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RESEARCH PAPER

TAS2R5 and TAS2R38 are bitter taste receptors whose colonic expressions could play important roles in age-associated processes

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Abstract

Ageing disrupts how our bodies process nutrients, leading to deregulation of nutrient-sensing and increased inflammation. Dietary interventions can promote healthy ageing, which demonstrates the importance of both metabolism and the gastrointestinal tract for our health. Bitter taste receptors (TAS2R) present in the intestine are key members of metabolic regulation. TAS2R are involved in controlling enterohormonal secretion, detect phenolic compounds in our diet, and potentially have a great impact on the ageing process.

Here, we aimed to analyze the potential role of intestinal TAS2R on the ageing process and establish potential impact of these receptors on the biomarkers.

Healthy subjects were divided into two age cohorts: young (38.9±6) and aged (63.6±6). TAS2R expression was analyzed in the colon. Analyses of metabolomics and of phenolic markers were performed in plasma. Best discriminatory parameters were obtained using three machine-learning methods. Finally, Spearman's rank correlation was performed.

The best separators of the age cohorts were docosahexaenoic acid and multiple lipoprotein fractions. Two TAS2R were also identified: TAS2R5 and TAS2R38. TAS2R5 correlated with multiple lipoprotein-derived fractions, inflammatory marker IL-6 and polyunsaturated fatty acids. TAS2R38 was much more selective, correlating with a few parameters, including membrane lipid sphingomyelin, ketone body acetone, and omega acids. Both TAS2R5 and TAS2R38 correlated with β-hydroxybutyrate.

The parameters that correlated with TAS2R have known effects on the ageing process. This suggests that TAS2R5 and TAS2R38 are the bitter receptors most likely to play a role in the development and progress of ageing.

Keywords: Bitter taste receptors; TAS2R5 and TAS2R38; Ageing; Metabolomics; Phenolic metabolites; Gastrointestinal tract.

Abbreviations: CCK, cholecystokinin; CD36, cluster of differentiation 36; CPMG, carr-purcell-meiboom-gill; Glyc-A, N-acetyl glucosamine/galactosamine; GlycB, Sialic acid; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LMWMs, low-molecular-weight metabolites; MCP-1, monocyte chemoattractant protein-1; MS, mass spectrometry; NMR, nuclear magnetic resonance; PLS-DA, partial least squares-discriminant analysis; Ppb, parts per billion; PROP, 6-n-propylthiouracil; PSI, pounds per square inch; PUFAs, polyunsaturated fatty acids; RF, random forrest; sMRM, scheduled multiple reaction monitoring; VLDL, very-low-density lipoprotein.

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1. Introduction

Ageing is characterized as a progressive loss of physiological functions, a deterioration that poses a significant risk of developing serious health disorders [1]. One aspect that is greatly affected as we age is metabolic regulation. As we progress through life and our physiological makeup changes, a shift is observed in how our cells process nutrients and how energy production is conducted [2]. One hallmark of the ageing process is the deregulation of nutrient-sensing [3]. When food is ingested, the signaling cascades are activated by the components of our diet, such as glucose and amino acids, and can achieve an anabolic response that is characterized by changes in cellular activity. Autophagy, protein synthesis, glucose, and nucleotide and lipid metabolism are just a few noticeable effects [4–6]. However, these mechanisms are often affected by ageing-induced changes that potentially produce negative effects on our health. These changes lead to excessive anabolic signaling and, finally, increased inflammation, which is one of the most prominent causes of age-related health issues [7,8]. Further evidence of the importance of metabolism in the progression of ageing is provided by the fact that dietary interventions such as caloric restriction, which have been widely studied over the years, have displayed a positive effect in delaying the ageing process or achieving healthier ageing [9,10].

An important component of this nutrient-sensing complex are the numerous chemo-sensing receptors found along the gastrointestinal tract (GIT) that form important signaling pathways with the whole range of physiological systems [11,12]. These receptors possess a wide range of functions. One family of receptors found here are taste receptors [13]. Although their name provides a clue as to their roles, there is much more to these receptors—as we deduce from their appearance in a myriad of diverse tissues. One large group of these receptors are the bitter taste receptors, or TAS2R. We currently know of 26 different forms of TAS2R in humans, which makes them the most diverse taste receptors in our species [14]. These receptors have evolved to perform a protective function (prevent the ingestion of potentially poisonous foods), which was the first role discovered for them. However, in the last few decades, researchers have found that they appear in a highly diverse set of tissues from the brain to the reproductive system [15]. Bitter taste receptors became a point of interest for researchers seeking to explain why they are so ubiquitous. One area in which they have been studied recently, mostly as components of the immune system [16,17]. Evidence also exists that they are involved in numerous pathophysiological processes [18–20]. One of the most interesting TAS2R locations for researchers in recent years, however, is the gastrointestinal wall [21], where they appear in a range of cells that make up the GIT. Some of the most interesting of these cells are the enteroendocrine cells, which secrete and regulate enterohormones [22,23]. These hormones play a key role in the regulation of metabolism, nutrient uptake, insulin secretion and, therefore, glucose regulation and the body's overall systemic response to the presence or absence of nutrients. The prevalent theory in the literature is that the presence of bitter taste receptors in these cells is a key factor in their function. Beyond the fact that many components of our daily diet contain polyphenolic compounds that bind to these receptors, it is clear that the interplay between food and TAS2R is important for our well-being [24].

However, the novelty of this research area means that much is yet to be established. The interplay between the bitter receptors located in the GIT, the metabolic pathways, and the consequences of this network for our health are yet to be determined. Moreover, although we know that phenolic compounds do interact with TAS2R, we lack overall knowledge regarding how these compounds from our daily diet influence TAS2R and their expression in the intestine. Importantly for us, we also lack knowledge on the role that TAS2R play in the metabolic changes that take place during ageing. Although we have some data on ageing and TAS2R [25,26], and there is evidence that bitter agonists have antiageing benefits [27–29–30], our overall knowledge in this area is wanting.

To shed light on these uncertainties, and possibly create a starting point for further research, we designed the present study in which, working with two age cohorts, we analyze the potential role of intestinal bitter receptors on the ageing process. First, we present a comprehensive metabolomic analysis accompanied by an analysis of enterohormonal secretion, anti-inflammatory markers, and food-derived phenolic markers in plasma. Then, building on our previous results for the expression of TAS2R present in the GIT [31], we used machine-learning models and computer analysis to determine the importance of these receptors and establish their possible connection with changes in the parameters that occur during the ageing process.

2. Materials and methods

2.1. Study cohorts

All the samples in this study were kindly provided by the Gastroenterology Department of the Hospital Clínic of Barcelona, Spain. 23 healthy men and 25 healthy women participated in this study. Subjects were divided into one a young cohort (range 20–50 years: mean age ± SD =38.9±6 years) and one an aged cohort (range 50–80; mean age ± SD =63.6±6 years). The exclusion criteria for participation in the study were: (1) a body mass index (BMI) below 18.5 or above 35; (2) a previous diagnosis of diabetes mellitus type 1 or 2; (3) chronic treatment with anti-inflammatory medicines; and 4) previously diagnosed illnesses. This experimental procedure was approved by the Drug Research Ethics Committee of Hospital Clínic in Barcelona (HCB/2019/1115). All participants were informed before they provided their written consent to take part in the study. The procedures performed were in compliance with local laws.

2.2. Colon mucosa samples

All participants underwent a colonoscopy performed for several indications (i.e. colorectal cancer screening, polyp surveillance or investigation of gastrointestinal symptoms) where colonic mucosa sampling was performed. Samples from the ascending and descending colon were collected. All individuals had no colorectal cancer at the time of colonoscopy. Colonic samples were stored at −80°C until processing. Briefly, RNA extraction was performed using a RNeasy Plus Mini Kit (Cat. No.: 74134, Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The quality and purity of the extracted RNA were evaluated using a NanoDrop® ND-1000 spectrophotometer (Fisher Scientific, Madrid, Spain). cDNA was obtained using a High-Capacity cDNA Reverse Transcription Kit (Cat. No: 4368814, Fisher Scientific, Madrid, Spain). Quantitative PCR amplification was performed using specific TaqMan probes (list of probes: supplementary appendix A1; Applied Biosystems, Waltham, USA). For further processing and subsequent analysis, relative gene expression was calculated as $1/\Delta Ct$, where RPS9 was used as a reference.

2.3. Plasma samples

Plasma samples were collected from the participants on the same day as the tissue samples. The samples were stored at −80°C until further use. Various kits were used to measure enterohormones and inflammatory markers. CCK (intra-assay variation:

<10%; inter-assay variation: <15%; Cat No.: EKE-069-04, Phoenix Europe GmbH, Karlsruhe, Germany) and PYY (intra-assay variation: <10%; inter-assay variation: <15%; Cat No.: FEK-059-02, Phoenix Europe GmbH, Karlsruhe, Germany) were measured individually whereas the other parameters were analyzed together using Milliplex® Human Metabolic Hormone Panel V3 kit (Cat. No.: HMH3-34K, Millipore, Madrid, Spain). All analyses were performed strictly in accordance with manufacturers' instructions.

2.4. Analysis of metabolomic biomarkers in plasma

Metabolomic analysis was performed by Biosfer Teslab (Reus, Spain) using ^{1}H-NMR spectroscopy to analyze the samples (306 K, proton frequency 600.20 MHz, 14.1 T).

Lipoprotein profile was analyzed using the NMR-based Liposcale® test. The lipid concentrations, size and particle number of the four main classes of lipoproteins (intermediate-density lipoprotein or IDL, very-low-density lipoprotein or VLDL, LDL and HDL) as well as the particle number were determined as previously described [32]. Glycoprotein profile was determined by analyzing the glycoprotein-specific region of the ^{1}H-NMR spectrum using several analytical functions according to previously published procedures [33,34]. Low-molecular-weight metabolites (LMWMs) were identified and quantified in the 1D Carr-Purcell-Meiboom-Gill (CPMG) spectra using an adaptation of Dolphin [35]. After ^{1}H-NMR metabolomic characterization, the diluted serum samples were lyophilized and then diluted with 100 μL of 50 mM PBS at pH 7.4 before lipid extraction using the BUME method with slight modifications [36]. The upper lipophilic phase was completely dried in Speedvac until the organic solvents evaporated. This phase was then frozen at –80°C until NMR analysis. Lipid signals in ^{1}H-NMR spectra were quantified using LipSpin [37], an in-house program based on Matlab.

2.5. Analysis of markers of intake of phenolic compounds in plasma

[38–41] The plasma samples were subjected to a protein precipitation procedure with minor modifications using a Sirocco Plate (Waters, Milford, MA, USA) as previously described [42]. Briefly, 100 μL of plasma samples were spiked with 10 μL of 1 mg/L myristoyl-L-carnitine d9 and ferulic acid 13C3 in water. The samples were subsequently mixed with 500 μL of cold acetonitrile (−20°C) containing 1.5 M formic acid and 10 mM ammonium formate in the plate, vortexed for 1 min, and kept at −20°C for 10 min to promote protein precipitation. A Waters Positive Pressure-96 Processor was used to collect the extracts in 96-well collection plates, which were taken to dryness under a stream of nitrogen gas. Finally, the samples were reconstituted in 100 μL of water: acetonitrile (80:20, v/v) containing 0.1% formic acid (v/v) and 100 μg/L of the taxifolin and caffeine 13C3 and centrifuged at 1000 g for 5 min. Clean extracts then were transferred to 96-well plates for further analysis.

Analyses were performed on a 1,290 Infinity UHPLC system (Agilent, Santa Clara, CA, USA) coupled to a QTRAP 6500 mass spectrometer equipped with Ion Drive Turbo V ion source (Sciex, Framingham, MA, USA). Chromatographic separation was achieved on a Luna Omega Polar C18 column (100 mm × 2.1 mm (i.d. 1.6 μm)) using a fully porous polar C18 security guard cartridge (Phenomenex, Torrance, CA, USA). The chromatographic conditions can be found here [42]. Mass spectrometry detection was performed under positive and negative ionization in separate runs by using the scheduled multiple reaction monitoring (sMRM) mode. The general MS parameters were as follows: ion spray voltage, +4500/−3500 V; source temperature, 600°C; curtain gas, 30 psi; ion source gas 1 and gas 2, 50 psi each; collision-activated dissociation gas, 3 psi; entrance potential, ±10 V; and target scan time, 0.05 s.

Calibration curves were prepared at 12 concentration levels in the 0.1–4000 μg/L range. Compounds lacking the corresponding commercial standard were semiquantified using the calibration curves of structurally similar metabolites.

2.6. Statistical analysis

All our results are expressed as mean ± standard error of the mean (SEM). P-values < .05 were considered statistically significant. For parametric data, we used Student's T test when comparing two groups of parametric data, or Mann–Whitney U test when comparing two groups of nonparametric data. All calculations were performed using Lumivero XLSTAT 2023.1.5 software (Addinsoft, New York, NY, USA). Each figure or table indicates the analysis performed.

2.6.1. Integrative analysis and variable selection

The whole process of data processing, integration, variable selection pipeline and statistical analysis outlined in this section was conducted using RStudio version 2023.06.0 Build 421 (2009–2023 Posit Software, PBC).

All initial data were subjected to preprocessing. This included elimination of parameters with more than seven missing values and median imputation for missing values of the others. Once the data were processed, we continued with our analysis. To analyze the parameters that best discriminate between our groups, we applied the following procedure: for the results of gene expression, we used $1/\Delta Ct$ as the value expressing the quantity of gene expression and for the other parameters, we used the values obtained from the processing. To rank the variables, we used three machine-learning methods: Elastic Net, Random Forrest (RF) and Partial Least Squares-Discriminant Analysis (PLS-DA). [43–45] For Elastic Net and PLS-DA, the data were scaled using the built-in "ScaleData" function. Briefly, Elastic Net is a linear regression method used to regularize a wide range of parameters; Random Forrest is an algorithm used for classification and regression problems; and PLS-DA is a dimensionality-reduction method. The parameters for each method underwent optimization through 100-times-repeated 5-fold cross-validation. To avoid overfitting, we used the glmnet R package and followed a rule that adds a little flexibility. We applied the above machine-learning algorithms to compare all parameters and determine how well the parameters distinguished between the young and the aged cohorts. Each method therefore produced scores that reflect the importance of variables in this task. These scores were treated as individual values for each variable within each method. The sum of the values obtained from the three methods yielded the final value. This value indicates the overall importance of the variables in the context of our study, i.e. how well an individual parameter differentiates between these age groups. The one with the highest score was identified as the variable with the most significance in our study. With these results we conducted an integrative analysis that ranked all variables based on their order of importance. This selection method enabled us to concentrate our analysis on variables with the greatest discriminatory power between the groups.

For the correlation analysis we used Spearman rank-order correlation with all the data that underwent the above processing steps without separating the patients by age.

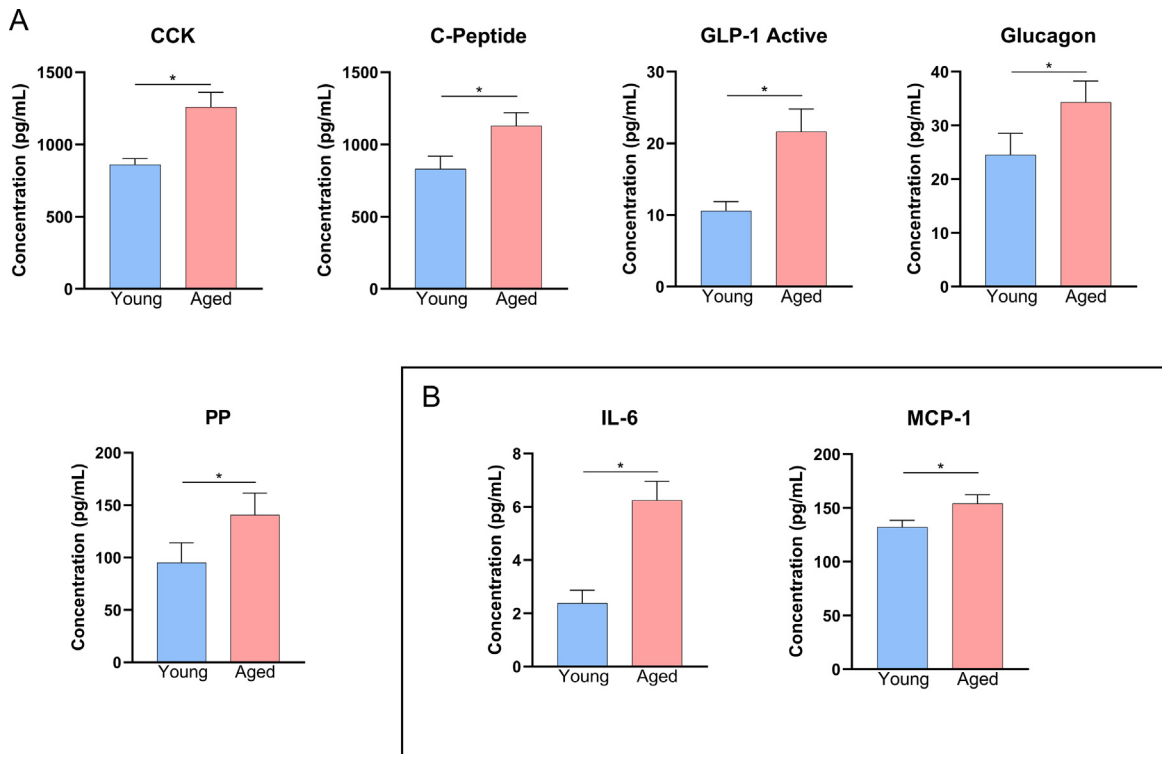

Fig. 1. Parameters analyzed in plasma that were significantly modified by ageing. (A) Enterohormones; and (B) Inflammatory markers (all concentrations are in pg/mL; $^{*}P < .05$; T-test or Mann–Whitney U test was used depending on the distribution of the data; n=15–25).

3. Results

3.1. The concentrations of most endogenous plasma biomarkers increased with age

Our first aim was to analyze endogenous plasma biomarkers and define the ageing-induced changes on metabolic parameters and inflammatory markers in the two age cohorts. To do so we conducted a panel analysis of various parameters in the plasma obtained from patients during biopsy sampling and compared a young group (38.9±6 years) with an aged group (63.6±6 years).

Figure 1 shows the results for several essential enterohormonal parameters and inflammatory markers. The enterohormones that significantly increased in the aged cohort were C-peptide, CCK, active GLP-1, PP, and glucagon (Fig. 1A). The same trend was observed in relation to the inflammatory markers IL-6 and MCP-1, which were also elevated in the aged group.

To obtain a more complete metabolic picture of the changes that are produced as we age, we then conducted a comprehensive metabolomic analysis of 60 different parameters with additional focus on lipoprotein characterization (supplementary appendix A2). Table 1 shows the 23 parameters with statistically significant differences between the age groups.

As we can see, most of the statistically significant parameters were increased in the aged cohort. In the group of low-molecular-weight molecules, an increase with ageing was observed in the levels of creatine, glucose and lactate, while the levels of glycine and histidine were significantly lower in the aged group. In the panel of lipoproteins, the levels of IDL and LDL constituents, their particle number and concentrations clearly tended to be higher in the aged cohort with only the number of HDL particles displaying an opposite trend. The concentrations of glycoproteins N-acetylglucosamine/galactosamine (Glyc-A), sialic acid (GlycB) and their aggregation states (height/weight; H/W) were again higher in the aged cohort. Finally, all lipid molecules analyzed (free cholesterol, polyunsaturated fatty acids, omega-3, docosahexaenoic acid, arachidonic acid and eicosapentaenoic acid) except linoleic acid were significantly higher in the aged group than in the young group.

3.2. TAS2R5 and TAS2R38 were among the internal parameters that best distinguished between the young and the aged cohort

The next aim of the study was to use the results we obtained together with the expression analysis of TAS2Rs [46,47] we had previously published [31] to create a list of the most relevant parameters for distinguishing between the age groups and identify whether bitter taste receptors are important in this ranking. Our analysis began with a total of 82 parameters. To do so we used three machine-learning methods to characterize the optimal parameters for achieving this goal. We then combined the results from all three methods and created a final list of the most important discriminatory parameters (Fig. 2).

At first glance the parameters appear to be divided into two groups depending on their importance—one with the first five parameters and one with the rest. Of all the groups of parameters we quantified (receptors, enterohormones, inflammatory markers, lipoproteins, glycoproteins, low-molecular-weight metabolites and lipids), only glycoproteins did not appear in this analysis.

If we take a closer look at the biomarkers, we see that docosahexaenoic acid is the most important distinguisher between the ages with all three methods used. In this first group of biomarkers, some lipoproteins (IDL-Triglycerides, LDL-Triglycerides and IDL-Cholesterol) and, interestingly, alanine were the biomarkers selected.

Table 1
Statistically different metabolic parameters analyzed in plasma. Data are shown as mean ± SEM ($P < .05$; the higher values are indicated in bold; $.05 < P < .1$ is indicated in italics; T-test or Mann–Whitney U test was used depending on the distribution of the data; n=20–25).

Group	Parameter	Young	Aged	*P*-value
Low-Molecular-Weight Molecules	Creatine (μmol/L)	48.40 (± 2.53)	**58.99 (± 3.33)**	.015
	Glucose (mmol/L)	3.02 (± 0.10)	**3.46 (± 0.15)**	.021
	Glycine (μmol/L)	**155.03 (± 6.17)**	132.64 (± 5.33)	.003
	Histidine (μmol/L)	**50.80 (± 1.36)**	45.03 (± 2.20)	.036
	Lactate (μmol/L)	153.51 (± 10.17)	**190.48 (± 11.20)**	.019
Lipoproteins	IDL-Cholesterol (mg/dL)	6.03 (± 0.31)	**9.71 (± 0.65)**	9.4E-06
	LDL-Cholesterol (mg/dL)	125.80 (± 2.97)	**142.66 (± 4.37)**	.003
	IDL-Triglycerides (mg/dL)	7.43 (± 0.28)	**9.89 (± 0.44)**	2.8E-05
	LDL-Triglycerides (mg/dL)	10.81 (± 0.28)	**14.45 (± 0.67)**	1.5E-05
	LDL-Particle Number (nmol/L)	1197.43 (± 27.68)	**1378.36 (± 41.35)**	.001
	Medium LDL-Particle Number (nmol/L)	335.43 (± 11.88)	**437.97 (±21.55)**	.000
	Small LDL-Particle Number (nmol/L)	638.86 (± 12.77)	**717.97 (± 19.78)**	.001
	Medium HDL-Particle Number (μmol/L)	**11.41 (± 0.33)**	10.14 (± 0.26)	.003
Glycoprotein	Glyc-B (μmol/L)	288.45 (± 7.99)	**320.18 (± 7.85)**	.007
	Glyc-A (μmol/L)	559.25 (± 15.21)	**629.12 (± 16.85)**	.004
	H/W Glyc-B	3.63 (± 0.1)	**4.03 (± 0.1)**	.007
	H/W Glyc-A	12.85 (± 0.26)	**14.99 (± 0.39)**	2.2E-05
Lipid	Free cholesterol (mmol/L)	2.39 (± 0,05)	**2.60 (± 0.06)**	.010
	Polyunsaturated fatty acids (mmol/L)	14.72 (± 0.45)	**17.38 (± 0.58)**	.001
	Linoleic Acid (mmol/L)	*4.15 (± 0.13)*	3.86 (± 0.10)	.079
	omega-3 fatty acids (mmol/L)	0.30 (± 0.02)	**0.40 (± 0.02)**	.001
	Docosahexaenoic acid (mmol/L)	0.11 (± 0.01)	**0.19 (± 0.01)**	1.1E-05
	Arachidonic acid + Eicosapentaenoic acid (mmol/L)	1.23 (± 0.04)	**1.38 (± 0.05)**	.023

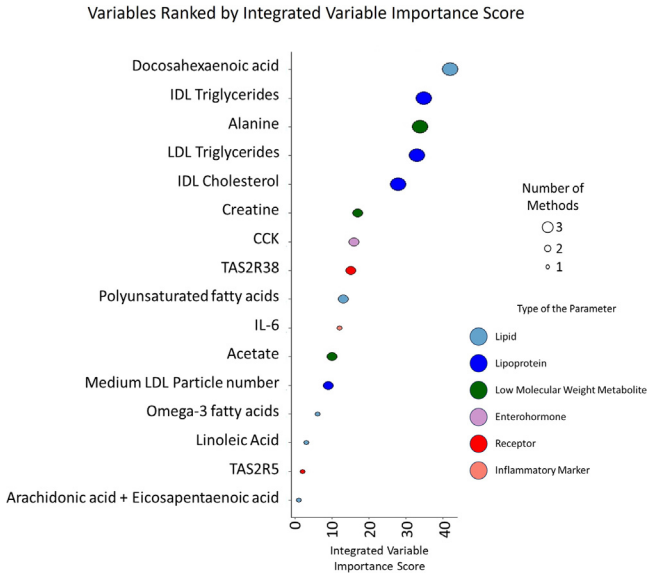

Fig. 2. Ranking of the best variables for discriminating between the age cohorts. The methodology used to obtain this figure is explained in Materials and Methods chapter 2.6.1.

The second observed group in the Figure 2 contained more parameters. Especially interesting for us was the appearance of two bitter receptors (TAS2R38 and TAS2R5) that were described as significant by two methods and one method, respectively.

All the parameters selected in Figure 2 appear as statistically significant in Figure 1 and Table 1, with the notable exception of acetate, which was labelled as a good distinguishing biomarker by two of the three techniques.

3.3. All TAS2R displayed at least one significant correlation with the measured biomarkers

Once we had established which parameters were interesting for further studies in the context of ageing, we focused on the interplay between these, and other molecules and the bitter receptors found in the GIT.

At first, we aimed to establish the possible connection between the TAS2R present in the GIT and the biomarkers measured in plasma in the overall population.

To better understand this and the potential importance of interactions between bitter taste receptors and the endogenous biomarkers we measured, we conducted a correlation analysis. Figure 3 shows the correlation heatmap of TAS2R with biomarkers that had at least one significant correlation as calculated by Spearman's rank correlation.

A closer look at this heatmap reveals that most of the significant correlations are characterized as positive (illustrated by the predominantly red color in Fig. 3). If we focus on the receptors, we see that all nine TAS2R analyzed here displayed at least one correlation with at least one biomarker. Interestingly, TAS2R13 was the receptor with the highest number of biomarkers (14), most of which were positive (MCP-1, various lipoproteins, CCK, triglycerides) though some were significantly negative (several HDL particles and sphingomyelin). The next two receptors with the highest numbers of correlations were TAS2R4 and TAS2R5, with largely similar correlation profiles to that observed for TAS2R13.

Switching our focus to biomarkers, we found that several lipoproteins correlated with five TAS2Rs. The most important biomarker for distinguishing between the age groups (docosahexaenoic acid) only correlated with one receptor (TAS2R5), which was also one of the significant discriminators between age groups (Fig. 2). Several other biomarkers labelled as important in that

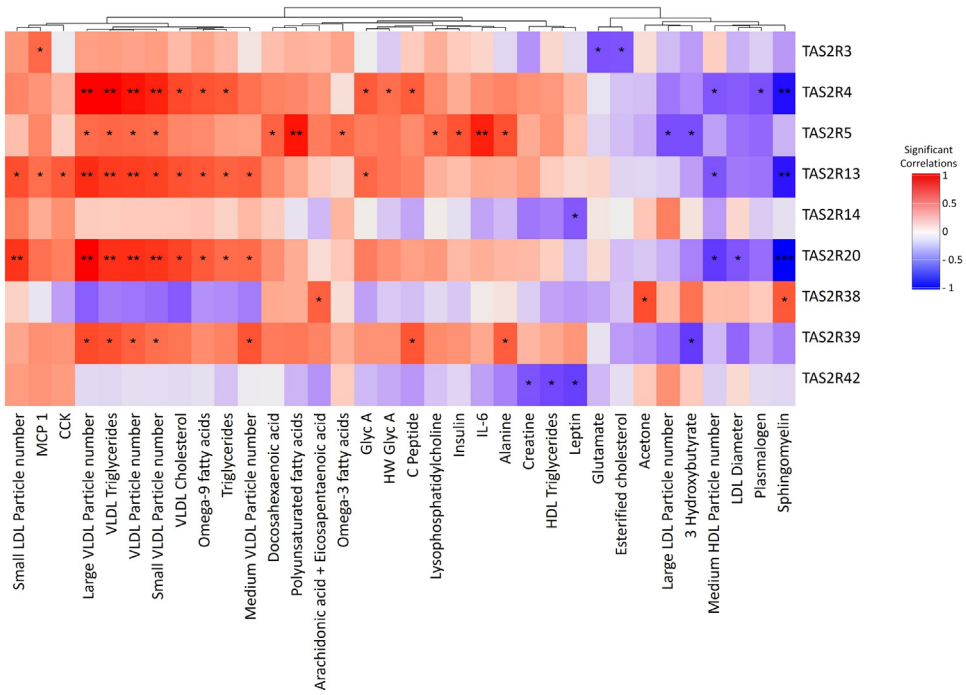

Fig. 3. Correlation heatmap. Results of the correlation analysis between the TAS2R and the endogenous biomarkers (Spearman rank-order correlation; $^{*}P < .05$; $^{**}P < .01$; n=26).

Table 2
Analysis of phenolic markers in plasma. Only parameters that were significantly different statistically are shown. Data are shown as mean ± SEM. The higher values are indicated in bold (* indicates $p < 0.05$; # indicates $0.05 < p < 0.1$; T-test; n=20–25).

Parameter	Young (ppb)	Aged (ppb)
3-hydroxyphenylacetic Acid	11.37 (± 0.96)	**16.49**$^{\#}$ (± 1.96)
Catechol Sulphate	1540.98 (± 289.7)	**3351.34*** (±785.0)
Lactic Acid	58783.91 (± 2812.9)	**70692.80*** (± 3506.2)
Pyrogallol Sulphate 1	**2735.09**$^{\#}$ (± 1235.7)	780.00 (± 256.6)

analysis (IDL and LDL triglycerides, IDL cholesterol, alanine, creatine, CCK, polyunsaturated fatty acids, IL-6, numerous medium LDL particles, omega-3 fatty acids, and arachidonic and eicosapentaenoic acid) were also found to correlate with several TAS2R, all of which were positive except with creatine, and most of which had correlations with TAS2R5.

3.4. TAS2R5 and TAS2R38 were the bitter taste receptors among the metabolite markers of phenolic consumption that best discriminated between the young and the aged cohorts

TAS2R located in intestinal mucosa are a clear target for food-derived compounds. Since phenolic molecules have been shown to be TAS2R ligands, we also aimed to investigate how different polyphenolic components in the diets of our participants may have interacted with local bitter receptors and sought to establish their potential effect on the other, more global, parameters.

Our analysis encompassed 370 phenolic markers with a wide range of origins (supplementary appendix A3) [48]. The signal was detected for 75 of these markers. Table 2 shows that both catechol sulphate and lactic acid were significantly different between the age cohorts (with higher levels in the aged group), while pyrogallol sulphate (with higher levels in the young group) and 3-hydroxyphenylacetic acid (with higher levels in the aged group) displayed a trend to increase in aged cohort.

Since we were again interested in determining the most suitable parameters for differentiating between the two age groups, we conducted the same analysis as we did with the other markers. In this case we considered the same TAS2R as in the previous analysis plus the 75 phenolic plasma markers. Figure 4A show that, this time, our analysis highlighted four receptors overall. The first two of these were again TAS2R5 and TAS2R38 and the two new ones were TAS2R4 and TAS2R3. Of the parameters considered here, TAS2R5 and TAS2R38 best discriminated between the two age groups.

To identify which TAS2R showed some correlation with these phenolic plasma markers we also ran a correlation analysis in which all groups were considered together. This analysis again highlighted TAS2R5 with three negative correlations, followed by TAS2R20, TAS2R38 and TAS2R39 with two (Figure 4B). Two parameters worth emphasizing were cis-resveratrol-3-sulphate and 3-hydroxybutyric acid, each of which had three correlations with TAS2R.

4. Discussion

The broad approach we adopted in this study enabled us to describe the relationship between the ageing process and colonic bitter taste receptors. We ran two integrative analyses in which the gene expressions of the nine TAS2R in the colon were considered—one analysis with endogenous plasma components

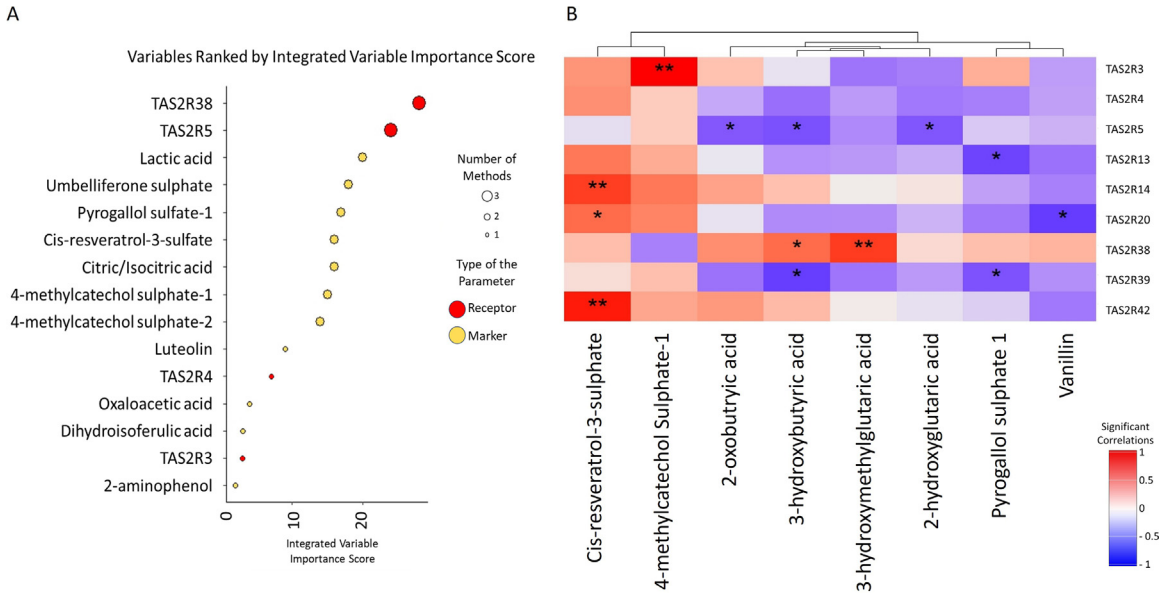

Fig. 4. Ranking of the best phenolic markers for distinguish age cohort, and correlation heatmap with TAS2R. (A) Parameters ranked according to their capacity to differentiate between the young and aged the older groups; (B) Significant correlations of the polyphenolic markers in plasma parameters (Spearman rank-order correlation; $^{*}P < .05$; $^{**}P < .01$; n=26). How the ranking was calculated is described in Materials and Methods chapter 2.6.1.

and the other with exogenous phenolic plasma markers—to determine the most important parameters for differentiating between the age cohorts. TAS2R5 and TAS2R38 were identified in both analyses.

TAS2R5 is one of the most explored receptors [49,50]. However, as with all bitter receptors, most of their roles in extraoral tissues are yet to be determined. We have previously shown that the expression of this receptor in the descending colon is increased by ageing [31]. Now we clearly show that it plays an important role in the ageing process. Unlike previous studies in which TAS2R showed no significant correlations with HDL, LDL, and triglycerides [49], we have shown that TAS2R5 correlated with lipoprotein constituents in plasma. Kim et al. [51] recently discovered a novel form of interaction between TAS2R and their ligands when they identified cholesterol as a key modulator of ligand recognition by TAS2R14 [51]. They also showed that cholesterol, which is abundant in the cellular membrane, binds to the extracellular binding site of this receptor, while the bitter compound binds to the intracellular allosteric binding site. Total activation of this bitter receptor was possible only when both cholesterol and the bitter ligand were bound. Together with the fact that bile acids, which are structurally similar to cholesterol since they share common steroid motif, activates bitter receptors (–1, –4, –14, –39), cholesterol and its carriers probably play an important role in the activation and signal transduction of TAS2R [52]. Overall, these correlations between TAS2R5 and the lipoprotein particles and their constituents are interesting to further reflect on especially when we consider that, with ageing, dysregulation of lipoproteins contributes greatly to the manifestation of age-related disorders.

TAS2R5 also correlated with polyunsaturated fatty acids (PUFAs), which, interestingly, are detected by another member of the G protein-coupled receptors family, GPR120 [53]. Moreover, GPR120 shares both the type of cells in which it is expressed (enteroendocrine cells) and the function (GLP-1 regulation) with other TAS2R [54,55]. Some PUFAs, such as docosahexaenoic acid (the best for differentiating between the young and the aged cohorts), have been found to bind to TAS2R [56]. PUFAs also have multiple beneficial effects on the elderly [57,58], and it would be interesting to see whether the impact of docosahexaenoic acid and other PUFAs is due to their interactions with various TAS2R.

We also observed strong and significant correlations between TAS2R5 and inflammatory marker IL-6. In the intestine, the activation of bitter receptors induces the release of antimicrobial peptides and regulates the expression of other innate immune factors [59]. TAS2R have also been observed in tuft cells of the GIT, where such cells can detect potential helminthic and protozoan infections [60]. While evidence of the precise role of TAS2R regulation of inflammation in the GIT is still inconclusive, TAS2R5 may be an interesting point of investigation as a target for inflammatory modulation.

We found significant correlation between TAS2R5 and three markers of phenolic consumption. 2-oxobutyric acid is an endogenous molecule that is formed during the metabolism of cysteine [61]. Its conjugate base, α-ketobutyrate, has been shown to increase lifespan in *C. elegans* [62]. 3-hydroxybutyric acid and its conjugate base 3-hydroxybutyrate (β-hydroxybutyrate) have been proposed as antiageing metabolites [63]. In an animal model, a bitter compound elicited an increase in the level of β-hydroxybutyrate [64]. Little is known about 2-hyodroxyglutaric acid, but it has been shown that its accumulation due to the mutation of isocitrate dehydrogenase leads to a hypermethylated state of DNA and histones, which activates oncogenes and ultimately leads to cancer [65]. Although initial results suggest that these markers play a role in the ageing process, whether they interact with TAS2R5 is still unknown.

The second receptor that was labelled a good discriminator of age cohorts is TAS2R38. Unlike with TAS2R5, we had previously observed no age-related differences in its expression [31]. Researchers investigating the distribution of homozygous variants of this receptor in the aged population have reported a connection between the genetic variance of TAS2R38 and longevity [26]. Some studies have associated genetic variations of this specific receptor with the clinical outcomes of medical conditions such as obesity [66].

In our analysis TAS2R38 had one of the fewest significant correlations since it correlated positively only with sphingomyelin, acetone and arachidonic and eicosapentaenoic acid (measured together). Sphingomyelin is an important membrane lipid that is significant for membrane fluidity and involved in the creation of ceramide, a lipid inductor of apoptosis [67]. Sphingomyelin accumulates during ageing, which induces cellular dysfunction and some inflammatory cytokines [68]. As an important membrane lipid that regulates the function of G protein-coupled receptors such as TAS2R, sphingomyelin was studied for its potential effects on the signaling of TAS2R14, but no significant effect was found. However, its potential influence on other receptors has not been assessed [69]. Acetone, one of the ketone bodies produced by the liver, is usually elevated in diabetes, a component of the metabolic syndrome whose prevalence increases with age [70]. Interestingly, in cellular models that evaluated the response to different bacterial metabolites, it was shown that TAS2R38 was activated by acetone [71]. With regard to fatty acids such as arachidonic and eicosapentaenoic acids, in addition to the GPR120 described above, the cluster of differentiation 36 (CD36) glycoprotein has also been identified as a possible receptor for fatty acids [72]. There is also evidence that TAS2R38 may play a role in fatty acid signaling by modulating CD36 functionality. Firstly, people with greater sensitivity to the TAS2R38 ligand 6-n-propylthiouracil (PROP) were also more sensitive to detecting fat [73]. Further studies have also shown that specific haplotypes of TAS2R38 and sensitivity to PROP are linked with the polymorphism of CD36 [74], which indicates that sensitivity to detecting bitter and sensitivity to detecting fat (such as fatty acids) are interlinked [75–77].

While TAS2R5 and TAS2R38 were the receptors that best discriminated between the two age cohorts, our results showed that TAS2R13 is the receptor with the highest number of correlations (fourteen) in the analysis of endogenous biomarkers. TAS2R13 was also the only receptor that correlated with one of the phenolic consumption markers that was significantly different between the age groups: pyrogallol-1-O-sulphate. This compound is the result of the microbial metabolism of catechol-moiety-containing polyphenols, which are structural motifs often found in bitter agonists (such as epigallocatechin gallate) [56,78]. Changes to the composition of microbiota are strongly affected by both internal and external factors, one of the most impactful of which is ageing [79]. It has been demonstrated that by influencing the microbiome in our gut, we can modulate and lower inflammation, which is an important factor in achieving healthier ageing [80,81]. It is also known that the secretome of the microbiota is important in controlling food intake and, therefore, body weight [82]. Researchers have also recently shown that microbes modulate the taste receptors in the GIT through the molecules they secrete and suggested that correlations exist between specific strains in the gut and taste perception [83]. Finally, an animal model of obesity showed that a high-fat diet can influence the microbiota as well as the expression of bitter receptors, which raises the idea that the luminal microbes influence the expression of bitter taste receptors [84].

TAS2R13 also correlated with CCK, an anorexigenic enterohormone that regulates the metabolic response to the intake of nutrients and whose levels increase with ageing [85,86]. The role of TAS2R in the modulation of enterohormone secretion has been well described [87,88] and some enterohormones have been associated with the onset of age-associated diseases [89].

We have also found that several fractions of lipoproteins are good separators of the age cohorts. This is in line with previous studies in which higher levels of certain lipoprotein constituents, such as IDL cholesterol and IDL- and LDL-triglycerides, were associated with myocardial disfunction, diabetes, diabetic retinopathy and many other metabolic disorders [90–93]. The metabolic syndrome and its associated disorders are known to affect health in the aged population.

5. Conclusions

In conclusion, we have investigated the potential of bitter taste receptors present in the GIT as markers for age discrimination. TAS2R5 and TAS2R38 are identified as the most promising candidates based on their ability to distinguish between younger and older individuals. Interestingly, TAS2R5 correlated with several lipoproteins and potentially plays a role in inflammatory response, which suggests there are possible connections to age-related metabolic changes. TAS2R38, on the other hand, showed significant correlations with factors potentially linked to liver function. However, further research is needed to elucidate the precise roles of these TAS2Rs and to determine whether their expression changes are a cause or a consequence of the ageing process.

Declaration of generative AI in scientific writing

The authors of this manuscript confirm that generative AI tools have not been used in the generation, drafting, or editing of this work. All content, including the research, data analysis, interpretation, and manuscript writing, has been conducted entirely by the authors without AI assistance.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2025.109872.

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