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# Low levels of salivary lactoferrin may affect oral dysbiosis and contribute to Alzheimer's disease: A hypothesis

Ingar Olsen<sup>1</sup>, Sim K. Singhrao<sup>2</sup>

Ingar Olsen

ingar.olsen@odont.uio.no

<sup>1</sup>*Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway*

<sup>2</sup>*Brain and Behavior Centre, Faculty of Clinical and Biomedical Sciences, School of Dentistry, University of Central Lancashire, Preston, UK*

## ABSTRACT

Recently it has been reported that reduced levels of salivary lactoferrin (LF) can be a plausible biomarker for amyloid beta (A $\beta$ ) accumulation [as in the brain of Alzheimer's disease \(AD\) brains](#). This could mean that reduced levels of salivary LF act as a trigger for oral dysbiosis and that low LF levels could change the oral microbiota. A chemical change in the composition of saliva has not yet been considered as a cause for microbial dysbiosis but does present an opportunity to view oral dysbiosis as a plausible contributory factor in the development of AD pathophysiology. Oral dysbiosis has largely been reported as a result of inadequate oral hygiene and dry mouth in elderly subjects. Here we discuss if the deficiency of LF in saliva and gingival fluid of AD patients can facilitate proliferation of oral pathogens, and as a result their spread elsewhere in the body. Additionally, we ask if LF in the AD brain could be overexposed as a result of chronic infection. Together these outcomes will indicate if reduced levels of salivary LF can act as a trigger of oral dysbiosis.

*Keywords:*

Lactoferrin

30 Saliva

31 Brain

32 *Porphyromonas gingivalis*

33 Gingipains

34 Dysbiosis

35

## 36 **Introduction**

37 On the question of whether Alzheimer's disease (AD) is an infectious (communicable)  
38 disease the research of Olsen and Singhrao [1] and Singhrao and Harding [2] support the  
39 plausibility of AD being a polymicrobial dysbiosis of the host's microbiome.

40 Inadequate oral hygiene and dry mouth are accepted reasons for oral dysbiosis.

41 However, a change in the composition of saliva has not previously been considered [as](#) a cause  
42 for microbial dysbiosis. The reasons for dysbiosis in a host's oral microbiome could be due to  
43 unknown reasons whilst including those already linked to poor oral hygiene and xerostomia.

44 Carro et al. [3], using mass spectrometry and ELISA, [it was demonstrated](#)~~showed~~ for the first  
45 time that early diagnosis of mild cognitive impairment (MCI) and subsequent AD can be  
46 associated with impairment of salivary lactoferrin (LF). Later, González-Sánchez et al. [4]  
47 suggested that in salivary deficiency of LF, amyloid beta ( $A\beta$ ) could be a biomarker of AD as  
48 it correlates with the  $A\beta$  load in the brain following its visualization with amyloid-Position-  
49 Emission Tomography (PET) neuroimaging. We hypothesize that salivary LF deficiency may  
50 act as an unknown trigger of oral microbial dysbiosis. LF is a glycoprotein present in the  
51 human saliva. It is also found in secretions such as milk, tears and gingival fluid, and in cells  
52 like neutrophils [5] and has a broad spectrum antimicrobial activity. Being an antimicrobial  
53 peptide, LF is considered part of the first line or innate immune defense against infections in  
54 man [6] as it targets bacteria, viruses, fungi, yeasts and protozoa. LF is also an iron chelator  
55 and hence prevents iron deposition. It has the ability to block aggregation of both  $A\beta$  and  
56 phosphorylated tau, and rescues neuronal damage in AD brains [7, 8]. For a summary of the  
57 biological functions of LF, see Table 1.

58 When  $A\beta$  accumulation reaches a plateau possibly from both local and peripheral  $A\beta$   
59 pools [9], it indicates the MCI stage or prodromal AD. Following this stage, the pathological  
60 cascade of progressive AD takes over. As an antimicrobial peptide, LF can modulate immune  
61 reactions and inflammation (for a review see Farah et al. [10]). A plethora of reports

62 implicate the immune system as a major player in AD manifestation [11-14]. There is  
63 probably an association between systemic infection and AD where salivary LF is down-  
64 regulated like several other factors of systemic immunity [6].

65 The present paper will discuss (1) if deficiency of LF in saliva and gingival fluid of  
66 AD patients can facilitate proliferation of oral pathogens, (2) if this proliferation can result in  
67 transfer of oral pathogens and tissue inflammatory mediators to the brain, and (3) if LF in the  
68 brain of AD patients could be overexposed as a result of chronic infection.

69

### 70 **Decreased salivary lactoferrin is specific to Alzheimer's disease**

71 In a recent study González-Sánchez et al. [4], who examined the relationship between  
72 salivary levels of LF and cerebral A $\beta$  load by using PET neuroimaging, found that LF could  
73 be used to detect MCI or prodromal AD and distinguish AD from other frontotemporal  
74 dementias (FTDs), with sensitivities and specificities over 87% and 91%, respectively. This  
75 study also indicated that LF represents one of the main first lines of defense against pathogens  
76 and confirmed previous findings that there is an association between AD and the immune  
77 system, and brain infections with bacteria, viruses ~~and~~ yeasts. These microorganisms can all  
78 be related to increased signs of neuroinflammation in the brain [1, 4, 15]. The study of  
79 González-Sánchez et al. [4] was the first to show the diagnostic performance and specificity  
80 of a single saliva-based biomarker for detecting MCI and AD. It demonstrated that salivary  
81 LF levels are reduced in AD and, noteworthy, are associated with the amyloid-PET imaging  
82 profile, even in the prodromal stage. An independent cross-sectional study confirmed  
83 simultaneously the presence of low saliva LF levels in AD, as shown previously [3].

84

### 85 **Low salivary lactoferrin might be an effect of immunological disturbances in** 86 **Alzheimer's disease**

87 AD subjects have long been recognized to suffer from poor oral health and xerostomia  
88 which is thought to be a side effect of their medication. However, this view is changing as  
89 Bermeji-Pareija et al. [6] proposed that reduced levels of salivary LF might be an effect of  
90 immunological disturbances associated with AD. Two pathways could be responsible for this:  
91 first, AD could be a systemic disorder (or disorders) related to early immunological and low  
92 inflammatory changes, and secondly, systemic immunity changes in AD manifestation could

93 be a downstream effect of early AD brain involvement. The authors emphasized that the  
94 general acceptance of low LF as an early AD biomarker would rely on validation of LF levels  
95 in other clinical and population-based studies.

96

### 97 **Deficiency in salivary lactoferrin in Alzheimer's disease could contribute to dysbiosis of** 98 **the oral microbiota**

99 LF is secreted by the serous acinar cells of the major and minor salivary glands. In  
100 whole saliva it also originates from neutrophil granulocytes and from the gingival crevicular  
101 fluid. LF plays an important role in regulating the oral microbiota and the inflammatory state  
102 of the oral mucosa [16]. It contributes to the maintenance of symbiosis in the host-  
103 microbiome relationship. In dysbiosis, however, certain bacteria are able to flourish at the  
104 demise of others. Particularly the oral pathogen *Porphyromonas gingivalis* will take  
105 advantage of iron released from haem in inflamed tissues, and increase in number (Figure 1).  
106 This bacterium has a remarkable effect to initiate dysbiosis even in low concentration [17]. In  
107 dysbiosis, levels of salivary LF are expected to increase whilst the body resolves  
108 inflammation and restores symbiosis [18].

109 However, when LF levels are low, as seen in AD, dysbiosis is expected to proceed  
110 freely. In a study on the subgingival microbiota of people with cognitive dysfunction  
111 participants with periodontitis had a greater abundance of several bacteria: the highest log<sub>2</sub>-  
112 fold changes were seen for *Porphyromonas* and *Peptostreptococcaceae* [19]. Even in aged  
113 subjects with oral dryness, salivary levels of LF and chromogranin A were low [20] and this  
114 may aid spread of oral bacteria to the brain.

115 **Dysbiosis can also contribute to a dormant blood microbiome (atopobiosis) and**  
116 **directly promote systemic inflammation through amyloidogenic formation and shedding of**  
117 **inflammagens such as lipopolysaccharides (LPSs) [21]. Dormant, non-growing bacteria are**  
118 **important features in AD. Their growth *in vivo* is usually limited by a lack of free iron and**  
119 **this iron dysregulation could be an important factor in their resuscitation [22]. A simultaneous**  
120 **iron dysregulation and microbial aberrations could affect the hematological system by**  
121 **promoting fibrin amyloidogenesis and pathological clotting [23].**

122

### 123 **Lactoferrin in the gingival crevicular fluid**

124 LF is part of the gingival crevicular fluid secreted from the inflamed periodontium  
125 around teeth harboring supra- and sub-gingival biofilms. Studies have shown that LF can be a  
126 biofilm inhibitor of periodontopathic bacteria *in vitro* and *in vivo* [24]. These authors reported  
127 that LF reduced the established biofilm at physiological concentrations. The adjunct use of LF  
128 for the prevention and treatment of periodontal diseases has therefore been suggested [25]. LF  
129 was raised in stimulated whole saliva in subjects with “chronic” periodontitis where it  
130 correlated with probing pocket depths  $\geq 6$  mm [26]. In a study by Daspher et al. [27], LF  
131 inhibited *P. gingivalis* biofilm formation by 80% at concentrations above 0.625  $\mu$ M. *P.*  
132 *gingivalis*, which is a Gram-negative anaerobic rod, is considered a keystone bacterium in  
133 periodontitis [28-30]. The antimicrobial protection exerted by LF could be reduced when it is  
134 present in low concentrations, as in AD. Maintaining the flow of saliva and the presence of  
135 antimicrobial substances are important to preserve oral health. As mentioned, in the older  
136 population salivary flow is often reduced, for example as a side effect of drug intake. This  
137 could predispose these persons to systemic infection with periodontal bacteria.

138

### 139 **Periodontal bacteria can degrade lactoferrin by its proteases**

140 LF binds to a high-affinity receptor on periodontal bacteria such as *P. gingivalis*,  
141 *Prevotella intermedia* and *Prevotella nigrescens*. In the case of *P. gingivalis*, all strains  
142 completely degraded LF under the investigative conditions used, whereas only partial  
143 degradation was seen with *P. intermedia* and *P. nigrescens* [31]. The proteases (gingipains) of  
144 *P. gingivalis* may protect this bacterium against LF in periodontal and systemic sites and thus  
145 serve as important virulence factors. Alugupalli and Kalfas [32] found in an *in vitro* study  
146 that the degradation of LF was more extensive by *P. gingivalis* and *Capnocytophaga*  
147 *sputigena*, slow by *Capnocytophaga ochracea*, *Aggregatibacter (Actinobacillus)*  
148 *actinomycetemcomitans* and *P. intermedia*, and very slow or absent by *P. nigrescens*,  
149 *Campylobacter rectus*, *Campylobacter sputorum*, *Fusobacterium nucleatum ssp. nucleatum*,  
150 *Capnocytophaga gingivalis*, *Tannerella (Bacteroides) forsythia* and *Peptostreptococcus*  
151 *micros*. All the *P. gingivalis* strains tested degraded LF. The degradation was sensitive to the  
152 protease inhibitors cystatin C and albumin. These studies indicated that periodontopathogens  
153 can degrade LF. This could facilitate proliferation of some of the most virulent bacteria in  
154 periodontal infections, and possibly promote AD by systemic spread of these bacteria and  
155 their inflammagens to the brain. **Inflammagens from *P. gingivalis* such as gingipain R1  
156 (RgpA) and LPS have been reported to have major effects on blood clot morphology and**

157 **mechanics thereby driving systemic inflammation [33].** Interestingly, intake of tablets  
158 containing LF (60 mg/d) and lactoperoxidase (7.8 mg/d) improved gingival inflammation and  
159 oral health-related quality of life in healthy adults [34] supporting the concept that low levels  
160 of LF are indicators of dysbiosis.

161

### 162 ***Porphyromonas gingivalis* in Alzheimer's disease**

163 Recent work has increasingly focused on AD as a microbial disease, for example  
164 Sochocka et al. [15] and Itzaki et al. [35]. In the oral microbiota, *P. gingivalis* has attracted  
165 much attention for its possible role in AD (Figure 1) [13, 36-40]. However, it may take a long  
166 time for *P. gingivalis* to promote development of AD. Thus, Sparks Stein et al. [41], Tzeng et  
167 al. [42] and Chen et al. [43] found that gingivitis and chronic periodontal disease could take  
168 up to 10 years for AD to occur. This may also be the time it takes for A $\beta$  to reach a plateau to  
169 become MCI.

170

### 171 **Low salivary lactoferrin could promote transfer of oral bacteria and tissue** 172 **inflammatory mediators to the brain**

173 Each time we chew on a periodontitis-affected tooth there will be a bacteremia. During  
174 a day this can last for a total of 3 hours [44]. The spectrum of oral bacteria in this bacteremia  
175 is wide [45] (Figure 1). Also viruses, bacteriophages and yeasts in the periodontal pocket  
176 could follow the bacteria into the blood stream as well as inflammatory mediators from the  
177 inflamed periodontal tissues [1]. In an elderly person with deteriorated blood-brain barrier,  
178 periodontal microorganisms and inflammatory mediators can reach the brain. Several other  
179 ways than the blood stream can also be used by oral microorganisms for brain transfer [1].  
180 Periodontal pathogens like *P. gingivalis* and their main virulence factors, like LPS and  
181 gingipains have been demonstrated in the brain of AD patients and in animal models of AD  
182 [37, 46-48]. It is therefore highly plausible that low salivary LF levels, by reducing innate  
183 immunity, can promote dissemination of periodontitis-related microorganisms and  
184 inflammatory tissue mediators to the brain. In addition, salivary LF is transferred into the  
185 brain via the sublingual route [49]. Low levels of salivary LF may therefore affect the  
186 concentration of LF in the AD brain.

187

188 **High concentrations of lactoferrin have initially a protective effect on Alzheimer's**  
189 **disease**

190 LF has been considered to have a beneficial effect in AD subjects, but the mechanism  
191 is unclear. A possible way could be through its ability to alleviate the AD pathological  
192 cascade and cognitive decline via modulation of the p-Akt/PTEN (phosphatidylinositol-4,5-  
193 bisphosphate 3-kinase (PI3K)/protein kinase B (PKB or Akt)/phosphatase and tensin homolog  
194 (PTEN) pathway [50]. These authors reported a possible protective mechanism of post-LF  
195 administration for 3 months in AD patients' changes in this pathway. LF probably caused this  
196 by affecting key players of inflammation and oxidative stress involved in AD pathology. **It**  
197 **should also be mentioned that iron dysregulation, which is seen in AD, contributes to**  
198 **oxidative stress [21]. LF could reduce inflammation and stress by binding iron.**

199 The spread of microorganisms to the brain is controlled by several factors, including  
200 LF which, as mentioned, also has an anti-inflammatory effect, especially associated with the  
201 down-regulation of pro-inflammatory cytokines like IL-6. This reduces local and/or systemic  
202 inflammation [51]. Excessive iron contributes to the deposition of A $\beta$  and the formation of  
203 neurofibrillary tangles, which in turn, promotes the development of AD [7]. LF blocks A $\beta$ -  
204 aggregation, tauopathy spread and neuronal damage [7]. It also acts as an iron-binding protein  
205 and is strongly up-regulated in the brains of patients with AD [52]. In transgenic mice with  
206 AD these authors used double-immunofluorescence labelling with antibodies directed against  
207 A $\beta$  and LF, and found LF depositions localized to A $\beta$  plaques and regions of amyloid  
208 angiopathy. Both the intensity and number of LF-positive depositions increased with age. The  
209 up-regulation of LF in the brains of both AD patients and transgenic mice with AD indicated  
210 an important protective role for LF in infected AD-brain tissue [53]. It is tempting to  
211 speculate that the high consumption of LF in AD could lead to reduced LF levels over time,  
212 particularly when AD is promoted by long-term chronic infection. Interestingly, Bermejo-  
213 Pareja et al. [6] suggested that LF was downregulated in the saliva of AD patients like several  
214 other factors of systemic immunity.

215

216 **Concluding remarks**

217 If LF is a trigger of oral dysbiosis this makes it plausible that it could be a factor in the  
218 etiology or pathophysiology of AD. It is remarkable that the levels of LF are increased in the  
219 brains of AD patients, at least initially, and reduced in their whole saliva. It may be that the  
220 long-term fight against chronic infection in the brain tends to reduce the level of LF. The  
221 latter scenario could aggravate brain infection. It is also possible that low levels of LF in the  
222 whole saliva of AD patients may affect the LF concentration in the brain since salivary LF is  
223 transferred into the brain via the sublingual route. In mice dietary LF supplementation  
224 prevented memory impairment and reduced A $\beta$  generation, and post LF-administration for 3  
225 months in AD patients alleviated the AD pathological cascade and cognitive decline by  
226 modulating the p-Akt/PTEN pathway. Furthermore, tablets containing LF and lactoperoxidase  
227 improved gingival inflammation and oral health-related quality of life in healthy adults  
228 suggesting LF supplements may be a plausible therapy for AD subjects, together with  
229 effective periodontitis prophylaxis and treatment to prevent systemic spread of periodontal  
230 bacteria.

231 Another intriguing aspect is that *P. gingivalis*, which is a keystone bacterium in  
232 periodontitis, and recently has been associated with AD, has the ability to reduce LF levels  
233 through its gingipains. This could take place in the periodontal pocket, but could also occur in  
234 the brain of AD patients where both *P. gingivalis* and its gingipains have been detected.  
235 Noteworthy in this context is also the finding that *P. gingivalis* was the most powerful LF-  
236 degrading bacterium of several periodontal pathogens tested *in vitro*. It is plausible that *P.*  
237 *gingivalis*' effect on LF could be added to its wide capacity of immune suppression, acting  
238 both in the periodontal pocket and in the AD brain. There are also other proteins and peptides  
239 in saliva but their functions and interactions with the oral microbiome remain to be  
240 determined. Clearly, when the level of whole saliva is reduced, its composition is changed and  
241 this could promote dysbiosis and the risk of associated oral diseases such as caries, gingivitis,  
242 periodontitis and fungal infections, and possibly AD. For now, Carro et al. [3] and González-  
243 Sánchez et al. [4] have highlighted LF as an A $\beta$  biomarker of AD, and the authors of the  
244 current paper have suggested it to be a plausible trigger of oral dysbiosis. Further *in vivo*  
245 research on LF and its functions in causing dysbiosis of host mechanisms in the periodontal  
246 pocket and in the brain of AD patients is required to support our hypothesis.

247

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257

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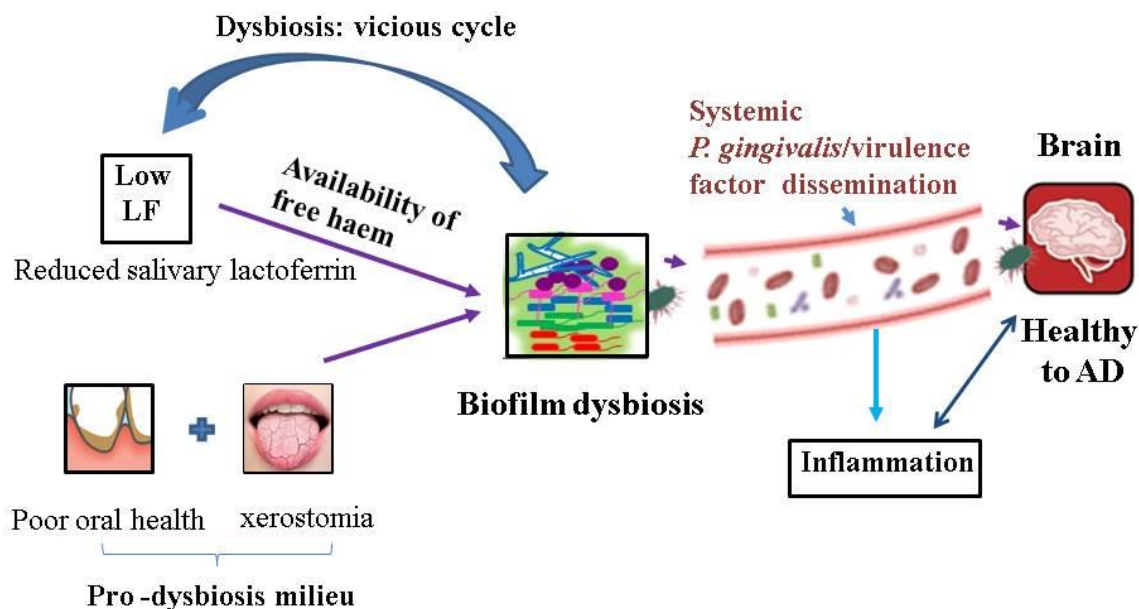
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412 **Figure 1.** Schematic to show how reduced levels of salivary lactoferrin (LF) may be a  
 413 plausible trigger of oral biofilm dysbiosis. Oral dysbiosis has largely been seen as a result of  
 414 inadequate oral hygiene and xerostomia in elderly subjects. Once the LF level begins to

415 decrease, this becomes a vicious cycle for sustained dysbiosis. From here *P. gingivalis* can  
416 spread, via bacteremia, to disparate body organs, for example the brain. This destabilize the  
417 immune balance, and inflammatory disease such as AD (Alzheimers'disease) develops.

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428 **Table 1.** Physiological properties of lactoferrin (adopted from [5])

Physiological actions	Mechanisms
Iron-binding protein	Iron absorption, transport and sequestration
<b>Host defence</b>	Activities against pathogens: antibacterial, antifungal, antiparasitic, antiviral
	Anti-inflammatory and alarming
	Anti-endotoxin
	Anticancer
	Inhibition of prion accumulation
<b>Host activities</b>	Brain development and neuroprotection: alleviating psychological stress
	Bone formation
	Gastrointestinal development
	Immune actions (innate and adaptive): enhancer and modulator
	Wound healing
<b>Metabolic</b>	Adipocytes differentiation
	Antioxidant: inhibiting lipid peroxidation
	Association with other proteins: osteopontin and others
	Decreasing vasoconstriction
	Enzymatic activities
	Glucose regulation (decreasing hyperglycemia)
	Gut microbiota modulation
	Transcriptional regulator
<b>Miscellaneous</b>	Compounds or metabolites carrier (mainly into brain)
	Vaccine adjuvant
	Possible sAD biomarker

