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## The potential of salivary biomarkers of nutritional status and dietary intake: A Systematic Review

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1 **Title:** The potential of salivary biomarkers of nutritional status and dietary intake: A Systematic  
2 Review

3 **Abstract**

4 **Objectives:** To explore whether nutritional salivary biomarkers could be used to aid nutritional  
5 status assessment and/or support traditional dietary assessment methods for patients.

6  
7 **Data and Sources:** Searches were performed using four electronic databases; MEDLINE,  
8 EMBASE, Scopus and Web of Science. Trial registers (i.e. Cochrane), grey literature and  
9 reference lists were searched.

10  
11 **Study Selection:** Studies which measured nutritional salivary biomarkers related to nutritional  
12 status and/or dietary intake outcome were included. No restrictions on participants' age, study  
13 design, publication date, setting or health status. Animal studies, non-English language studies,  
14 commentaries, and conference abstracts were excluded.

15  
16 **Results:** Study titles and abstracts were screened (n=7982), full-texts assessed (n=176) and 85  
17 studies included in a narrative synthesis. The most promising salivary biomarkers for nutritional  
18 status included: glucose, where saliva and serum levels were positively correlated in those with  
19 type 2 diabetes (T2D), higher salivary calcium levels in post-menopausal women in general and  
20 specifically those with lower bone mineral density (BMD), and salivary vitamin D to assess  
21 vitamin D status in healthy volunteers. Higher salivary total antioxidant capacity (TAC),  
22 nitrate/nitrite and fluoride were observed with increased antioxidant, nitrate/nitrite and fluoride  
23 dietary intake, respectively. A meta-analysis found significantly higher mean salivary glucose  
24 (n=12) in T2D compared with healthy controls, but there was substantial heterogeneity ( $I^2=94\%$ )  
25 and evidence of publication bias.

26  
27 **Conclusions:** The most promising salivary biomarkers identified were, glucose, vitamin D,  
28 calcium, TAC, nitrate/nitrite and fluoride. However, this was based on a small number of studies

29 of varying quality, with many lacking a salivary assay performance assessment.

30

31 **Clinical Significance:** At present, nutritional salivary biomarkers cannot be used alone to assess  
32 nutritional status or dietary intake. Further research into the most promising nutritional salivary  
33 biomarkers is required.

34

### 35 **Introduction**

36 Nutritional status is defined as the state of an individual's health, in terms of the intake and  
37 utilisation of nutrients [1]. The traditional assessment of nutritional status is complex, as it  
38 typically involves a combination of different measures including, anthropometry, clinical  
39 assessment of disease state, dietary intake assessment, a blood test interpreted alongside a clinical  
40 examination and environmental assessment relating to factors affecting eating behaviours [2].

41 Individuals with a combination of unsatisfactory assessment results such as, blood results outside  
42 of normal ranges or inadequate dietary intake, may be at risk of for example, malnutrition, iron-  
43 deficiency anaemia (IDA), osteoporosis or T2D, if nutritional intervention does not take place.

44 Dietary data collection as part of a nutritional status assessment and additionally the individual  
45 collection of dietary data to assess dietary intake, is not without challenge. This is because the  
46 usual methods of dietary data collection including, a food frequency questionnaire (FFQ), 24-hour  
47 recall or food diary, are subjective, at risk of recall bias and are prone to under- and over-reporting  
48 due to difficulty associated with reporting portion sizes [3,4]. Therefore, one method to help  
49 overcome these limitations, is using dietary biomarkers to objectively support traditional dietary  
50 assessment methods and aid nutritional status assessment [4]. Dietary biomarkers can more  
51 accurately associate dietary intake with disease risk and nutritional status, in comparison to self-  
52 reported methods [4]. Similarly, biomarkers can help detect, monitor and diagnose conditions [5].

53 Blood is generally considered the best body fluid to evaluate biomarkers of systemic health and  
54 dietary intake, however it is not without its limitations, as collection is inherently invasive, it can  
55 cause discomfort and induce patient anxiety from a fear of needles, and a risk of infection exists

56 for both the patient and practitioner [6]. Blood collection in older adults can also prove difficult  
57 due to poor venous access, while it also tends to be less favoured amongst children in research [6].  
58 Therefore, the process of assessing nutritional status is currently complex and invasive, creating a  
59 need for a non-invasive, timely and cost-effective method to provide a biological sample which  
60 could help assess nutritional status [7,8]. Similarly, the use of a quick method to support  
61 traditional dietary assessment methods would be helpful in dietary research.

62 This is where the use of saliva has come to attention as it is often referred to as a “window on  
63 health status” or a “mirror of the body” [9]. Saliva mainly consists of water (99%), but the  
64 remaining 1% consists of various inorganic compounds, organic compounds and proteins/  
65 polypeptide compounds [10]. Collection of saliva is considered the least invasive of all biological  
66 samples [11] and may offer a credible means to evaluate health status [12]. Saliva collection is  
67 relatively simple with specialised training not required and a biological sample produced which is  
68 easier to ship and store, compared to serum samples, which need processed relatively immediately  
69 after collection [7]. One previous systematic review [13] plus narrative literature reviews [9,14–  
70 17] have suggested the possible clinical usefulness of different salivary biomarkers in the early  
71 diagnosis and treatment of various oral or systemic disorders including, dental caries, cancer and  
72 cardiovascular disease. Previous research also suggested that saliva may be a source of nutritional  
73 biomarkers, as nutritional deficiencies may affect salivary function and composition [18,19].

74 To date, however, no systematic review has investigated the available range of potential nutritional  
75 salivary biomarkers in relation to different nutritional states and dietary intake. The aim of this  
76 systematic review was to explore whether nutritional salivary biomarkers in humans of any age  
77 could aid nutritional status assessment and/or support traditional dietary assessment methods in  
78 comparison to corresponding nutritional serum biomarkers or control groups (where applicable).

79

## 80 **Methods**

### 81 **Protocol and registration**

82 This systematic review was conducted and reported according to the Preferred Reporting Items for

83 Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20] (Supplementary File 1). The  
84 review protocol was prospectively registered with PROSPERO: International Prospective Register  
85 of Systematic Reviews (CRD42018107667).

### 86 **Eligibility criteria**

87 Broad criteria were predefined to select papers for inclusion, using the participants, interventions,  
88 outcomes and study design reporting system (PICOS). The predefined list of inclusion and  
89 exclusion criteria used for this systematic review are detailed in Table 1.

### 90 **Information sources**

91 Four electronic bibliographic databases (MEDLINE, Web of Science, Scopus and EMBASE) were  
92 searched by the principal author (DL) to identify eligible studies. Trial registers (i.e. Cochrane) and  
93 other grey literature sources (open grey, google and google scholar) were searched using the key  
94 words, as well as relevant reference lists. The literature search was updated on January 9, 2021.

### 95 **Search strategy**

96 The literature was initially scoped in order to develop the search strategy. The search terms  
97 employed were either Medical Subject Headings (MeSH) terms or key words classified under  
98 general (all fields) category. The search terms were then combined with an “OR”, and PICO  
99 categories combined using “AND” to produce the search query. Appropriate adaptations were  
100 made to allow search strategies to be carried out on all the databases (Table 2). All searches were  
101 limited to include published, English and human studies and the library at Queen’s University  
102 Belfast retrieved any full-texts of identified articles that were not able to be accessed online.

### 103 **Study selection**

104 Study titles and abstracts were screened against the eligibility criteria. Duplicates were excluded  
105 automatically on an online programme (Covidence, Veritas Health Innovation Ltd, Australia) and  
106 manually checked. Those not meeting the inclusion criteria were removed and reasons noted. Full  
107 texts were retrieved if studies were considered potentially eligible. Studies meeting inclusion  
108 criteria were included in the narrative synthesis. If multiple studies measured the same salivary  
109 biomarker and nutritional state, with the same study design and outcome, they were considered

110 appropriate for a meta-analysis. Reviewer disagreements relating to study selection were resolved  
111 through discussion.

### 112 **Data collection process and data items**

113 Data extraction was carried out independently by two researchers (DL and SW) for those studies  
114 meeting inclusion criteria. The following data were extracted including: first author's name, year  
115 of publication, study design, country, study population, demographics (sample size, age range and  
116 gender), salivary collection methods and analysis, primary outcome, as well as the comparator (i.e.  
117 serum) if available, covariates and main conclusions. Any disagreements between reviewers during  
118 data extraction were resolved through discussion and a consensus reached prior to finalising  
119 extracted data. Data items extracted from the included studies are detailed in Supplementary File 2.

### 120 **Missing data**

121 As this review included studies which either had the identification and/or level of nutritional  
122 salivary biomarkers, corresponding authors were not contacted for missing data.

### 123 **Risk of bias and quality assessment of included studies**

124 Risk of bias was assessed by the principal author (DL) according to individual study design. The  
125 Newcastle-Ottawa Scale (NOS) was used to assess the quality of non-randomised studies [21].  
126 However, adaptations ensured responses were suitable in relation to non-response rate and  
127 ascertainment of exposure (Supplementary File 3). For a good quality case-control study, 7-9 stars  
128 were required, 5-7 stars for a fair quality study and anything lower suggested a poor quality study.  
129 For cross-sectional studies an existing adapted NOS version was used [22]. This was further  
130 adapted with the same changes as case-control studies. A maximum of 9 stars were given, where 0-  
131 3 was unsatisfactory, 4-5 was satisfactory, 6-7 was good and 8-9 was very good [22]. Randomised  
132 controlled trials (RCT) used the Cochrane risk of bias tool [23] and randomised cross-over trials  
133 used an existing adapted Cochrane risk of bias tool [24]. For before and after studies with no  
134 control group, the National Institute of Health quality assessment tool was used [25].

### 135 **Summary measures**

#### 136 *Outcome measures*

137 The primary outcomes in this review were the identification and/or level of nutritional salivary

138 biomarkers in relation to nutritional status or dietary intake outcomes. Secondary outcomes were  
139 the comparison of the corresponding serum biomarkers to the salivary biomarkers if available.

#### 140 **Synthesis of results**

141 Salivary glucose was the only biomarker appropriate for a meta-analysis to identify any glucose  
142 differences in those with and without T2D. This was presented as a cumulative forest plot with  
143 standardised mean differences and 95% confidence intervals (CI), using a random-effects model.  
144 Statistical heterogeneity was quantified using I-squared ( $I^2$ ) statistics from the RevMan meta-  
145 analysis (Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration,  
146 2014). The other nutritional salivary biomarkers were not suitable for meta-analyses due to data  
147 heterogeneity, thus a narrative synthesis was carried out for these. A league table was also devised  
148 to determine which salivary biomarkers were the most promising and could be explored further in  
149 additional future research (Supplementary File 5).

#### 150 **Risk of publication bias across studies and additional analyses**

151 Publication bias across the meta-analysis studies was assessed visually using a funnel plot of  
152 salivary glucose levels in those with and without T2D. No additional analyses was conducted.

153

### 154 **Results**

#### 155 **Study selection**

156 The initial search retrieved 6585 possible studies and, following the removal of duplicates, 4836  
157 studies were identified and screened. Of these, 134 full texts were assessed and 64 studies were  
158 eligible. The search was updated in March 2020 and this identified a further 1906 studies, of which  
159 16 studies met the inclusion criteria after full text review. A third search update was conducted in  
160 January 2021 and a further 1240 studies were identified, of which five met the inclusion criteria  
161 after full text review, leaving a total of 85 studies for inclusion in the review. Full details of the  
162 process are presented in the PRISMA Flowchart including reasons for exclusions (Figure 1).

#### 163 **Study characteristics**

##### 164 ***Studies included for narrative analysis***

165 The data from each included study (n=85) were summarised in Supplementary File 2. 63 studies

166 identified salivary biomarkers relating to nutritional status and 22 related to dietary intake. Of the  
167 85 included studies, 53 were case-control studies, 20 cross-sectional, six randomised controlled  
168 crossover trials, four before and after studies (with no control group), one RCT, and one study which  
169 included aspects of a cross-sectional study and a before-after study (with no control group). Studies  
170 were conducted across different continents including; 35 in Asia, 19 in Europe, 14 in South  
171 America, eight in North America, five in Africa, three in Australia and one in Oceania. The total  
172 sample size was 16486 participants, ranging from six up to 8317 participants. Participants' age  
173 varied from six weeks to >65 year olds. Studies were published between 1956 up until 2020.

#### 174 ***Studies included for meta-analysis***

175 Of the salivary biomarkers identified, glucose had the greatest number of studies with the same  
176 study design and outcome measure, compared to other biomarkers. A total of 12 case-control  
177 studies were identified as eligible for a salivary glucose meta-analysis [26,27,36,37,28–35]. These  
178 studies measured glucose in saliva of children and adolescents [37], adults [26–30,32,36],  
179 adolescents, adults and older adults [35], adults and older adults [31,34] and one study the age was  
180 not stated [33]. These studies ranged in sample size from 40 [30] up to 127 participants [31].

#### 181 **Risk of bias within studies**

182 Risk of bias within each study was summarised according to study design (Supplementary File 4).  
183 The quality of case-control studies varied, ranging from one to eight and a 4.28 average out of nine  
184 (poor quality). This average is reflected by only 10 studies having a consecutive or representative  
185 set of cases [29,30,38–45]. The quality of cross-sectional studies ranged between two and five,  
186 with a 3.86 average out of nine (unsatisfactory). This average is reflected by only nine studies  
187 having a truly representative sample [73–81]. All case-control and cross-sectional studies lacked at  
188 least one or more of the ascertainment of exposure criteria. The one RCT was of fair quality due to  
189 two unclear criteria [93]. Of the six randomised controlled crossover trials, three were poor [94–  
190 96], one fair [97] and two good [98,99] quality. Of the five before and after studies, two studies  
191 achieved six [85,100], two achieved seven [101,102] and one achieved nine [103] out of 12.

#### 192 **Synthesis of results**



193 ***Meta-analysis of the included studies: salivary glucose levels in all ages***

194 A meta-analysis was performed for salivary glucose levels in those with T2D and controls  
195 [26,27,36,37,28–35]. The random-effects model using standardised mean difference revealed that  
196 the mean glucose levels were significantly higher in individuals with T2D by on average 1.26  
197 standard deviations (SD) compared to controls ( $p < 0.0001$ ) (Figure 2). However, substantial  
198 heterogeneity existed as the  $I^2$  statistic was 94% (Figure 2). According to the funnel plot analysis,  
199 potential publication bias was illustrated by the non-symmetrical funnel plot (Figure 3).

200 ***Narrative synthesis of nutritional salivary biomarkers***

201 This systematic review identified 34 nutritional salivary biomarkers in relation to nutritional status  
202 and/or dietary intake, as summarised in the league table (Supplementary File 5). The results for the  
203 most promising biomarkers including, glucose, vitamin D, TAC, calcium, fluoride and  
204 nitrate/nitrite are presented in this section. A summary of the results for the less promising salivary  
205 biomarkers is included in Supplementary File 6.

206 ***Glucose***

207 In addition to the glucose meta-analysis, ten studies out of 17 reported on the correlation between  
208 salivary glucose and serum glucose; a positive correlation was found in six studies including, adults  
209 with T2D and controls [26,29,32] and in healthy men [89]. However, salivary and serum glucose  
210 only correlated amongst those with T2D, as salivary glucose was not detected in controls [50], whilst  
211 a correlation only existed between salivary glucose and fasting plasma glucose in those with T2D  
212 [33]. However, three studies found no significant correlation between salivary and serum glucose in  
213 adults with T2D [31,36] or in 19-72 year olds [78] and no significant correlation between salivary  
214 glucose and serum fasting plasma glucose in older adults with T2D and controls [30].

215 ***Vitamin D***

216 Two studies investigated the relationship between salivary and serum 25-hydroxyvitamin D<sub>3</sub>  
217 [25(OH)D<sub>3</sub>] in adults. One study found a significant positive linear relationship between salivary  
218 and serum 25(OH)D<sub>3</sub>, as well as an increase in salivary 25(OH)D<sub>3</sub> levels after taking 10 days of  
219 25(OH)D<sub>3</sub> supplementation [85]. Similarly, a positive correlation was found between salivary and

220 serum 25(OH)D<sub>3</sub> after adjusting for salivary flow rate in adults [102].

#### 221 *Total antioxidant capacity*

222 An increasing trend in salivary TAC was found with an increased dietary antioxidant intake in  
223 adults [93] and a positive association and an inverse association between salivary TAC and simple  
224 carbohydrate and complex carbohydrate, respectively [74]. However, salivary TAC was not  
225 associated with micronutrient or macronutrient intake during pregnancy [61].

#### 226 *Calcium*

227 Two studies found that post-menopausal women with a lower BMD had higher salivary calcium  
228 than post-menopausal women without bone mineral changes [88] or pre-menopausal or pregnant  
229 women [45]. Long-term supplementation of vitamin D combined with calcium, did not  
230 significantly affect salivary calcium in osteoporotic women [40].

#### 231 *Fluoride*

232 Three studies investigated dietary fluoride in relation to salivary fluoride, with salivary fluoride  
233 levels significantly increasing after inclusion of a sodium fluoride supplement in children [52], a  
234 significant correlation in young children [86], but not repeated in adolescents [75].

#### 235 *Nitrate/Nitrite*

236 Two studies reported salivary nitrate/nitrite in relation to diet. One study showed that adults had  
237 significantly higher salivary and plasma nitrate and nitrite levels after consuming a high nitrate diet,  
238 compared to a low-nitrate diet [97]. The other study found that salivary nitrate and nitrite levels  
239 returned to baseline levels seven days after cessation of the high-nitrate diet [103].

240

### 241 **Discussion**

242 This systematic review explored the potential of nutritional salivary biomarkers to aid nutritional  
243 status assessment and/or support traditional dietary assessment methods for patients. The results for  
244 the six most promising biomarkers, based on consistent evidence and biological plausibility, are  
245 discussed in this section. For nutritional status this included; glucose for T2D, vitamin D for  
246 vitamin D status and calcium related to BMD. For dietary intake this included; TAC, nitrate/nitrite

247 and fluoride. This evidence, even when considered promising, was based on a small number of  
248 studies of varying quality. Current literature, however, does not suggest a role for a large number of  
249 nutritional salivary biomarkers identified in the scoping exercise for this review, due to the  
250 inconsistent direction of significant associations across studies or the lack of evidence to inform a  
251 conclusion (see Supplementary Files 5 and 6).

252

### 253 *Salivary biomarkers and nutritional status*

254 One of the most promising biomarkers identified was salivary glucose in relation to T2D. The data  
255 generated in the meta-analysis, supports previous evidence which suggested that salivary glucose  
256 levels are significantly elevated in patients with T2D [112]. The positive correlation between  
257 salivary and serum glucose mirrors the increased serum glucose in T2D [26,29,32,33,50,89].  
258 Increases in salivary glucose may reflect microvascular structural changes within the salivary  
259 glands which increase the glucose diffusion rate from the blood to oral cavity [112]. However, the  
260 meta-analysis was subject to publication bias as shown in Figure 3 and substantial heterogeneity ( $I^2$   
261 =94%) as the absolute values varied between studies. High heterogeneity may be due to the  
262 different age groups including, adults and children [26,27,36,37,28–35]. All studies in the meta-  
263 analysis analysed unstimulated saliva using colorimetric glucose kits, but one study was not  
264 included as glucose was only detected in adults with T2D [50]. The reason is unclear but perhaps  
265 levels were below the limit of detection in controls. Overall, the collection and analyses of glucose  
266 in unstimulated saliva is promising for T2D diagnoses and glucose monitoring. However, this was  
267 based on poor and fair quality studies and should be interpreted with caution. Higher quality studies  
268 are needed to confirm the use of salivary glucose to monitor and diagnose T2D.

269

270 Despite limited evidence, vitamin D (25(OH)D<sub>3</sub>) in unstimulated saliva, positively correlated with  
271 serum 25(OH)D<sub>3</sub> in healthy adults [85] and after adjusting for salivary flow rate [102]. This  
272 corresponds to low serum 25(OH)D<sub>3</sub> in those with a vitamin D deficiency [113]. Therefore, the  
273 current analysis suggests that reduced salivary 25(OH)D<sub>3</sub> may also indicate this deficiency. This is  
274 important as measuring serum 25(OH)D<sub>3</sub> is expensive and universal screening is not supported

275 [113]. However, saliva was collected on three consecutive days which may restrict community use  
276 [102]. The results may not be representative due to small study samples sizes [85,102]. Larger  
277 studies are required to confirm the use of salivary 25(OH)D<sub>3</sub> for assessing vitamin D status.

278  
279 Limited, but promising, evidence was reported for salivary calcium and BMD in post-menopausal  
280 women, as those with lower a BMD had higher unstimulated calcium levels compared to pre-  
281 menopausal or pregnant women [45] or post-menopausal women without bone mineral changes  
282 [88]. This suggests that salivary calcium may be a useful screening tool for bone mineral changes  
283 and has the potential for early diagnoses of osteoporosis [88]. Unfortunately, as the included  
284 studies did not assess serum calcium levels, the relationship between salivary and serum calcium is  
285 currently unclear. However, recent research found that adults in Sweden with elevated serum  
286 calcium at baseline developed osteoporosis more often than controls [114]. This finding  
287 corresponds with increased salivary calcium in those with a low BMD. Longitudinal studies with  
288 larger sample sizes are required, to confirm the use of salivary calcium in relation to BMD.

289

### 290 *Salivary biomarkers and dietary intake*

291 Despite limited evidence, salivary TAC was promising, as a RCT showed an increasing trend in  
292 salivary and plasma TAC amongst intervention adults, who increased fruit and vegetable intake  
293 compared to controls [93]. Samples were analysed using the Trolox Equivalent Antioxidant  
294 Capacity (TEAC) assay [93]. Results suggested that both salivary and plasma TAC respond to an  
295 increased antioxidant intake similarly. However, no differences in salivary TAC [93] or nutrient  
296 intake during pregnancy [61] were detected using the ferric reducing antioxidant power (FRAP)  
297 assay. Therefore, the TEAC assay may be more sensitive than the FRAP assay in detecting salivary  
298 TAC changes. It is unclear which saliva collection is most appropriate, as this information was not  
299 included [93]. Cross-sectionally, a higher intake of simple carbohydrate was associated with higher  
300 unstimulated TAC in adults, using the Oxygen Radical Absorbance Capacity (ORAC) assay [74].  
301 Results may differ if diet was recorded for longer, instead of 24 hours [61,74] or three days [93].  
302 Further evidence is required to confirm the use of salivary TAC and the best assay.

303 Limited, yet promising evidence, suggested that salivary and plasma nitrate and nitrite respond in  
304 the same way to an increased dietary intake, as levels increased amongst those with a greater  
305 consumption of spinach and green leafy vegetables in adults and older adults compared to those on  
306 a low-nitrate diet [97]. Salivary nitrate and nitrite levels also returned to baseline levels seven days  
307 after cessation of the high-nitrate diet [103]. This increase in salivary nitrate and nitrite may be  
308 explained by dietary intake, along with nitrate produced from NO, both entering the enterosalivary  
309 nitrate-nitrite-NO-pathway, and absorbed from plasma into the salivary glands [97]. It was unclear  
310 how saliva was collected, but gas chromatography mass-spectrometry was used to analyse saliva  
311 samples and a food diary used to record diet [97,103]. Based on good quality evidence, salivary  
312 nitrate and nitrite was successful in validating dietary intake, but further studies are required.

313  
314 The use of salivary fluoride to reflect fluoride intake was also promising, as unstimulated fluoride  
315 levels increased with higher fluoride intake in children living in higher fluoride areas, via food  
316 (moderate correlation) [86] or sodium fluoride supplementation over a 12 month period [52],  
317 compared to those living in lower fluoride areas. Similar results were achieved using a fluoride  
318 selective electrode [52] and acid diffusion [86]. Salivary and plasma fluoride levels may respond in  
319 the same way, as a strong positive correlation existed between plasma fluoride and total daily  
320 fluoride intake [86]. However, stimulated fluoride levels did not correlate with dietary fluoride  
321 intake in adolescents using a fluoride selective electrode [75]. Therefore, 24-hour dietary recalls  
322 may not be long enough to reflect salivary fluoride [75]. Further higher quality studies are needed  
323 to confirm the use of salivary fluoride in reflecting dietary fluoride intake.

324

### 325 **Strengths and limitations**

326 This is the first systematic review to investigate a comprehensive range of nutritional salivary  
327 biomarkers in relation to nutritional status and dietary intake. Broad inclusion criteria meant that all  
328 age groups, publication years, study design (with or without controls), and settings (any health  
329 condition), maximised the number of studies. Data extraction involved two independent reviewers,  
330 although only one reviewer conducted the risk of bias assessment. Six salivary biomarkers were

331 promising based on consistent significant evidence and biological plausibility. However, this  
332 review was limited by the quality of the evidence, as the majority of studies lacked an assessment  
333 of salivary assay performance, so results may be based upon unreliable methods. Comparisons  
334 were also limited due to high variability in salivary collection and analysis methods. Many studies had  
335 small sample sizes which may have lacked power to detect significant findings. A meta-analysis was  
336 only possible for salivary glucose, but substantial heterogeneity and publication bias existed.

337

### 338 **Conclusions**

339 Saliva represents a biological matrix that is easily accessible, considered the least invasive of all  
340 biological samples and its collection does not require specialised training. It can also be collected  
341 by patients themselves, thus offering an alternative to invasive blood tests in an era of limited  
342 access to healthcare facilities. At present, the evidence is generally limited, inconclusive and  
343 inconsistent as the change in the direction of significant associations was not always consistent.  
344 Despite this, some results were judged as promising, with the potential use of salivary glucose for  
345 monitoring and diagnosing T2D, vitamin D for assessing vitamin D status, calcium levels related to  
346 BMD, TAC for assessing dietary antioxidant intake, nitrate/nitrite for assessing dietary  
347 nitrate/nitrite intake and fluoride for assessing dietary fluoride intake. In general, these promising  
348 salivary biomarkers responded to nutritional state or dietary intake in the same way as their  
349 corresponding serum/plasma biomarker. Therefore, using nutritional salivary biomarkers is still  
350 largely uncertain and requires further research. At present they should not be used alone to assess  
351 nutritional status or reflect dietary intake. Future studies should include salivary assay performance  
352 to ensure reliable results and explore the methodology of commercially available serum kits for  
353 detecting nutritional salivary biomarkers. More specific and sensitive methods may be required to  
354 evaluate potential salivary biomarkers as indicators of nutritional status or dietary intake.

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