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# Salivary fingerprint of simple obesity

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ABSTRACT

*Background:* The nature of a link between poor oral health and obesity is not fully understood. It is also unclear if saliva contributes to it and whether the properties of saliva change as a result of an increase in body mass or rather as a consequence of obesity-associated comorbidities. This pilot study was undertaken in an attempt to determine if salivary biomarkers can identify obesity per se.

*Methods:* Whole mixed saliva was analysed for 16 soluble parameters covering 4 categories (inflammation, oxidative stress, endothelial dysfunction, adipokines). In the discovery group, 19 obese and 25 non-obese women matched for age, with similar hygiene habits, with no comorbidities and not taking any medication known to affect saliva secretion were analysed. In the validation group, a cohort of no-preselected 81 individuals (34 obese) were analysed.

*Results:* Individuals with obesity had significantly higher salivary concentrations of several cytokines and adipokines, of which TNF-R1, serpin A12 and PAI-1 were identified as parameters discriminating between obese and non-obese subjects with the highest sensitivity and specificity.

*Conclusions:* Obesity per se leads to distinct changes in the concentration of several parameters in saliva. These findings may have diagnostic implications for distinguishing the effects of obesity and obesity-linked comorbidities on oral health.

## 1. Introduction

Obesity is a well-recognized global health problem. It is directly implicated in the development of type 2 diabetes, cardiovascular disease, and some forms of cancer. The detrimental impact of obesity is largely related to obesity-induced chronic low grade inflammation [1]. In addition to metabolic and cardiovascular abnormalities, obesity has been linked with an impairment in oral health status [2,3]. However, the mechanism underlying the association between oral health and obesity is unclear. This is because there is a number of common socio-economic and lifestyle factors that contribute both to the weight gain and to the deterioration of oral health. Furthermore, the relationship between obesity and oral health status may be bidirectional and form a vicious circle of interactions [4–6]. On the one hand, poor oral health may determine food choices that favour the development of obesity. On the other hand, obesity-associated inflammation may deregulate the host immune defence and thus create a favourable milieu for the development periodontal infection. Periodontitis, in turn, may further add to the systemic inflammatory burden and promote the development of obesity-related complications.

One of the mechanisms by which obesity can impact on oral health is through altering the properties and output of saliva. Previous studies have reported on a reduced flow rate of saliva in obese individuals [7–9]. It has been hypothesised that this effect might be related to an imbalance in neuroendocrine regulation of salivary glands [10]. Moreover, it has been observed that morbid obesity is associated with a

*Abbreviations:* API, approximal plaque index; AUC, area under the curve; BMI, body mass index; CAL, clinical attachment level; CRP, C-reactive protein; DMF-S, caries prevalence index (decayed, missing, and filled surfaces); DMF-T, caries prevalence index (decayed, missing, and filled teeth); GI, gingival index; MCP-1, monocyte chemoattractant protein-1; NPV, negative predictive value; PAI-1, plasminogen activator inhibitor-1; PD, probing depth; PL-I, plaque index; PPV, positive predictive value; PTX-3, pentraxin 3; ROC, receiver operating characteristic; SBI, sulcus bleeding index; sICAM-1, soluble intercellular adhesion molecule-1; SOD, superoxide dismutase; TNFα, tumour necrosis factor-α; TNFR1, tumour necrosis factor receptor 1; VEGF, vascular endothelial growth factor

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decrease in total salivary antioxidant status [11]. Given the key role of saliva in maintaining oral health [12], both hyposalivation and a reduction in salivary antioxidant capacity may indeed facilitate the development of dental and gingival lesions.

Changes in salivary concentrations of mediators relevant to obesity are largely obscure. One may expect that changes evoked by obesity in the systemic circulation will be mirrored by similar changes in the saliva. However, the composition of saliva is also affected by local factors and formation of gingival crevicular fluid. Furthermore, the majority of obese patients suffer from comorbidities that may modulate saliva secretion either directly or through a required treatment. For these reasons it is difficult to dissect out which changes in salivary parameters could be attributed purely to an increase in body mass. Here, we have sought to determine whether there is a specific salivary immune signature of obesity per se.

## 2. Methods

### 2.1. Subjects

Subjects of the study were recruited from patients of the Poznan University of Medical Sciences seeking weight-loss treatment for obesity or those presenting for routine dental check-up. Patients willing to participate in the study underwent physical examination and basic blood tests. For the discovery group only patients without associated diseases, taking no medication known to affect salivary secretion [13,14], and with fasting blood sugar < 100 mg/dl and HDL > 40 mg/dl [15] were considered (Fig. 1). All blood test were performed routinely in the hospital central laboratory. The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the Poznan University of Medical Sciences (decision #189/14).

### 2.2. Clinical examination and saliva collection

Oral health was assessed by the same experienced dentists using routine methods [16]. The examination included the parameters of dental and periodontal status, as well as the parameters of oral hygiene.

Unstimulated and paraffin-stimulated whole mixed saliva was collected under standardized conditions at least 2 h after a meal and always at the same time (between 10 am and 1 pm) to minimise diurnal variations. Stimulated saliva was collected over a 15-min period, during which the patient was asked to chew on a 1 g paraffin pellet. The samples of saliva were collected, processed, and stored as recommended [17] and as previously described in detail [18]. The saliva was immediately analysed for pH and centrifuged (10,000 × *g* for 5 min at 4 °C) to remove any debris. The samples were then aliquoted and stored at −80 °C until assayed.

### 2.3. Salivary measurements

The saliva was assessed for parameters falling into four categories: (1) inflammation (TNFα, TNFR1, MCP-1, PTX-3), (2) oxidative stress (total antioxidant capacity, catalase, superoxide dismutase, uric acid), (3) endothelial cell function (VEGF, sICAM-1, PAI-1, E-selectin), (4) adipokines (leptin, adiponectin, serpin A12, resistin). The choice of parameters was based on our previous experience [18] and preliminary experiments, which allowed us to select the parameters readily detectable in saliva.

Salivary total antioxidant capacity, and the concentrations of catalase, superoxide dismutase and uric acid were measured with the assay kits from Cayman Chemical (Ann Arbor, MI, USA). All other analytes were measured with enzyme-linked immunosorbent assays using either Quantikine High Sensitivity kit (TNFα) or DuoSet Immunoassays (R&D Systems, Minneapolis, MN, USA USA).

### 2.4. Statistical analysis

Statistical analyses were performed using Statistica v.12 software (StatSoft, Krakow, Poland). A P value of less than 0.05 was considered significant. Categorical variables were expressed as numbers or percentages, and analysed by the $\chi^2$ test with Yates' correction or Fisher-Freeman-Halton test, when applicable. Continuous data were checked for normal distribution by the Lilliefors' test and presented as means ± SDs. The data were compared using the t-Student test or the Mann-Whitney *U* test for normally and non-normally distributed data, respectively. Receiver operating characteristic (ROC) curves were analysed by evaluating the area under the curve (AUC) to detect the sensitivity and specificity of each parameter and to select parameters that best discriminated the studied groups. For the parameters selected,

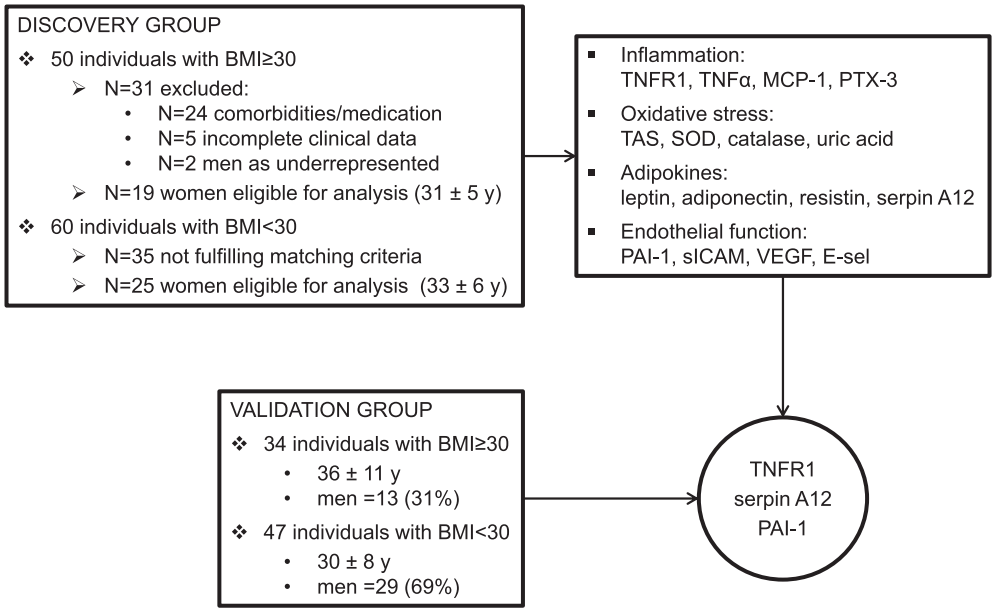


**Fig. 1.** Study design. The *discovery group* served to identify salivary parameters associated with simple obesity. i.e. without comorbidities. Three candidate markers were selected and further verified in a *validation group* of non-preselected individuals with and without obesity.