The Laryngoscope

Saliva Pepsin Concentration of Laryngopharyngeal Reflux Patients Is Influenced by Meals Consumed Before the Samples

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Objectives/Hypothesis: To assess the impact of diet on the saliva pepsin concentration of patients with laryngopharyngeal reflux (LPR).

Study Design: Non-controlled Prospective Study.

Methods: Patients with positive LPR regarding hypopharyngeal–esophageal impedance-pH monitoring (HEMII-pH) were enrolled from three European Hospitals. Patients collected three saliva samples, respectively, in the morning (fasting), and 1 to 2 hour after lunch and dinner. Patients carefully detailed foods and beverages consumed during meals and before the pepsin samples. The 3-month treatment was based on the association of diet, proton pump inhibitors, alginate, or magaldrate regarding the HEMII-pH characteristics. Reflux Symptom Score (RSS) and Reflux Sign Assessment (RSA) were used for assessing the pre- to posttreatment clinical evolution. The Refluxogenic Diet Score and the Refluxogenic Score of a Dish (RESDI) were used to assess the refluxogenic potential of foods and beverages. The relationship between saliva pepsin concentration, HEMII-pH, RESDI, RSS, and RSA was investigated through multiple linear regression.

Results: Forty-two patients were included. The saliva pepsin concentration of the 24-hour period of testing was significantly associated with foods and beverages consumed during the testing period and the evening dinner (r_s = 0.973, P < .001). RSS and RSA significantly improved throughout treatment. The level of saliva pepsin in the morning was a negative predictive factor of the therapeutic response regarding RSA and RSS (P < .036).

Conclusions: Foods and beverages may significantly impact the saliva pepsin concentration of patients with LPR. Patients with high-level saliva pepsin in the morning had lower therapeutic response compared with those with low-level saliva pepsin.

Key Words: Reflux, laryngopharyngeal, laryngitis, pepsin, saliva, diet, foods, beverages.

Level of Evidence: 4

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INTRODUCTION

Laryngopharyngeal reflux (LPR) is an inflammatory condition of the upper aerodigestive tract tissues related to direct and indirect effect of gastroduodenal content reflux, which induces morphological changes in the upper aerodigestive tract.[1] The inflammatory process of the laryngopharyngeal mucosa is mainly due to the refluxate pepsin, which induces injuries through intra- and extracellular mechanisms.[2,3] The key role of pepsin in LPR development led some authors to develop the Peptest, which is a noninvasive diagnostic approach based on the detection of pepsin in the saliva of LPR patients.[3,4] Currently, the saliva pepsin measurement is not considered as a gold standard. According to a recent meta-analysis, the sensitivity and the specificity of saliva pepsin measurement are 64% and 68%, respectively,[5] the sensitivity depending on the method used for the pepsin measurement and many unknown factors.[1,6] The diet of the

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Reflux Symptom Score

Within the last month, I suffered from one/several followed symptoms

Severity: 0= problem is not severe, 5 = problem very troublesome when it occurs

Frequency: 0= I don't have this complaint over the past month, 1;2;3;4 = I had 1-2;2-3;3-4;4-5 weekly over the past month; 5= complaint occurs daily

	Disorder Frequency	Disorder Severity		Quality of Life impact	
Ear Nose and Throat Disorders			Total score		Total score
1. Hoarseness or a voice problem	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
2. Throat pain	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
3. Pain during swallowing time	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
4. Difficulty swallowing (pills, liquids or solid foods)	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
5. Clearing your throat	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
6. Sensation of something sticking in the throat	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
7. Excess mucous in the throat or post nasal drip sensation	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
8. Ear pressure/pain (daytime or nigh-time)	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
9. Tongue burning	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
10. Other:	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
Abdominal Disorders					
1. Heartburn, stomach acid coming up	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
2. Regurgitations of liquids, solid foods or burps	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
3. Abdominal pain	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
4. Diarrheas	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
5. Constipation	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
6. Indigestion	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
7. Abdominal distension and/or flatus	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
8. Halitosis	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
9. Nausea	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
10. Other:	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
Chest/respiratory Disorders					
1. Cough after eating or lying down	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
2. Cough (daytime)	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
3. Breathing difficulties,breathlessness, or wheezing	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
4. Chest pain	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....
5. Other:	0 - 1 - 2 - 3 - 4 - 5	0 - 1 - 2 - 3 - 4 - 5	.....	0 - 1 - 2 - 3 - 4 - 5	.....

Do you think that this questionnaire well assesses your current complaints ? YES - NO **RSS total score**:..... **Quality of Life score**:.....

Fig. 1. Reflux Symptom Score (RSS). The questionnaire is subdivided into three parts according to the complaints: ear, nose, and throat (part 1, nine items), digestive (part 2, nine items), and respiratory (part 3, four items) symptoms. The frequency and severity of each symptom are rated with a five-point scale. Regarding the frequency, 0 = patient did not have the complaint over the past month; 1, 2, 3, 4 = patient had the complaint 1 to 2, 2 to 3, 3 to 4, or 4 to 5 times weekly over the past month; 5 = patient had the complaint daily over the past month. Regarding the severity, 0 = the complaint is absent, 5 = the complaint is very troublesome when it occurs. For each item, the severity score is multiplied by the frequency score to obtain a symptom score ranging from 0 to 25. The sum of these symptom scores is calculated to obtain the RSS final score (ranging from 0 to 550, with the possibility for the physician and the patient to add three symptoms not identified in the RSS, leading to a maximal possible score of 625). The RSS also assesses the symptom impact on quality of life. The total quality of life score is calculated by the sum of each item score.

patient could be one of these unknown factors regarding a recent study reporting a significant association between the patient's diet and the occurrence of hypopharyngeal reflux episodes at the hypopharyngeal–esophageal intraluminal multichannel impedance-pH monitoring (HEMII-pH).[7] To date, there are no data in the literature about the potential impact of foods and beverages consumed by LPR patients on the measurement of the saliva pepsin concentration.

The aim of this study was to investigate the impact of foods and beverages consumed by LPR patients on the saliva pepsin concentration.

MATERIAL AND METHODS

The local ethics committee approved the study protocol (CHU Saint-Pierre, Université Libre de Bruxelles (ULB), No. BE076201837630). Patients were invited to participate, and informed consent was obtained.

Subjects and Setting

Patients with LPR symptoms and signs were enrolled from three European hospitals (University Hospital Center Saint-Pierre, Cesar De Paepe Hospital, Brussels, Belgium; Elsan Private Hospital of Poitiers, Poitiers, France) from January 2018 to June 2019. The LPR diagnostic was based on the occurrence of one or more acid or nonacid hypopharyngeal reflux episodes at the HEMII-pH.[8] Elderly patients (≥60 years old) and those with gastrointestinal (GI) symptoms benefited from GI endoscopy for excluding esophagitis. Patients with the following conditions were excluded: smoker, alcohol dependence, pregnancy, neurological or psychiatric illness, upper respiratory tract infection within the last month, current use of antireflux treatment, previous history of neck surgery or trauma, benign vocal fold lesions, malignancy, history of ear, nose, and throat radiotherapy, and active seasonal allergies or asthma.

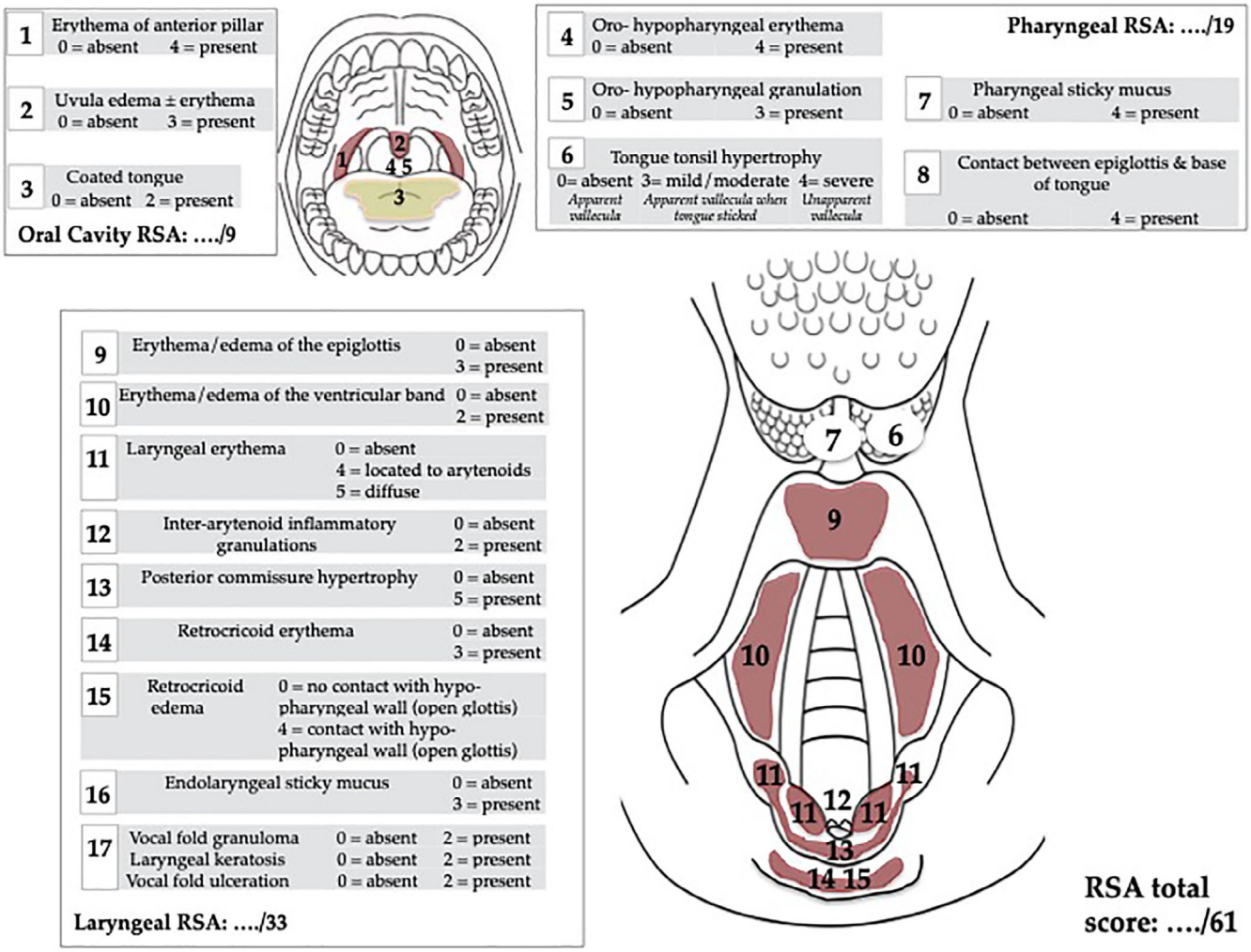

Fig. 2. Short version of the Reflux Sign Assessment (RSA). The tool is subdivided into three parts according to the sign localization: oral cavity, pharynx, and larynx. The occurrence of vocal fold granuloma (+2), keratosis (+2), or ulceration (+2) may be considered in the last item of the score. Because of low prevalence, the following items were removed from the initial version of the RSA: edema/erythema of the vocal folds, nasopharyngeal erythema, and subglottic edema/erythema. The total score is calculated by the sum of each item score. The maximum score is 61.

Hypopharyngeal–Esophageal Multichannel Intraluminal Impedance-pH Monitoring

The characteristics of the HEMII-pH device, placement, and analyses have been described in previous publications.[9,10] In summary, eight impedance segments and two pH electrodes composed the HEMII-pH (Versaflex Z, Digitrapper pH-Z Testing System; Medtronic, Minneapolis, MN). The impedance segments were placed along the esophagus zones (Z1 to Z6; centered at 19, 17, 11, 9, 7, and 5 cm above the lower esophagus sphincter (LES)). Two additional impedance segments were placed 1 and 2 cm above the upper esophagus sphincter (UES) in the hypopharynx. The pH electrodes were placed 2 cm above LES and 1–2 cm below the UES, respectively. A proximal/hypopharyngeal reflux event consisted of an episode reaching two impedance sensors in the hypopharynx. An acid reflux episode consisted of an episode with pH ≤ 4.0. A nonacid reflux episode consisted of an episode with pH > 4.0. The device was placed in the morning (8:00 A.M.), and was removed the next morning (8:00 A.M.).

Saliva Pepsin Detection

The patients collected three saliva samples, respectively, in the morning (fasting), and 1 to 2 hours after lunch and dinner, during 24-hour HEMII-pH testing. Patients carefully detailed foods and beverages consumed during the meals before the saliva sample collection. The saliva sample was collected into a 30-mL universal sample collection tube containing citric acid for preserving the action of any pepsin present. The saliva samples were stored in a refrigerator after the collection. The measurement of saliva pepsin level was performed through the Peptest device (RD Biomed, Hull, United Kingdom) by a trained lab technician. The steps of pepsin measurement were performed in a standardized procedure, which has been previously described.[9] The saliva pepsin concentration was measured using the Cube Reader

TABLE I.
Categories of the Refluxogenic Potential of Foods.

Very Low Reflux Foods	REDS	Cat.	Low Reflux Foods	REDS	Cat.	Moderate Reflux. Foods	REDS	Cat.	High Reflux. Foods	REDS	Cat.	Very High Reflux. Foods	REDS	Cat.
Artichoke	0.086	1	Eggplant	0.166	2	Apricot	0.391	3	Apple	0.534	4	Avocado	5.610	5
Asparagus*	0.072	1	Banana	0.227	2	Blueberry	0.472	3	Blackberries	0.640	4	Bacon	25.40	5
Baked spinach	0.025	1	Carrots	0.132	2	Boiled egg	0.348	3	Brie, blue, bread cheeses	1.001	4	Butter	—	5
Beetroot	0.082	1	Cherry	0.243	2	Camembert	0.495	3	Cake	1.850	4	Candy or sweets	5.216	5
Broccoli	0.077	1	Chicken fillet	0.148	2	Cereals (corn flakes)	0.470	3	Cauliflower	0.596	4	Chocolate (dark)	4.171	5
Brussels sprouts	0.030	1	Chile	0.171	2	Zucchini	0.289	3	Cheddar	1.068	4	Chocolate (milk)	3.787	5
Celery	0.101	1	Corn	0.244	2	Cucumber	0.274	3	Chocolate cookies	1.920	4	Chocolate (white)	4.543	5
Cooked mushrooms	0.103	1	Fat chicken	0.236	2	Dried plum	0.252	3	Cookies	1.695	4	Chocolate croissant	2.911	5
Crabs	0.088	1	Fennel	0.131	2	Duck (without skin and fat)	0.350	3	Cracker	0.952	4	Chocolate eclairs	2.079	5
Egg white	0.006	1	Ketchup†	0.166	2	Fat fish	0.368	3	Egg yolk	1.334	4	Croissant	2.860	5
Endive	0.014	1	Kidneys	0.192	2	Fig	0.267	3	Feta	1.501	4	Curry	2.985	5
Fresh and thin fish	0.058	1	Lamb	0.232	2	Fish oil (sardines, cod)	—	3	Fontina	0.946	4	French fries and frying	2.836	5
Garlic	0.035	1	Lamb chops or shoulder	0.201	2	Fish oil (herring)	—	3	Goat cheese	1.061	4	Ice cream	3.364	5
Green beans	0.054	1	Leek	0.139	2	Fish sauce	0.428	3	Gouda	1.193	4	Macadamia nut	7.074	5
Green peas	0.095	1	Melon	0.189	2	Ginger	0.362	3	Ground meat	0.704	4	Mayonnaise	56.80	5
Green salad*	0.074	1	Oat	0.243	2	Grapefruit	0.392	3	Gruyere	0.992	4	Meat sauce (Bearnaise)	45.04	5
Honey	0.000	1	Onion*	0.129	2	Guava	0.376	3	Hard cheese, full-fat cheese	1.093	4	Meat sauce (pepper)	3.839	5
Horse	0.076	1	Parsley	0.139	2	Lamb cutlets	0.462	3	Kiwi	0.540	4	Meat sauce (Roquefort)	3.060	5
Lentil	0.064	1	Pepper	0.186	2	Mandarin	0.478	3	Lychee	0.512	4	Milk (coco)	6.521	5
Low fat cheese	0.003	1	Pork tenderloin	0.208	2	Milk (goat, semiskim)	0.272	3	Mango	0.536	4	Nut, cashew, hazelnut	3.585	5
Milk (skim)	0.030	1	Rib steak	0.153	2	Milk (soy)	0.298	3	Meat sauce (mushroom)	1.116	4	Olive (black)	7.478	5
Mollusk	0.060	1	Ribs	0.246	2	Milk (semiskim)	0.363	3	Milk (whole)	0.690	4	Oliver (green)	12.92	5
Pork roast	0.110	1	Rice (brown)	0.188	2	Mint	0.302	3	Mozzarella	1.025	4	Pasta sauce (carbonara)	2.071	5
Pumpkin	0.085	1	Rindless, fatless	0.131	2	Nectarine	0.292	3	Munster	1.223	4	Pasta sauce (pesto)	8.331	5
Red cabbage	0.046	1	Cooked ham	0.131	2	Olive oil	-	3	Mustard	1.839	4	Pesto	8.331	5
Rice (red)	0.121	1	Rye bread	0.166	2	Orange	0.381	3	Noodles	0.565	4	Potato chips	2.830	5
Rice (white)	0.089	1	Shallot*	0.201	2	Peach	0.361	3	Orange jam	0.623	4	Sauerkraut	5.696	5
Roast veal	0.090	1	Steak, fillet, striploin	0.208	2	Pear	0.364	3	Parmesan	0.836	4	Spicy§	0.000	5

(Continues)

TABLE I.
Continued

Very Low Reflux Foods	REDS	Cat.	Low Reflux Foods	REDS	Cat.	Moderate Reflux. Foods	REDS	Cat.	High Reflux. Foods	REDS	Cat.	Very High Reflux. Foods	REDS	Cat.
Shrimp or lobster	0.033	1	Tofu	0.248	2	Pickle	0.270	3	Pasta sauce (Bolognese)	1.134	4			
Spaghetti (cooked)	0.060	1	Turnip	0.186	2	Plum	0.471	3	Pâté	1.612	4			
Sweet potato	0.073	1	Veal chop	0.181	2	Pork chops and shoulder	0.316	3	Peanut	1.618	4			
Tuna (low fat)	0.043	1	Watermelon	0.175	2	Potato	0.357	3	Pomegranate	0.725	4			
Turkey fillet	0.026	1	White bread	0.187	1	Raspberry	0.307	3	Raisin	0.758	4			
Veal cutlet	0.059	1	Whole ham	0.236	2	Rhubarb	0.362	3	Raspberry jam	0.566	4			
Wheat	0.079	1				Salmon	0.375	3	Red currant	0.922	4			
						Sardines	0.290	3	Ricotta	1.030	4			
						Strawberry	0.340	3	Roquefort	1.288	4			
						Sugar‡	0.000	3	Salami	1.177	4			
						Tomato (raw)	0.297	3	Sausages	0.722	4			
						Tripe	0.255	3	Sorbet	1.942	4			
						Whole wheat/brown bread	0.264	3	Strawberry jam	0.618	4			
									Tomato sauce	1.538	4			
									Vinaigrette	-	4			
									Yogurt and ice cream	0.674	4			

In practice, based on this table, laryngopharyngeal reflux patients selected the foods and beverages that they consumed once or more over the past 2 weeks, and the physician may add the categories corresponding of the consumed foods or beverages to get a score, called the Global Reflux Score. Several foods may be upgraded or downgraded according to characteristics.

*Raw vegetables are less digestible and may be associated with low gastric emptying time; in case of raw consumption, the food has to be upgraded 1 category. Except for green salad, the addition of vinegar or vinaigrette upgrades the category.

†In cases of the addition of spicy (for example, spicy ketchup), these foods have to be upgraded.

‡For sugar, only the pH and the glycemic index have been considered regarding the lack of fat.

§Because spicy has no lipid and no pH, the authors based the classification of this food on the literature. If the patients only eat industrial foods (ready-made food), the foods may be upgraded regarding the acidifying potential of industrial conservative.

REDS = Refluxogenic Diet Score.

TABLE II.
Categories of Refluxogenic Potential of Beverages.

Juice, Water, and Alcohol	pH	GI > 40	Cat.	UCat.
Alcohol (strong and liquor)*†	4	+	3	5
Aloe vera	6.1	0	2	2
Apple juice	3.65	+	4	5
Beer†‡	4	+	3	5
Cacao (hot chocolate)	6.3	+	2	3
Chamomile	6.5	0	2	2
Chicory	5.95	0	3	3
Coffee§	5	0	3	4
Grapefruit juice	3.05	+	4	5
Lemon juice	2.3	+	4	5
Multifruit juice	3.8	+	4	5
Orange juice	3.5	+	4	5
Soda (sugar free)‡	2.5	0	4	5
Soda (with sugar)‡	2.5	+	4	5
Syrup (mint, lemon, grenadine)	2.15	+	4	5
Tea§	5	0	3	4
Tea (blackberry)§	2.5	0	4	5
Tea (black)§	5.3	0	3	4
Tea (green)§	7	0	2	3
Tea (lemon)§	2.9	0	4	5
Tomato juice	4.35	0	3	3
Water (sparkling)‡	7	0	2	3
Water (still)	7	0	2	2
Water (alkaline)	8	0	1	1
Wine (red)†	4	0	4	5
Wine (rose)†	4	0	4	5
Wine (white)†	4	0	4	5

In practice, based on this table, laryngopharyngeal reflux patients selected the foods and beverages that they consume once or more over the past 2 weeks and the physician may add the categories corresponding of the consumed foods or beverages to get a score, called the Global Reflux Score. The classification of beverages depends on pH. For hot chocolate, the category is upgraded for additional sugar.

*GI high sugar-related osmolarity.

†The alcohol degree (>3% = upgrade).

‡Sparkling (upgrade).

§Presence or lack of caffeine or theine (upgrade or downgrade).

+ beverage exhibits a GI>40.

Cat. = category at baseline; GI = glycemic index; UCat. = upgraded category.

TABLE III.
Characteristics of Patients.

Characteristics	Value
Age, yr, mean ± SD (range)	47.5 ± 15.8 (20–75)
BMI, mean ± SD (range)	26.4 ± 6.2 (18.6–44.5)
Gender, no. (%)	
Male	20 (47.6%)
Female	22 (52.4%)
Gastrointestinal endoscopy (n = 28), no. (%)	
Normal	11 (39.3%)
Esophagitis (LA grading system)	6 (21.4%)
Los Angeles grade A	5 (17.9%)
Los Angeles grade B	1 (3.6%)
Los Angeles grade C	0 (0%)
Los Angeles grade D	0 (0%)
Hiatal hernia	7 (25.0%)
LES insufficiency	9 (32.1%)
Gastritis	8 (28.6%)
Duodenitis	2 (7.1%)
Helicobacter pilori infection	2(7.1%)
HEMII-pH, mean ± SD (range)	
Proximal reflux episodes (acid/nonacid)	20.3 ± 18.2/16.7 ± 21.8
Upright reflux episodes	30.5 ± 25.7
Recumbent reflux episodes	9.1 ± 14.6
DeMeester Score	20.9 ± 34.9
% of acid distal reflux	5.3 ± 11.5
GRES (pre/posttreatment)	50.7 ± 23.8/27.3 ± 23.2
RESDI, dinner before Peptest 1	24.6 ± 9.7 (10–54)
RESDI, lunch before Peptest 2	24.1 ± 11.4 (13–540
RESDI, dinner before Peptest 3	24.6 ± 9.7 (8–57)
Saliva pepsin level, mean ± SD (range)	
Morning	79.5 ± 91.4 (0–500)
After lunch	141.7 ± 133.0 (0–500)
After dinner	124.1 ± 119.9 (0–500)

BMI = body mass index; GRES = global refluxogenic score; HEMII-pH = multichannel intraluminal impedance-pH monitoring; LA = Los Angeles; LES = lower esophageal sphincter; RESDI = refluxogenic potential score of a dish; SD = standard deviation.

(RD Biomed, Hull, United Kingdom), which detects pepsin down to 16 ng/mL. If the results did not reach 16 ng/mL, the test was considered negative.

Treatment and Clinical Outcomes

The therapeutic algorithm was based on recent recommendations of the LPR Study Group of Young Otolaryngologists of the International Federation of Oto-Rhino-Laryngological Societies.[1] Based on the HEMII-pH characteristics of LPR (daytime, nighttime, acid, nonacid, or mixed LPR), patients received a personalized therapeutic scheme associating diet, behavioral changes, and use of proton pump inhibitors (PPIs) (pantoprazole) ± alginate (Gaviscon Advance; Reckitt Benckiser, Slough, United Kingdom) ± magaldrate (Riopan; Takeda, Zaventem, Belgium) for 3 months. Medication intake was evaluated posttreatment through a visual analog scale ranging from 0 ("I did not take the medication") to 10 ("I never forgot the medication"). Patients who did not take medication were excluded.

Symptoms and findings were assessed from pre- to posttreatment with the Reflux Symptom Score (RSS)[10] and Reflux Sign Assessment (RSA).[11] The RSS is a 22-item, validated patient-reported outcome questionnaire assessing frequency, severity, and the impact of LPR symptoms on quality of life (Fig. 1).[10] The RSA is a validated finding score rating both laryngeal and extralaryngeal signs associated with reflux (Fig. 2).[11] The RSA was rated by three blinded laryngologists (J.R.L., F.B., C.F.) regarding the pre- to posttreatment status (videolaryngostroboscopy recordings and oral cavity photos).

Diet Evaluation

At the first consultation, patients were invited to report their diet habits through two standardized diet grids describing both foods and beverages usually consumed in Western Europe (Tables I and II).[12] Western European foods and beverages were classified in five categories from "very low refluxogenic food/beverage" (category 1) to "very high refluxogenic food/beverage" (category 5). This classification of foods and beverages was based on the calculation of a score assessing the refluxogenic potential of foods and beverages (Refluxogenic Diet Score [REDS]). The REDS considers pH, fat, protein, sugar composition, and other specific factors.[12] From the patient anamnesis, the authors calculated the Global Refluxonic Score (GRES), which consists of the addition of REDS of foods and beverages that have been consumed by patients over the past 2 weeks. The refluxogenic potential of foods and beverages that have been consumed during the 24-hour HEMII-pH testing (and before the saliva sample collections) was evaluated through the Refluxogenic Score of a Dish (RESDI), which consists of the weighted sum of the REDS of foods and beverages consumed during a meal.[12] The RESDI may be calculated as absolute (sum of all RESDIs of the testing period) or mean (mean of all RESDIs of the testing period). At the end of the consultation, patients received a personalized diet grid identifying the foods and beverages to avoid (i.e., a diet therapeutic scheme).

Statistical Methods

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (SPSS version 22.0; IBM, Armonk, NY). The relationship between GRES, RESDI of meals preceding the saliva pepsin collection, HEMII-pH findings, pre- and posttreatment RSS, and RSA was analyzed through multiple linear regression. Pre- to posttreatment changes in RSS, RSA, and GRES were evaluated using the Wilcoxon signed rank test. A level of significance of $P < .05$ was used.

TABLE IV.
Evolution of Reflux Symptom Score Throughout Treatment.

RSS Items	Pretreatment	Posttreatment	*P* Value
Ear, nose, and throat symptoms			
1. Voice disorder	4.21 ± 6.45	3.31 ± 6.11	.121
2. Throat pain	7.40 ± 7.73	2.54 ± 4.35	.001
3. Pain during swallowing time	4.81 ± 6.84	1.04 ± 1.66	.003
4. Dysphagia	2.95 ± 4.71	.73 ± 1.66	.017
5. Throat clearing	8.95 ± 7.87	8.27 ± 8.75	.313
6. Globus sensation	8.64 ± 7.77	8.85 ± 9.92	.423
7. Excess throat mucus	11.21 ± 9.83	8.88 ± 9.80	.011
8. Ear pressure/pain	5.10 ± 7.40	3.38 ± 6.42	.010
9. Tongue burning	2.33 ± 5.75	1.38 ± 4.96	.293
Ear, nose and throat total score	56.71 ± 42.97	38.38 ± 32.56	.006
Digestive symptoms			
1. Heartburn	8.67 ± 8.50	3.27 ± 5.32	.011
2. Regurgitations or burps	4.21 ± 5.68	1.54 ± 2.55	.040
3. Abdominal pain	3.64 ± 6.63	2.19 ± 5.67	.059
4. Diarrheas	1.64 ± 3.67	1.23 ± 3.25	.163
5. Constipation	4.43 ± 7.51	2.88 ± 6.04	.277
6. Indigestion	2.36 ± 5.16	1.19 ± 5.53	.444
7. Abdominal distension/flatus	6.05 ± 7.24	4.15 ± 6.89	.097
8. Halitosis	7.14 ± 8.55	3.63 ± 6.84	.006
9. Nausea	2.74 ± 5.17	1.50 ± 4.99	.181
Digestive total score	40.90 ± 31.64	21.58 ± 31.60	.005
Respiratory symptoms			
1. Cough after eating/lying down	5.19 ± 6.63	2.46 ± 4.76	.001
2. Cough	5.17 ± 7.14	2.92 ± 6.69	.021
3. Breathing difficulties	1.62 ± 4.08	2.00 ± 5.19	.953
4. Chest pain	5.21 ± 7.65	3.15 ± 6.01	.208
Respiratory total score	17.21 ± 17.81	10.54 ± 15.27	.003
RSS total score	114.60 ± 77.36	70.50 ± 63.67	.001

RSS = Reflux Symptom Score.

RESULTS

A total of 42 patients completed the evaluations. The characteristics of patients are described in Table III. There were 20 acid, 13 mixed, and nine non-acid LPRs. Twenty-four patients (57.1%) had both LPR and gastroesopheal reflux disease (GERD) according to the Montreal criteria.[13] HEMII-pH findings, GI endoscopy characteristics, GRES, RESDI, and saliva pepsin levels are reported in Table I. Note that no patient had heterotopic gastric mucosa in the esophagus. Regarding HEMII-pH, 82.4% of pharyngeal reflux episodes occurred in the daytime and while upright. The mean pretreatment GRES significantly decreased posttreatment ($P = .01$), meaning that patients generally respected diet advices.

Clinical Evolution

The mean RSS total and subscores (otolaryngological, digestive, and respiratory RSS) significantly decreased from pre- to posttreatment (Table IV). The RSA total score significantly decreased from pre- to posttreatment (Table V). The pre- to posttreatment decreases of RSA subscores were significant for oral, pharyngeal and laryngeal subscores. There were no vocal lesions (e.g., nodules, polyps, granuloma) in our cohort.

Associations Between Diet, Peptest, and Clinical Outcomes

According to the multiple linear regression analysis, the morning level of saliva pepsin was significantly associated with foods and beverages (RESDI) consumed during the previous evening dinner ($r_s = 0.552$, $P < .001$). In the same vein, the concentration of saliva pepsin after dinner of the testing day was significantly associated with 1) the dinner RESDI of the testing day ($r_s = 0.547$, $P < .001$), 2) the mean RESDI of all meals consumed during the 24-hour testing period ($r_s = 0.647$, $P = .001$), and 3) the absolute RESDI considering the addition of RESDI of the dishes of the testing day ($r_s = 0.426$, $P = .024$). When considering the 24-hour mean level of saliva pepsin, we found a significant positive association between the 24-hour mean level of saliva pepsin and the mean RESDI of the testing period (i.e. dinner of the day before and the meals of the testing day [$r_s = 0.414$, $P = .004$]).

TABLE V.
Evolution of Reflux Sign Assessment Throughout Treatment.

Reflux Sign Assessment	Pretreatment	Posttreatment	*P* Value
Oral cavity findings			
Anterior pillar erythema	2.39 ± 1.91	2.70 ± 1.87	.748
Uvula erythema ± edema	1.52 ± 1.46	1.42 ± 1.35	.474
Coated tongue	1.22 ± 0.89	1.31 ± 0.87	.202
Oral cavity subscore	5.75 ± 3.03	4.31 ± 2.36	.019
Pharyngeal findings			
Posterior oro- or hypopharyngeal wall erythema	2.92 ± 1.65	1.88 ± 1.90	.229
Posterior oro- or hypopharyngeal wall inflammatory granulations	0.90 ± 1.70	0.30 ± 0.75	.527
Tongue tonsil hypertrophy	2.18 ± 1.33	2.28 ± 1.24	.258
Contact between epiglottis and tongue tonsils	2.25 ± 1.90	2.74 ± 1.79	.269
Pharyngeal sticky mucus	2.03 ± 1.87	1.70 ± 1.88	.501
Pharyngeal cavity subscore	10.46 ± 4.42	6.32 ± 3.29	.001
Laryngeal findings			
Sub- and supraglottic areas			
Ventricular band erythema ± edema	0.93 ± 0.92	1.04 ± 0.88	.076
Epiglottis redness ± edema	1.33 ± 1.41	0.34 ± 0.92	.016
Posterior commissure			
Commissure posterior/arytenoid erythema	3.12 ± 1.67	1.60 ± 2.08	.008
Interarytenoid granulatory tissue	0.31 ± 0.69	0.12 ± 0.44	.276
Posterior commissure hypertrophy	3.27 ± 2.08	1.78 ± 2.05	.142
Retrocricoid erythema	0.49 ± 1.10	0.24 ± 0.71	.131
Retrocricoid edema	1.15 ± 1.67	1.22 ± 1.78	.788
Vocal folds			
Endolaryngeal sticky mucus deposit	0.87 ± 1.22	1.13 ± 1.36	.586
Vocal fold lesions	0.00 ± 0.00	0.00 ± 0.00	1.00
Laryngeal subscore	10.74 ± 5.87	5.71 ± 4.05	.004
RSA total	23.03 ± 9.41	16.24 ± 6.49	.012

RSA = Reflux Sign Assessment.

The mean level of saliva pepsin of the testing day was more significantly associated with RESDI of both dinner of the day before and the first meals of the testing day than the RESDI of the last meal of the testing day (dinner, $P = .022$).

The saliva pepsin level in the morning (first sample) was a negative predictive factor of the posttreatment reduction of RSS ($r_s = -0.518$, $P = .035$), laryngeal RSA ($r_s = -0.720$, $P = .003$), and RSA total score ($r_s = -0.665$, $P = .007$). In other words, patients with a high level of saliva pepsin in the morning had a lower therapeutic clinical response compared with patients with a low saliva pepsin concentration.

DISCUSSION

The role of diet in the development of LPR has been studied intensively in recent years,[7,14–16] but currently, the impact of diet on the results of the diagnostic approaches, such as HEMII-pH or Peptest, remain poorly investigated. A recent study found that the consumption of high-fat, low-protein, high-sugar, acidic foods and beverages was associated with a high number of pharyngeal reflux episodes at the HEMII-pH.[7] The highlight of this association was possible through the recent development of clinical scores assessing the refluxogenic potential of foods and beverages of LPR patients.[12]

First, the main result of the present study supports the existence of a relationship between refluxogenic foods and beverages and the deposit of pepsin in the mucosa of the upper aerodigestive tract. However, this relationship seems to be more complex than appears at first sight. If the 24-hour concentration of saliva pepsin was strongly associated with the consumed meals, the consumption of a refluxogenic meal did not necessary lead to an immediate postmeal increase of saliva pepsin concentration. In the same way, there are no associations between the measured saliva pepsin concentration and the HEMII-pH characteristics (number and duration of hypopharyngeal reflux episodes). These data suggest that the increase of the saliva pepsin concentration would be more subtle than initially presumed, and could involve both extra- and intracellular pepsin. Johnston et al. demonstrated that a substantial proportion of refluxate pepsin may be internalized in the mucosa cells, reactivated in the Golgi apparatus, and externalized a second time.[3] According to this mechanism, it seems conceivable that the level of the saliva pepsin, which is measured by the Peptest, reflects only a certain proportion of the refluxate pepsin over the past few hours/days. This hypothesis could explain the lack of significant association between the saliva pepsin concentration and the number and duration of pharyngeal reflux episodes.

To better understand the Peptest results, the study of the variation of the saliva pepsin concentration throughout the 24-hour day may be linked to a detailed study of the profile of the occurrence of pharyngeal reflux episodes at the 24-hour HEMII-pH. In other words, it would be interesting to better determine what pharyngeal reflux episodes do determine the saliva pepsin concentration at a given point of time.

Second, our data revealed that the morning Peptest was significantly associated with the intake of refluxogenic foods and beverages during the dinner of the day before. Because the majority of pharyngeal reflux episodes occur the daytime and while upright,[17,18] the pepsin would be mainly deposited in the daytime in the upper aerodigestive tract mucosa, making its concentration variable throughout the day regarding the numerous pharyngeal episodes and the cell internalization mechanism. The level of saliva pepsin in the morning would further reflect the quantity of refluxate pepsin of the last 12 to 24 hours, which may be associated with the foods and beverages consumed for dinner the day before. This explanation makes particular sense in the context of the lack of or low number of nighttime pharyngeal reflux episodes in the majority of patients, and our results show an association between the mean pepsin concentration and the absolute RESDI of the testing day. It is important to note that this association does not consider one isolated meal, but the addition of many foods and beverages consumed over the past 12 to 24 hours. This additional observation makes it conceivable that the saliva pepsin concentration requires many hours to increase after the consumption of a refluxogenic diet and the occurrence of pharyngeal reflux episodes. This hypothesis is strengthened by recent data that did not observe a significant association between the level of saliva pepsin and the pharyngeal reflux episodes occurring in the 2 hours before the saliva collection.[9]

The last significant result of the present study is the potential negative predictive value of morning saliva pepsin concentration on the therapeutic response. Patients with a higher saliva pepsin concentration in the morning would have a stronger inflammatory reaction in the upper aerodigestive tract mucosa, which could require more time to cure. This explanation is, however, counterbalanced by the lack of significant association between saliva pepsin concentration and the severity of both symptoms and findings.[7,19]

Hope has been placed in the development of the Peptest as a noninvasive diagnostic tool for LPR. However, currently, the reliability of the Peptest is still controversial, and both sensitivity and specificity remain low.[5,6] The results of the present study do not contraindicate the use of the Peptest but shed light the possible impact of the patient's diet on the Peptest's results. Not surprisingly, the majority of otolaryngologists usually observe that diet plays a critical role in the development of LPR.[20] It is probable that LPR patients have a more refluxogenic diet than healthy individuals, and consequently, a higher saliva pepsin level.

The role of diet on the saliva pepsin concentration has to be elucidated in future controlled studies considering LPR patients and healthy individuals. Because the acid expression is higher after meals, future studies could investigate the relationship between saliva pepsin concentration and acid secretion after meals.

The lack of a control group is the main weakness of the present study, but it is due to the difficulty to utilize HEMII-pH in healthy subjects because of the cost of the technique and the inconveniences associated with the

probe in subjects without a complaint. Moreover, the HEMII-pH is not a gold standard, and therefore, it is possible that a few false-positive patients have been included in this study.

Because pepsin is probably not the only refluxate enzyme involved in the development of the inflammatory reaction of the upper aerodigestive tract mucosa, future studies may also consider the measurement of other gastroduodenal enzymes (e.g., trypsin, elastase, lipase, or amylase).

CONCLUSION

The diet of the LPR patient may have a significant impact on the saliva pepsin concentration measured with the Peptest. The saliva sample in the morning would be the more representative sample of the mean level of the refluxate pepsin in the upper aerodigestive tract mucosa over the previous hours. The level of pepsin in the morning would be a negative predictive factor of the therapeutic response. Future controlled studies are needed to determine the place and the usefulness of the Peptest in the management of LPR.

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