related to some extent to body composition measures associated with cardiovascular health, such as WHR. Contrarily to what we expected, the analysis revealed a significant and positive association between sAA and two indicators of cardiovascular health in adults: body adiposity and WHR [36–38]. However, according to the hypothesis of amylase, this relationship should be inverse.

Some studies suggest that greater sAA activity indicates a better adaptation to starchy foods, as carbohydrates can be more rapidly digested and blood glucose more easily regulated [30,39]. This opens the door to considering this enzyme as an indicator of lacking specific nutrients, maybe acting as a motivational trigger towards foods high in such missing components. This is consistent with the observation of food preference and health factors. Since lower levels of sAA would increase (subjective) preference for certain foods, the probability of consuming them (behaviour) rises, finally contributing to the intensification of such factors.

It is also important to note various other limitations in this study. First, in relation to the absent relationship between sAA and self-reported behavioural preference, this could be explained by participants exerting some kind of restrained eating strategy [20,40]. Interestingly, self-reported behavioural preference did not correlate with the cognitive control measure from the FEV, although it did positively correlate with the disinhibition subscale (data not shown). Another reason for this subjective/behavioural discrepancy could be palatability (more palatable foods may present better chance to be approached), or the opportunity to consume a particular food. However, this seems unlikely given that all the categories presented highly palatable and available food items.

Second, regarding body composition, we acknowledge the deficiencies of methods based on mathematical formulas compared with more precise techniques. Although we found a positive relationship between sAA and body composition, the utility of these procedures is still a matter of debate, with some studies showing no difference between BMI and other anthropometric measures [41]. On the other hand, previous research has shown that these WHR and BAI efficiently predict the risk of developing obesity-related conditions. Segheto and collaborators demonstrated that in the absence of more sophisticated methods (i.e., DEXA scan), BAI is an effective estimator of adiposity [42]. In addition, WHR has proved a better predictor of dyslipidaemia, hypertension, and diabetes, when compared with other anthropometric measures, like BMI [43]. In addition, regardless of the adequacy of the methods used, most of the anthropometric values measured fell within a range considered healthy. Therefore, the clinical relevance of this finding is unclear at best. Regarding this, studies in a clinical population could help to better elucidate our findings.

Finally, we did not take into consideration the phase of the menstrual cycle. A higher appetence for foods high in fat and sugars has been reported during the menstrual cycle [44]. Unfortunately, we did not register information on the participants' cycle phase. Nevertheless, it is very unlikely that this factor would make a difference in our results, given the reduced chance that all women, during different days, were under the same cycle by the time they participated in the study. Also, although the time of the day was considered, the ultradian variations in amylase levels were not introduced as a confounding variable in the analysis. We also acknowledge this as a limitation. However, considering that the time variable did not show a significant effect, we believe that for the purposes of this pilot study, it is not a major issue. Nevertheless, we reckon that future investigations should not neglect this variable in the design.

5. Conclusions

To the best of our knowledge, this is the first study exploring the relationship between sAA and food preference, understood as a motivational construct. Our findings suggest that sAA could influence eating behaviour towards consuming certain food groups (i.e., high in sugar), and body composition, particularly in women. However, given the limitations mentioned before, conclusions

should be taken carefully. Regardless of this, we believe that the results presented here point to factors worth considering further. Taking basal levels of sAA as a biomarker associated with relevant health measures could be a promising tool to consider when approaching eating behaviour, whether it is in a clinical or research context. Therefore, we trust that this work reports interesting results on the relationship between psychophysiological aspects of eating behaviour and could be of use in future studies that further explore this relationship between salivary enzymes and food preference.

Author Contributions: E.T. designed the study, redacted the results, the introduction and the discussion; J.S. collected the data, redacted the methodology and the results; J.M. helped to design the study, proofread, and contributed to the introduction and the discussion.

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Conflicts of Interest: Authors declare no conflict of interest.

Appendix A. Food Item Inventory. Subjective Scale

"Craving" ist ein Fachbegriff aus der Psychologie, der das nahezu unbezwingbare Verlangen einer Person um schreibt, ein bestimmtes Nahrungsmittel zu sich zu nehmen.

Bitte markieren Sie, wie oft Sie im letzten Monat "Craving" auf die folgenden Lebensmittel gefühlt haben.

	Nie	Selten	Manchmal	Häufig	Immer/Fast Immer
Backfisch					
Frühstücksspeck (Bacon)					
Belegte Brötchen					
Brezeln					
Brötchen					
Brownies					
Chips					
Cracker					
Döner					
Donuts					
Eiscreme					
Frikadellen					
Gesalzene Erdnüsse					
Haferflocken					
Hamburger					
Honig					
Kekse					
Kuchen					
Marzipan					
Müsli					
Nudeln					
Nussnougatcreme					
Ofenkartoffeln					
Pfannkuchen					
Pizza					
Pommes frites					
Reis					
Schokolade, Pralinen					
Steak					
Süße Backwaren					
Süßigkeiten					
Toastbrot					
Waffeln					
Wurst					

Table A1. List of foods and frequency to which they are craved.

Appendix B. Food Item Inventory. Behavioural Scale

Am diese Mal im letzten Monat wenn Sie auf ein besonderes Lebensmittel "Craving" gefühlt haben, wie oft haben Sie nachgegeben und aßen?

Table A2. List of foods and frequency to which cravings are given in.

	Nie	Selten	Manchmal	Häufig	Immer/Fast Immer
Backfisch					
Bacon (Frühstücksspeck)					
Belegte Brötchen					
Brezel					
Brötchen					
Brownies					
Chips					
Cracker					
Döner					
Donuts					
Eiscreme					
Frikadellen					
Gesalzene Erdnüsse					
Haferflocken					
Hamburger					
Honig					
Keks					
Kuchen					
Marzipan					
Müsli					
Nudeln					
Nussnougatcreme					
Ofenkartoffeln					
Pfannkuchen					
Pizza					
Pommes frites					
Reis					
Schokolade, Pralinen					
Steak					
Süße Backwaren					
Süßigkeiten					
Toastbrot					
Waffel					
Wurst					

References

- Dsamou, M.; Palicki, O.; Septier, C.; Chabanet, C.; Lucchi, G.; Ducoroy, P.; Chagnon, M.C.; Morzel, M. Salivary protein profiles and sensitivity to the bitter taste of caffeine. *Chem. Senses* 2012, 37, 87–95. [CrossRef] [PubMed]
- Salles, C.; Chagnon, M.-C.; Feron, G.; Guichard, E.; Laboure, H.; Morzel, M.; Semon, E.; Tarrega, A.; Yven, C. In-mouth mechanisms leading to flavor release and perception. *Crit. Rev. Food Sci. Nutr.* 2011, 51, 67–90. [CrossRef] [PubMed]
- 3. De Wijk, R.A.; Prinz, J.F.; Engelen, L.; Weenen, H. The role of α-amylase in the perception of oral texture and flavour in custards. *Physiol. Behav.* **2004**, *83*, 81–91. [CrossRef] [PubMed]
- 4. Prindiville, E.A.; Marshall, R.T.; Heymann, H. Effect of Milk Fat on the Sensory Properties of Chocolate Ice Cream. *J. Dairy Sci.* **1999**, *82*, 1425–1432. [CrossRef]
- Prindiville, E.A.; Marshall, R.T.; Heymann, H. Effect of Milk Fat, Cocoa Butter, and Whey Protein Fat Replacers on the Sensory Properties of Lowfat and Nonfat Chocolate Ice Cream. J. Dairy Sci. 2000, 83, 2216–2223. [CrossRef]

6. Berthoud, H.R. Metabolic and hedonic drives in the neural control of appetite: Who is the boss? *Curr. Opin. Neurobiol.* **2011**, *21*, 888–896. [CrossRef] [PubMed]

- 7. Falchi, M.; El-Sayed Moustafa, J.S.; Takousis, P.; Pesce, F.; Bonnefond, A.; Andersson-Assarsson, J.C.; Sudmant, P.H.; Dorajoo, R.; Al-Shafai, M.N.; Bottolo, L.; et al. Low copy number of the salivary amylase gene predisposes to obesity. *Nat. Genet.* **2014**, *46*, 492–497. [CrossRef] [PubMed]
- 8. Viljakainen, H.; Andersson-Assarsson, J.C.; Armenio, M.; Pekkinen, M.; Pettersson, M.; Valta, H.; Lipsanen-Nyman, M.; Mäkitie, O.; Lindstrand, A. Low Copy Number of the AMY1 Locus Is Associated with Early-Onset Female Obesity in Finland. *PLoS ONE* **2015**, *10*, e0131883. [CrossRef] [PubMed]
- 9. Bray, G.A.; Popkin, B.M. Dietary Sugar and Body Weight: Have We Reached a Crisis in the Epidemic of Obesity and Diabetes? *Diabetes Care* **2014**, *37*, 950–956. [CrossRef] [PubMed]
- 10. Incledon, E.; Wake, M.; Hay, M. Psychological predictors of adiposity: Systematic review of longitudinal studies. *Int. J. Pediatr. Obes.* **2011**, *6*, e1–e11. [CrossRef] [PubMed]
- 11. Stenvinkel, P. Obesity—A disease with many aetiologies disguised in the same oversized phenotype: Has the overeating theory failed? *Nephrol. Dial. Transplant.* **2015**, *30*, 1656–1664. [CrossRef] [PubMed]
- 12. Fogelholm, M.; Anderssen, S.; Gunnarsdottir, I.; Lahti-Koski, M. Dietary macronutrients and food consumption as determinants of long-term weight change in adult populations: A systematic literature review. *Food Nutr. Res.* **2012**, *56*, 19103. [CrossRef] [PubMed]
- 13. Ley, S.H.; Hamdy, O.; Mohan, V.; Hu, F.B. Prevention and management of type 2 diabetes: Dietary components and nutritional strategies. *Lancet* **2014**, *383*, 1999–2007. [CrossRef]
- 14. Rouhani, M.H.; Haghighatdoost, F.; Surkan, P.J.; Azadbakht, L. Associations between dietary energy density and obesity: A systematic review and meta-analysis of observational studies. *Nutrition* **2016**, *32*, 1037–1047. [CrossRef] [PubMed]
- 15. Yang, Q.; Zhang, Z.; Gregg, E.W.; Flanders, W.D.; Merritt, R.; Hu, F.B. Added sugar intake and cardiovascular diseases mortality among US adults. *JAMA Intern. Med.* **2014**, *174*, 516–524. [CrossRef] [PubMed]
- 16. Stanhope, K.L. Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit. Rev. Clin. Lab. Sci.* **2015**, *53*, 52–67. [CrossRef] [PubMed]
- 17. Larsen, T.M.; Dalskov, S.; Van Baak, M.; Jebb, S.; Kafatos, A.; Pfeiffer, A.; Martinez, J.A.; Handjieva-Darlenska, T.; Kunešová, M.; Holst, C.; et al. The diet, obesity and genes (diogenes) dietary study in eight European countries—A comprehensive design for long-term intervention. *Obes. Rev.* 2010, 11, 76–91. [CrossRef] [PubMed]
- 18. Schwingshackl, L.; Hobl, L.P.; Hoffmann, G. Effects of low glycaemic index/low glycaemic load vs. high glycaemic index/high glycaemic load diets on overweight/obesity and associated risk factors in children and adolescents: A systematic review and meta-analysis. *Nutr. J.* 2015, 14, 87. [CrossRef] [PubMed]
- 19. Schwingshackl, L.; Hoffmann, G.; Lampousi, A.-M.; Knüppel, S.; Iqbal, K.; Schwedhelm, C.; Bechthold, A.; Schlesinger, S.; Boeing, H. Food groups and risk of type 2 diabetes mellitus: A systematic review and meta-analysis of prospective studies. *Eur. J. Epidemiol.* **2017**, *32*, 363–375. [CrossRef] [PubMed]
- 20. Larsen, J.K.; van Strien, T.; Eisinga, R.; Herman, C.P.; Engels, R.C.M.E. Dietary restraint: Intention versus behavior to restrict food intake. *Appetite* **2007**, *49*, 100–108. [CrossRef] [PubMed]
- 21. Anschutz, D.J.; Engels, R.C.M.E.; van der Zwaluw, C.S.; Van Strien, T. Sex differences in young adults' snack food intake after food commercial exposure. *Appetite* **2011**, *56*, 255–260. [CrossRef] [PubMed]
- 22. Deurenberg, P.; Weststrate, J.A.; Seidell, J.C. Body mass index as a measure of body fatness: Age- and sex-specific prediction formulas. *Br. J. Nutr.* **1991**, *65*, 105–114. [CrossRef] [PubMed]
- 23. Bergman, R.N.; Stefanovski, D.; Buchanan, T.A.; Sumner, A.E.; Reynolds, J.C.; Sebring, N.G.; Xiang, A.H.; Watanabe, R.M. A Better Index of Body Adiposity. *Obesity* **2011**, *19*, 1083–1089. [CrossRef] [PubMed]
- 24. Farran, A.; Zamora, R.; Cervera, P. *Tablas de Composición de ALIMENTOs del CESNID*; Reixach, M., Ed.; McGraw-Hill Interamericana: Barcelona, Spain, 2004; ISBN 84-486-0590-X.
- 25. Atkinson, F.S.; Foster-Powell, K.; Brand-Miller, J.C. International Tables of Glycemic Index and Glycemic Load Values: 2008. *Diabetes Care* 2008, 31, 2281–2283. [CrossRef] [PubMed]
- 26. Selya, A.S.; Rose, J.S.; Dierker, L.C.; Hedeker, D.; Mermelstein, R.J. A practical guide to calculating Cohen's f 2, a measure of local effect size, from PROC MIXED. *Front. Psychol.* **2012**, *3*, 111. [CrossRef] [PubMed]
- 27. Lee, C.M.Y.; Huxley, R.R.; Wildman, R.P.; Woodward, M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: A meta-analysis. *J. Clin. Epidemiol.* **2008**, *61*, 646–653. [CrossRef] [PubMed]

28. Ashwell, M.; Gunn, P.; Gibson, S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: Systematic review and meta-analysis. *Obes. Rev.* **2012**, *13*, 275–286. [CrossRef] [PubMed]

- 29. Gallagher, D.; Heymsfield, S.B.; Heo, M.; Jebb, S.A.; Murgatroyd, P.R.; Sakamoto, Y. Healthy percentage body fat ranges: An approach for developing guidelines based on body mass index. *Am. J. Clin. Nutr.* **2000**, 72, 694–701. [CrossRef] [PubMed]
- 30. Mandel, A.L.; Peyrot des Gachons, C.; Plank, K.L.; Alarcon, S.; Breslin, P.A.S. Individual differences in AMY1 gene copy number, salivary α -amylase levels, and the perception of oral starch. *PLoS ONE* **2010**, *5*, e13352. [CrossRef] [PubMed]
- 31. Mandel, A.L.; Breslin, P.A.S. High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. *J. Nutr.* **2012**, *142*, 853–858. [CrossRef] [PubMed]
- 32. White, M.A.; Whisenhunt, B.L.; Williamson, D.A.; Greenway, F.L.; Netemeyer, R.G. Development and validation of the food-craving inventory. *Obes. Res.* **2002**, *10*, 107–114. [CrossRef] [PubMed]
- 33. White, M.A.; Grilo, C.M. Psychometric properties of the Food Craving Inventory among obese patients with binge eating disorder. *Eat. Behav.* **2005**, *6*, 239–245. [CrossRef] [PubMed]
- 34. Carpenter, D.; Dhar, S.; Mitchell, L.M.; Fu, B.; Tyson, J.; Shwan, N.A.A.; Yang, F.; Thomas, M.G.; Armour, J.A.L. Obesity, starch digestion and amylase: Association between copy number variants at human salivary (AMY1) and pancreatic (AMY2) amylase genes. *Hum. Mol. Genet.* **2015**, *24*, 3472–3480. [CrossRef] [PubMed]
- 35. Kahlhöfer, J.; Lagerpusch, M.; Enderle, J.; Eggeling, B.; Braun, W.; Pape, D.; Müller, M.J. Bosy-Westphal, a Carbohydrate intake and glycemic index affect substrate oxidation during a controlled weight cycle in healthy men. *Eur. J. Clin. Nutr.* **2014**, *68*, 1060–1066. [CrossRef] [PubMed]
- 36. Schneider, H.J.; Glaesmer, H.; Klotsche, J.; Böhler, S.; Lehnert, H.; Zeiher, A.M.; März, W.; Pittrow, D.; Stalla, G.K.; Wittchen, H.-U. Accuracy of Anthropometric Indicators of Obesity to Predict Cardiovascular Risk. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 589–594. [CrossRef] [PubMed]
- 37. Spahillari, A.; Mukamal, K.; DeFilippi, C.; Kizer, J.R.; Gottdiener, J.S.; Djoussé, L.; Lyles, M.F.; Bartz, T.M.; Murthy, V.L.; Shah, R.V. The association of lean and fat mass with all-cause mortality in older adults: The Cardiovascular Health Study. *Nutr. Metab. Cardiovasc. Dis.* **2016**, *26*, 1039–1047. [CrossRef] [PubMed]
- 38. Vogel, P.; Stein, A.; Marcadenti, A. Visceral adiposity index and prognosis among patients with ischemic heart failure. *Sao Paulo Med. J.* **2016**, *134*, 211–218. [CrossRef] [PubMed]
- 39. Bornet, F.R.J.; Bizais, Y.; Bruley des Varaness, S.; Pouliquen, B.; Delort Laval, J.; Galmiche, J.P. α-Amylase (EC 3.2.1.1) susceptibility rather than viscosity or Gastric Emptying Rate Controls Plasma Responses to Starch in Healthy Humans. *Br. J. Nutr.* **1990**, *63*, 207–220. [CrossRef] [PubMed]
- 40. Sniehotta, F.F.; Scholz, U.; Schwarzer, R. Bridging the intention–behaviour gap: Planning, self-efficacy, and action control in the adoption and maintenance of physical exercise. *Psychol. Health* **2005**, *20*, 143–160. [CrossRef]
- 41. Ortega, F.B.; Sui, X.; Lavie, C.J.; Blair, S.N. Body Mass Index, the Most Widely Used but Also Widely Criticized Index Would a Criterion Standard Measure of Total Body Fat Be a Better Predictor of Cardiovascular Disease Mortality? *Mayo Clin. Proc.* **2016**, *91*, 443–455. [CrossRef] [PubMed]
- 42. Segheto, W.; Coelho, F.A.; Cristina Guimarães da Silva, D.; Hallal, P.C.; Marins, J.C.B.; Ribeiro, A.Q.; Pessoa, M.C.; Morais, S.H.O.; Longo, G.Z. Validity of body adiposity index in predicting body fat in Brazilians adults. *Am. J. Hum. Biol.* **2016**, 1–8. [CrossRef] [PubMed]
- 43. Despreś, J.P. What is "metabolically healthy obesity"? From epidemiology to pathophysiological insights. *J. Clin. Endocrinol. Metab.* **2012**, 97, 2283–2285. [CrossRef] [PubMed]
- 44. Gorczyca, A.M.; Sjaarda, L.A.; Mitchell, E.M.; Perkins, N.J.; Schliep, K.C.; Wactawski-Wende, J.; Mumford, S.L. Changes in macronutrient, micronutrient, and food group intakes throughout the menstrual cycle in healthy, premenopausal women. *Eur. J. Nutr.* **2016**, *55*, 1181–1188. [CrossRef] [PubMed]



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