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Eating disorders and biochemical composition of saliva: a retrospective matched case–control study

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This study aimed to compare the biochemical composition of saliva from patients with eating disorders (EDs) with saliva from control subjects with no ED. All patients who initiated outpatient treatment in an ED clinic during a 12-month period were invited to participate. Of the 65 patients who started treatment during the period, 54 (50 female patients/four male patients; mean age: 21.5 yr) agreed to participate. The controls were 54 sex- and age-matched patients from a dental health clinic. All participants completed a questionnaire and underwent dental clinical examinations, including laboratory analyses of saliva. The proportion of subjects with unstimulated salivary hyposalivation was lower in the ED group and not correlated with intake of xerogenic drugs. Significant differences in the biochemical composition of saliva were found almost exclusively in the unstimulated state, with albumin, inorganic phosphate, aspartate aminotransferase (ASAT), chloride, magnesium, and total protein all being significantly higher in the ED group. Conditional logistic regression showed that higher ASAT and total protein concentrations were relatively good predictors of ED, with sensitivity and specificity of 65% and 67%, respectively. In conclusion, elevated salivary concentrations of ASAT and total protein may serve as indicators of ED as well as of disease severity. Future studies are needed to corroborate these initial findings.

Key words: eating disorders; hyposalivation; saliva; xerostomia

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Eating disorders (ED) are psychosomatic disorders that may influence oral health because dental hard tissue, as well as salivary conditions, may be affected. In this regard, increased prevalences of dental erosion and caries have been reported, in addition to xerostomia, hyposalivation, and enlargement of salivary glands (1–8).

Eating disorders are characterized by a specific psychopathology focused on eating behavior, body weight, and shape, and the individual's efforts at controlling them (9). In the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition* (DSM-IV) (10), which is used in the present study, the EDs are divided into three main diagnoses: anorexia nervosa (AN), bulimia nervosa (BN), and ED not otherwise specified (ED-NOS). Anorexia nervosa is mainly characterized by food restriction leading to underweight, and BN by binge eating and inappropriate behaviors to compensate for the binge eating (e.g. self-induced vomiting, use of laxatives/diuretics, and excessive exercise). Eating disorders not otherwise specified is a very heterogeneous category, comprising inter alia AN-like and BN-like atypical EDs, as well as a preliminary diagnosis called binge eating disorder (10, 11). There is frequently an overlap in symptoms and behavior among the ED diagnoses. Self-induced vomiting can, for example, be found in almost all patients with EDs, irrespective of diagnosis. Diagnostic shifts over the course of the ED is common, as are repeated relapses after healthy periods (12).

There are well-established connections between EDs in general, their behavioral specifiers (e.g. binge eating and vomiting), and oral complications. The connection is especially apparent between bulimic behaviors and dental erosion, but is also present with salivary and other oral-related factors (3, 7, 8, 13–16). In addition, the duration of the ED may have a significant impact on these factors (8).

Eating disorders are likely to affect oral health and the saliva in different ways, depending on the expression, intensity, and duration of the ED symptoms. It may therefore be of interest to study salivary factors – both the quantity and the composition – and analyze the results in terms of duration of the disease and behavioral symptoms, such as vomiting or binge eating.

The aim of the study was therefore to investigate the biochemical composition of saliva from patients with EDs and in the different ED subgroups, and to compare it with that of saliva from sex- and age-matched

healthy controls. It is hypothesized that the composition of saliva from patients with EDs may be affected and that specific markers for ED disease may exist.

Material and methods

Participant selection and inclusion/exclusion criteria

During a 12-month period, a total of 65 patients accepted and initiated psychiatric/medical outpatient treatment for EDs at the Eating Disorder Clinic, Örebro County Council, Örebro, Sweden. The ED diagnoses were made by the expert team at the clinic. All 65 patients were invited to participate in this study and, of these, 54 (83%) agreed to participate at their first physical examination. A control group of 54 subjects, matched for sex and age, was selected from the standard recall patients at a Public Dental Health Clinic, Örebro, Sweden. The selection was made from the consecutive list of patients who were to be given an appointment for a routine check-up. The matching of controls was based on gender and date of birth. The sex- and age-matched controls were examined during February to June 2006.

In order to identify and exclude, from the controls, patients with risk for having an ED, each potential control subject was assessed using the Symptom Index of the Eating Disorder Inventory-2 (EDI-2) (17). The EDI-2 is a 91-item questionnaire that is widely used in the study of EDs and is divided into 11 subscales, three of which are subscales of central symptoms (the symptom index, i.e. includes the subscales drive for thinness, bulimia, and body dissatisfaction). In the present study, only the symptom index was used, which includes 23 of the 91 items in the questionnaire. Among the 54 controls first selected, two subjects with risk for EDs (i.e. a score of >14, according to the EDI-2 symptom index) were found. These subjects were excluded from the control group and two additional controls were selected, evaluated using the EDI-2, and found to be suitable for inclusion.

Of the 11 patients who declined to participate in the study ($\bar{x}$ = 20.5 yr, range 14–38 yr, all women), four were diagnosed with AN and seven were diagnosed with ED-NOS. The reasons given for not participating were: did not consider herself to have any dental problem (one patient), failed to attend the appointment (one patient), did not have the time (three patients), and was not comfortable with participation (e.g. long travel time and did not like to answer questions, etc.) (six patients). There was no statistically significant difference regarding age, gender, and ED diagnoses between the non-participants and the study group.

The study was approved by the Ethics Committee of the Örebro region, Sweden, and informed consent was obtained from all participants. In the case of children, parental consent was also obtained. As an incentive to participate in the study, all control subjects were offered cinema tickets (children) or a free ordinary recall (adults).

Questionnaire and clinical examination

A questionnaire was constructed in collaboration with the staff at the ED clinic. It comprised 196 questions on socio-demographic factors, general and oral health, and lifestyle. The questionnaire was given to participants of both study and control groups, except for 20 questions that were specifically related to the ED and were therefore not given to the controls. All medication intake was recorded and its possible xerogenic effect was classified according to 1177 VÅRDGUIDEN's (18) list of drugs that are known to cause mouth dryness. The clinical examination of the study group was performed at the specialist clinic for ED in an ordinary medical examination room using mobile dental equipment and an operating light. The controls were examined using the operating light from the same mobile equipment but in the dental clinic. The status of the salivary glands was assessed by visual examination and palpation. The examiner was blinded to the medical history and to the results of the questionnaires for both ED patients and controls. Full details of the questionnaire, clinical examination, and the results of oral health status and symptoms related to oral health and temporomandibular disorders (TMDs) in this sample have previously been published (8, 19).

Salivary analyses

The patients were instructed to refrain from eating and drinking, as well as from oral hygiene and smoking, for 1 h before the appointment. At the appointment, unstimulated whole saliva and paraffin-stimulated whole saliva were collected in precooled tubes on ice, for periods of 15 min and 5 min, respectively. After determining the secretion rate (ml/min), a small aliquot from the sample of stimulated whole saliva was used to determine buffer capacity (Dentobuff; Orion Diagnostica, Espoo, Finland). The remainder of the collected saliva was transported in dry ice to a freezer and was stored at −80°C until required for further analyses.

Analyses of the biochemical composition of both unstimulated and stimulated saliva were carried out at Sahlgrenska University Hospital, Göteborg, Sweden. A BM/Hitachi 917 instrument (Boehringer Mannheim, Indianapolis, IN, USA) was used to analyze calcium (mmol/l), inorganic phosphate (mmol/l), α-amylase (μkat/l), lactate dehydrogenase (LDH, μkat/l), aspartate aminotransferase (ASAT, μkat/l), magnesium (mmol/l), creatinine (μmol/l), and urea (mmol/l) using photometric assays. Total protein concentration (g/l) was analyzed using turbidity measurements. An ion-selective electrode was used for determination of potassium (mmol/l), chloride (mmol/l), and sodium (mmol/l). Nephelometry (IMMAGE 800 Immunochemistry System; Beckman Coulter, Brea, CA, USA; or Nephelometer II, Dade Behring, Deerfield, IL, USA) was used for analyses of albumin (mg/l). The number of moles of solute that contribute to the osmotic pressure of a solution – osmoles (mOsm/kg) – was measured by freezing point depression (Fiske 2400 Multi Sample Osmometer; Norwood, MA, USA).

Statistical methods

Differences between patients with ED and controls were calculated using the Wilcoxon signed-rank test (SPSS version 20.0; Statistical Package for the Social Sciences, IBM SPSS, Armonk, NY, USA). In addition to comparing the ED group with its matched control, comparisons were also made between subgroups of patients with EDs and their respective controls. The subgroups comprised: (i) duration of ED of ≤1 yr or >1 yr; and (ii) present self-induced vomiting and/or binge eating behavior or not. *P*-values of <0.05 were considered statistically significant. Conditional

logistic regression, using the nomreg and the Cox procedures, in SPSS was used to test for statistically significant differences in salivary variables between ED patients and controls in unstimulated saliva. Associations within the ED and control groups were tested using the Pearson correlation coefficient. For variables significant in the regression analysis, a receiver–operating characteristic (ROC) curve was plotted and the area under the curve (AUC) was calculated.

Results

Distribution of ED diagnoses and body mass index

The mean age ±SD for both patients and controls was 21.5 ± 6.8 yr (range: 10–50 yr; 100 female subjects and eight male subjects). The distribution of ED diagnoses among the patients was: AN, 28% (14/54 patients); BN, 14% (eight of 54 patients); and EDNOS, 58% (32/54 patients). The mean age at onset of EDs in the study group was 16 (SD 3.8, range: 9–26) yr, and the mean duration of the disease was 4.5 (SD 6.1, range: 0.3–35) yr. Sixty-one per cent of patients had had an ED for more than 1 yr. The mean body mass index (BMI) of subjects in the ED group was 19 ± 4.2 (range: 12–36) and in the controls was 23 ± 4.7 (range: 18–44) ($P = 0.001$). A more detailed analysis of oral health status and symptoms related to TMDs in this sample has previously been reported (8, 19).

Questionnaire

In the ED group, 34 patients (five with AN, eight with BN, and 21 with EDNOS) reported present vomiting or binge eating, whereas 20 (nine with AN and 11 with EDNOS) did not. Seventeen per cent of patients with EDs reported daily xerostomia compared with 6% of control subjects, whereas xerostomia once a month or more was reported by 52% and 30% of patients with ED and control subjects, respectively ($P = 0.004$). Swelling in front of the ear (parotid enlargement) was only reported in the ED group (four patients). Stomach problems were reported by 59% of patients with EDs (four with AN, five with BN, and 23 with EDNOS) and by 28% of the controls ($P = 0.002$). The frequency of intake of xerogenic medications was not significantly different between patients with ED and controls. This was true for both psychotropic (nine patients with ED and three controls, respectively) and anti-allergic (nine patients with ED and 11 controls) drugs taken separately, as well as in combination (18 patients with ED and 13 controls).

Clinical findings and salivary analyses

Parotid gland enlargement was found in 31% ($n = 17$) of patients with EDs (one with AN, four with BN, and 12 with EDNOS) ($P = 0.001$) but in none of the controls. The unstimulated salivary secretion rate was lower in patients with EDs ($\bar{x} = 0.22$ ml/min, SD = 0.19) compared with controls ($\bar{x} = 0.27$ ml/min, SD = 0.21), but not significantly so ($P = 0.148$). During stimulation, no clinical or significant differences were found between the study groups (ED group: $\bar{x} = 0.64$ ml/min, SD = 0.88; control group: $\bar{x} = 0.66$ ml/min, SD = 0.90). The proportion of subjects with unstimulated hyposalivation (a secretion rate of ≤0.1 ml/min) was significantly higher in the ED group compared with the control group (39% vs. 21%, respectively; $P = 0.025$). Buffering capacity did not differ significantly between the ED and control groups, and salivary secretion rate and buffering capacity did not differ significantly between ED subgroups and controls. Intake of xerogenic drugs and unstimulated and stimulated salivary flow showed no significant correlation within the ED group. Among the controls, a tendency for significant correlation was found between lower unstimulated saliva secretion rate and intake of psychotropic and anti-allergic drugs (Pearson $R = -0.27$; $P = 0.05$) but not for each drug taken separately.

The biochemical compositions of unstimulated and stimulated saliva in the ED and control groups are shown in Tables 1 and 2. Significant differences were found almost exclusively for the unstimulated state, in which albumin, phosphate (Table 1), ASAT, chloride, magnesium, and total protein (Table 2) were all significantly higher among patients with EDs ($P = 0.042$ to $P = 0.003$). In stimulated saliva, only LDH was significantly different, being higher in the ED group ($P = 0.043$) (Table 2). There was a significant, negative correlation between BMI and ASAT in the ED group (Pearson $R = -0.42$, $P = 0.006$), but no such correlation was found among the controls.

Symptoms and signs as a function of duration of disease and behavior

Unstimulated and stimulated salivary secretion rates and buffer capacity were not significantly different with respect to ED subgroups (i.e. duration of disease and vomiting and/or binge eating behavior or not) compared with matched controls. Moreover, there was no difference between frequencies of intake of xerogenic drugs in ED subgroups. The significant differences found in biochemical composition relating to duration of ED and behaviors are shown in Table 3, and all numerical values for the salivary composition of subgroups compared with those of matched controls are given in Tables S1–S8. A pattern emerged in which most differences compared with controls were found in those in the ED group who had had a duration of disease of >1 yr (six with AN, seven with BN, and 20 with EDNOS) and in the group not reporting vomiting and/or binge eating (nine with AN and 11 with EDNOS). Furthermore, these differences were almost exclusively in unstimulated saliva, with significantly higher ion concentrations compared with matched controls. In stimulated saliva, only LDH differed significantly, in the subgroup with a duration of the disease of >1 yr.

Table 1

*Biochemical composition of unstimulated and stimulated whole saliva in patients with an eating disorder (ED) compared with that in sex- and age-matched controls**

	Study group						
	ED			Control			
Saliva variable	*n*	Mean ± SD	Range	*n*	Mean ± SD	Range	*P*
Unstimulated saliva							
α-Amylase (μkat/l)	48	1385 ± 917	7–4334	51	1277 ± 1142	14–5612	0.85
Albumin (mg/l)	44	72 ± 81	12–460	47	49 ± 48	6–249	**0.003**
Calcium (mmol/l)	48	0.82 ± 0.21	0.46–1.28	51	0.76 ± 0.17	0.47–1.23	0.25
Osmoles (mOsm/kg)	46	62 ± 16	33–115	51	56 ± 14	32–90	0.07
Phosphate (inorganic) (mmol/l)	48	6.4 ± 2.2	2.8–13.7	51	5.5 ± 1.8	2.9–10.8	**0.04**
Potassium (mmol/l)	48	22.3 ± 4.6	14.8–36.9	51	21.0 ± 4.7	13.4–30.7	0.37
Urea (mmol/l)	48	5.15 ± 1.93	0.90–10.20	51	4.68 ± 1.53	2.30–9.80	0.79
Stimulated saliva							
α-Amylase (μkat/l)	52	2181 ± 1359	57–6078	53	2400 ± 2154	77–11330	0.73
Albumin (mg/l)	52	52 ± 43	12–236	52	61 ± 57	7–278	0.24
Calcium (mmol/l)	52	0.75 ± 0.18	0.42–1.20	53	0.76 ± 0.14	0.49–1.02	0.86
Osmoles (mOsm/kg)	52	76 ± 18	52–119	53	72 ± 16	50–113	0.20
Phosphate (inorganic) (mmol/l)	52	4.1 ± 1.1	2.4–7.9	53	3.8 ± 0.8	2.2–5.6	0.33
Potassium (mmol/l)	52	20.2 ± 3.2	12.8–30.1	53	20.2 ± 3.0	15.4–27.0	0.76
Urea (mmol/l)	52	3.46 ± 1.20	0.90–7.30	53	3.42 ± 0.97	1.40–6.20	0.79

Descriptive statistics: Mean salivary variables of numerical variables (Wilcoxon signed-rank test).
**P*-value in bold indicate a statistically significant difference.

Table 2

*Biochemical composition of unstimulated and stimulated whole saliva from patients with an eating disorder (ED) compared with that in sex- and age-matched controls**

	Unstimulated					Stimulated				
	ED		Controls			ED		Controls		
Salivary variable	*n*	%	*n*	%	*P*	*n*	%	*n*	%	*P*
ASAT (μkat/l)										
<0.10	19	39.6	31	62.0	**0.004**	1	1.9	0	0	0.66
0.10–0.60	26	54.2	19	38.0		38	73.1	43	81.1	
>0.60	3	6.3	0	0.0		13	25.0	10	18.9	
Chloride (mmol/l)										
<15	3	7.0	12	24.5	**0.03**	2	3.8	4	7.7	0.52
15–27	36	83.7	37	75.5		41	78.8	42	80.8	
>27	4	9.3	0	0.0		9	17.3	6	11.5	
Creatinine (μmol/l)										
<3	11	22.9	23	45.1	0.09	27	51.9	20	37.7	0.23
3–5	26	54.2	19	37.3		22	42.3	30	56.6	
>5	11	22.9	9	17.6		3	5.8	3	5.7	
LDH (μkat/l)										
<0.2	28	58.3	36	70.6	0.13	24	46.2	31	58.5	**0.04**
0.2	12	25.0	11	21.6		12	23.1	15	28.3	
>0.2	8	16.7	4	7.8		16	30.8	7	13.2	
Mg (mmol/l)										
<0.1	3	6.3	12	23.5	**0.005**	31	59.6	29	54.7	0.70
0.10–0.16	21	43.8	25	49.0		18	34.6	22	41.5	
>0.16	24	50.0	14	27.5		3	5.8	2	3.8	
Protein (total) (g/l)										
<0.2	4	9.3	12	24.5	**0.016**	9	17.3	8	15.4	0.98
0.2–0.5	27	62.8	34	69.4		32	61.5	33	63.5	
>0.5	12	27.9	3	6.1		11	21.2	11	21.2	
Sodium (mmol/l)										
<10	42	87.5	45	88.2	0.50	14	26.9	21	39.6	0.76
10–15	3	6.3	5	9.8		10	19.2	12	22.6	
>15	3	6.3	1	2.0		28	53.8	20	37.7	

Categorical variables (Wilcoxon signed-rank test).
ASAT, aspartate aminotransferase; LDH, lactate dehydrogenase.
**P*-value in bold indicate a statistically significant difference.

Albumin, ASAT, chloride, magnesium, phosphate, and protein from unstimulated saliva were significantly different according to the bivariate analysis (Tables 1 and 2), and they were entered into the conditional logistic regression. The final model constituted two variables: ASAT (OR = 3.5) and protein (total; OR = 2.5). The sensitivity and specificity for correct classification into the ED group and the control group were 65.1% and 67.3% for ASAT and protein, respectively, and Nagelkerke $R^2 = 0.32$ (Table 4).

The AUC based on the ROC curve (Fig. 1) was 0.73 (95% CI: 0.62–0.84) for ASAT alone, and 0.75 (95% CI: 0.65–0.86) for protein alone. With both variables in the model, the AUC was 0.83 (95% CI: 0.74–0.92). Including all variables in the model gave an AUC of 0.87 (95% CI: 0.78–0.95).

Table 4

Conditional logistic regression (stepwise forward entry method) with eating disorder (ED) and matched control as the dependent variable and six independent variables [unstimulated saliva albumin (mg/l), aspartate aminotransferase (ASAT) (μkat/l), chloride, (mmol/l), magnesium (mmol/l), phosphate (inorganic) (mmol/l), and protein (total; g/l)]

	B	*P*	OR	95% CI for OR
ASAT	1.2	0.044	3.5	1.03–11.5
Protein (total)	0.9	0.038	2.5	1.05–5.9

Nagelkerke $R^2 = 0.32$.
B, regression coefficient; *P*, significance level.

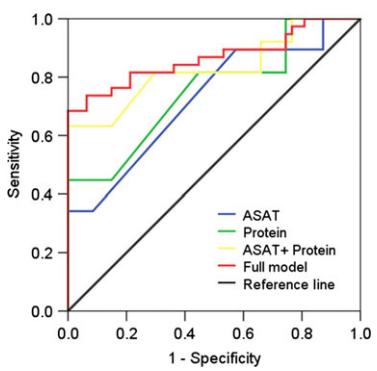

Fig. 1. Receiver–operating characteristic (ROC) curve for significant variables in the regression analysis. The area under the curve (AUC) was 0.73 for aspartate aminotransferase (ASAT), 0.75 for protein, and 0.83 for combined ASAT + protein.

Discussion

Of the total of 65 patients who accepted and initiated psychiatric/medical outpatient treatment for ED at the Eating Disorder Clinic, 54 agreed to participate in the study. This constitutes a participation rate of 83%, which is acceptable considering that this is a 'difficult' patient group in terms of management and because of the serious nature of the disease. There were no statistically significant differences in age, gender, and ED diagnoses between participating and non-participating patients. It is therefore probable that the group examined is largely representative of patients with EDs referred to an outpatient ED clinic in Sweden. The distributions of the ED diagnoses and BMI are largely in agreement with the normal distribution among patients for ongoing ED treatment, although there was a slight over-representation of AN and EDNOS and an under-representation of BN. The sex- and age-matched controls from the public dental health clinic were considered to represent a healthy group of individuals.

Table 3

Differences in unstimulated (UWS) and stimulated (SWS) salivary composition in relation to the duration of disease and present vomiting and/or binge eating (VOM/BE or not (N-VOM/BE)

	ED ≤1 yr (*n* = 21)		ED >1 yr (*n* = 33)		VOM/BE (*n* = 34)		N-VOM/BE (*n* = 20)	
Salivary variable	UWS	SWS	UWS	SWS	UWS	SWS	UWS	SWS
α-Amylase	0.65	0.46	0.91	0.75	0.97	0.70	0.72	0.90
ASAT	0.07	0.48	0.02↑	0.25	0.09	0.37	0.01↑	0.74
Albumin	0.24	0.38	0.005↑	0.47	0.12	0.70	0.004↑	0.14
Calcium	0.91	0.26	0.12	0.59	0.87	0.27	0.04↑	0.26
Chloride	0.13	0.37	0.13	0.06	0.13	0.76	0.13	0.21
Creatinine	0.92	0.44	0.03↑	0.35	0.30	0.66	0.17	0.17
LDH	0.05	0.01↑	0.57	0.72	0.40	0.12	0.18	0.19
Mg	0.12	0.80	0.02↑	0.78	0.15	0.37	0.008↑	0.89
Osmoles	0.30	0.39	0.16	0.01↑	0.81	0.44	0.01↑	0.27
Phosphate (inorganic)	0.30	0.44	0.06	0.59	0.39	0.21	0.03↑	0.94
Potassium	0.56	0.66	0.45	0.43	0.76	0.96	0.04↑	0.57
Protein (total)	0.31	0.56	0.02↑	0.69	0.13	0.86	0.04↑	0.80
Sodium	0.79	0.34	0.53	0.01↑	1.0	0.48	0.45	0.19
Urea	0.47	0.59	0.21	0.89	0.77	0.94	0.07	0.57

P-values indicate significant differences with respect to matched controls. Arrows indicate a higher (↑) mean/categorical *P*-value compared with matched controls.
ASAT, aspartate aminotransferase; ED, eating disorder; LDH, lactate dehydrogenase.

Even though studies of EDs are often related to their specific sub-diagnoses, we decided to study EDs mainly in relation to their duration and behavioral factors because the different sub-diagnoses frequently overlap during the course of the disease.

The concentrations of saliva constituents were generally higher in patients with EDs than in controls, and those variables that showed significant differences were almost exclusively found in unstimulated saliva. This applied both to the total group as well as to the subgroups. It is difficult to explain these differences fully but a few comments can be made. The patients with EDs reported, and presented clinically with, enlargement in the area of the parotid glands significantly more frequently than did controls, and this is in agreement with previous studies (20–22). In the present study, a great proportion of patients with EDs reported self-induced vomiting or binge eating. Although the etiology of parotid enlargement in patients with EDs is not yet clarified, it is believed to be associated with vomiting and binge-eating behavior (23, 24), even though an absence of salivary gland hypertrophy cannot exclude the presence of vomiting behavior (25). Parotid enlargement has been noted in a variety of conditions besides eating disorders, including alcoholism, malnutrition, diabetes, cirrhosis, hypothyroidism, obesity, and pregnancy. The effect may also be related to the amount of saliva secreted, mechanical factors, inflammatory changes, and centrally induced mechanisms (24, 26). Patients with EDs generally experience xerostomia and hyposalivation, which are considered to be related to the intake of xerogenic drugs, at least in BN (7). In this study, no significant differences between patients with EDs and controls were found regarding intake of xerogenic medication, and similar results were found in the subgroups related to duration of the disease and presence of vomiting/binge eating. Salivary secretion rates were not significantly correlated with intake of xerogenic drugs in the ED group but were so in the controls. From the findings of this study, it can therefore be concluded that impaired salivary secretion in patients with EDs is not a function of xerogenic medication only. In this regard, other factors, such as the practise of purging behaviors (e.g. combinations of self-induced vomiting, misuse of laxatives, diuretics, etc.) and/or excessive exercise, resulting in body dehydration, may contribute to the manifestations of xerostomia and hyposalivation observed in patients with EDs (24). Consequently, the significantly altered biochemical composition of saliva in patients with EDs compared with healthy controls, observed in this study, must be seen in the light of the aforementioned influences and may be a direct effect of impaired salivary function and/or an indirect expression of the ED itself.

Compared with their matched healthy controls, subgroups of patients with EDs [i.e. those with a longer duration of the disease together with those who did not present with vomiting/binge eating (anorexic behavior)], differed significantly in salivary composition compared with those with a shorter duration and vomiting/binge eating behavior (bulimic behavior). It is possible that the difference in salivary flow and composition could be attributed to the effect of longer disease activity and anorexic behavior, which is likely to have a more severe impact on bodily physiologic functions. That the differences between the subgroups and controls is only caused by hyposalivation (i.e. a higher proportion of subjects with an unstimulated salivary flow rate of $\geq$0.1 ml/min was found in the total ED group) is refuted by the fact that there was no significant difference in the salivary secretion rate between subgroups of ED and their matched controls. Nevertheless, anorexic behavior and longer duration of ED seem to have a significant impact on salivary composition.

In the conditional regression analysis performed on the total sample, the model identified ASAT and total protein content to the best predictors in the set of variables analyzed for ED patients (OR = 3.5 and 2.5, respectively), with sensitivity and specificity of 65% and 67%, respectively. In female subjects with EDs, 'increased blood serum activities of ASAT was significantly correlated to lower BMI (27), had a high incidence in critically-ill anorexic patients and was considered to be a sign of multi-organ failure requiring urgent caloric supplementation' (28). Elevated levels of serum transaminases are not a typical feature of starvation alone, but this may be the case when occurring in patients with EDs in combination with alcohol, drugs, and/or medication abuse (29). Salivary ASAT in this study, in addition to being a significant predictor of ED disease, also had a significant, negative correlation with BMI in the ED group, similar to that found in blood serum (27). Consequently, it seems that salivary levels of ASAT could be an important factor and this deserves further investigation.

An elevated total protein level in unstimulated saliva was another predictor for ED. In bulimic patients a similar correlation has been reported (30), and it has been speculated that the increase of salivary protein concentrations in bulimic patients is caused by an abnormal parotid sympathetic innervation resembling that of stimulatory proteodyschylia (31). Nevertheless, salivary and parotid gland affection was common among the patients with EDs in this study and it is therefore not unlikely that the elevated concentrations of salivary total protein mirror these conditions. In bivariate analyses, ASAT and total protein concentrations were also significantly higher in subgroups of patients with EDs with a longer duration of the disease and in those who did not report vomiting/binge eating behavior. If one agrees that these groups represent a more severe presentation of ED disease, increased salivary ASAT and protein levels may be an indication of this increased severity.

In conclusion, elevated salivary concentrations of ASAT and total protein may serve as indicators for patients with EDs as well as of the severity of the disease. Future studies are needed to corroborate these preliminary findings.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Tables S1–S8. All numerical variables for saliva measurements in subgroup comparisons.