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Effect of physical exercise on taste perception and saliva composition: An exploratory study

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ABSTRACT

Physical exercise has known physiological effects. Although it is empirically assumed that these effects extend to changes in salivation and even in appetite, the influence of exercise on oral biochemistry – and its potential interplay with taste perception - remains poorly understood. This exploratory study investigated the impact of physical exercise on salivary composition and intensity perceived for sweet and bitter tastes, considering high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), in both active and sedentary individuals. Unstimulated saliva samples and taste intensity rating were assessed in 36 participants (18 men, 18 women), 18 sedentary and 18 active, before and after each of the two training sessions (MICT and HIIT). Saliva analysis included secretion rate, total protein concentration, and proteomic profiles using electrophoresis techniques (SDS-PAGE and 2-DE, in this last case for 9 male active individuals), while sweet and bitter taste intensities were evaluated using sucrose and quinine taste strips. Results revealed that MICT significantly increased sweet taste intensity rating in active individuals, while HIIT had this effect in sedentary individuals. No changes were observed for bitter taste intensity. Salivary secretion rate decreased, and protein concentration increased after exercise. However, the effects of exercise in salivary protein profile depended on whether participants were active or sedentary and on the type of exercise. Only in active individuals, levels of albumin and zinc-α2-glycoprotein + carbonic anhydrase VI decreased following acute exercise; notably, the latter was negatively associated with changes in both bitter and sweet taste intensities. Moreover, in these individuals, amylase increased, but only after HIIT. Results from this exploratory study indicate that the effect of physical activity on saliva composition and taste perception may vary according to exercise intensity and lifestyle (active or sedentary). Therefore, nutritional management strategies involving physical activity need to take these aspects into account.

1. Introduction

Taste sensitivity differs between individuals and might affect food choices. Among basic tastes, sweet and bitter tastes are key to promote healthy and sustainable diets by reducing sugar overconsumption and increasing the acceptance of nutrient-rich bitter vegetables or plant-based products (Louro et al., 2021; Rodrigues et al., 2020). A better understanding of sweet and bitter taste perception could help to adapt dietary preferences toward more balanced and plant-based eating habits.

Taste perception may be affected by several factors related to the individual, including its physiology, genetics, emotional state, diet, etc. (Bae & Kang, 2024; Louro et al., 2021; Martin et al., 2023; Shanmugamprema et al., 2024; Subramanian et al., 2024; Zushi et al., 2023). In addition to these factors, physical exercise has also been suggested as a modifiable factor influencing taste perception (Feeney et al., 2019; Wardhani et al., 2011). The effect of exercise in sweet, bitter, salty, sour and umami has been evaluated by different authors, although studies

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differ in methodological aspects related with both the type of physical activity considered, population and sensory evaluation (e.g. sensitivity, preference, etc.). Even so, decreases in umami and increases in salty, sour and sweet preferences have been reported (Gauthier et al., 2020). For the particular cases of sweetness and bitterness, for instance, it have been observed differences in the sweet taste perception according to the level of physical activity, being active persons those reported to perceive a higher intensity of sweet taste in comparison to inactive ones, either in the case of women (Crystal et al., 1995) or men (Feeney et al., 2019; Vidanage et al., 2022). One study performed in male individuals showed that the practise of exercise for some weeks (4–8) increased the sweet taste sensitivity comparatively to the period before the physical activity (Wardhani et al., 2011). On the other hand, the type of exercise may be not irrelevant for the effect at taste sensitivity level: a systematic review performed on 2020 Gauthier et al. (2020) concluded that the sensitivity and preference to sweet taste is increased by acute physical exercise, whereas chronic (long term) practice of exercise, seems to decrease this preference. The authors also looked for studies about the effect of physical activity/exercise on bitter taste, but in this case they did not take straightforward conclusions, with no observation of significant variations obtained after the practise of physical exercise in the reviewed studies (Gauthier et al., 2020). Thus, a better understanding about the relationship between physical exercise and sweet and bitter taste preferences, is still necessary to allow for better strategies for promoting healthier diets and active lifestyles.

It is known that saliva is associated with the oral perception of food, namely taste perception. This fluid, which our group previously observed to be associated with bitter (Rodrigues et al., 2019; Rodrigues et al., 2017a) and sweet (Lamy et al., 2021; Rodrigues et al., 2019; Rodrigues et al., 2017b) tastes sensitivities also undergoes changes in response to individual and environmental conditions, including physical exercise. These alterations produced in saliva by physical exercise are explained, at least in part, to the higher activation of the autonomic nervous system and leads to variations in the secretion and expression of various salivary constituents (Leicht et al., 2018; Ligtenberg et al., 2016; Ventura et al., 2022). For instance, it has been observed an increased in salivary protein concentration following physical exercise (Ventura et al., 2022), what can be due to the sympathetic activation, predominant during exercise, that results in smaller volume of a saliva that is protein-rich. This distinction in signalling can enhance protein expression and secretion even when fluid secretion decreases (Ligtenberg et al., 2016; Sant'Anna et al., 2019). On the other hand, salivary changes induced by physical exercise might result also from to the passage of molecules from blood. Acute exercise can impact muscle tissue, leading to the release of intracellular components like creatinine, kinase, myoglobin, and troponin into the bloodstream, which can then reach the saliva through active or passive transport (Franco-Martínez et al., 2020).

Among the salivary changes caused by physical exercise it has been reported increased levels of salivary proteins like amylase (De Pero et al., 2021; Honceriu et al., 2021; Rutherfurd-Markwick et al., 2017; Weiss et al., 2019), mucins like MUC5B, thus enhancing the salivary viscosity at high exercise intensities (Ligtenberg et al., 2016). The levels of other proteins in saliva like mucin MUC7, lysozyme C, albumin and proline-rich proteins (PRPs) have been also observed to be modified in a different way (increased or decreased) due to physical exercise (Ventura et al., 2022). Physical exercise also impacts salivary proteins related to the immune system as immunoglobulins and cystatins. Moderate exercise enhances salivary IgA (sIgA) secretion, whereas high-intensity or prolonged exercise suppresses it (Leicht et al., 2018; Rodrigues de Araujo et al., 2018; Ventura et al., 2022), and high-intensity exercise elevates the levels of both type C and S cystatins (Sant'Anna et al., 2019).

Despite the growing interest in the relationship between the changes induced in taste perception and saliva composition by physical exercise, to our knowledge, there is a lack of studies characterising the changes in the protein profile of saliva and correlating them with the changes occurring in taste perception (sensitivity and/or intensity), both resulting from the practise of physical exercise. Moreover, the physiological changes that may occur in response to exercise may vary according to the level of activity and the individual characteristics (Noone et al., 2024). For example, at the level of appetite regulation, active individuals are more sensitive to the energy density of food than inactive ones (for more detail please see (Takai & Shigemura, 2020)). Differences in body composition (fat mass and fat-free mass), as well as in the levels of satiety gastrointestinal peptides and hormones, were suggested as potential causes. Since these satiety factors, such as leptin (Rodrigues et al., 2017c), insulin (Beaulieu et al., 2016) have been previously referred as affecting taste sensitivity, different effect of physical activity in active or sedentary individuals cannot be excluded. As such, studies considering the intensity of the exercise and the profile of the individual (active or inactive) can be of interest and have potential implications for dietary and hydration behaviour, particularly in individuals who engage in regular physical activity, or the ones for which changes in lifestyle are needed.

In view of all the above, the aim of this study was to explore how two types of exercise produce changes in salivary protein composition and in the intensity perceived for sweet and bitter tastes, in individuals who exercised regularly (active individuals) and those who did not (sedentary individuals). Given the limited existing research on this topic, the study was designed to identify potential patterns and inform future hypothesis-driven investigations.

2. Materials and methods

2.1. Participants

Forty volunteers between 18 and 29 years were recruited for this study in the University of Évora (Portugal). This age range was selected to ensure a relatively homogeneous population. Taste sensitivity has been shown to change with age (Methven et al., 2012) and by focusing on young adults, we aimed to minimize age-related variability in taste perception. Additionally, this age group represents a period of peak physical capacity, making it an ideal target for investigating the interaction between physical activity levels and sensory outcomes. Additional inclusion criteria were to be non-smokers, not have oral pathology, chronic illness or take any medication. From the 40 participants included, only 36 (18 men and 18 women) completed the entire protocol, although 3 of them (1 sedentary men, 1 sedentary woman and 1 active woman) did not collect enough volume of saliva to allow to perform all laboratory analysis. As such only 33 [17 men (9 active, 8 sedentary) and 16 women (8 active, 8 sedentary)] were considered for statistical analysis.

The anthropometric measures of height and weight were measured for each participant at the beginning of the study and Body Mass Index (BMI) was calculated as kg/m-2.

The participants were divided in two groups according to their frequency of practising physical exercise: a group with regular physical exercise, called "active individuals"; and a group without regular physical exercise, called "sedentary individuals". A regular practice was considered 4 or more physical exercise sessions (at least 40 min) per week, while the absence of regular practice was considered one or less physical exercise session per week, according to the classifications employed previously by other authors (Beaulieu et al., 2017; Feeney et al., 2019; Long et al., 2002). Like this, the participants were divided in active individuals ($n = 9$ men, $n = 8$ women) and sedentary individuals (n = 8 men and n = 8 women).

This study was approved by the Ethics Committee of the University of Évora (Document 22025). Study was conducted only after the participants gave their approval and informed consent, being also informed about the study procedures.

2.2. Study design and sample collection

This study was carried out from December 2021 to July 2022 in the Gym/Sports facilities of the University of Évora, Evora, Portugal. The study was based in the collection of saliva samples and assessment of the intensity perception of sweet and bitter taste before, and after each of two training sessions: a high-intensity interval training (HIIT), and a moderate-intensity continuous training (MICT) (see Section 2.3). Training sessions. An overview of the study design is shown in Fig. 1.

The exercise program took place consistently in the morning period, between 9 a.m. and 11 a.m. Participants were not allowed to drink or eat anything before the assay (except water), and were instructed to 1 h before the training, not take other breakfast and eat a cereal bar (total energy content 120 Kcal), which was equal to all, to avoid potential variations in saliva due to short-time effect of diet (Simões et al., 2021).

Taste strips were prepared by cutting filter paper strips in strips, with the top squared (1 cm × 1 cm). The top of each strip was soaked in 0.20 g/mL of sucrose or 0.0024 g/mL of quinine hydrochloride dihydrate (Merck, Darmstadt, Germany) solutions and dried in a drying chamber at 50 °C. Dry strips were stored in plastic bags and kept at 4 °C until the day of test, never more than 3–4 days after preparation. These concentrations were confirmed, in a previous study (Louro et al., 2021), to allow taste intensity evaluation using filter paper strips (taste strips). Before the training, unstimulated saliva was collected during 4 min, spiting all the volume produced during that time to a tube maintained on ice. Immediately after saliva collection, the perceived intensity of sweet and bitter taste was evaluated. The top of the strip was placed at the top of the tongue, centrally, and participants instructed to close the mouth and allow the strip to wet in saliva for 10 s. Participants registered the intensity perceived, using a LMS (Labelled Magnitude Scale) (Green et al., 1996), i.e., a 100 mm vertical line, marked with labels, with a quasi-logarithmic spaces, ranging from "barely detectable" (0) to "strongest imaginable sensation" (100). Participants were allowed to mark in any point of the line that best represented what they were feeling. Between taste strips, individuals drink water and wait 30 s until the next evaluation. All participants were asked to evaluate their taste perception (intensity of response to sweet and bitter stimuli) before and after each exercise.

Each participant was tested for both exercise protocols on the same day, with 1 h rest between them, as represented in Fig. 1. At the end of each of the two training sessions (HIIT and MICT), unstimulated saliva was collected, and the perceived intensity was evaluated following the same procedure. In total, in the day of experiment, each participant collected saliva 4 times and performed 4 tests for assessing taste intensity (before and after each of the two training sessions).

2.3. Training sessions

Two training sessions of 20 min each were performed in a treadmill (Medisoft, 870 A; Padova. Italy). All participants underwent two types of training: HIIT and MICT. Prior each training, participants had 5 min warming through relaxed walk. Half of the participants randomly started with one type of training and the other half with the other type. HIIT training consisted in the alternation of high intensity with medium intensity exercise. Participants performed repeated periods of continuous running (1-min intensity peaks) to reach 80–90 % of maximum heart rate (MHR), and 1 min of active recovery at 70–75 % of MHR, up to complete the 20 min session (Roy et al., 2018). In MICT training participants performed 20 min of continuous running/walking at an intensity of 65–75 % of MHR (Wewege et al., 2017).

During training sessions, heart rate (HR) was controlled using a heart rate monitor (Polar T31 - Coded Chest Strap; Kempel, Finland). Since the study was performed in individuals in the same age range, the MHR was calculated using the following formula as a function of age: MHR = 220 – age (Roy et al., 2018).

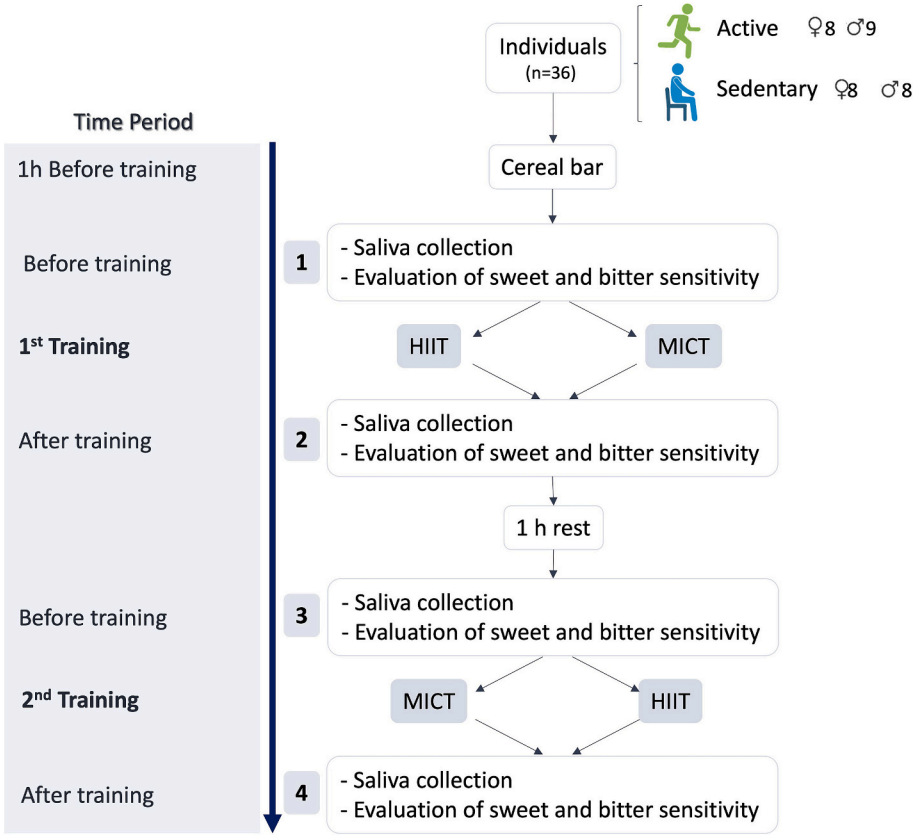

Fig. 1. Diagram of the study design.

2.4. Saliva flow rate, protein quantification and amylase activity

Saliva samples were centrifuged at 13000*g* for 15 min at 4 °C, and the supernatants were aliquoted and stored at −28 °C until analysis. Salivary parameters related with the general functions of saliva, like volume secreted, total protein and enzymatic activity, were evaluated. Saliva flow rate was determined by measuring the saliva volume collected during the 4-min collection period and dividing by this time, assuming a saliva density of 1 g/mL.

Saliva collected was assayed for total protein concentration by the Bradford method (Bradford, 1976), using bovine serum albumin (BSA) as standard, and plates were read at 600 nm in a microplate reader (Glomax, Promega Corporation, Madison, WI, USA).

Alpha-amylase enzymatic activity was determined using a colorimetric commercial kit (Salimetrics®, Cambridge, UK), using p-nitrophenol as substrate, according to the manufacturer's recommendations.

2.5. Salivary protein profile by one-dimensional electrophoresis (SDS-PAGE)

Proteins were separated according to their molecular masses by sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). For each saliva sample, a volume corresponding to 7 μg of total protein was mixed with sample buffer and run on each lane of a 14 % polyacrylamide gel using a Laemmli buffer system, as described in (Lamy et al., 2015). The electrophoretic run was performed at a constant voltage of 150 V. Gels were fixed for 1 h in 40 % methanol/10 % acetic acid, followed by staining for 1 h with Coomassie Brilliant Blue (CBB) R-250 (BioRad, Hercules, CA, USA). Gels were scanned by ImageScanner III (Epson, Suwa, Nagano, Japan) using LabScan software (GE Healthcare, Chicago, IL, USA) and images analysed using ImageLab software (BioRad, Hercules, CA, USA).

2.6. Salivary protein profile by two-dimensional electrophoresis (2-DE)

To go deeper in the possible changes in the salivary protein profile induced by physical exercise, 2-DE analysis was carried out. The 9 male active individuals were considered as a sub-sample, since the limited amount of saliva samples, and the technical limitations of the 2-DE procedure (considering the total number of samples collected - 4 samples per individual) made impossible to use this proteomics approach in all the samples. The selection of male active individuals was made to avoid variations due to usual physical activity practice, as well as for avoid sex and/or higher hormonal variation. The selected samples were pooled and tested in duplicate. Thus, 24 gels (3 pools, each constituted by saliva from 3 different individuals x 4 collection moments x 2 technical repetitions) were analysed in total. The volume of each saliva sample in the pools was adjusted based on its protein concentration to ensure that each pool contained the same amount of protein from each individual sample.

For 2-DE, a saliva volume corresponding to 125 μg of total protein was lyophilized. After this, saliva samples were mixed with rehydration buffer [7 M urea, 2 M thiourea, 4 % (*w/v*) CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonatehydrate), 2 % (*v/v*) IPG buffer, 40 mM DTT (Dithiothreitol)] at a final volume of 125 μL, incubated for one hour at room temperature, then centrifuged at 10000 rpm for 10 min (Eppendorf centrifuge 5424). The supernatants were applied to gel strips with an immobilized pH gradient (3−10) of 7 cm (IPG strips, GE Healthcare, Chicago, IL, USA), covered with mineral oil, and were kept overnight at room temperature. After rehydration, the strips were placed in the Multiphor II system (GE Healthcare, Chicago, IL, USA) for isoelectric protein focusing (first dimension) at 18 °C for 8 h. Focusing program was divided into 4 stages: 1) 0–150 V for 15 min; 2) 150–300 V for 1 h; 3) 300–3500 V for 3 h; 4) 3500 V for 4 h 30 min. After focusing, strips were equilibrated with equilibration buffer [6 M urea; 75 mM Tris–HCl, pH 8.8; 29.3 % (v/v) glycerol; 2 % (w/v) sodium dodecyl sulphate (SDS); 0.002 % (w/v) bromophenol blue] with 1 % (w/v) DTT for 15 min, then, were equilibrated with the same equilibration buffer and 2.5 % iodoacetamide. After equilibration, the strips were applied in SDS-PAGE gels 14 % acrylamide, and electrophoresis was run as previously described for SDS-PAGE gels (Section 2.5). The two-dimensional profiles were analysed using SameSpots software (TotalLab, Newcastle Upon Tyne, UK). Spot editing and matching were performed automatically and corrected manually. Spot volume was normalized to the total spot volume.

2.7. Statistical analysis

The perceived intensity ratings of sweet and bitter, as well as salivary parameters, were assessed for normal distribution using the Shapiro-Wilk test. When data did not follow a normal distribution, transformed values were used (logarithmic transformation). In order to evaluate the effect of exercise on taste and salivary composition, the values of each parameter evaluated [sweet and bitter taste intensity rating, total protein concentration, salivary secretion rate, amylase enzyme activity and levels (% volume) of each protein band] were compared before exercise with the values of this parameter after practicing each type of exercise (HIIT or MICT). This evaluation was based on a GLM model (general linear model), considering period as within-subjects factor (2 levels – before and after) and the factors exercise intensity (2 levels - HIIT or MICT), and individual's usual level of exercise (2 levels - active or sedentary) as between-subjects factors. This allowed to access the effect of each factor, as well as the existence of interactions among factors. The partial eta squared (η^2_p) was calculated as a measure of effect size for the main effects and interactions.

When GLM revealed a statistically significant effect, pairwise comparisons (e.g., before vs. after exercise) were performed using paired Student *t*-test to identify the specific conditions under which significant changes occurred. To complement statistical significance, effect sizes were calculated, for these comparisons, using Cohen's d, to assess the magnitude of the observed differences.

To test the existence of an association between variation in the protein composition of saliva and variation in taste intensity, correlation tests (Pearson) were carried out between the intensity parameters (for sweet or bitter) and each of the salivary parameters. For this, the salivary variation was considered as the ratio between the value after and the value before the exercise (this for each salivary parameter considered), whereas for taste variation it was considered the difference between the intensity perceived after and the one perceived before the exercise (named delta sweet or delta bitter).

For protein spots percentage volumes (obtained after 2-DE analysis), a 2-way ANOVA was applied considering the period (before and after), as within-subjects and the type of training (HIIT and MICT) as between-subjects factors.

The analysis was carried out using SPSS v.29 software. A value of $\alpha = 0.05$ was considered.

3. Results

3.1. Individuals' characteristics

The characteristics of participants (age, weight, height and body mass index) are shown in Table 1.

As it can be observed, all the characteristics of participants except the age (which was slightly higher in active individuals) were similar between active and sedentary individuals, since no significant differences were observed in weight, height or BMI.

3.2. Effect of physical exercise on taste intensity rating

Regarding the effect of physical exercise on taste intensity rating, the interaction between the factors period (before and after) and type of

Table 1
Participants' demographic and anthropometric characteristics (values represent mean ± standard deviation).

	Active individuals			Sedentary individuals			P-value[1]
	Men (n = 9)	Women (n = 8)	Total (n = 17)	Men (n = 8)	Women (n = 8)	Total (n = 16)	
Age	20.1 ± 1.1	22.0 ± 4.0	21.0 ± 2.9	18.8 ± 0.7	19.2 ± 1.4	19.0 ± 1.1	**<0.001**
Weight (W, kg)	68.3 ± 7.0	56.5 ± 6.0	62.8 ± 8.8	66.3 ± 9.8	58.3 ± 9.0	62.3 ± 10.0	0.822
Height (H, m)	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	0.518
BMI (W/H^2, kg/m^2)	22.1 ± 2.4)	22.4 ± 3.3	22.3 ± 2.7	20.9 ± 2.4	22.3 ± 2.5	21.6 ± 2.4	0.296

[1] p-value refers to comparison between the total active and total sedentary individuals.

training (MICT and HIIT) and frequency of physical exercise practice (active or sedentary individuals) was assessed.

Sweet taste intensity was significantly changed by the practice of physical exercise, but type of training (HIIT or MICT) and the level of usual physical activity practice (sedentary vs active individuals) influenced this effect, as observed by the statistically significant interaction observed among these factors (P = 0.037; partial η^2 = 0.066) and moderate effect size. Pairwise comparisons (through t-test) evidenced that in the active individuals, it was the MICT that significantly increased the perceived intensity of sweet taste, with medium effect size (Fig. 2a). While in the case of sedentary individuals, it was the HIIT that produced a slightly increase in sweet taste, with a small effect size (Fig. 2a).

In bitter taste intensity perception significant differences were observed in the interaction of period and type of training (P = 0.042; partial η^2 = 0.065), indicating a moderate effect size. Specifically, HIIT tended to increase and MICT tended to decrease the bitter taste intensity. Nevertheless, not statistically significant within-group differences were observed, neither for sedentary nor active, after HIIT or MICT (Fig. 2b).

3.3. *Effect of physical exercise on salivary secretion, total protein concentration and amylase activity*

The effects of physical exercise on salivary secretion rate, total protein concentration and amylase activity are shown in Table 2. To evaluate these effects, the following factors were considered: training period (before and after), as a within-subjects factor and both the type of training (HIIT or MICT) and the frequency of physical exercise practice (active or sedentary individuals), as between-subjects factors. This design also allowed testing for potential interactions between the factors.

Although, for secretion rate, period had a significant effect, with this salivary parameter presenting decreases with exercise, a statistically significant interaction between period and type of training was observed, with a medium-to-large effect size (Table 2). When pairwise

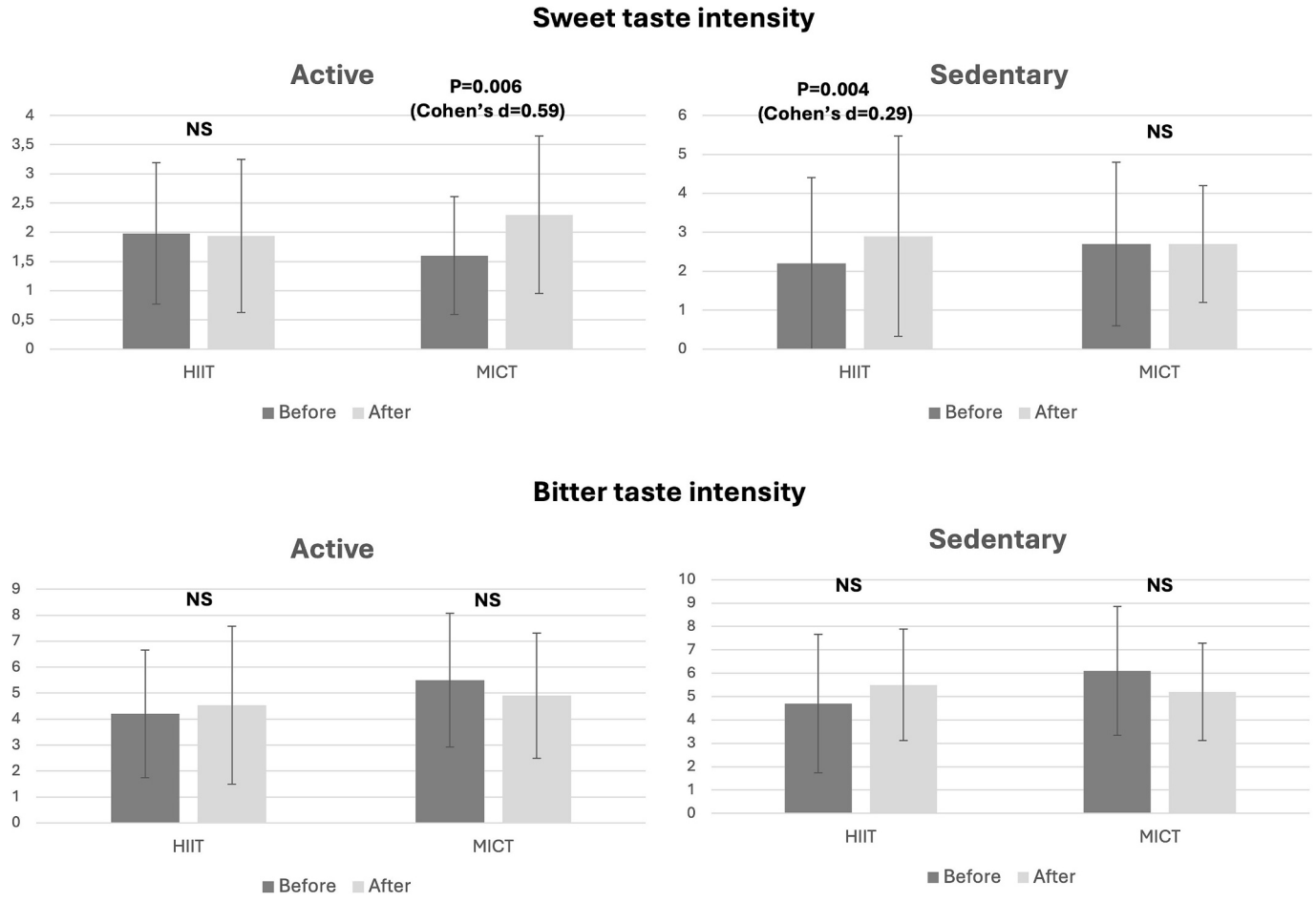

Fig. 2. Variation in the perceived intensities of sweet (2a) and bitter (2b) tastes before and after each training session (HIIT or MICT) in active and sedentary individuals.

Table 2
Effect of exercise on salivary secretion, protein concentration and amylase activity.

Parameter	Before	After	Period[a]	Period*TT[b]	Period*Freq[c]
	Mean ± Standard Deviation	Mean ± Standard Deviation	P-value (Cohens'd)	*P*-value (partial η^2)	P-value (partial η^2)
Secretion rate (mL/min)	0.39 ± 0.29	0.35 ± 0.23	**0.013** (0.1)	**0.010** (0.103)	0.529 (0.006)
Protein concentration (μg/mL)	595.6 ± 391.0	696.3 ± 578.6	**0.04** (−0.19)	0.255 (0.021)	0.537 (0.006)
Amylase activity (U/mL)	126.4 ± 110.0	143.0 ± 122.7	0.078 (0.20)	0.771 (0.009)	0.562 (0.002)

[a] Period: before and after training.
[b] Interaction between the factors period and type of training (TT); TT was considered HIIT or MICT.
[c] Interaction between the factors period and frequency (Freq); Freq was considered as active or sedentary.

comparisons were made, it was only HIIT that resulted in significant decreases in salivary secretion rate (0.42 ± 0.31 to 0.33 ± 0.21 mL/min; $P = 0.012$).

Concerning the total protein concentration, this showed a significant increase after physical exercise, being this not affected by type of training, nor by the level of usual physical exercise practice, as observed by the lack of significant interactions (Table 2). Finaly, amylase enzymatic activity, per unit of volume, did not vary significantly with exercise (Table 2).

3.4. Effect of physical exercise on salivary protein profile by SDS-PAGE

Through SDS-PAGE analysis, it was possible to observe 25 protein bands constantly present in the salivary protein profiles from all individuals (Fig. 3). The identifications of some of these bands are shown in Table 3, according to what was previously obtained in our laboratory, by mass spectrometry, for saliva SDS-PAGE profiles run in the same conditions (Carreira et al., 2020).

The effect of physical exercise, in the different protein bands, for active and sedentary individuals, is presented in Table 3. This table shows the expression levels of protein bands for which exercise-induced variations were observed.

Bands B, B1, C and F, corresponding to polymeric Ig receptor, a non-identified protein (n.i.), albumin, and zinc-α2-glycoprotein + carbonic anhydrase VI (CA VI), respectively, significantly diminished with physical exercise (Table 3). On the other hand, band I (n.i.) increased with exercise. Looking at this effect of physical exercise, but considering to be active or sedentary, significant differences (with medium effect sizes) were observed for active individuals in bands B1 (n.i.), C (albumin) and F (CA VI + zinc-α2 glyco-protein), which showed a decrease after physical exercise. While, in the sedentary group, only band C (albumin) decreased after physical exercise, and this effect was smaller than that observed in active individuals (Cohen's d of 0.34 vs.0.56, for sedentary and active, respectively).

A tendency ($P = 0.05$) for interaction between period and type of training (Period*TT) was observed for band J (n.i.), with medium effect size (partial $\eta^2 = 0.07$), whereas the interaction period and frequency (Period*Freq) of physical activity practice was observed for band B1 (n.i.), also with a medium effect size (partial $\eta^2 = 0.087$).

3.5. Relationship between salivary protein profile and taste intensity rating

Given the effects that physical exercise had on both taste intensity

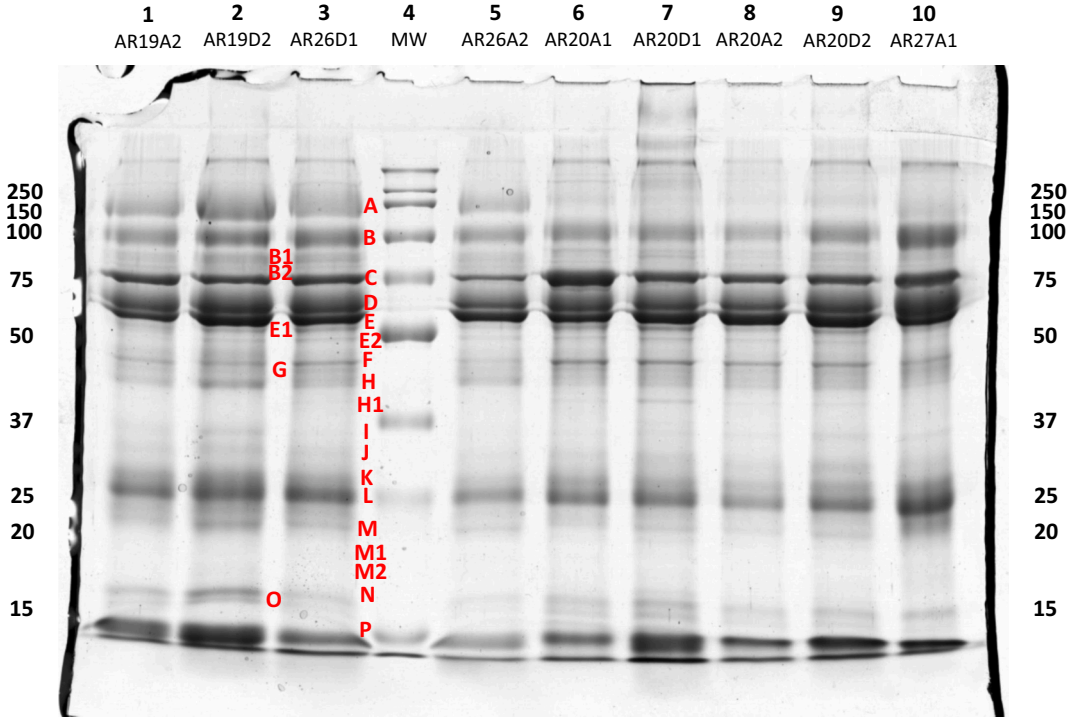

Fig. 3. Representative gel of the salivary protein profile obtained by SDS-PAGE. The letters from A to P represent the different protein bands present in most of the profiles. Values on left and right side represent molecular masses (MW - kDa).

Table 3
Effect of physical exercise on the salivary protein profile.

Band	Protein ID	Group	Before	After	Period[a]	Period*TT[b]	Period*Freq[c]
	UNIPROT Assession number[1]		Mean (± Standard Deviation)	Mean (± Standard Deviation)	P-value (Cohen's d)	P-value (partial η^2)	P-value (partial η^2)
B	Polymeric immunoglobulin receptor (P01833)	Total	6.39 ± 1.70	5.99 ± 1.47	**0.044** (0.25)		
		Active	6.93 ± 1.87	6.43 ± 1.53	0.091 (0.29)	0.701 (0.003)	0.622 (0.005)
		Sedentary	5.88 ± 1.36	5.57 ± 1.29	0.276 (0.23)		
B1	n.i[2].	Total	1.32 ± 0.97	1.00 ± 0.77	**0.006** (0.37)		
		Active	1.52 ± 1.12	0.95 ± 0.59	**0.002** (0.64)	0.673 (0.003)	**0.031** (0.087)
		Sedentary	1.12 ± 0.75	1.05 ± 0.92	0.634 (0.08)		
C	Albumin (P02768)	Total	8.74 ± 4.43	7.04 ± 3.48	**<0.001** (0.43)		
		Active	10.39 ± 4.34	8.11 ± 3.85	**0.001** (0.56)	0.944 (0.000)	0.138 (0.040)
		Sedentary	7.22 ± 4.00	6.05 ± 2.82	**0.003** (0.34)		
F	Zinc-α2-glycoprotein (P25311) + Carbonic anhydrase VI (CA VI) (P23280)	Total	1.81 ± 0.70	1.56 ± 0.50	**0.003** (0.41)		
		Active	1.91 ± 0.85	1.55 ± 0.46	**0.020** (0.53)	0.204 (0.030)	0.145 (0.039)
		Sedentary	1.71 ± 0.51	1.56 ± 0.55	0.111 (0.28)		
I	n.i[2].	Total	1.10 ± 1.29	1.38 ± 1.37	**0.049** (−0.21)		
		Active	0.83 ± 0.59	1.02 ± 0.71	0.135 (−0.29)	0.547 (0.007)	0.613 (0.005)
		Sedentary	1.37 ± 1.68	1.73 ± 1.73	0.153 (−0.21)		
J	n.i[2]	Total	1.36 ± 0.58	1.37 ± 0.67	0.859 (−0.02)		
		Active	1.34 ± 0.54	1.55 ± 0.75	0.143 (−0.32)	**0.050** (0.070)	0.781 (0.001)
		Sedentary	1.37 ± 0.63	1.20 ± 0.54	0.221 (0.20)		

[a] Period: before and after training.
[b] Interaction between the factors period and type of training (TT); TT was considered HIIT or MICT.
[c] Interaction between the factors period and frequency (Freq); Freq was considered as active or sedentary.
[1] UNIPROT assession number of the proteins that were previously identified [34] for SDS-PAGE profiles of saliva obtained in similar conditions.
[2] n.i. – "not identified", means a protein band for which identification was not obtained from previous studies.

rating and salivary protein profile, as observed in previous sections, the existence of correlations between sweet and bitter taste intensities and the salivary protein profile was assessed.

Pearson correlation test showed that, variations in protein band G (CA VI + zinc-α2 glycoprotein) were negatively correlated with variations in the intensity of sweet taste ($r = -0.333$, $P = 0.011$); this, considering together saliva and taste data obtained both performing HIIT and MICT. When looking specifically for the correlation, considering each type of training separately, the correlation between variations in sweet taste ratings and band G ($r = -0.469$; P = 0.01) was statistically significant for HIIT, being not for MICT ($r = -0.102$; $P = 0.605$). This means that with HIIT, individuals with higher negative variations in band G are the ones who tend to increase more the sweet taste intensity rating.

Taking into account that to be active or sedentary was previously observed as impacting the effect of physical exercise on taste perception, these groups of individuals were assessed separately. For sweet taste, in both active and sedentary individuals a significant negative correlation between the variation in band G (CA VI + zinc-α2 glycoprotein) and the variation in sweet taste intensity rating was only observed for HIIT (active: $r = -0.605$, $P = 0.022$, $N = 14$; sedentary: $r = -0.604$, $P = 0.017$, $N = 15$) (Fig. 4).

Regarding bitter taste, results showed that variations in bitter taste intensity were negatively correlated with variations in the protein band F (CA VI + zinc-α2 glyco-protein) ($r = -0.272$; $P = 0.038$) and positively correlated with the protein band H (CA VI + zinc-α2 glyco-protein) ($r = 0.303$; $P = 0.021$). However, when looking at the impact of the type of training, it was only with HIIT that variations in the intensity of bitter taste perception correlated with the changes in saliva. In this case, a moderate positive correlation was observed between the variations in bitterness and the changes in band H ($r = 0.549$; $P = 0.002$) and in band I ($r = 0.409$, $P = 0.029$), this last a non-identified band.

Looking separately at the groups of active and sedentary individuals, variation in bitter taste after HIIT was correlated with changes in band H (CA VI + zinc-α2 glyco-protein) ($r = 0.653$, P = 0.011) only in active participants, whereas for sedentary individuals after HIIT, the band I (not identified, n.i.) was the one significantly correlated with variations in bitter taste ($r = 0.591$, $P = 0.020$). In the case of MICT exercise, the active participants had variations in bitter taste intensity rating negatively correlated with the variation in band C (albumin) ($r = -0.569$, $P = 0.034$).

3.6. Effect of physical exercise on salivary protein profile by 2-DE

To deepen into the impact of physical exercise on salivary protein profiles, 2-DE profiles were obtained for pooled saliva samples from 9

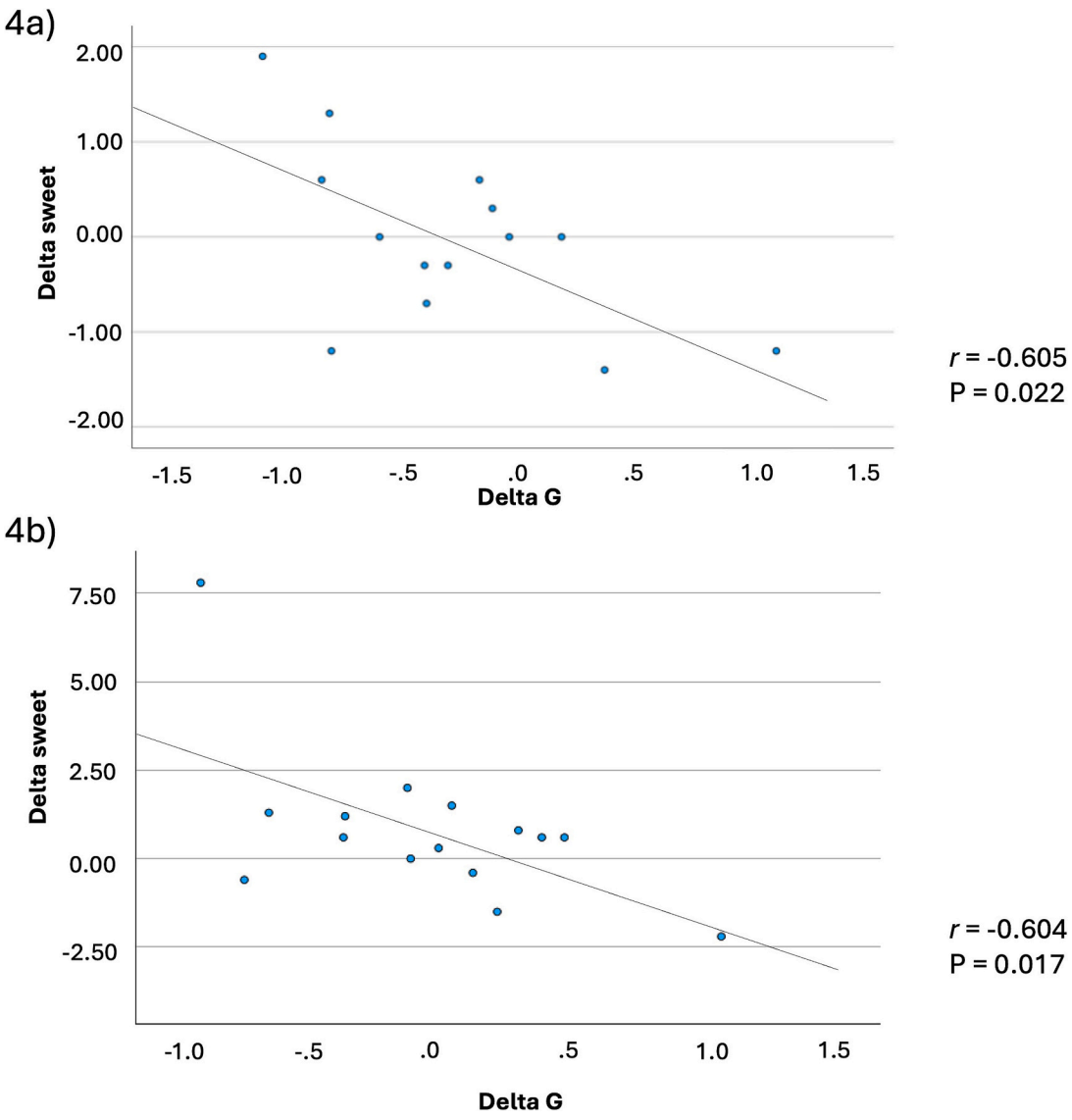

Fig. 4. Correlation between the variations (delta) in taste sensitivity and the variations in the levels of protein band G (CA VI + Zinc-α2 glycoprotein) in active (**4a**) and sedentary (**4b**) individuals.

active men collected before and after exercise. A total of 94 spots were observed (Fig. 5) and considered for the statistical analysis.

Results showed significant variation due to the physical exercise practice (taking HIIT and MICT together) in the spots 89 and 134, corresponding to amylase and carbonic anhydrase VI (CA VI), respectively. Both protein spots experienced a decrease in their expression levels due to exercise (Table 4).

In a further step, a 2-way ANOVA was applied considering the period (before and after) and the type of training (HIIT and MICT) as the two factors. Results showed significant differences in 7 protein spots (spots 55, 67, 69, 106, 116, 134 and 135), induced only by HIIT. Based on the identifications made by other researchers (Jessie et al., 2010; Rodrigues et al., 2019; Rodrigues et al., 2017a), it was possible to identify these protein spots (Fig. 6). Spots 55 (albumin), 67 and 69 (both of amylase) increased only after HIIT training (*p*-values of 0.036, 0.046 and 0.028, respectively) (Fig. 6a). On the other hand, spots 106 (n.i.), 116, 134 (corresponding to CA VI) and 135 (n.i.) decreased with HIIT (p-values of 0.025, 0.044, 0.009, 0.049, respectively), while they did not experienced variations in their levels with MICT (Fig. 6b).

4. Discussion

This study aimed to assess the changes induced by physical exercise on the salivary protein profile and perception of sweet and bitter tastes. Results showed that sweet taste intensity rating was affected by physical exercise. This effect was dependent on the individual being active or sedentary and on the type of training (HIIT or MICT), with sweet taste intensity rating increased in active individuals after MICT session, whereas in sedentary only HIIT results in slight increases in this.

In a systematic review evaluating the impact of exercise on sweet taste sensitivity and preferences and including 16 papers from 1995 to 2020, authors concluded that physical exercise increased the sensitivity to sweet taste (Gauthier et al., 2020), which agrees with results from present study. The different effect of exercise that we observed between active and sedentary individuals goes also in line with previous studies that compared individuals who are physically active with individuals who are not active (sedentary) (Crystal et al., 1995; Feeney et al., 2019), observing that active people were more sensitive to sweet taste than sedentary people (Gauthier et al., 2020). It is possible that the effect of exercise in increasing taste intensity can be maintained with regular practice, explaining this higher intensity perception in active

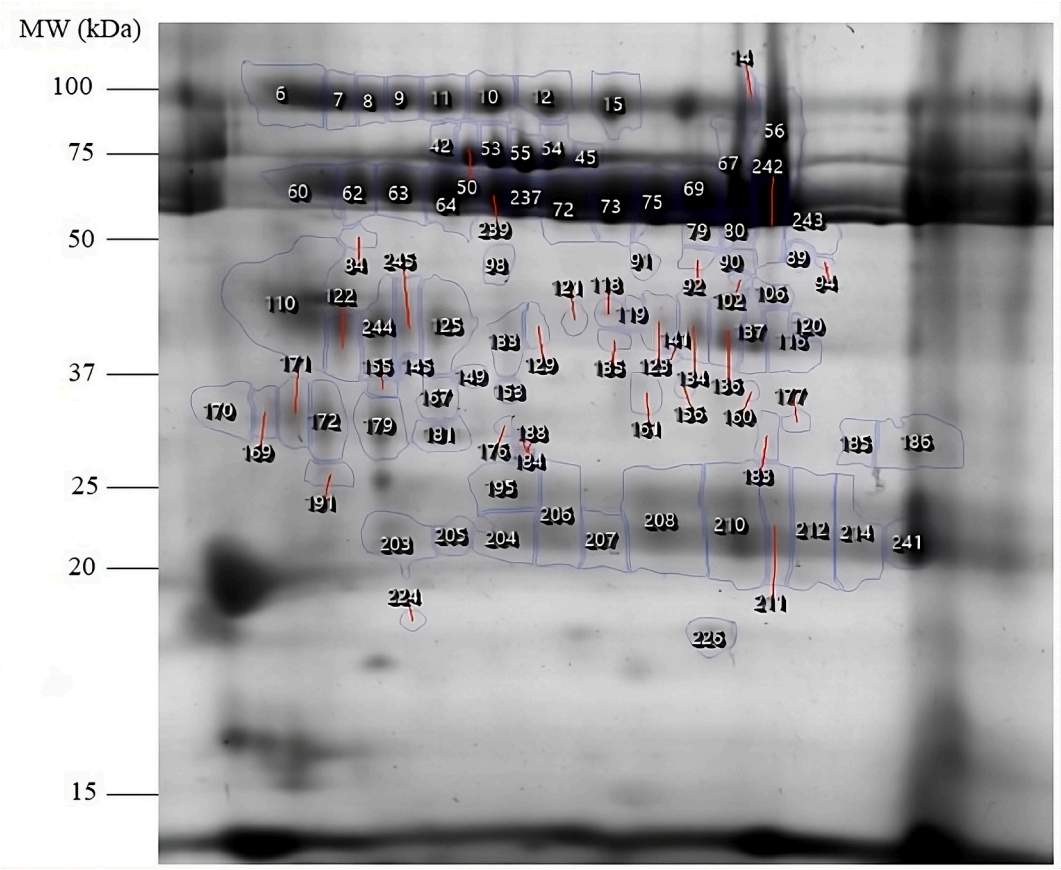

Fig. 5. Representative image of a 2-DE gel obtained from the saliva samples from active men collected before and after exercise. The numbers represent the protein spots (n = 94) considered for the statistical analysis. MW – molecular mass (kDa).

Table 4
Variations in the two-dimensional electrophoresis protein spots with the practice of physical exercise (values represent the mean ± standard deviation of normalized spot volume).

Spot	ID[1]	Before (N = 3 pools)[2]	After (N = 3 pools)[1]	P-value
89	Amylase fragment	$165.4 \times 10^4 \pm 37.7 \times 10^4$	$37.4 \times 10^4 \pm 33.7 \times 10^4$	**0.023**
134	Carbonic anhydrase VI	$557.3 \times 10^4 \pm 40.5 \times 10^4$	$400.4 \times 10^4 \pm 36.2 \times 10^4$	**0.011**

[1] Spots identification were based on previous studies (Jessie et al., 2010; Rodrigues et al., 2017a).
[2] Each pool was run in duplicate (technical replicate).

individuals. Nevertheless, this idea needs to be further validated.

As an explanation to the increase in intensity perception of sweet taste after exercise, this could be attributed to the need to higher detection of sugar in food following the physical activity. During exercise, active muscles require energy, and they use blood glucose as fuel, which result in decreasing blood glucose levels. While, during prolonged or intense exercise, muscles use the glycogen stored in muscles and liver as a source of energy. Additionally, physical exercise improves insulin sensitivity, turning the body cells more efficient to bind to glucose molecules from the bloodstream (Ashcroft et al., 2024). This fact makes that the preference to sweet taste increase to rapidly satisfy the energy needs(Kimmeswenger & Lieder, 2024). Thus, it makes sense that the sensitivity to sweet taste increase to maintain a balance in blood glucose levels by using glucose from food, thus replenish glycogen stores. All this can also suggest an explanation for the different effect of exercise types for sedentary and active participants. The moderate increase in sweetness intensity after MICT, in active individuals, by opposition to the need of high intensity training in sedentary, may be a reflex of the adaptation to exercise that active individuals have, which "activate" mechanisms involved in a higher efficiency of replacing energy comparatively to no trained individuals. In fact, scientists started to see that subjects with high physical activity start to adapt to exercise, not increasing the total energy expended (Pontzer et al., 2016). A potential synergic effect of increased sweetness intensity, at lower activity signals, may be hypothesized, although further studies are needed to test this hypothesis. It is necessary to carefully consider the hypothesis raised, since the relationship between sweet taste perception and carbohydrate intake reach no consensus through studies. On one hand, different levels of sweetness can exist in products containing carbohydrate, whereas on the other hand many factors affect food final choices. There are even studies observing inverse relationship between sweet intensity perception and sweet foods intake (Jayasinghe et al., 2017).

Regarding bitter taste, no significant differences were observed in active and sedentary individuals when comparing the intensity perceived before and after both trainings (HIIT and MICT). Although a limited number of studies evaluated the effect of exercise on bitter taste perception, the results are not consensual. In some studies authors did not found significant impact on the habitual physical exercise practice on bitter taste perception (Feeney et al., 2019), whereas a more recent study, considering a high number of participants (N > 3000) refers an impact of frequent vigorous physical activity in increasing bitter taste sensitivity for normal-weight males (Gauthier et al., 2023). In the present study, a significant effect was observed in the interaction between period and type of exercise, suggesting a tendency for an increase in bitter taste intensity rating after HIIT and a decrease after MICT, in both active and sedentary individuals. Considering than a lower sensitivity to bitter taste might favour the acceptance of vegetable foods (Louro et al.,

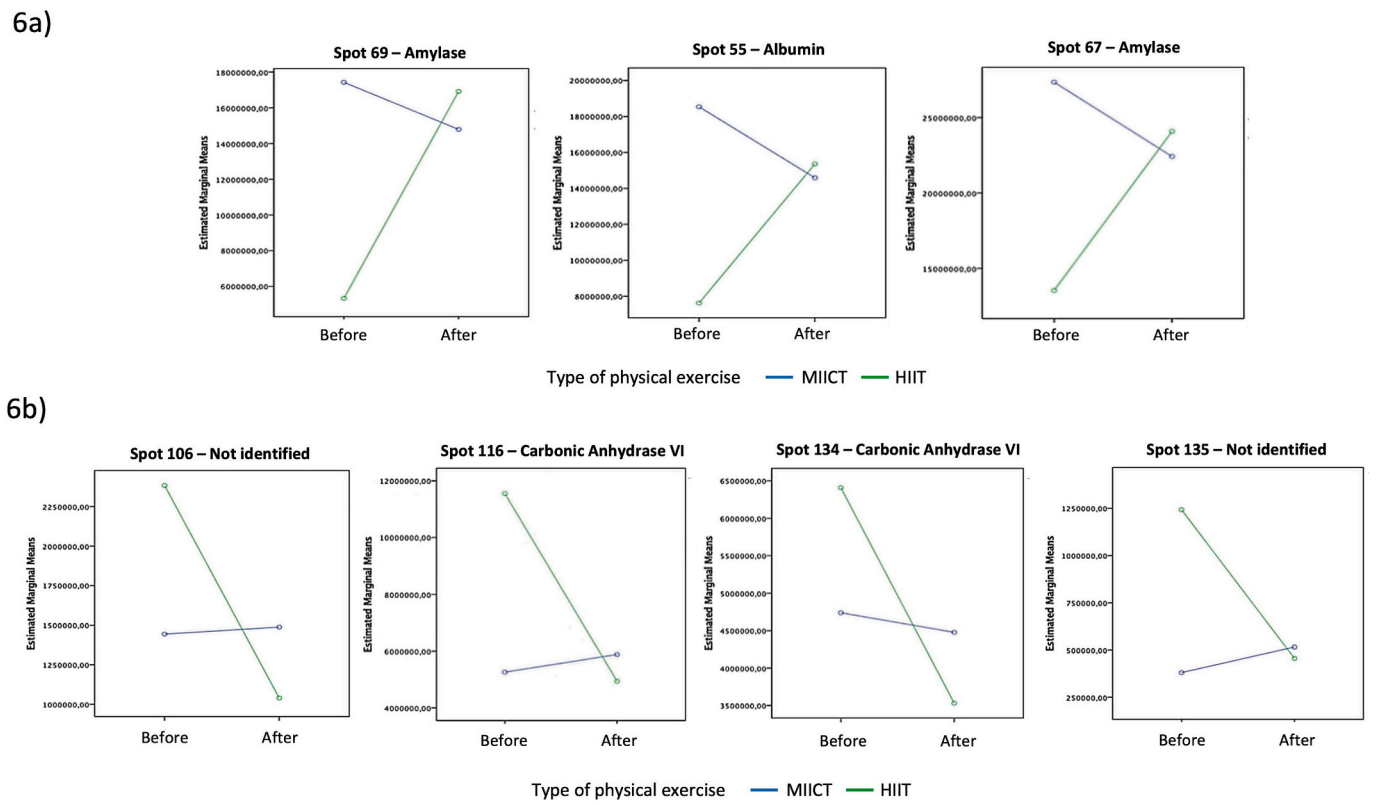

Fig. 6. Protein spots with significant differences from 2-way ANOVA considering the period (before and after) and the type of training (HIIT and MICT). Fig. 6a and b shows the spots that increased and decreased, respectively due to HIIT physical exercise practice. Identifications of protein sports were based on previous studies (Jessie et al., 2010; Rodrigues et al., 2019; Rodrigues et al., 2017a).

2021; Rodrigues et al., 2020), it would be interesting to develop more studies to elucidate the characteristics of exercise that could affect bitter taste, in order to develop further strategies that lead to modulate bitter taste and to a greater acceptance of healthier vegetable foods with lower sugar levels.

Regarding the effect of physical exercise on saliva, changes were observed in the secretion rate and protein concentration. Salivary secretion rate decreased, while the protein concentration increased after the practise of physical exercise. Although previous studies have reported a decrease in secretion rate after exercise (Chicharro et al., 1998; Mulic et al., 2012; Walsh et al., 2004), in a more recent review investigating effect of physical exercise in saliva composition, authors concluded that salivary flow increased during the practise of exercise, while it decreased at the end of the practise when recovered (Ntovas et al., 2022). In the present study, saliva was collected after finishing the training, so the results agree with previous findings. The decrease in salivary flow after physical exercise has been explained by the increase in the sympathetic nervous system activity during exercise (Mulic et al., 2012). In this case, sympathetic activation and adrenergic activity cause significant vasoconstriction in the arterioles supplying the salivary glands, reducing secretion (Ntovas et al., 2022; Trochimiak & Hübner-Woźniak, 2014). Another explanation to the reduced salivary secretion rate after physical exercise has been a certain level of dehydration caused by the exercise (Ligtenberg et al., 2015). Although in the present study, hydration status was not monitored, and participants were allowed to drink water whenever they needed, a variable level of loss of hydration is expected.

Regarding salivary total protein increase after physical exercise, is also in line with reports by other authors (Chicharro et al., 1998; Ligtenberg et al., 2015; Ntovas et al., 2022; Sant'Anna et al., 2019). This also agrees with the previously mentioned physical exercise stimulation of sympathetic activity, activating the β-adrenergic receptors. An increased β-adrenergic activation in the salivary glands stimulates the release of protein-rich vesicles without significantly increasing saliva volume, with the consequent increase in saliva total protein concentration. Curiously, no statistically significant increases in amylase enzymatic activity occurred in response to the two types of exercise tested, although a tendency was seen. Other potential physiological and hormonal changes resultant from exercise cannot be excluded, as for example a decrease in insulin release, during the practice of exercise. In animal models, this hormone was previously observed to acutely affect salivary glands secretion (DeVito-Moraes et al., 2021). Moreover, other receptors, for molecules that can be affected by acute exercise (e.g. aldosterone, testosterone) are also present in salivary glands (Laine et al., 1993).

Looking at the impact of physical exercise on salivary protein profiles, it was observed that protein bands corresponding to salivary albumin decreased after physical exercise in both sedentary and active individuals, while the levels of CA VI + zinc-α2 glyco-protein decreased only in the saliva from active individuals.

Regarding the impact of exercise in salivary albumin levels, various studies have investigated this fact and found diverse results. Some authors have observed an increase in salivary albumin after high intensity exercise. For instance, Ligtenberg et al. (2015) observed an increase in salivary albumin bands from SDS-PAGE analysis, in unstimulated saliva samples collected after high intensity exercise (running for 10 min as fast as participants could), however, saliva samples taken after moderate exercise intensity and after recovery showed relatively little variation in the levels of albumin. More recently, Ventura et al. (2022), observed an increased in salivary albumin determined by LC–ESI–MS/MS in stimulated and non-stimulated saliva samples collected after low intensity exercise (interval exercise in a treadmill with a medium speed of 4.6 ± 0.4 km/h during 40 min) compared to those samples collected before exercise. On the other hand, some studies did not find any effect of

physical exercise on the levels of salivary albumin. As is the study of Borchers et al. (2022), in which authors did not observed significant differences in the levels of salivary albumin (determined by ELISA) collected in non-stimulated saliva before, after an ultramarathon, and after recovery period (8–11 days after). The inconsistency of the results, from the different studies can be due to the use of different techniques (e.g. electrophoresis, chromatography, immunochemistry), which can be assessing different proteoforms. Moreover, the population and the time of the day are all factors that may affect saliva composition (Rocha et al., 2024) and that can affect the different conclusions. Nevertheless, those investigations suggest that the levels of albumin in saliva, in response to the practice of physical exercise may be not constant, with more studies needed to understand the dynamic of this salivary protein with exercise.

CA VI has been described as a trophic factor that supports the growth and development of taste buds, also protecting them from apoptosis (Morzel et al., 2014). Indeed, it has been observed that individuals with lower taste sensitivity or with taste disorders present under expressed levels CA VI and zinc-α2 glycoprotein in saliva (Igarashi et al., 2008; Shatzman & Henkin, 1981). The association of CA VI with sweet taste intensity has been also observed in previous studies, in which authors showed a higher expression of CA VI in individuals with low sweet sensitivity compared to those with high sensitivity (Rodrigues et al., 2017c). The decrease in CA VI, together with an increase in sweet taste intensity rating, reported above, agree with these results. This is reinforced by the present observation of a significant correlation between this protein and sweet taste perception after the high intensity training. As previously explained, physical exercise increased the sensitivity to sweet taste to satisfy the energy needs produced by exercise (Kimmeswenger & Lieder, 2024). Thus, altogether these results suggest that physical exercise could modulate the saliva composition, relating with the sweet taste perception.

The interest about this relationship between exercise and CA VI is also increased by the association of this protein with the sensitivity and acceptance of bitter taste (Patrikainen et al., 2014; Subramanian et al., 2024). For instance, higher abundance of bands containing CA VI and zinc-α2 glyco-protein in saliva have been associated to a lower acceptance of bitter taste in 3–6 month babies (Morzel et al., 2014). In children (8–9 years old), higher levels of salivary CA VI have been observed in boys low-sensitive to bitter taste and in girls sensitive to bitter taste (Rodrigues et al., 2019). However, the relationship between CA VI is not totally understood, with some studies suggesting that variations in the sensitivity to bitterness (evaluated for the bitter PROP compound) were not related to the levels of CA VI in saliva, but with the functionality of the protein influenced by genetic polymorphism (Padiglia et al., 2010). Results from our study are somehow difficult to interpret, since bands F, G and H are all identified as potentially containing CV IV and/or zinc α-2 glycoprotein and the changes in each of them do not correlate in the same way with changes in bitterness perception. One important aspect is that the proteomics approach used in the present study (gel-based protein separation followed by mass spectrometry identification of protein bands and spots) does not allow to be sure about which of these proteins (zinc-α2-glycoprotein or CA VI) are more abundant than the other, neither if this proportion is different among the different protein bands mentioned. In active participants, HIIT resulted in changes in band H (containing CA VI + zinc α-2 glycoprotein) positively correlated with changes in bitterness intensity, indicating that increased the salivary levels of these proteins associate with an increased perception of bitter taste after exercise, in line with the studies that suggest that CA VI can be involved in increased bitterness sensitivity. These results reinforce that exercise might be linked to taste perception, and consequently with dietary preferences or food acceptance post-exercise.

Additionally, results from this study also showed a negative correlation between changes in bitter taste intensity rating and changes in the levels of salivary albumin in active individuals after less intense effort (MICT session). This seems to occur in opposition to previous studies, where albumin was observed to be positively related with bitterness. For instance, in children aged 8–9 years, it was observed that increased levels of albumin spots (determined by 2-DE) were present in saliva from children sensitive to bitter taste, although this correlation was influenced by the BMI percentile of children (Rodrigues et al., 2019). Moreover, in male adults, it have also been observed higher levels of salivary albumin in individuals hypersensitive to bitter taste (< 0.5 mM of caffeine) (Dsamou et al., 2012). In spite of these findings, the relationship between albumin, with bitter taste is still unclear and will deserve more research.

To explore in deep the impact of exercise on salivary proteome, 2-DE analysis were run in pooled saliva from 9 active male individuals. Only active males were chosen, to minimize variability in physic conditions and hormonal parameters. Results showed that HIIT increased the levels of albumin and amylase but decreased CA VI levels in saliva. While MICT decreased salivary albumin and amylase levels. Concerning CA VI, these results were in line with what was observed in individual SDS-PAGE gels, where CA VI containing bands decreased after exercise, as discussed above. For albumin, it can seem contradictory, however, in 2-DE this variation was observed only in one albumin spot, indicating the need to better know the differences in the proteoforms of each protein, to better interpret the results.

Regarding salivary amylase, several studies have reported an increase in this protein due to physical exercise, which has been explained by the activation of the sympathetic nervous system and the hypothalamic pituitary adrenal axis in response to exercise (Chicharro et al., 1998; Koibuchi & Suzuki, 2014; Ntovas et al., 2022), which produce a saliva lower in volume but rich in proteins (like amylase) (Chicharro et al., 1998). Additionally, higher levels of salivary amylase have been shown with more intensity (>70 % VO2) and longer trainings (Koibuchi & Suzuki, 2014; Ntovas et al., 2022; Rutherfurd-Markwick et al., 2017), while for light or moderate physical exercise (e.g. relaxed walking or cycling) results are less consensual, including observations of no significant effects in the variations of salivary amylase (Borchers et al., 2022; Chicharro et al., 1998; Ligtenberg et al., 2015; Rutherfurd-Markwick et al., 2017) or a significant increase but less acute than for high intensity activities (Koibuchi & Suzuki, 2014; Yamaguchi et al., 2006). Some studies have reported that trained individuals tend to show a more moderate response in salivary amylase levels compared to sedentary individuals, which was explained because their autonomic system is better regulated, and the perceived stress on the body during exercise is lower (Ntovas et al., 2022). Results from present study showed an increase in amylase spots after HIIT. It is interesting to see that increases in amylase, with HIIT, were only evident in 2-DE analysis, with analysis to total amylase enzymatic activity or SDS-PAGE resulting in no statistic significant variations. This highlight the need to have in mind that salivary amylase is present in different proteoforms, which may be differently regulated and may have different activities and/or functions (Contreras-Aguilar et al., 2021). Being this a protein frequently used in a diversity of studies to assess stress (sympathetic activity) or food oral perception, metabolism and diet, it is important to reinforce the need to deeply study its different forms.

In spite of this, this study presents some limitations that should be considered when interpreting the results, such as the size of the subgroup for 2-DE analysis, and the fact of this to consist only in male active individuals, which could limit the generalizability of the findings about the responses at proteoform level. Another limitation is the short-term nature of the intervention, which not allow to evaluate the long-term effects of regular exercise on taste perception and salivary proteome. Moreover, although the same food has been provided to all participants, to avoid salivary variations due to potential short-time effect of intake, it is not possible to exclude potential variations in dietary habits among participants. Participants performed the two training sessions in the same day, to avoid variations due to daily uncontrollable factors (e.g. mood, etc.). However, this introduced the limitation of potential carry-over effects that, although minimized by the randomized design, cannot be fully excluded.

An important point of this study is the decision of not applying corrections to control for Type I error in repeated measures analyses, since these can be overly conservative, in studies with limited sample sizes and exploratory aims, like this one, increasing the risk of Type II errors. As such, we prioritized sensitivity to potential effects by reporting uncorrected *p*-values. This approach allowed us to identify trends and group-specific changes in taste intensity rating and/or salivary protein levels that may be relevant for future hypothesis-driven research. However, the possibility of inflated Type I error should be acknowledged, and these findings should be interpreted as preliminary and hypothesis-generating rather than conclusive.

Despite these limitations, this study offers valuable directions for future research on the interactions between exercise, saliva, and taste perception and to identify potential opportunities to improve dietary behaviour through physical activity.

5. Conclusions

This exploratory study shows that physical exercise influences salivary composition and taste perception, with different effects depending on lifestyle (active or sedentary) and exercise intensity (moderate or high). These results suggest potential influences of physical activity for food perception and acceptance, which require further investigation. Results showed that physical exercise reduced salivary flow, increased the total protein concentration and changed the levels of different proteins. These changes indicate that regular physical activity can modulate salivary protein composition in ways that can have implications for food oral processing and/or sensory functions.

One of the main advances of this study, over others previously made, is that it explores how both intensity and regularity of exercise jointly affect taste and salivation. Until now, the studies focus on exercise effect in each of these parameters independently. A negative relationship between variations in CA VI + zinc-α2 glyco-protein levels and variations in sweet and bitter taste intensity rating suggest that changes in salivation may mediate taste perception after exercise, or vice versa. Further studies need to be performed to understand the existence and direction of causality.

Overall, this study provides novel insights about the relationship between physical exercise, salivary proteome, and taste. Despite being an exploratory study, the results add contribution to understand about how physical activity modulates dietary preferences and metabolic responses, offering a basis for future research aimed to further explore this topic. Deeper comprehension of this issue can aid the management and personalization of diets for individuals engaged in physical activity, including high-performance athletes, by providing more effective strategies to promote healthier dietary patterns and active lifestyles.

CRediT authorship contribution statement

Ana Roque: Methodology, Investigation, Formal analysis. **Maria Perez-Jimenez:** Writing – original draft, Visualization, Formal analysis. **Carla Simões:** Writing – review & editing, Investigation. **Laura Carreira:** Writing – review & editing, Investigation. **Fernando Capela e Silva:** Writing – review & editing, Supervision. **Nuno Batalha:** Writing – review & editing, Supervision. **Armando Raimundo:** Writing – review & editing, Supervision. **Elsa Lamy:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Elsa Lamy reports equipment, drugs, or supplies was provided by University of Évora. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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