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Evaluation of metabolic profile and C-reactive protein concentrations in brachycephalic dogs with upper airway obstructive syndrome

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Abstract

Background: Brachycephalic dogs have abnormal breathing patterns similar to those in humans with obstructive sleep apnea syndrome. Obstructive sleep apnea syndrome is associated with dyslipidemia, hyperglycemia, and insulin resistance. Despite the fact that anatomic and functional alterations are well described in brachycephalic dogs, little is known about the consequences of upper airway obstruction on systemic inflammatory response and metabolic profile.

Objectives: To describe history, clinical presentation, and anatomic abnormalities; to evaluate systemic inflammatory response and metabolic profile; and to identify possible associations among clinical signs, anatomic abnormalities, inflammatory response, and metabolic profile in brachycephalic dogs with airway obstruction.

Animals: Thirty purebred brachycephalic dogs with brachycephalic airway obstructive syndrome (BAOS).

Methods: Prospective study. The following information was recorded and studied: respiratory and digestive signs, airway and digestive endoscopic anomalies, presence or absence of tracheal hypoplasia, histologic evaluation of gastrointestinal tract biopsy specimens, serum concentrations of C-reactive protein (CRP), fructosamine, insulin, glucose, triglyceride, cholesterol, and plasma concentrations of lipoprotein classes.

Results: A high proportion of dogs (76.7%) had gastrointestinal signs. Esophageal deviation, atony of the cardia of the stomach, and distal esophagitis were the most common endoscopic anomalies detected. Twenty-six (86.6%) dogs had different degrees of laryngeal collapse. Gastrointestinal histologic evaluation identified mostly chronic inflammation. Glucose, fructosamine, triglycerides, cholesterol, CRP, pre-beta, beta lipoproteins, and chylomicrons were increased to a variable extent. Significant associations among clinical signs, anatomic abnormalities, CRP, and metabolic profile were not found.

Abbreviations: BAOS, brachycephalic airway obstructive syndrome; BCS, body condition score; CRP, C-reactive protein; HDL, high-density lipoproteins; IH, intermittent hypoxia; LDL, low-density lipoproteins; OSAS, obstructive sleep apnea syndrome; SDB, sleep-disordered breathing; VLDL, very low-density lipoproteins.

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Conclusion and Clinical Importance: Despite the presence of inflammation and some mild metabolic derangements, the clinicopathological variables evaluated did not offer valuable information in dogs with BAOS.

KEY WORDS

cholesterol, fructosamine, glucose, plasma lipoprotein electrophoresis, triglycerides

1 | INTRODUCTION

Brachycephaly, or a shortened facial structure, is a common condition of dogs that is magnified by selective breeding. Breeds most commonly affected by brachycephalic airway obstructive syndrome (BAOS) are English and French Bulldogs, Pugs, and Boston Terriers.¹ Most dogs with this condition develop BAOS, a chronic upper airway obstruction clinically characterized by heat stress and exercise intolerance, snoring, inspiratory dyspnea, cyanosis, and, in more severe cases, syncopal episodes.² In addition, gastrointestinal signs such as dysphagia, vomiting, and regurgitation can develop as a consequence of the negative intrathoracic pressure generated by increased respiratory effort and aerophagia, and worsen upper esophageal, pharyngeal, and laryngeal inflammation.²⁻⁴ Excessive flatulence as a consequence of aerophagia also is often present. French Bulldogs often have more frequent and severe digestive signs than do Pugs.⁴⁻⁷

Because medical management may only temporarily improve clinical signs and provide palliation, permitting progression of the disease to a more advanced stage,⁸ surgical treatment consisting of rhinoplasty, turbinectomy, staphylectomy, laryngeal sacculectomy, or some combination of these often is required.^{9,10} Brachycephalic airway obstructive syndrome shares features of obstructive sleep apnea (OSA) in people, a form of sleep-disordered breathing (SDB) characterized by recurrent collapse of the upper airway during sleep leading to intermittent hypoxia (IH), oxygen desaturation, sleep fragmentation, and arousal from sleep.^{11,12} Obstructive sleep apnea is prevalent in obese individuals,¹³ and is associated with decreased survival as a result of development of cardiovascular and thromboembolic disorders.^{11,14} Moreover, growing epidemiological evidence suggests that OSA is linked to metabolic abnormalities, such as hyperglycemia, insulin resistance, type 2 diabetes, and dyslipidemia.^{15,16} Although spontaneous OSA is very uncommon in animals, the English Bulldog has been used as an animal model of OSA because of its disordered breathing and episodes of oxygen desaturation associated with abnormal upper airway anatomy.^{17,18} Despite the fact that anatomic and functional alterations are well described in brachycephalic dogs,^{1-9,18-22} little is known about the consequences of upper airway obstruction on the systemic inflammatory response, parameters of glucose regulation, and lipid metabolism.²²⁻²⁴ Thus, our prospective study was designed to: (1) describe history, clinical presentation, and anatomic abnormalities; (2) evaluate the systemic inflammatory response, parameters of glucose regulation, and lipid profiles; and (3) identify possible associations among clinical signs, anatomic abnormalities, inflammatory response, parameters of glucose

regulation, and lipid profiles in a series of brachycephalic dogs with BAOS.

2 | MATERIALS AND METHODS

Purebred brachycephalic dogs with BAOS presented to different veterinary teaching hospitals and referral clinics over a 2-year period were included in the study. At admission, each dog's case history was obtained from the owner. The frequency and nature of upper respiratory (eg, snoring, inspiratory efforts, stress or exercise intolerance, and syncope) and digestive (eg, ptyalism, regurgitation, and vomiting) signs were assessed according to a previously described grading system.²⁵ The frequency of both respiratory and digestive signs was classified as follows: never, occasionally (less than once monthly), regularly (once weekly), daily (once daily), often (more than once daily), and constantly. Inclusion of at least 1 sign in a higher grade determined the classification assigned. On the basis of the frequency of each respiratory sign, a global classification of 3 grades was assigned: grade 1 (absent or minimal), grade 2 (moderate), and grade 3 (severe). The same was done for the frequency of each digestive sign.²⁵ For each dog, a final symptomatic score from the global classifications of both upper respiratory and digestive signs was obtained. In addition to upper respiratory and digestive signs, SDB history (eg, nighttime arousals, respiratory signs and attitude after arousals, owner's perception that sleep disruption was caused by airway obstruction, and abnormal sleeping positions) was collected for each dog. The information about abnormal sleeping positions was retrospectively gleaned by telephone interview at the time of the manuscript drafting. Dogs were classified according to the presence (score 1) or absence (score 0) of at least 1 clinical sign or abnormal attitude during sleep. The frequency of nighttime arousals was classified arbitrarily as follows: never (score 0), mild (1-3 times per night; score 1), moderate to severe (>4 times per night; score 2). A clinical examination was performed, body weight was recorded, and a 9-point body condition score (BCS) was assigned.²⁶ Physical examination and cardiac auscultation findings, echocardiographic and radiographic abnormalities, and blood test results provided evidence that no other clinically relevant diseases were present in dogs included in the study.

Aliquots of serum and plasma obtained from centrifugation of blood collected from each dog for preanesthetic investigations were separated and immediately stored at -20°C for biomarker analysis. Sample storage varied from 1 to 5 days. Biomarker analyses were performed in 2 different laboratories (BiEsseA S.r.l., Milan, Italy, and Ematos Vet Lab S.r.l., Rome, Italy) and included evaluation of serum

concentrations of fructosamine, insulin, and glucose (BiEsseA S.r.l.), and total protein, C-reactive protein (CRP), triglycerides, and cholesterol (Ematos Vet Lab S.r.l.). In addition, plasma lipoprotein agarose gel electrophoresis (Hydrasis, Sebia, UK Ltd) was performed using a dedicated kit (Hydragel protein, Sebia, UK Ltd).

Upon signed owner consent for diagnostic and therapeutic procedures, all dogs underwent presurgical endoscopic evaluation of the airways (nares, rhino-pharynx and oro-pharynx, larynx, trachea, and bronchi) and, if digestive signs were present, the esophagus, stomach, and duodenum. Premedication protocols were decided on a case-by-case basis (.2 mg/kg methadone alone or in combination with 10 µg/kg acepromazine). Preoxygenation was provided for 5 minutes before endoscopy using a face mask. General anesthesia was induced with 2-4 mg/kg propofol IV and maintained by gas anesthesia (isoflurane or sevoflurane in 100% oxygen). Intravenous methylprednisolone sodium succinate (1 mg/kg) was given to control laryngeal edema at anesthesia induction. All endoscopic procedures were performed in standardized fashion by 3 of the authors (C.R., B.E., and P.M.).²⁷⁻²⁹ In the event of gastrointestinal endoscopic evaluation, mucosal biopsy samples were collected and submitted for histologic evaluation. Rigid (2.7 mm × 18 cm, 30°; model 64 029, Karl Storz Endoscopia Italia S.r.l., Verona, Italy) and flexible (6.0 mm × 103 cm; EG-1840, Pentax Italia S.r.l., Milano, Italy; 7.8 mm × 140 cm, model PV-SG 28-140, Karl Storz Endoscopia Italia S.r.l., Verona, Italy; 5.0 mm × 55 cm fiberscope; Olympus BF-P40, Olympus Medical Systems Europe GmbH, Hamburg, Germany) video endoscopes were used. Images and movies were acquired using video recording devices (Pinnacle Studio 21.5; Corel Corporation, Ottawa, Canada; Tele Pack Vet X Led, Karl Storz Endoscopia Italia S.r.l.).

Balanced isotonic crystalloid fluids were administered IV to all dogs during the entire procedure; ECG, blood pressure, and pulse oximetry were monitored continuously. During recovery, supplemental oxygen was provided as needed. The definitive diagnosis of BAOS was made by combining both upper airway respiratory signs and anatomic abnormalities, as has been described elsewhere.³⁰ Laryngeal paralysis (unilateral or bilateral) was defined as a total lack of abduction of the corniculate processes of the arytenoids during inspiration.

To describe anatomic abnormalities, a quantitative anatomical scoring system was used by evaluating the following variables recorded at presentation and at the time of radiography and endoscopy: tracheal hypoplasia (tracheal diameter to thoracic inlet ratio < 0.16),³¹ tracheal collapse, bronchial collapse, turbinate hypertrophy, stenotic nares, macroglossia or elongated tongue, elongated soft palate, thickened soft palate, nasopharyngeal collapse, altered rhinopharyngeal mucosa (hypertrophy, erosions, erythema), increased laryngopharyngeal secretions, laryngopharyngeal erosions, laryngopharyngeal nodules, and hypertrophy and eversion of the tonsils. Each variable was classified as present (score 1) or absent (score 0). A final quantitative anatomical score was obtained by the sum of each single score. In addition, the extent of laryngeal collapse was assessed endoscopically as mild (eversion of laryngeal saccules, first degree, score 1), moderate (medial displacement or overlap of the cuneiform processes, second degree, score 2), or severe (medial displacement or overlap of the corniculate processes, third degree, score 3), based on a previous classification.³²

2.1 | Statistical analysis

Preliminarily, the symptomatic score was dichotomized on the basis of the median value (≤ 4 versus ≥ 5) and a 2-stage analysis was applied. In the first stage, all of the variables were tested using the χ^2 test. Quantitative data were divided arbitrarily into 2 categories based on the medians of values. The proportion of dogs with symptomatic score ≥ 5 was evaluated by breed, sex, BCS, body weight, age, mean arterial pressure (mm Hg), percent saturation of oxygen (SpO_2), SDB history (ie, nighttime arousals, respiratory signs and attitude after arousals, owner's perception that sleep disruption was caused by airway obstruction, abnormal sleeping positions, presence of at least 1 clinical sign or abnormal attitude during sleep regardless of the type of clinical sign or abnormal attitude), quantitative anatomical score, laryngeal collapse score, serum concentrations of cholesterol (mg/dL), triglycerides (mg/dL), total protein (g/dL), glucose (mg/dL), insulin (μ U/mL), CRP (mg/dL), and fructosamine (μ mol/L), and plasma pre-alpha (%), alpha 1 (%), alpha 2 (%), pre-beta (%), beta (%), and chylomicrons (%) lipoprotein classes.

In the second stage, factors that had P values $< .20$ then were evaluated using binary logistic regression. The model was based on the simultaneous entry of all variables, and its efficacy was assessed based on the likelihood ratio and the Hosmer-Lemeshow statistic. Values of $P < .05$ were considered significant. The odds ratio (OR) and 95% confidence intervals (95% CIs) were calculated from the final binary logistic model.

All statistical analyses were performed using statistical software (IBM SPSS 25.0.0, Armonk, New York).

3 | RESULTS

3.1 | Signalment, clinical signs, and clinicopathologic data

Thirty dogs affected by BAOS were included in the study, subdivided as follow: 10 Pugs (33.3%), 13 French Bulldogs (43.3%), and 7 English Bulldogs (23.3%). Twenty-two dogs were intact males and 8 were females (4 spayed). The median age was 36.4 months (range, 10-93). The median body weight was 13.4 kg (range, 6-34). The median BCS was 6.6 (range, 5-8). The most common presenting respiratory clinical signs were snoring (28 dogs, 93.3%), increased inspiratory efforts (28 dogs, 93.3%) and stress or exercise intolerance (28 dogs, 93.3%), and syncope (7 dogs, 23.3%). Among the dogs with snoring, 16 (57.1%) snored constantly, whereas 12 (42.9%) snored with a frequency equal to or more than once daily. Among the dogs with increased inspiratory efforts, 12 (42.9%) were affected constantly, 12 (42.9%) with a frequency equal to or more than once daily, 2 (7.1%) regularly, and 2 (7.1%) occasionally. Among the dogs with stress or exercise intolerance, 5 (17.9%) had it constantly, 19 (67.9%) with a frequency equal to or more than once daily, 1 (3.6%) regularly, and 3 (10.7%) occasionally. Among the dogs with syncope, 4 (66.7%) fainted regularly and 3 (42.6%) occasionally. For 1 dog, the frequency of respiratory signs could not be precisely defined. Twenty-three (76.7%) dogs had gastrointestinal clinical signs in addition to respiratory clinical signs. Nineteen

(76%) dogs had vomiting, 2 (10.5%) with a frequency equal to or more than once daily, 8 (42.1%) regularly, and 9 (47.4%) occasionally. Fourteen dogs (60.9%) had regurgitation, 1 (7.1%) of which regurgitated constantly, 3 (21.4%) with a frequency more than once daily, 3 (21.4%) regularly, and 7 (50%) occasionally. Eleven (47.8%) dogs had ptalism, 1 (9.1%) of which at a frequency of once daily, 5 (45.4%) regularly, and 5 (45.4%) occasionally. Twenty (66.7%) dogs experienced nighttime arousals, with mild and moderate to severe frequency in 12 (60%) and 8 (40%) dogs, respectively. For 17 (85%) dogs, the owner's perception was that disruption of sleep was caused by the airway obstruction. Three (15%) dogs had coughing bouts after arousal, 3 (15%) panting and pacing at night, 2 (10%) loud snoring, apnea followed by abrupt arousal and reverse sneezing, and 1 (5%) reverse sneezing. Two (10%) dogs assumed abnormal sleeping positions with elevated chin. Signalment, respiratory and digestive clinical signs, and SDB history can be found in Supporting Information. Tables 1 and 2 show clinicopathologic data. Results of the univariate and logistic regression analysis are found in Tables 3 and 4, respectively.

Odds ratio of symptomatic score ≥ 5 was not significantly associated with the factors evaluated.

3.2 | Evaluation of airway anatomic abnormalities and quantitative scoring system

Twenty-nine dogs underwent airway endoscopic evaluation. In 1 dog, because of clinical sign severity, endoscopic evaluation was not completed,

and was followed by tracheal stent placement and surgical correction of BAOS. Twenty-nine (100%) dogs had stenotic nares and elongated soft palate, 21 (72.4%) turbinate hypertrophy, 19 (65.5%) macroglossia, 17 (58.6%) increased laryngopharyngeal secretions, 16 (55.2%) thickened soft palate, 13 (44.8%) nasopharyngeal collapse, 11 (37.9%) hypertrophy and eversion of the tonsils, 8 (27.6%) altered rhinopharyngeal mucosa, 6 (20.7%) laryngopharyngeal nodules, and 4 (13.8%) tracheal collapse, tracheal hypoplasia, and bronchial collapse. Laryngopharyngeal erosions were not observed. Normal laryngeal function was observed in all dogs. Twenty-six (86.6%) dogs had laryngeal collapse, which was evaluated as grade 3, 2, and 1 in 2 (7.7%), 15 (57.7%), and 9 (34.6%) dogs, respectively. The remaining 3 dogs had a larynx without any degree of collapse. The median final quantitative anatomical score was 6 (range, 4-9). Results of univariate analysis of quantitative anatomical score, SDB history, and laryngeal collapse score showed no statistically significant differences ($P > .05$, Table 3).

3.3 | Digestive endoscopic anomalies and histological evaluation

Gastroduodenal endoscopy was performed in 21 (91.3%) of 23 dogs that had gastrointestinal clinical signs in addition to respiratory clinical signs. In the remaining 2 dogs, gastroduodenal endoscopy was not performed because of lack of owner consent.

Overall, all 21 dogs had ≥ 1 endoscopic esophageal, gastric, or duodenal anomalies. Esophageal deviation, atony of the cardia of the

TABLE 1 Cholesterol, triglyceride, glucose, total protein, fructosamine, insulin, and C-reactive protein serum concentration results

Variables	Median values (range)	Mean values ($\pm SD$)	Normal values % (n/t)	Values above the R.R. % (n/t)	Values below the R.R. % (n/t)
Cholesterol	210.5 mg/dL (101-373)	221.8 mg/dL (± 68.3)	73.4 (22/30)	20 (6/30)	6.6 (2/30)
Triglyceride	88.5 mg/dL (29-208)	101.2 mg/dL (± 50.0)	67.9 (19/28)	32.1 (9/28)	-
Glucose	100.5 mg/dL (72-227)	102.6 mg/dL (± 27.8)	85.7 (24/28)	14.3 (4/28)	-
Total protein	5.6 g/dL (4.5-7.3)	5.7 g/dL (± 0.8)	65.5 (19/29)	-	34.5 (10/29)
Fructosamine	237 μ mol/L (100-329)	241.5 μ mol/L (± 52.1)	64.3 (18/28)	32.1 (9/28)	3.6 (1/28)
Insulin	1.9 μ U/mL (1.9-10.8)	2.5 (± 2.0)	7.4 (2/27)	-	92.6 (25/27)
C-reactive protein	0.6 mg/dL (0.0-19.10.10)	1.7 mg/dL (± 3.8)	48.1 (13/27)	51.9 (14/27)	-

Abbreviations: n, number of dogs in which the variable was normal, above, or below the reference range; R.R., reference range; t, total number of dogs.

TABLE 2 Plasma lipoprotein electrophoresis results

Lipoprotein classes (R.R.)	Median values (range)	Mean values ($\pm SD$)	Normal values % (n/t)	Values above the R.R. % (n/t)	Values below the R.R. % (n/t)
Pre-alfa (α 1) (8.2-2.5%)	1.8% (0.9-9.1)	2.3% (± 1.8)	80 (16/20)	20 (4/20)	-
Alfa 1 (α 1) (30.9-40.6%)	32.6% (25.6-43)	32.7% (± 3.7)	75 (15/20)	5 (1/20)	20 (4/20)
Alfa 2 (α 2) (21.5-35.0%)	25.2% (17.5-33.6)	25.8 (± 4.5)	85 (17/20)	-	15 (3/20)
Pre-beta (β 1) (4.9-11.0%)	12.9% (5.4-23.6)	13.8 (± 5.9)	40 (8/20)	60 (12/20)	-
Beta (β) (11.1-19.0)	19.7% (10.8-26.0)	18.7 (± 4.7)	40 (8/20)	55 (11/20)	5 (1/20)
Chylomicrons (κ) (1.3-3.4)	5.7% (1.6-16.2)	6.7 (± 4.1)	35 (7/20)	65 (13/20)	-

Abbreviations: n, number of dogs in which the lipoprotein class was normal, above, or below the reference range; R.R., reference range; t, total number of dogs.

TABLE 3 Results of univariate analysis

Variables	Symptomatic score			Total	P
	Score ≤ 4	Score ≥ 5			
Breed	French Bulldogs	6	7	13	.75
	English Bulldogs	2	5	7	
	Pugs	4	6	10	
Sex	Male	8	14	22	.68
	Female	4	4	8	
BCS	≤6	7	6	13	.26
	>6	5	12	17	
Body weight (kg)	≤11.7	7	8	15	.71
	>11.7	5	10	15	
Age (months)	≤36	6	12	18	.46
	>36	6	6	12	
MAP (mm Hg)	≤70	7	3	10	.05*
	>70	5	15	20	
SpO ₂	≤98	7	4	11	.06*
	>98	5	14	19	
Quantitative anatomical score	≤6	8	9	17	.47
	>6	4	9	13	
Nighttime arousal	Never	5	5	10	.72
	Mild	4	8	12	
	Moderate/severe	3	5	8	
Respiratory signs and attitude after arousals	Present	10	11	11	.25
	Absent	2	7	9	
Owner's perception that sleep disruption was caused by airway obstruction	Present	7	6	13	.26
	Absent	5	12	17	
Abnormal sleeping positions	Present	12	16	28	.50
	Absent	0	2	2	
Presence of at least 1 clinical sign or abnormal attitude during sleep	Yes	5	5	10	.46
	No	7	13	20	
Laryngeal collapse score	≤2	5	7	12	1
	>2	7	10	17	
Cholesterol (mg/dL)	≤210.5	8	7	15	.26
	>210.5	4	11	15	
Triglyceride (mg/dL)	≤88.5	8	7	15	.26
	>88.5	4	11	15	
Total protein (g/dL)	≤5.6	5	10	15	.71
	>5.6	7	8	15	
Glucose (mg/dL)	≤100.5	6	8	14	1
	>100.5	6	10	16	
C-reactive protein (mg/dL)	≤0.55	7	7	14	.46
	>0.55	5	11	16	
Fructosamine (μmol/L)	≤237	6	8	14	1
	>237	6	10	16	
Insulin (μU/mL)	≤1.9	9	14	23	1
	>2.0	3	4	7	

(Continues)

TABLE 3 (Continued)

Variables		Symptomatic score		Total	P
		Score ≤ 4	Score ≥ 5		
Pre-alpha (%)	≤1.80	7	5	12	.14*
	>1.80	5	13	18	
Alpha 1 (%)	≤32.60	6	4	10	.14*
	>32.60	6	14	20	
Alpha 2 (%)	≤25.25	3	7	10	.69
	>25.25	9	11	20	
Pre-beta (%)	≤12.95	5	5	10	.46
	>12.95	7	13	20	
Beta 1 (%)	≤19.75	5	5	10	.46
	>19.75	7	13	20	
Chylomicrons (%)	≤5.70	4	6	10	1
	>5.70	8	12	20	

Abbreviations: BCS, body condition score; MAP, mean arterial pressure; SpO₂, percent saturation of oxygen.

*Statistical association P < .20.

TABLE 4 Results of logistic regression analysis

Factors	Dogs with symptomatic score ≥ 5/total (%)	OR	95% CI for OR	P
MAP				
≤70	3/10 (30.0)	-		
>70	15/20 (75.0)	4.45	0.31-64.87	.27
SpO ₂				
≤112	4/11 (36.4)	-		
>112	14/19 (73.7)	1.02	0.07-14.00	.99
Pre-alpha				
≤2.46	5/12 (41.7)	-		
>2.46	13/18 (72.2)	2.16	0.46-14.72	.28
Alpha 1				
≤32.60	4/10 (40.0)	-		
>32.60	14/20 (70.0)	1.97	0.32-12.15	.46
Constant		0.01		.03

Abbreviations: MAP, mean arterial pressure; SpO₂, percent saturation of oxygen.

stomach, distal esophagitis, and precardial erosions were observed in 9 (42.9%), 3 (14.3%), 7 (33.3%), and 2 (9.5%) of dogs, respectively. Gastric stasis, pyloric mucosal hyperplasia, duodenogastric reflux, diffuse erythema, punctiform inflammation, erosions, and edema were observed in 6 (28.6%), 4 (19%), 1 (4.8%), 7 (33.3%), 3 (14.3%), 1 (4.8%), and 10 (47.6%) dogs, respectively. Diffuse duodenal erythema, edema, mucosal irregularity, and whitish tips of swollen villi were observed in 16 (76.2%), 2 (9.5%), 9 (42.9%), and 2 (9.5%) dogs, respectively.

Mucosal biopsy samples were collected from all dogs undergoing gastroduodenal endoscopy and submitted for histologic evaluation. Histologic examination was performed in all dogs according to a quantitative simplified scoring system.³³

Lymphocytic-plasmacytic inflammation and squamous metaplasia of the esophagus were found in 1 (4.8%) and 1 (4.8%) dogs,

respectively. Lymphocytic-plasmacytic inflammation, erosions with neutrophilic inflammation, fibrosis, and mucosal hyperplasia of the stomach were found in 12 (57.1%), 2 (9.5%), 2 (9.5%), and 1 (4.8%) dogs, respectively. Lymphocytic-plasmacytic inflammation, neutrophilic inflammation, lacteal dilatation, and epithelial injury of the duodenum were found in 10 (47.6%), 7 (33.3%), 9 (42.9%), and 1 (4.8%) dogs, respectively.

The anatomical lesions observed can be found in the Supporting Information.

4 | DISCUSSION

Our study provides a systematic description of the clinical presentation, as well as clinicopathological and airway anatomic abnormalities

in a population of brachycephalic dogs with BAOS, and explores their possible association with systemic inflammatory response, parameters of glucose regulation, and lipid profiles.

Obstructive sleep apnea describes recurrent collapse of the upper airway during sleep leading to IH.¹¹ Growing evidence from animal models of OSA suggests that IH is independently associated with metabolic dysfunction, including dyslipidemia and insulin resistance.³⁴

The precise mechanisms by which IH induces metabolic disturbances are not fully understood. Dyslipidemia may be caused by excessive lipolysis supplying free fatty acids to the liver, upregulation of hepatic triglyceride biosynthesis, and lipoprotein secretion and suppression of lipoprotein clearance.³⁵ Insulin resistance may be caused by activation of hepatic lipid biosynthesis, activation of the sympathetic nervous system with consequent lipolysis, the hypothalamic-pituitary-adrenal axis, and systemic inflammation.^{34,36}

Although BAOS in brachycephalic dogs is not identical to OSA in humans, important similarities exist and, because English Bulldogs have been used as a spontaneous animal model for human OSA, it would be reasonable to presume that other brachycephalic dogs may similarly be at risk for IH.

Within the study population, median age was 3 years, median BCS was 6.6, and male dogs were more common than female dogs, consistent with available literature data.^{22-24,37} In humans, obesity is a major risk factor for OSA and the effect of BCS has been reported as a potential aggravating factor for brachycephalic syndrome, although no correlation between BCS and the severity of upper airway obstruction has been found, as in our study.^{8,38,39} In human medicine, waist circumference, neck circumference, and deposition of fat around specific parts of the body are considered in addition to the use of body mass index.^{40,41} On the contrary, in veterinary medicine, with the exception of neck girth evaluation,^{42,43} obesity is defined based on BCS. Therefore, additional studies considering other obesity-related parameters are needed to explore a possible correlation with increased BAOS risk, as recently found for neck girth ratio in male bulldogs.⁴³

Consistent with available data, snoring, inspiratory efforts, and exercise intolerance were the most common presenting clinical signs, followed by syncope.^{5,25} Moreover, 76.7% of dogs with respiratory problems also had gastrointestinal clinical signs, specially vomiting and regurgitation.^{7,22,25,44,45} Although it is not frequently described, SDB may be consequence of BAOS.^{17,37} Indeed, sleep is thought to exacerbate BAOS because the muscles that dilate the upper airways relax.² Sleep-disordered breathing has been described in Pugs, French Bulldogs, English Bulldogs, and, more recently, in Cavalier King Charles Spaniels.^{17,30,46} In our study, more than half of the dogs showed clinical signs of SDB. However, without polysomnography and follow-up information, it is not possible to better characterize SDB and conclude that BAOS at least contributed to sleep apnea.^{17,47} With regard to the airway anatomic abnormalities, our findings were consistent with those of other studies, but a higher number of dogs showed laryngeal collapse (86.6%).^{9,25,44} Tracheal and bronchial collapse were detected only in a minority of dogs (13.8%). These results are not surprising because laryngeal collapse is considered a common secondary functional change occurring in brachycephalic dogs, along with pharyngeal

and tonsillar hyperplasia and bronchial collapse.⁴⁸⁻⁵⁰ However, the severity of respiratory and digestive clinical signs seen at presentation and at the time of radiography and endoscopy was not influenced by the number of anatomic abnormalities or severity of laryngeal collapse, whereas a correlation between laryngeal collapse and bronchial abnormalities already has been described elsewhere.⁵⁰ This lack of relationship, however, could be explained by the presence of other anomalies that are not routinely evaluated, such as obstructing turbinates, deviation of the nasal septum, or narrowing or distortion of the retropharyngeal space, in addition to a different combination of anatomic abnormalities and breed-specific anatomical airway differences.^{51,53}

As reported elsewhere, esophageal deviation, distal esophagitis, atony of the cardia of the stomach, gastric stasis, pyloric mucosal hyperplasia, and gastrointestinal inflammation were the most common endoscopic anomalies recorded, although esophageal deviation and pyloric mucosal hyperplasia were observed more and less frequently, respectively.²⁵ Gastro-esophageal reflux and hiatal hernia were not identified. However, considering that sliding hiatal hernia is by far the most frequently observed in dogs and its frequency is underestimated by radiography or endoscopy, it cannot be definitively excluded here. In addition, hiatal hernia may not have been diagnosed because of the effects of tracheal intubation and anesthesia.^{4,7} Finally, the gastrointestinal tract of dogs without digestive signs was not evaluated, although brachycephalic dogs may have mild digestive anomalies and inflammation even without having digestive signs.²⁵

Consistent with available data, histologic evaluation of the stomach, and duodenum identified mostly chronic inflammation,^{25,44} in addition to lacteal dilatation, not previously reported. Because brachycephalic breeds are not overrepresented among dogs with intestinal lymphangiectasia, it can be hypothesized that the lacteal dilatation could have been a consequence of chronic gastrointestinal inflammation, rather than a primary gastrointestinal problem.⁵⁴ However, it cannot be determined because a specific diagnostic evaluation of intestinal signs was not undertaken. To our knowledge, ours is the first prospective clinical study of dogs with BAOS that has evaluated parameters of glucose regulation and lipid profiles and their relationship with clinical signs and anatomical abnormalities. Glucose, fructosamine, total cholesterol, and triglyceride concentrations were increased in variable proportions in dogs in our study. In human medicine, an association between obstructive sleep apnea syndrome (OSAS) and insulin resistance was observed in some studies, whereas it was not clear in others.⁵⁵ Moreover, SDB can be the cause of notable glycemic variability, which in turn is associated with complications of diabetes.⁵⁶ However, no relationship between increased blood glucose concentrations and severity of OSAS was detected in nondiabetic patients, as in dogs with BAOS described here.⁵⁷ Increased fructosamine concentrations were observed in almost one-third (32.1%) of the dogs, whereas hyperglycemia was only found in a smaller proportion (14%). Because hyperproteinemia as a possible cause of increased fructosamine concentrations was ruled out, this discrepancy might be explained by the presence of intermittent hyperglycemia, perhaps related to surges in sympathetic activity secondary to respiratory distress.^{34,36} None of the dogs in our study had hyperinsulinemia, whereas decreased serum

insulin concentrations were observed in almost all dogs (92.6%). Because no evidence of any other diseases was found in dogs included in the study, lower than normal serum insulin concentrations were considered to be caused by preanesthesia fasting. The majority of studies in human medicine suggest that triglyceride but not total cholesterol concentrations are increased in OSAS patients, and that continuous airway pressure has a positive effect on lowering the serum concentrations of both analytes.^{58,59} Total cholesterol and triglyceride concentrations were normal in approximately 70% of the dogs studied here, whereas lipoprotein classes were increased in the majority of the dogs, perhaps suggesting that serum lipoprotein electrophoresis is more accurate in detecting dyslipidemia in dogs than are total cholesterol and triglyceride concentrations.⁶⁰ In veterinary medicine, a gold standard validated method for the evaluation of canine lipoproteins is currently lacking, and many published reports have utilized automated electrophoretic protocols developed for human use, as in our study.^{61,62} However, the electrophoresis method used here was selected because some studies have documented that it might be more accurate than the wet chemistry method for identifying some lipoprotein classes, especially low-density lipoproteins (LDL), which are substantial in dogs.^{61,63} The alpha fraction is the sum of the pre-alpha, alpha-1, and alpha-2 components and is predominant in normal dogs, and the canine high-density lipoproteins seem to show this migratory pattern.^{64,65} In veterinary medicine, to date, a decrease in LDL was reported in chronic kidney disease, nephrotic syndrome, and pancreatitis in dogs.^{64,66,67} In our study, these lipoprotein classes were normal in the majority of the dogs (80%) whereas the pre-beta and beta classes, corresponding to the very low density lipoproteins and LDL, respectively, and the chylomicrons were increased in more than half of the dogs.⁶⁵ In veterinary medicine, to date, both classes are increased in chronic kidney disease, nephrotic syndrome, diabetes mellitus, and pancreatitis.^{63,66-68}

Limited information exists in dogs with BAOS with regard to the systemic inflammatory response. C-reactive protein concentration was found to be increased in 14% of dogs, but no statistical correlation was found with severity of clinical signs or after surgery.^{22,24} In another study, some proinflammatory cytokines and nitric oxide were found to be higher in brachycephalic dogs than control dogs and to be associated with disease severity.²³ In our study, half of the dogs showed increased CRP concentrations, suggesting that a systemic inflammatory response might be common in dogs with BAOS.

Our study failed to identify a significant association between severity of clinical signs and anatomic abnormalities, inflammatory response, parameters of glucose regulation, and lipid profiles.

Our study had some limitations, primarily related to relatively small sample size, considering the different degrees of BAOS severity addressed. Other limitations were that the quantitative scoring system was assessed by a single observer at each center and therefore was somewhat subjective, gastroduodenal endoscopy was only performed in dogs with gastrointestinal signs, and polysomnography and exercise testing were not performed to better characterize SDB and improve BAOS's assessment.^{50,69} Moreover, it was not possible to include a control group in our study because of the difficulty in finding

brachycephalic dogs with normal breathing pattern. Nearly all brachycephalic dogs have some degree of upper airway obstruction.^{2,69} Lastly, information about the gold standard method for lipoprotein evaluation as well as the pathophysiology of their metabolism in dogs is lacking. Without this information, the clinical utility of some data presented here is unknown and cannot be interpreted reliably.

In conclusion, it seems that the clinicopathological variables considered do not offer valuable information in dogs with BAOS. On the other hand, however, the presence of an inflammatory response and some mild metabolic derangements suggest the need to further explore the metabolic profile and inflammatory status of dogs with BAOS using studies including more animals with similar degrees of BAOS severity.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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