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## **The salivary proteome reflects some traits of dietary habits in diabetic and non-diabetic older adults**

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## Abstract

1 **Purpose** : Objective markers of usual diet are of interest as alternative or validating tools in  
2 nutritional epidemiology research. The main purpose of the work was to assess whether saliva  
3 protein composition can reflect dietary habits in older adults, and how type 2 diabetes impacted on  
4 the saliva-diet correlates.

5 **Methods** : 214 participants were selected from two European cohorts of community-dwelling older  
6 adults (3C-Bordeaux and Seniors-ENRICA-2), using a case-control design nested in each cohort. Cases  
7 were individuals with type 2 diabetes. Dietary information was obtained using the Mediterranean  
8 Diet Adherence Screener (MEDAS). Saliva was successfully obtained from 211 subjects, and its  
9 proteome analyzed by liquid chromatography-tandem mass spectrometry.

10 **Results** : The relative abundance of 246 saliva proteins was obtained across all participants. The  
11 salivary proteome differed depending on the intake level of some food groups (especially vegetables,  
12 fruits, sweet snacks and red meat), in a diabetic status- and cohort-specific manner. Gene Set  
13 Enrichment Analysis suggested that some biological processes were consistently affected by diet  
14 across cohorts, for example enhanced platelet degranulation in high consumers of sweet snacks.  
15 Minimal models were then fitted to predict dietary variables by sociodemographic, clinical and  
16 salivary proteome variables. For the food group « sweet snacks », selected salivary proteins  
17 contributed to the predictive model and improved its performance in the Seniors-ENRICA-2 cohort  
18 and when both cohorts were combined.

19 **Conclusion** : Saliva proteome composition of elderly individuals can reflect some aspects of dietary  
20 patterns.

21

22 **Keywords** : salivary biomarkers, proteomics, usual diet, ageing, diabetes, Gene Set Enrichment  
23 Analysis

24 **Declarations**

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29 **Conflicts of interests** : nothing to disclose

30 **Ethics approval** : Study protocols of the 3C and Seniors-ENRICA-2 cohorts were approved  
31 respectively by the Committee for Protection of Persons (CPP) participating in Biomedical Research of  
32 the Kremlin-Bicêtre University Hospital (Paris) and the Ethics Research Committee of the « La Paz »  
33 University Hospital (Madrid).

34 **Consent to participate / Consent to publish** : Participants provided written informed consent to  
35 participate and for the results of the study to be published.

36 **Availability of data and material** : Data described in the manuscript will be made available upon  
37 request addressed to the corresponding author.

38 **Code Availability** : The R scripts will be made available upon request addressed to the corresponding  
39 author.

40 **Author's contributions** : EN, CF, FRA, ELG, GP & MM conceived and designed the study. CC, TS & PB  
41 performed saliva analysis. RD, FH & DGCL performed statistical analyses. PA, EGGE, CF, FRA & ELG  
42 provided epidemiological data and sampled saliva. CC & MM drafted the manuscript. All authors  
43 revised the manuscript and approved its final version.

#### 44 **Introduction**

45 There is vast evidence linking intakes of nutrients, specific foods, food groups or even dietary  
46 patterns with health and well-being outcomes. Some studies have focused on the older population,  
47 targeting health issues more prevalent in this age group. For example, a link has been established  
48 between usual intake of protein and frailty [1] or between coffee consumption and risk of falling [2].  
49 Higher adherence to the so-called Mediterranean diet (MeDi) has also been associated with  
50 decreased cognitive decline [3] or decreased risk of frailty [4,5]. **Concerning** another pathology with  
51 higher prevalence among the elderly population, higher adherence to MeDi diet has been associated  
52 with lower risk of type 2 diabetes mellitus (T2DM) [6,7] and not only through its impact on body  
53 weight [8]. Recording one's diet can therefore be a useful tool for the clinician as a starting point to  
54 implement dietary guidelines and/or to follow adherence to dietary advice.

55 One challenge in nutritional epidemiology research is to capture real dietary intakes. Assessment  
56 usually relies on questionnaires (such as 24-hour recalls or Food Frequency Questionnaire) which  
57 may be prone to memory or social desirability bias. Therefore, objective markers of dietary intake  
58 are required as alternative or validating tools to increase the reliability and accuracy of diet  
59 information [9]. With this objective in mind, markers of dietary intake have been sought for mainly in  
60 serum and in urine. For example, metabolome markers of usual consumption of citrus fruit or fish  
61 were identified in serum of adult participants [10]. Urinary metabolome markers of usual nut intake  
62 were also identified and correlated to cognitive decline in older adults [11]. Recently, plasma  
63 metabolome markers of adherence to the Mediterranean diet have been associated with  
64 cardiovascular disease risk [12].

65 Saliva is a biological fluid which presents some advantages over blood or urine, particularly regarding  
66 its sampling which minimizes pain, privacy or safety issues. Saliva sampling also presents some  
67 limitations, for example in case of minimal saliva flow. Young children and the oldest or frail elderly  
68 subjects are thus two groups for which it may be difficult to obtain saliva samples [13]. However,  
69 saliva was successfully sampled and its protein or peptide composition analyzed on 3- and 6-month-

70 old infants [14], on premature babies [15] or on old persons with a mean age of 82 years [16]. Saliva  
71 composition was successfully associated with usual intake of carbohydrates in adults [17], with diet  
72 transition in infants [14,18] or with dietary patterns in children with or without eating difficulties  
73 [19]. In addition, saliva (together with blood) proved more resilient than urine to recent dietary  
74 intake when focusing on metabolome composition [20]. For all these reasons, the primary objective  
75 of this work was to evaluate whether saliva is a suitable source of objective protein markers of usual  
76 dietary intake in older adults. We evaluated this with participants from two different cohorts, in  
77 France and Spain, which provided information on the cross-cultural and/or geographical sensitivity of  
78 the results. Finally, participants were part of a wider project on salivary biomarkers of Mediterranean  
79 diet and type 2 diabetes mellitus [21]. The design of participants' selection therefore also enabled to  
80 address a secondary objective, namely to assess the impact of this pathology on the association  
81 between saliva composition and dietary habits.

## 82 **Methods**

### 83 Population-based cohorts

84 Participants originated from two population-based cohorts on ageing, the Bordeaux sample of the  
85 Three-City Study (3C) in France [22] and the Seniors-ENRICA-2 cohort in Spain [23]. Study protocols of  
86 the 3C and Seniors-ENRICA-2 cohorts were approved respectively by the Committee for Protection of  
87 Persons (CPP) participating in Biomedical Research of the Kremlin-Bicêtre University Hospital (Paris)  
88 and the Ethics Research Committee of the « La Paz » University Hospital (Madrid).

### 89 Participants

90 The 214 Individuals included in the study were selected using a case-control design nested in each  
91 cohort. Cases were individuals affected by T2DM based on self-reported physician's diagnosis and/or  
92 being on antidiabetic treatment (oral medication or insulin) at the time of data collection. Controls  
93 were selected concurrently and were free of diabetes at the time of data collection. For the purpose  
94 of the present study, we used the following information from each participant: cohort, sex, age

95 (years), body mass index (BMI; kg/m<sup>2</sup>), type 2 diabetic status, smoker status (never, former, current),  
96 saliva flow (g/min) and food consumption.

97 Dietary surveys and assessment of adherence to the Mediterranean diet

98 Food consumption data were collected using a FFQ in the 3C cohort [24] and a validated electronic  
99 diet history in the Seniors-ENRICA-2 cohort [25]. Adherence to the Mediterranean dietary pattern  
100 was assessed by calculating a MEDAS score [26] modified by omitting the question on *sofrito*, since  
101 this cooking technique is specific of the Spanish population. The MEDAS score could therefore range  
102 from 0 to 13, with higher values indicating higher adherence to the Mediterranean diet. Differences  
103 between cohorts or diabetic status was tested by a Wilcoxon test. In addition, to study the link  
104 between saliva proteome and diet, we restricted the analysis to the 11 MEDAS items describing the  
105 consumption frequency of food groups, namely olive oil, vegetables, fruits, red meat,  
106 butter/margarine/cream, sweet drinks, wine, legumes, fish/shellfish, sweet snacks (confectionary,  
107 biscuits and commercial pastries) and nuts. For each food group, the participants were classified as  
108 high or low consumers based on the cut-off points defined for MEDAS score calculation (Online  
109 Resource 1).

110 Saliva sampling

111 Sampling of unstimulated saliva was conducted at the participants' home. Sampling was proposed  
112 early in the morning after overnight fasting. Drinking water was permitted up to 5 minutes before  
113 saliva collection. Participants were instructed to sit comfortably and to tilt their head slightly  
114 downwards. At their own rythmn, they spat the saliva pooling on the floor of the mouth into 40mL  
115 polypropylene tubes. Sampling was performed for 10 minutes. In case a participant wished to stop  
116 before the end of the 10 minutes, the time was recorded in order to be able to calculate the saliva  
117 flow (expressed in g/min). Saliva samples were immediately placed on ice, transported to the  
118 laboratory and placed at -80°C as soon as possible (never after 4 hours on ice). At the end of the  
119 collection wave, samples were shipped to the analytical facilities in dry-ice.

120 Saliva proteome analyses

121 *Sample preparation*

122 Saliva was thawed at 4°C and vortexed. One mL of saliva (or the total volume of saliva when it was  
123 lower than 1 mL) was centrifuged at 14000 g for 20 minutes. The supernatant was used for proteome  
124 analyses. Protein concentration was measured by an infrared spectroscopy-based method using a  
125 Direct Detect® spectrometer (Merck). Samples were diluted in water in order to adjust all samples to  
126 the same protein concentration, then mixed with 1 volume of Laemmli denaturing buffer and heated  
127 at 90°C for 5 minutes. Sample volumes corresponding to 3.5 µg of protein were loaded onto SDS-  
128 PAGE gels containing 12% and 5% acrylamide in the resolving and stacking gels, respectively.  
129 Electrophoresis was performed using a Mini-Protean II unit (BioRad, Marnes-La-Coquette, France) at  
130 100 V until the dye front entered the resolving gel. Gels were stained for one hour in R-250  
131 Coomassie. Bands were manually excised, reduced in 10 mM dithiotreitol in 50 mM ammonium  
132 bicarbonate, and alkylated in 55 mM iodoacetamide in 50 mM ammonium bicarbonate. Destaining of  
133 the excised bands was obtained by successive rinses in 25 mM ammonium bicarbonate / acetonitrile  
134 (1 :1 v/v). Gel pieces were then dried by incubation in 100% acetonitrile for 10 min followed by  
135 vacuum-drying in a SpeedVac. Finally, gel pieces were incubated overnight at 37°C with 30 µL of a  
136 trypsin solution (V5111, Promega) at 10 ng/µL in 25 mM ammonium bicarbonate. Peptide extraction  
137 was performed by addition of 40 µL of acetonitrile 100%, 0.5% formic acid and sonication for 15 min.  
138 The trypsin digests were vacuum-dried in a SpeedVac and stored at 20 °C in a solution of 0.05%  
139 trifluoroacetic acid before Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis

140 *Mass Spectrometry analyses*

141 Five µL of the protein digests were injected into a nanoHPLC (Ultimate 3000, ThermoFisher  
142 Scientific). The peptide mixture was first concentrated and desalted on a microcolumn (Acclaim, 300  
143 µm, 5mm) equilibrated with trifluoroacetic acid (TFA) 0.05% in water. After 6 min, the microcolumn  
144 was switched on-line to an analytical C18 nanocolumn (Acclaim, 75 µm, 25 cm, Pepmap) equilibrated  
145 with 94.9 % H<sub>2</sub>O, 5% dimethyl sulfoxide (DMSO), 0.1% formic acid (FA) and peptides were separated

146 at 35°C according to their hydrophobicity with a 4 to 25% linear gradient of acetonitrile (94.9 % ACN,  
147 5 % DMSO, 0.1% FA) at a flow rate of 300 nL/min for 50 min. Peptides were electro-eluted with an  
148 ESI nanosource (1.6 kV) in the mass spectrometer (Orbitrap Velos, ThermoFisher scientific). The  
149 Orbitrap was used in top 15 data dependent mode, with gas phase fractionation (GPF1 400-480 m/z;  
150 GPF2 480-560 m/z; GPF3 560-660 m/z; GPF4 660-850 m/z, GPF5 850-1401 m/z).

151 Samples were analyzed in 6 different series (different days). To monitor and normalize LC-MS  
152 performance over time, a Quality Control (QC) was injected every 10 samples. This QC was prepared  
153 by diluting 20 μL of a commercial mixture of 15 synthetic peptides (Thermo Scientific™ Pierce™  
154 Peptide Retention Time Calibration Mixture) into 180 μL of a trypsin digest of a pool of 20 salivas. Five  
155 μL of the QC was injected and analyzed similarly to samples, except that gas phase fractionation was  
156 not performed.

157 Raw files were imported into ProgenesisQI Proteomics (Nonlinear dynamics) and the label-free  
158 quantification workflow was followed (see [www.nonlinear.com](http://www.nonlinear.com)). This includes peak alignment,  
159 establishment of a single ion map, normalization of intensities using data from 2 to 5x charged ions  
160 and XIC type quantification of all ions detected. Peptide identification were performed using Mascot  
161 interrogating the database homosapiens UniProt 201804 (71,600 sequences). Peptide mass tolerance  
162 was set to 10 ppm and fragment mass tolerance was set to 0.5 Da. Two miscleavages were  
163 authorized, and methionine oxidation, carbamidomethylation of cysteine and deamidated  
164 asparagine or acid aspartic were set as variable modifications. Protein identification was then  
165 validated when at least two unique peptides from one protein showed significant identification  
166 Mascot scores with False Discovery Rate (FDR < 1%). For protein quantification, the sum of all unique  
167 normalized peptide ion abundances for a specific protein was calculated for each sample. The final  
168 dataset consisted in 246 proteins quantified in 211 samples.

169 Proteome data handling

170 Protein abundance as obtained above was corrected for any series effect using QC data. For each  
171 synthetic peptide, the average abundance was calculated per series and for all runs combined. This

172 enabled calculating a correcting coefficient applicable to each series for normalization. The mean  
173 correcting coefficient for the 15 synthetic peptides was calculated per series, and it was applied to  
174 the protein abundance within that series. Missing values (0.3% of overall data) were handled  
175 following two distinct imputation methods as described by Wei et al. 2018 [27]. Eighty-two percent  
176 of the missing values corresponded to proteins which always showed low abundance values. These  
177 missing values were regarded as Missing Not At Random and thus handled using the quantile  
178 regression imputation of left-censored method. Remaining missing values were regarded as Missing  
179 At Random thus handled using the Random Forest imputation method.

## 180 Statistics

181 First, for each food group, we tested differences in the salivary proteome between high and low  
182 consumers Student t-tests corrected by the Benjamini-Hocberg method to control the False  
183 Discovery rate (FDR set at 5%). This was performed for the entire sample or when participants were  
184 stratified by diabetic status or by cohort.

185 Second, the Gene Set Enrichment Analysis (GSEA) method [28] was used to highlight biological  
186 processes enriched depending on the level of consumption of a food group. Given the case-control  
187 design of the study, we performed the analyses separately for diabetic and non-diabetic subjects. For  
188 each of the 246 proteins of the dataset, ontology terms and related annotations were retrieved from  
189 quickGO and the GO database. GO terms corresponding to at least two proteins (1850 in total) were  
190 selected. For each condition (one condition = one diabetic status, one food group), proteins were  
191 ranked in descending order of their p-values after attributing a negative sign to p-values when  
192 proteins were under-expressed in “high consumers”. This way, a GO term is regarded as of interest if  
193 its protein members are mostly in the top (over-expressed) or bottom (under-expressed) region of  
194 the list. GO terms were tested by unweighted GSEA estimation algorithm with at least a million  
195 permutations. With multifactorial dependencies occurring in the GO terms classification, (correlated  
196 and/or co-occurring annotations, co-occurring proteins ...), controlling false positive occurrence is  
197 challenging and usual methods may be over-restrictive. Post-hoc correction was therefore conducted

198 as follows: the community structure of the GO network (terms as nodes, relationships as edges) was  
199 determined using the Girvan-Newman algorithm [29]. This revealed 223 separated communities with  
200 a modularity of 0.85. GO terms within a community were considered as strongly dependent from  
201 each other whereas communities were considered as independent from each other. P-values of the  
202 223 communities were computed as the mean of p-values of its GO terms members, and the  
203 Benjamini-Hochberg correction was performed (calculation of a q-value). This generated a correcting  
204 factor for each community, which was then applied to p-values of all GO terms within this  
205 community to calculate individual q-values. GO terms were considered significant for q-values <0.01  
206 (FDR 1%).

207 Finally, we fitted minimal models predicting dietary variables by sociodemographic, clinical and  
208 salivary proteome variables. For that purpose, the workflow was as follows for each food group. First  
209 we reduced the number of protein variables by three successive selection steps : 1- a Kernel Partial  
210 Least Square Regression model was computed and proteins with high Variable Importance in  
211 Projection (VIP) values were selected (cutoff=1). 2- For these proteins, the difference in abundance  
212 between high and low consumers of the food group was tested by a Student t-test corrected by the  
213 Benjamini-Hochberg method to control the False Discovery rate (FDR). Variables with a low FDR were  
214 selected (cutoff=0.05). 3- Correlation among these proteins were calculated. Candidates sharing at  
215 least one high correlation (cutoff=0.7) were ordered by Ascendant Hierarchical Clustering, with  
216 adequate number of clusters determined by bootstrapping. The final set of proteins was made of all  
217 uncorrelated variables plus one variable per cluster (the one with the highest contribution). Second,  
218 we fitted the minimal model by logistic model learning using selected proteins and sociodemographic  
219 and clinical confounding factors (diabetic status, cohort, sex, age, BMI, smoker status and saliva  
220 flow). Subjects were split into a learning set (75%) and a validation set (25%). From the learning set, a  
221 logistic model was adjusted. Two stepwise procedures (Akaike Information Criterion, Bayesian  
222 Information Criterion) were performed separately : the AUC (Area under the Curve) of the ROC  
223 (receiver operating characteristic) curves were computed from the validation set and the model with

224 the highest AUC was selected. This process was repeated through 1000 simulations of  
225 learning/validation sets, and the final model retained the variables selected in at least half of the  
226 simulations. For the food groups where proteome variables were retained, we evaluated the  
227 performance of the models by calculating the AUC of the ROC curve. More specifically, three models  
228 were tested : Model 1 was the optimal model obtained as described above, Model 2 was Model 1  
229 into which we forced some basic descriptive variables (cohort, age, sex, diabetic status), and Model 3  
230 was Model 1 from which protein variables were removed. The comparison of Model 1 and Model 3  
231 allows documenting how much value the saliva proteome variables add to the model.  
232 Data management and statistical analyses were performed using the Rgui open-source software  
233 (<https://cran.r-project.org>).

## 234 **Results**

### 235 Participants' characteristics

236 Among 570 participants from wave 8 (2017-2018, 18y after baseline) of the 3C-Bordeaux cohort, 65  
237 were diabetics. Among them, 37 participants agreed to participate and a random sample of 71  
238 participants were selected as controls. Among 3273 participants from baseline in the Seniors-  
239 ENRICA-2 cohort, 669 were diabetics. A random **sample** of 53 diabetics and 53 matched controls  
240 were selected. Out of those 214 participants, 3 were excluded because of insufficient saliva  
241 production (Online Resource 2). The 211 remaining participant's characteristics are presented in  
242 Table 1. The French sample was older on average (87.3 vs 71.0 y) and comprised more women (61.3  
243 vs 36.2%) than the Spanish one. As expected, the diabetic participants exhibited higher BMI on  
244 average than controls in both cohorts.

### 245 Saliva amounts collected and salivary flows

246 The amounts of saliva collected varied from 0.09 to 8.68 g. As reported in Table 1, the average at-rest  
247 saliva flow varied from 0.18 to 0.26 g/min depending on the cohort and diabetic status. The  
248 difference between the two cohorts tested by a Wilcoxon test was significant ( $p < 0.001$ ), with higher

249 flow among Seniors-ENRICA-2 participants. The difference between diabetic and non-diabetic  
250 subjects was not significant either when combining the two cohorts or within each cohort.

251 MEDAS scores and dietary intakes

252 The mean MEDAS scores and the proportions of high vs low consumers of 11 food groups among all  
253 participants or stratified by diabetic status or cohort are presented in Table 2. Adherence to the  
254 Mediterranean dietary pattern was slightly (but not significantly) lower in diabetic participants  
255 compared to controls, while it was significantly ( $p < 0.05$ ) lower in 3C-Bordeaux participants compared  
256 to Seniors-ENRICA-2 participants. The dietary patterns were overall comparable between diabetic  
257 and non-diabetic individuals : the only two food groups which were clearly different according to the  
258 diabetic status were « nuts » and « sweets snacks » (confectionary, biscuits and commercial  
259 pastries), with a higher proportion of low consumers among diabetic subjects. In contrast, there  
260 were more differences between the two cohorts, with five food groups clearly different between  
261 Seniors-ENRICA-2 and 3C-Bordeaux. In the Spanish cohort there were more high consumers of olive  
262 oil, while in the French cohort there were more high consumers of vegetables, nuts, wine and butter.

263 Proteins differentially expressed between high and low consumers of 11 food groups

264 Table 3 provides the number of proteins significantly different (FDR 5%) between high and low  
265 consumer of the 11 food groups recorded. When considering all participants, there were  
266 differentially expressed proteins for three food groups : vegetables, butter and sweet snacks. The list  
267 of proteins significantly different, details of mass spectrometry identifications and abundance in high  
268 vs low consumers are available in Online resource 3. The intake level of vegetables, red meat and  
269 butter altered more the saliva of non-diabetic subjects (20, 58 and 5 differential proteins,  
270 respectively) than that of diabetic subjects (4, 0 and 0 differential proteins, respectively). In contrast,  
271 the intake level of sweet snacks had a major impact on saliva proteome (65 differential proteins) but  
272 only for diabetic subjects. More generally, the link between saliva proteome and diet was more  
273 pronounced among non-diabetic controls, with 85 proteins in total linked to 4 food groups  
274 (vegetables, legumes, red meat, butter), than among diabetic participants with 69 proteins linked to

275 2 food groups (vegetables, sweet snacks). The link between saliva proteome and diet was also more  
276 expressed among Seniors-ENRICA-2 participants with 77 proteins linked to two food groups (fruits,  
277 sweet snacks), than among 3C-Bordeaux participants with 2 proteins linked to the intake level of  
278 olive oil.

#### 279 Gene Set Enrichment Analysis (GSEA)

280 Based on protein expression levels in the entire proteomic dataset, GSEA identified the biological  
281 processes that were significantly enriched or depleted depending on the participants' intake level of  
282 a given food group. This was performed for diabetic and control participants separately and for the  
283 food group « sweet snacks » because of its major and robust impact on the salivary proteome (see  
284 Table 3). Results for all participants are presented in Figure 1 and results of the analyses performed  
285 for each cohort separately are available in Online resource 4.

286 For the two cohorts combined, some biological processes were in common and similarly affected for  
287 all participants. Thus, *regulation of cellular macromolecule biosynthetic process* was repressed while  
288 *platelet degranulation, leukocyte migration involved in inflammatory response and regulation of*  
289 *immune system* were enhanced in high consumers of sweet snacks, regardless of their diabetic  
290 status.

291 In addition, the impact of sweet snacks intake level was particularly evident among diabetic  
292 participants (Fig. 1). In those participants, three GO categories related to apoptosis were repressed in  
293 high consumers of sweet snacks, as well as the category regulation of peptidase activity. In contrast,  
294 all the other GO terms were enriched in diabetic high consumers of sweet snacks. These terms  
295 covered a very large panel of biological functions such as hemostasis, protein and peptide  
296 expression, modification or secretion, immunity, cell-cell communication or metabolism. Of special  
297 interest is a group of GO terms related to carbohydrate metabolism, (black box in Fig. 1). In controls,  
298 high consumption of sweet snacks also modified specifically some biological processes but to a lesser  
299 extent.

300 Analyses performed for each cohort separately confirmed that the impact of sweet snacks intake  
301 level was higher for diabetic participants than for controls in both cohorts. A vast majority of the  
302 biological processes significant for the two cohorts combined were also identified and significant in  
303 at least one of the two cohorts. Results were mostly consistent between the two cohorts combined  
304 and one of the cohorts (lines highlighted in blue in Online resource 4, e.g. *platelet degranulation*).  
305 Five biological processes (highlighted in yellow in Online resource 4, e.g. *regulation of*  
306 *phosphorylation*) were significant for diabetics and controls in the Seniors-ENRICA-2 cohort, while  
307 they were significant only for diabetics in the 3C-Bordeaux cohort and in the joint analysis. Finally, 14  
308 biological processes were consistent across cohorts for diabetics subjects: *regulation of apoptotic*  
309 *process, platelet degranulation, regulation of immune response, regulation of gene expression,*  
310 *regulation of transport, regulation of cellular component organization, regulation of protein/peptide*  
311 *secretion, regulation of cell morphogenesis involved in differentiation, interleukin-12-mediated*  
312 *signaling pathway, regulation of substrate adhesion-dependent cell spreading, Arp2/3 complex-*  
313 *mediated actin nucleation, actin polymerization-dependent cell motility and microtubule-based*  
314 *movement*. The latter four processes, related to cell spreading and motility, are likely linked to the  
315 oral epithelium repair potential.

316 Salivary markers of specific food groups' intake levels

317 Minimal models were sought for all food groups. Tables 4 and 5 present an overview of the variables  
318 retained in such models for all participants combined or cohort by cohort, respectively.

319 Considering both cohorts combined, apart from fruit for which no minimal model was retained, the  
320 descriptive characteristics of the subjects could predict the level of consumption (defined as meeting  
321 the MeDi requirements or not) for all food groups. The descriptor « cohort » was most frequently  
322 retained (4 occurrences for vegetables, olive oil, wine and butter) followed by sex (3 occurrences for  
323 wine, red meat and sweet snacks), diabetic status (nuts and sweet snacks) and smoker status  
324 (legumes and sweet drinks). Of special interest are the three food groups for which abundance of  
325 salivary proteins are predictors of intake : vegetables (5 proteins : P22079 lactoperoxidase, O00391

326    sulfhydryl oxidase 1, P10909 clusterin, P37802 transgelin-2 and Q8TAX7 mucin-7), butter (4 proteins :  
327    Q96DR5 BPI fold-containing family A member 2, P22079 lactoperoxidase, P07476 involucrin and  
328    P23280 carbonic anhydrase 6) and sweet snacks (2 proteins : E7EQB2 lactotransferrin, P02749 beta-  
329    2-glycoprotein 1). For the three food groups vegetables, butter and sweet snacks, the distribution of  
330    AUCs for 10000 simulations and the corresponding means and 95% confidence intervals are  
331    presented in Fig. 2. These show that compared to the minimal models (top panels), forcing into the  
332    model additional subjects' basic descriptors (middle panels) did not improve the model performance.  
333    Moreover, removing the protein descriptors from the minimal models (bottom panel) clearly  
334    reduced the model performance with an AUC mean shift of approximately -0.05 for vegetables and  
335    butter and -0.1 for sweet snacks. In other words, we confirmed that the salivary proteome added  
336    value to the prediction models.

337    Results obtained cohort by cohort (Table 5) did not directly confirm the protein markers identified  
338    when the two cohorts were combined. In agreement with the very low number of proteins  
339    significantly different between high and low consumers of the different food groups among 3C-  
340    Bordeaux participants (Table 3), no minimal models retained salivary proteins for French  
341    participants. Among Seniors-ENRICA-2 participants, salivary proteins were predictors of intake of  
342    fruits, red meat and sweet snacks.

### 343    **Discussion**

344    In this study, the salivary proteome differed depending on the intake level of some food groups  
345    (especially vegetables, fruits, sweet snacks and red meat), in a diabetic status- and cohort-specific  
346    manner. In addition, some saliva proteins were predictive of the intake level of sweet snacks after  
347    adjusting for several sociodemographic and clinical confounding factors.

348    The first challenge of the study was the collection of saliva. Only 3 participants out of 214 were  
349    excluded due to insufficient saliva production, and only one of the 211 donors requested to stop  
350    before the end of the 10 min collection time. The average saliva flows were below the 0.3 g-0.4/min  
351    generally reported [30,31], in accordance with the well-known reducing impact of ageing on both at-

352 rest and stimulated saliva flow [32]. Age is likely the main factor explaining the statistical difference  
353 between the cohorts, with lower average flow in French participants who are on average 16 years  
354 older than Spanish participants. The sex-ratio and the cases-controls proportions also differ between  
355 the two cohorts. However, several studies reported that gender had no significant impact on saliva  
356 flows of either healthy [33] or diabetic elderly subjects [34]. Furthermore, the diabetic status had no  
357 significant impact on saliva flow in our study, in each cohort or combining the two. To conclude on  
358 saliva collection, our study highlighted that it was feasible, well-tolerated and although the volumes  
359 were sometimes limited especially in the oldest elderlies, they remained compatible with the needs  
360 of proteome analytical methods.

361 The assessment of dietary intakes is also a challenge for any population. Diet history and Food  
362 Frequency Questionnaire are often used to capture usual nutrient intake or dietary habits with a high  
363 level of details. Here, we used two different tools which are standard and validated instruments for  
364 collecting food consumption data. In a previous study where these same cohorts and respective diet  
365 recording tools were used, consistent results for the association between fruit and vegetable intake  
366 and functional outcomes in older adults were obtained [35]. Based on the data obtained, we used  
367 the MEDAS score to represent adherence to the MeDi diet [26]. We confirmed previous  
368 observational studies reporting no difference in adherence to MeDi between persons with diagnosed  
369 diabetes and controls [36]. The MEDAS questionnaire also enabled to classify participants into high  
370 and low consumers for 11 food groups. There was a limited impact of the diabetic status on the  
371 intake levels, at the exception of nuts and sweet snacks. This latter finding is in accordance with  
372 previous results observed within the Seniors-ENRICA cohort, showing that the diet of diabetics differs  
373 mainly by a greater avoidance of sweet products while other food groups are little affected [36].  
374 Other studies are also in general agreement with this idea. For example, a cross-cultural study  
375 investigated the diet of diabetic and non-diabetic elderly men in Finland, the Netherlands and Italy  
376 and highlighted that the only food group that was significant different in all three countries was the  
377 consumption of added sugar [37]. In another study [38], the food group *sweets* differed largely  
378 between individuals with or without diabetes both in a pan-European cohort (the EPIC study) and a

379 USA-based cohort (the MEC study). The two latter articles also evidenced that differences between  
380 countries exceeded those between diabetics and non-diabetics, in line with our results on the  
381 MEDAS score or the individual food groups. Some dietary differences that we observed between  
382 cohorts corresponded to expected cultural specificities (more high consumers of wine in the  
383 Bordeaux area in France, more high consumers of olive oil in Spain), while others were more  
384 surprising. In particular, the proportion of high consumers of vegetables was extremely high in the 3-  
385 C Bordeaux cohort, well above other estimations for France [39]. In the 3-C Bordeaux cohort, fruits  
386 and vegetables consumption has already been associated with a lower risk of death [40] which may  
387 explain that high consumers of vegetables are over-represented in our sample of very old  
388 participants. In contrast, the proportion of high consumers of vegetables in the Seniors-ENRICA-2  
389 cohort was extremely low (3.8%) compared to the 12.3% or 17.6% in the general Spanish population  
390 [41] or in diabetic Spanish patients [36], respectively.

391 Keeping these issues in mind, and with the objective of describing broadly the impact of dietary  
392 intake on salivary proteome, we used the GSEA approach. Many salivary proteins are multifunctional  
393 [42] and thus GSEA provided an overwhelming wealth of information, but a first obvious result was  
394 that the biological processes enriched according to the intake level were mostly different between  
395 diabetic subjects and controls. The salivary proteome is therefore shaped differently by diet  
396 depending on the physiopathological status of the subject.

397 The second finding is that intake of sweet snacks had a major effect on biological processes in  
398 diabetics, **regardless of the cohort considered**. This is not surprising since dietary sugars have a  
399 profound physiological effect in diabetic individuals, who by definition have difficulty in controlling  
400 glycemia. For example 8 enzymes of the *glucose catabolic process to pyruvate* (out of the 10 of the  
401 core glycolysis pathway) were over-represented in high consumers of sweet snacks among diabetic  
402 participants. This is consistent with the long-known increase in glycolytic enzymes activity or  
403 expression induced by glucose in various tissues or cells such as pancreatic cells [43] or the jejunum  
404 [44]. Inter-individual variability in salivary glycolytic enzymes has been hypothesized to be at least  
405 partly related to dietary habits of the healthy adult saliva donors [45]. Finally, several glycolytic

406 enzymes were found in saliva of diabetic subjects and these were overexpressed in subjects with the  
407 most severe forms of retinopathy [46].

408 More unexpectedly, **when analyzing the two cohorts jointly**, the *endothelial cell apoptotic process*  
409 was repressed in diabetic high consumers of sweets, while high glucose is known to trigger apoptosis  
410 in endothelial cells [47]. However, the three proteins attached to this GO term in our dataset are the  
411 fibrinogen  $\alpha$ ,  $\beta$  and  $\gamma$ -chains. They are linked to negative regulation of the apoptotic process in GO,  
412 but their primary function is rather blood clotting: their over-expression could thus reflect the more  
413 frequent gum bleeding in diabetic individuals with inadequate intake of sweet snacks. This is then in  
414 accordance with a consensus report indicating that poor glycaemic control in diabetes is associated  
415 with poorer periodontal status and outcomes [48]. This example illustrates that GSEA should be  
416 regarded as indicative of biological processes to be investigated further, and findings should be  
417 examined considering representativity of the proteins attached to each GO category. In that respect,  
418 the example of *platelet degranulation* deserves special attention since it is associated with 30  
419 proteins in our dataset (out of 129 in the human genome). Increased platelet degranulation is  
420 observed in T2DM patients compared to healthy subjects [49,50] and a mechanistic study concluded  
421 that high glucose *per se* increased platelet reactivity in blood of both diabetics and controls [51]. In  
422 our study, **combining both cohorts**, the saliva proteome composition also suggested that high intake  
423 of sweet snacks was linked with enhanced platelet reactivity both in diabetics or controls. **This was**  
424 **confirmed in the Spanish cohort and among diabetic subjects in the French cohort.**

425 We also looked for markers of diet and adjusted the models for confounding factors (diabetic status,  
426 cohort, age, sex etc.). **Combining both cohorts**, salivary proteins were retained in the model for three  
427 food groups. **For « vegetables » and « butter », one should note that the proportions of high**  
428 **consumers are much higher in the 3C-Bordeaux cohort. Results should therefore be taken cautiously**  
429 **because there is a risk that results are partly confounded with a cohort effect. In addition, the**  
430 **analyses performed for each cohort separately highlighted the differences between the two cohorts**  
431 **and illustrated the difficulty of identifying universal markers.** Nevertheless, whatever the subjects'  
432 characteristics in terms of cohort, age, sex, diabetic status, smoker status, BMI or saliva flow, higher

433 abundance of lactotransferrin (E7EQB2) and beta-2-glycoprotein 1 (P02749) in saliva was associated  
434 with a higher chance of being a high consumer of sweet snacks. Interestingly, we had previously  
435 observed in healthy children that another protein of the transferrin family (serotransferrin) was  
436 positively associated with a number of food groups including biscuits & sweets [19]. There is also  
437 some biological coherence among some of the identified markers. For example, the two positive  
438 markers of vegetables intake (lactoperoxidase, sulhydryl oxidase 1) catalyze reactions involving H<sub>2</sub>O<sub>2</sub>  
439 and contribute to cellular redox homeostasis. However, the main general lesson from those results is  
440 that the abundance of some salivary proteins was linked to the consumption level of some food  
441 groups in elderly subjects after adjusting for several sociodemographic and clinical confounding  
442 factors. In addition, since including salivary proteins data improved the prediction models, this is the  
443 proof-of-concept that saliva might be a source of objective markers of usual diet.

444 To conclude, it should be reminded that the studied population originated from two very contrasted  
445 cohorts, especially in terms of age and dietary habits. This represented a challenge, particularly for  
446 statistical methods which can not fully account for confounding factors. Dietary intake was also here  
447 simply evaluated as compliance or not to the requirements of MeDi diet. However, the salivary  
448 proteome data suggested biological functions affected by the dietary intake level of **sweet** snacks,  
449 **across cohorts and diabetic status (e.g. platelet degranulation) or across cohorts and specific to one**  
450 **diabetic status (e.g. functions linked to cell spreading and cell motility).** Proteome data also added  
451 value to minimal models predicting the intake level of some food groups. It is now necessary to  
452 confirm these results on a validation population, but also to assess more finely the link between the  
453 potential markers' expression and the actual quantitative intakes.

## References

- 454 1. Coelho-Júnior H, Rodrigues B, Uchida M, Marzetti E (2018) Low Protein Intake Is Associated  
455 with Frailty in Older Adults: A Systematic Review and Meta-Analysis of Observational Studies.  
456 *Nutrients*. <https://doi.org/10.3390/nu10091334>
- 457 2. Machado-Fragua MD, Struijk EA, Ballesteros J-M, Ortolá R, Rodríguez-Artalejo F, Lopez-Garcia E  
458 (2019) Habitual coffee consumption and risk of falls in 2 European cohorts of older adults. *Am J*  
459 *Clin Nutr* 109:1431- 1438. <https://doi.org/10.1093/ajcn/nqy369>
- 460 3. Singh B, Parsaik AK, Mielke MM, Erwin PJ, Knopman DS, Petersen RC, Roberts RO (2014)  
461 Association of Mediterranean Diet with Mild Cognitive Impairment and Alzheimer’s Disease: A  
462 Systematic Review and Meta-Analysis. *J Alzheimers Dis* 39:271- 282.  
463 <https://doi.org/10.3233/JAD-130830>
- 464 4. Kojima G, Avgerinou C, Iliffe S, Walters K (2018) Adherence to Mediterranean Diet Reduces  
465 Incident Frailty Risk: Systematic Review and Meta-Analysis. *J Am Geriatr Soc* 66:783- 788.  
466 <https://doi.org/10.1111/jgs.15251>
- 467 5. Struijk EA, Hagan KA, Fung TT, Hu FB, Rodríguez-Artalejo F, Lopez-Garcia E (2020) Diet quality  
468 and risk of frailty among older women in the Nurses’ Health Study. *Am J Clin Nutr*  
469 111:877- 883. <https://doi.org/10.1093/ajcn/nqaa028>
- 470 6. The InterAct Consortium (2011) Mediterranean Diet and Type 2 Diabetes Risk in the European  
471 Prospective Investigation Into Cancer and Nutrition (EPIC) Study: The InterAct project. *Diabetes*  
472 *Care* 34:1913- 1918. <https://doi.org/10.2337/dc11-0891>
- 473 7. Salas-Salvadó J, Guasch-Ferré M, Lee C-H, Estruch R, Clish CB, Ros E (2015) Protective Effects of  
474 the Mediterranean Diet on Type 2 Diabetes and Metabolic Syndrome. *J Nutr* 146:920S-927S.  
475 <https://doi.org/10.3945/jn.115.218487>
- 476 8. André P, Proctor G, Driollet B, Garcia-Esquinas E, Lopez-Garcia E, Gomez-Cabrero D, Neyraud E,  
477 Rodríguez-Artalejo F, Morzel M, Féart C (in press) The role of overweight in the association  
478 between the Mediterranean diet and the risk of type 2 diabetes mellitus: a mediation analysis  
479 among 21 585 UK biobank participants. *Int J Epidemiol*. <https://doi.org/10.1093/ije/dyaa103>
- 480 9. Hu FB, Willett WC (2018) Current and Future Landscape of Nutritional Epidemiologic Research.  
481 *JAMA*. <https://doi.org/10.1001/jama.2018.16166>
- 482 10. Guertin KA, Moore SC, Sampson JN, Huang W-Y, Xiao Q, Stolzenberg-Solomon RZ, Sinha R, Cross  
483 AJ (2014) Metabolomics in nutritional epidemiology: identifying metabolites associated with  
484 diet and quantifying their potential to uncover diet-disease relations in populations. *Am J Clin*  
485 *Nutr* 100:208- 217. <https://doi.org/10.3945/ajcn.113.078758>
- 486 11. Rabassa M, Zamora-Ros R, Palau-Rodríguez M, Tulipani S, Miñarro A, Bandinelli S, Ferrucci L,  
487 Cherubini A, Andres-Lacueva C (2020) Habitual Nut Exposure, Assessed by Dietary and Multiple  
488 Urinary Metabolomic Markers, and Cognitive Decline in Older Adults: The InCHIANTI Study. *Mol*  
489 *Nutr Food Res* <https://doi.org/10.1002/mnfr.201900532>
- 490 12. Li J, Guasch-Ferré M, Chung W, Ruiz-Canela M, Toledo E, Corella D et al (2020) The  
491 Mediterranean diet, plasma metabolome, and cardiovascular disease risk. *Eur Heart J*  
492 41:2645- 2656. <https://doi.org/10.1093/eurheartj/ehaa209>

- 493 13. Granger DA, Kivlighan KT, Fortunato C, Harmon AG, Hibel LC, Schwartz EB, Whembolua G-L  
494 (2007) Integration of salivary biomarkers into developmental and behaviorally-oriented  
495 research: Problems and solutions for collecting specimens. *Physiol Behav* 92:583- 590.  
496 <https://doi.org/10.1016/j.physbeh.2007.05.004>
- 497 14. Morzel M, Jeannin A, Lucchi G, Truntzer C, Pecqueur D, Nicklaus S, Chambon C, Ducoroy P  
498 (2012) Human infant saliva peptidome is modified with age and diet transition. *J Proteomics*  
499 75:3665- 3673. <https://doi.org/10.1016/j.jprot.2012.04.028>
- 500 15. Castagnola M, Inzitari R, Fanali C, Iavarone F, Vitali A, Desiderio C, et al (2011) The Surprising  
501 Composition of the Salivary Proteome of Preterm Human Newborn. *Mol Cell Proteomics*.  
502 <https://doi.org/10.1074/mcp.M110.003467>
- 503 16. Wang K, Wang X, Zheng S, Niu Y, Zheng W, Qin X, Li Z, Luo J, Jiang W, Zhou X, Li W, Zhang L  
504 (2018) iTRAQ-based quantitative analysis of age-specific variations in salivary proteome of  
505 caries-susceptible individuals. *J Transl Med*. <https://doi.org/10.1186/s12967-018-1669-2>
- 506 17. Méjean C, Morzel M, Neyraud E, Issanchou S, Martin C, Bozonnet S, Urbano C, Schlich P,  
507 Herberg S, Péneau S, Feron G (2015) Salivary Composition Is Associated with Liking and Usual  
508 Nutrient Intake. *PLOS ONE*. <https://doi.org/10.1371/journal.pone.0137473>
- 509 18. Morzel M, Palicki O, Chabanet C, Lucchi G, Ducoroy P, Chambon C, Nicklaus S (2011) Saliva  
510 electrophoretic protein profiles in infants: Changes with age and impact of teeth eruption and  
511 diet transition. *Arch Oral Biol* 56:634- 642. <https://doi.org/10.1016/j.archoralbio.2010.12.015>
- 512 19. Morzel M, Truntzer C, Neyraud E, Brignot H, Ducoroy P, Lucchi G, Canlet C, Gaillard S, Nicod F,  
513 Nicklaus S, Peretti N, Feron G (2017) Associations between food consumption patterns and  
514 saliva composition: Specificities of eating difficulties children. *Physiol Behav* 173:116- 123.  
515 <http://dx.doi.org/10.1016/j.physbeh.2017.02.005>
- 516 20. Walsh MC, Brennan L, Malthouse JPG, Roche HM, Gibney MJ (2006) Effect of acute dietary  
517 standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans.  
518 *Am J Clin Nutr* 84:531- 539.
- 519 21. Proctor GB, André P, Lopez-Garcia E, Gomez Cabrero Lopez D, Neyraud E, Féart C, Rodriguez  
520 Artalejo F, Garcia-Esquinas E, Morzel M (2017) The SALAMANDER project: SALivAry bioMarkers  
521 of mediterraneanAN Diet associated with long-tERm protection against type 2 diabetes. *Nutr Bull*  
522 42:369- 374. <https://doi.org/10.1111/nbu.12298>
- 523 22. The 3C Study Group (2003) Vascular Factors and Risk of Dementia: Design of the Three-City  
524 Study and Baseline Characteristics of the Study Population. *Neuroepidemiology* 22:316- 325.  
525 <https://doi.org/10.1159/000072920>
- 526 23. Cabanas-Sánchez V, Esteban-Cornejo I, Migueles JH, Banegas JR, Graciani A, Rodríguez-Artalejo  
527 F, Martínez-Gómez D (2020) Twenty four-hour activity cycle in older adults using wrist-worn  
528 accelerometers: The seniors-ENRICA-2 study. *Scand J Med Sci Sports* 30:700- 708.  
529 <https://doi.org/10.1111/sms.13612>
- 530 24. Rahi B, Ajana S, Tabue-Tegu M, Dartigues J-F, Peres K, Feart C (2018) High adherence to a  
531 Mediterranean diet and lower risk of frailty among French older adults community-dwellers:  
532 Results from the Three-City-Bordeaux Study. *Clin Nutr* 37:1293- 1298.  
533 <https://doi.org/10.1016/j.clnu.2017.05.020>

- 534 25. Guallar-Castillón P, Sagardui-Villamor J, Balboa-Castillo T, Sala-Vila A, Ariza Astolfi MJ, Sarrión  
535 Pelous MD, Leon-Muñoz LM, Graciani A, Laclaustra M, Benito C, Banegas JR, Rodríguez Artalejo  
536 F (2014) Validity and Reproducibility of a Spanish Dietary History. *PLoS ONE*.  
537 <https://doi.org/10.1371/journal.pone.0086074>
- 538 26. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al (2011) A  
539 Short Screener Is Valid for Assessing Mediterranean Diet Adherence among Older Spanish Men  
540 and Women. *J Nutr* 141:1140- 1145. <https://doi.org/10.3945/jn.110.135566>
- 541 27. Wei R, Wang J, Su M, Jia E, Chen S, Chen T, Ni Y (2018) Missing Value Imputation Approach for  
542 Mass Spectrometry-based Metabolomics Data. *Sci Rep*. <https://doi.org/10.1038/s41598-017>  
543 19120-0
- 544 28. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A,  
545 Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005) Gene set enrichment analysis: A  
546 knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad*  
547 *Sci* 102:15545- 15550. <https://doi.org/10.1073/pnas.0506580102>
- 548 29. Girvan M, Newman MEJ (2002) Community structure in social and biological networks. *Proc*  
549 *Natl Acad Sci*. 99:7821- 7826. <https://doi.org/10.1073/pnas.122653799>
- 550 30. Humphrey SP, Williamson RT (2001) A review of saliva: Normal composition, flow, and function.  
551 *J Prosthet Dent* 85:162- 169. <https://doi.org/10.1067/mpr.2001.113778>
- 552 31. Dawes C, Pedersen AML, Villa A, Ekström J, Proctor GB, Vissink A, et al (2015) The functions of  
553 human saliva: A review sponsored by the World Workshop on Oral Medicine VI. *Arch Oral Biol*  
554 60:863- 874. <https://doi.org/10.1016/j.archoralbio.2015.03.004>
- 555 32. Affoo RH, Foley N, Garrick R, Siqueira WL, Martin RE (2015) Meta-Analysis of Salivary Flow  
556 Rates in Young and Older Adults. *J Am Geriatr Soc* 63:2142- 2151.  
557 <https://doi.org/10.1111/jgs.13652>
- 558 33. Vandenberghe-Descamps M, Labouré H, Prot A, Septier C, Tournier C, Feron G, Sulmont-Rossé  
559 C (2016) Salivary Flow Decreases in Healthy Elderly People Independently of Dental Status and  
560 Drug Intake. *J Texture Stud* 47:353- 360. <https://doi.org/10.1111/jtxs.12191>
- 561 34. Lima DLF, Carneiro SDRM, Barbosa FT de S, Saintrain MV de L, Moizan JAH, Doucet J (2017)  
562 Salivary flow and xerostomia in older patients with type 2 diabetes mellitus. *PLOS ONE*.  
563 <https://doi.org/10.1371/journal.pone.0180891>
- 564 35. García-Esquinas E, Rahi B, Peres K, Colpo M, Dartigues J-F, Bandinelli S, Feart C, Rodríguez-  
565 Artalejo F (2016) Consumption of fruit and vegetables and risk of frailty: a dose-response  
566 analysis of 3 prospective cohorts of community-dwelling older adults. *Am J Clin Nutr* 104:132-  
567 142.
- 568 36. Muñoz-Pareja M, León-Muñoz LM, Guallar-Castillón P, Graciani A, López-García E, Banegas JR,  
569 Rodríguez-Artalejo F (2012) The Diet of Diabetic Patients in Spain in 2008–2010: Accordance  
570 with the Main Dietary Recommendations—A Cross-Sectional Study. *PLoS ONE*.  
571 <https://doi.org/10.1371/journal.pone.0039454>
- 572 37. Virtanen S, Feskens E, Räsänen L, Fidanza F, Tuomilehto J, Giampaoli S, Nissinen A, Kromhout D  
573 (2000) Comparison of diets of diabetic and non-diabetic elderly men in Finland, The  
574 Netherlands and Italy. *Eur J Clin Nutr* 54:181- 186. <https://doi.org/10.1038/sj.ejcn.1600916>

- 575 38. Nöthlings U, Boeing H, Maskarinec G, Sluik D, Teucher B, Kaaks R, et al (2011) Food intake of  
576 individuals with and without diabetes across different countries and ethnic groups. *Eur J Clin*  
577 *Nutr* 65:635- 641. <https://doi.org/10.1038/ejcn.2011.11>
- 578 39. Péneau S, Galan P, Jeandel C, Ferry M, Andreeva V, Hercberg S, Kesse-Guyot E, the SU.VI.MAX 2  
579 Research Group (2011) Fruit and vegetable intake and cognitive function in the SU.VI.MAX 2  
580 prospective study. *Am J Clin Nutr* 94:1295- 1303. <https://doi.org/10.3945/ajcn.111.014712>
- 581 40. Letois F, Mura T, Scali J, Gutierrez L-A, Féart C, Berr C (2016) Nutrition and mortality in the  
582 elderly over 10 years of follow-up: the Three-City study. *Br J Nutr* 116:882- 889.  
583 <https://doi.org/10.1017/S000711451600266X>
- 584 41. León-Muñoz LM, Guallar-Castillón P, Graciani A, López-García E, Mesas AE, Aguilera MT,  
585 Banegas JR, Rodríguez-Artalejo F (2012) Adherence to the Mediterranean Diet Pattern Has  
586 Declined in Spanish Adults. *J Nutr* 142:1843- 1850. <https://doi.org/10.3945/jn.112.164616>
- 587 42. Proctor GB, Carpenter GH (1998) The function of salivary proteins and the regulation of their  
588 secretion by salivary glands. *Biomed Rev* 9:3- 9. <https://doi.org/10.14748/bmr.v9.132>
- 589 43. Roche E, Yaney G, Corkey B, Asfari M, Prentki M (1997) Induction by Glucose of Genes Coding  
590 for Glycolytic Enzymes in a Pancreatic beta-Cell Line (INS-1). *J Biol Chem* 272:3091- 3098.  
591 <https://doi.org/10.1074/jbc.272.5.3091>
- 592 44. Stifel FB, Herman RH, Rosenweig NS (1969) Dietary regulation of glycolytic enzymes. III.  
593 Adaptive changes in rat jejunal pyruvate kinase, phosphofructokinase, fructosediphosphatase  
594 and glycerol-3-phosphate dehydrogenase. *Biochim Biophys Acta* 184:29- 34.  
595 [https://doi.org/10.1016/0304-4165\(69\)90094-4](https://doi.org/10.1016/0304-4165(69)90094-4)
- 596 45. Quintana M, Palicki O, Lucchi G, Ducoroy P, Chambon C, Salles C, Morzel M (2009) Inter-  
597 individual variability of protein patterns in saliva of healthy adults. *J Proteomics* 72:822- 830.  
598 <https://doi.org/10.1016/j.jprot.2009.05.004>
- 599 46. Chee CS, Chang KM, Loke MF, Angela Loo VP, Subrayan V (2016) Association of potential  
600 salivary biomarkers with diabetic retinopathy and its severity in type-2 diabetes mellitus: a  
601 proteomic analysis by mass spectrometry. *PeerJ*. <https://doi.org/10.7717/peerj.2022>
- 602 47. Baumgartner-Parzer SM, Wagner L, Pettermann M, Grillari J, Gessl A, Waldhäusl W (1995) High-  
603 glucose-triggered apoptosis in cultured endothelial cells. *Diabetes* 44:1323- 1327.  
604 <https://doi.org/10.2337/diab.44.11.1323>
- 605 48. Sanz M, Ceriello A, Buysschaert M, Chapple I, Demmer RT, Graziani F, et al (2018) Scientific  
606 evidence on the links between periodontal diseases and diabetes: Consensus report and  
607 guidelines of the joint workshop on periodontal diseases and diabetes by the International  
608 diabetes Federation and the European Federation of Periodontology. *Diabetes Res Clin Pract*  
609 137:231- 241. <https://doi.org/10.1016/j.diabres.2017.12.001>
- 610 49. Cerbone AM, Macarone-Palmieri N, Saldalamacchia G, Coppola A, Di Minno G, Rivellese AA.  
611 (2009) Diabetes, vascular complications and antiplatelet therapy: open problems. *Acta Diabetol*  
612 46:253- 261. <https://doi.org/10.1007/s00592-008-0079-y>
- 613 50. Randriamboavonjy V, Isaak J, Elgheznavy A, Pistrosch F, Frömel T, Yin X, Badenhop K, Heide H,  
614 Mayr M, Fleming I (2012) Calpain inhibition stabilizes the platelet proteome and reactivity in  
615 diabetes. *Blood* 120:415- 423. <https://doi.org/10.1182/blood-2011-12-399980>

616 51. Keating FK, Sobel BE, Schneider DJ (2003) Effects of increased concentrations of glucose on  
617 platelet reactivity in healthy subjects and in patients with and without diabetes mellitus. *Am J*  
618 *Cardiol* 92:1362- 1365. <https://doi.org/10.1016/j.amjcard.2003.08.033>

**Table 1.** Main characteristics of the 211 participants whose saliva was analyzed in the study

	<b>Seniors-ENRICA-2</b>		<b>3C-Bordeaux</b>	
	diabetics (n=52)	controls (n=53)	diabetics (n=37)	controls (n=69)
<b>Age (years, mean <math>\pm</math> SD)</b>	70.6 $\pm$ 4.3	71.4 $\pm$ 3.7	87.4 $\pm$ 4.1	87.3 $\pm$ 2.9
<b>Sex (% men)</b>	69	58	48	33
<b>BMI (mean <math>\pm</math> SD)</b>	30.0 $\pm$ 5.0	26.6 $\pm$ 3.9	29.0 $\pm$ 3.9	24.4 $\pm$ 4.0
<b>Smoker (never/former/current) <sup>a</sup></b>	9/37/6	15/33/5	17/7/5	33/20/5
<b>Saliva flow (g/min, mean <math>\pm</math> SD)</b>	0.26 $\pm$ 0.17	0.23 $\pm$ 0.16	0.18 $\pm$ 0.13	0.20 $\pm$ 0.18

<sup>a</sup> 19 missing values (3C-Bordeaux)

**Table 2.** Modified MEDAS score (0 to 13 points) and percentage of low and high consumers of 11 food groups among the 211 older adults, altogether or when individuals are stratified by diabetic status or cohort. The cut-off points to define low and high intake are provided in Online Resource 1. Cells shaded in gray highlight the food groups for which there is a large (>10%) difference between the two sub-samples.

		All subjects (n=211)	Diabetic status		Cohort	
			Diabetics (n= 89)	Controls (n=122)	Seniors-ENRICA-2 (n=105)	3C-Bordeaux (n=106)
<b>13-point MEDAS score</b>		6.24 ± 1.57	6.13 ± 1.52	6.32 ± 1.61	6.47 ± 1.39	6.02 ± 1.70
<b>Vegetables</b>						
	Low	56.4	60.7	53.3	<b>96.2</b>	<b>17.0</b>
	High	43.6	39.3	46.7	<b>3.8</b>	<b>83.0</b>
<b>Fruits</b>						
	Low	54.0	58.4	50.8	53.3	54.7
	High	46.0	41.6	49.2	46.7	45.3
<b>Olive oil</b>						
	Low	88.2	85.4	90.2	<b>80.0</b>	<b>96.2</b>
	High	11.8	14.6	9.8	<b>20.0</b>	<b>3.8</b>
<b>Legumes</b>						
	Low	91.0	88.8	92.6	88.6	93.4
	High	9.0	11.2	7.4	11.4	6.6
<b>Nuts</b>						
	Low	82.9	<b>91.0</b>	<b>77.0</b>	<b>89.5</b>	<b>76.4</b>
	High	17.1	<b>9.0</b>	<b>23.0</b>	<b>10.5</b>	<b>23.6</b>
<b>Wine</b>						
	Low	65.4	69.7	62.3	<b>82.9</b>	<b>48.1</b>
	High	34.6	30.3	37.7	<b>17.1</b>	<b>51.9</b>
<b>Fish</b>						
	Low	68.2	70.8	66.4	64.8	71.7
	High	31.8	29.2	33.6	35.2	28.3
<b>Red meat</b>						
	Low	92.4	91.0	93.4	90.5	94.3
	High	7.6	9.0	6.6	9.5	5.7
<b>Butter</b>						
	Low	61.6	66.3	58.2	<b>89.5</b>	<b>34.0</b>
	High	38.4	33.7	41.8	<b>10.5</b>	<b>66.0</b>
<b>Sweet snacks</b>						
	Low	63.5	<b>70.8</b>	<b>58.2</b>	63.8	63.2
	High	36.5	<b>29.2</b>	<b>41.8</b>	36.2	36.8
<b>Sweet drinks</b>						
	Low	91.5	86.5	95.1	88.6	94.3
	High	8.5	13.5	4.9	11.4	5.7

**Table 3.** Number of proteins significantly differently expressed (FDR<5%) in saliva of low vs high consumers of 11 food groups, altogether or when individuals are stratified by diabetic status or by cohort. The cut-off points to define low and high intake are provided in Online Resource 1.

	Vegetables	Fruits	Olive oil	Legumes	Nuts	Wine	Fish	Red meat	Butter	Sweet snacks	Sweet drinks
<b>All participants (n=211)</b>	22	0	0	0	0	0	0	0	3	8	0
<b>Diabetics (n=89)</b>	4	0	0	0	0	0	0	0	0	65	0
<b>Controls (n=122)</b>	20	0	0	2	0	0	0	58	5	0	0
<b>Seniors-ENRICA-2 (n=105)</b>	0	63	0	0	0	0	0	0	0	14	0
<b>3C Bordeaux (n=106)</b>	0	0	2	0	0	0	0	0	0	0	0

**Table 4.** Variables retained in minimal models of prediction of intake of 11 food groups (two cohorts combined). Quantitative variables are separated into positive (chance of being a high consumer increases with value) or negative (chance of being a high consumer decreases with value) predictors. For categorial variables, the category associated with high intake is reported. Proteins are identified by their UniProt entry reference.

Food groups	Quantitative variables		Categorial variables
	positive	negative	
Vegetables	P22079, O00391	P10909, P37802, Q8TAX7	French cohort
Fruits			
Olive oil	BMI		Spanish cohort
Legumes			Former or current smokers
Nuts			Non-diabetics
Wine			French cohort, Men
Fish		Saliva flow	
Red meat			Men
Butter	Q96DR5, P22079	P23280, P07476	French cohort
Sweet snacks	E7EQB2, P02749		Non-diabetics, Men
Sweet drinks		Age	Former or current smokers

**Table 5.** Variables retained in minimal models of prediction of intake of 11 food groups (for each cohort separately). Quantitative variables are separated into positive (chance of being a high consumer increases with value) or negative (chance of being a high consumer decreases with value) predictors. For categorical variables, the category associated with high intake is reported. Proteins are identified by their UniProt entry reference.

Food groups	Quantitative variables		Categorical variables
	positive	negative	
<b>Seniors-ENRICA-2</b>			
Vegetables	BMI	Age	Women
Fruits	Q86YZ3, P0DMV8	P06753, P01024	
Olive oil	BMI		
Legumes			Women
Nuts			Non-diabetics
Wine			Men
Fish			
Red meat		Q96DR5, Q8TDL5, P01024, Age	Men
Butter			
Sweet snacks	P61158, P07195, P02763, P61769, saliva flow	P06744	Non-diabetics
Sweet drinks		Age	
<b>3C-Bordeaux</b>			
Vegetables	Age		
Fruits		Age, saliva flow	
Olive oil			Non-diabetics
Legumes			Men
Nuts		Saliva flow	
Wine			Men
Fish		Saliva flow	
Red meat			Diabetics
Butter			
Sweet snacks	BMI		Non-diabetics, Men
Sweet drinks			

## Figure Captions

**Fig. 1** Biological processes (Gene Ontology terms) associated with saliva proteins in high consumers of sweet snacks. Gene Set Enrichment Analysis was conducted separately for diabetic participants or controls. A « positive » or « negative » effect refers to the situation where proteins attached to the GO term are over-expressed and under-expressed, respectively, in high consumers of sweet snacks. The size of the dots indicates the number of proteins attached to each GO term in the experimental dataset, and the colour of the dots translates the gene ratio, i.e. the number of proteins attached to each GO term in the experimental dataset divided by the total number of proteins linked to that GO category in the human genome. In addition, four biological processes are common to all high consumers of sweet snacks, regardless of their diabetic status : *regulation of cellular macromolecule biosynthetic process* (negative effect), *platelet degranulation*, *leukocyte migration involved in inflammatory response* and *regulation of immune system* (positive effect).

**Fig. 2** Estimation of the performance of minimal models predicting the consumption level of vegetables, butter and sweet snacks : distribution of AUCs (Area under the Curve) of the ROC (receiver operating characteristic) curves for 10000 simulations. For each food group, results are presented for three models: minimal model selected as described in the Material & Methods section (upper panel), minimal model with systematic inclusion of age, sex, cohort, diabetic status (middle panel), minimal model without protein variables (lower panel).