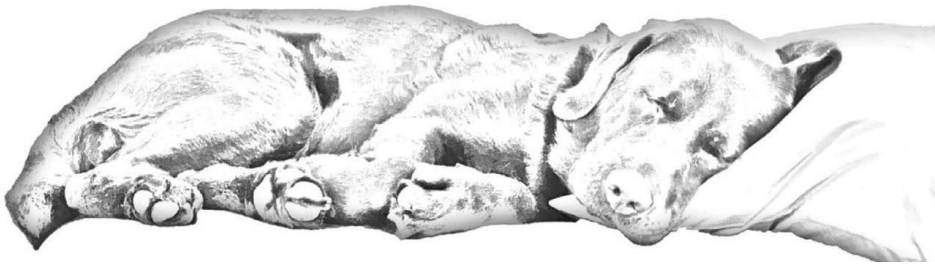
Department of Equine and Small Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki
Finland

SLEEP-DISORDERED BREATHING AND INFLAMMATORY RESPONSE IN DOGS

Iida Niinikoski



ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Veterinary Medicine of the University of Helsinki, for public examination in Porthania PIII, at City Centre Campus, on June 15th 2024, at 12 o'clock.

Helsinki 2024

University of Helsinki
Dissertationes Universitatis Helsingiensis 118/2024

Director of studies
Docent Minna Rajamäki
Department of Equine and Small Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki, Finland

Supervisors
Docent Minna Rajamäki
Department of Equine and Small Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki, Finland

Docent Liisa Lilja-Maula
Department of Equine and Small Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki, Finland

PhD Ninna Koho
Department of Equine and Small Animal Medicine
Faculty of Veterinary Medicine
University of Helsinki, Finland

Reviewed by
Professor Eleanor C. Hawkins
Department of Clinical Sciences
College of Veterinary Medicine
North Carolina State University

Professor Sigrid C. Veasey
Division of Sleep Medicine
University of Pennsylvania

Opponent
BSc (Hons) PhD PGCert(VetEd) FHEA Rowena Packer
Department of Clinical Science and Service
Royal Veterinary College
University of London

Cover: art by Aapo Niinikoski
Publisher: University of Helsinki
Series: Dissertationes Universitatis Helsingiensis 118/2024

ISBN 978-951-51-9800-6 (print)
ISBN 978-951-51-9799-3 (online)
ISSN 2954-2898 (print)
ISSN 2954-2952 (online)
PunaMusta, Joensuu 2024

To my family, for everything.

Abstract

Sleep is vital to life and fundamental to the welfare of dogs. Brachycephaly, the radically reduced length of the snout in the absence of a concurrent reduction in the soft tissues of the upper airways, generates a myriad of welfare issues for dogs. Brachycephalic obstructive airway syndrome (BOAS) is frequent in brachycephalic dogs, and results in various clinical signs, ranging from mild respiratory distress to syncope and death. Furthermore, sleep-disordered breathing (SDB) is described in brachycephalic dogs. In obstructive SDB, periods of partial or complete cessation of respiration occur due to a physical obstruction of airflow. This leads to intermittent hypoxemia, the decreased arterial partial pressure of oxygen. Research into SDB in dogs has been restricted, primarily due to the arduous diagnostic methods currently available. The condition is similar to obstructive sleep apnea in humans, which is associated with various comorbidities, decreased quality of life, and increased mortality. Additionally, obstructive sleep apnea is connected to low-grade inflammation in man. Low-grade inflammation is an independent risk factor for numerous chronic diseases and decreased survival. At present, the evaluation of SDB in dogs is not possible in clinical practice, and basic knowledge, such as predisposing factors for SDB, is lacking.

This thesis investigated the use of a novel neckband system for evaluation of SDB in dogs in their home environment. Risk factors for and owner-perceived signs of SDB in dogs were examined to add to our understanding of the condition. The low-grade inflammatory status of brachycephalic dogs was evaluated to further acquire insight into the consequences of BOAS.

The first study showed that SDB can be evaluated in dogs using the neckband device in their home surroundings. The obstructive respiratory event index was used to summarize the rate of obstructive SDB events during recording time. The neckband system was easy to use for owners and accepted by dogs. Brachycephalic dogs had a significantly higher rate of SDB events and a higher proportion of snoring compared to non-brachycephalic dogs. The proportion of snoring did not correlate with the severity of SDB in brachycephalic dogs, implying that the presence of snoring cannot be used as an exclusive sign of SDB in short-snouted dogs.

In the second study, risk factors for SDB were analyzed in both brachycephalic and non-brachycephalic dogs. Brachycephaly, increasing severity of BOAS, and excess weight predisposed to SDB. Ageing, gender, or neuter status were not identified as risk factors for SDB. SDB occurred also in non-brachycephalic dogs, which has not been reported previously. Owner-perceived disturbances in sleeping position, apneic episodes during sleep and nighttime restlessness were identified as potential signs of SDB in dogs.

The third study revealed a proinflammatory condition in brachycephalic English Bulldogs, observable as changes in the concentration of the proinflammatory mediator vascular endothelial growth factor A in serum and bronchoalveolar lavage fluid compared to non-brachycephalic control dogs. The proinflammatory condition resembles that seen in obstructive sleep apnea in man and is suspected to result from intermittent hypoxemia due to SDB. However, evaluation of breathing during sleep was not performed in these dogs.

Our findings add meaningful new knowledge on SDB in dogs. Together the three studies reveal that brachycephaly affects the welfare of dogs in a plethora of ways, including the disruption and fragmentation of sleep. The proinflammatory condition in English Bulldogs supports low-grade inflammation due to intermittent hypoxemia. The evaluation of SDB is possible in dogs without laboratory equipment, using an at-home neckband device. Snoring cannot be used as the sole indicator of SDB, and other indications of SDB may include changes in sleeping position, apneic episodes during sleep, and restlessness during nighttime. SDB is not limited to brachycephalic dogs and occurs also in non-brachycephalic dogs. Obesity should be avoided in all dogs to enhance improve breathing during sleep.

Acknowledgements

The studies included in this thesis were carried out at the Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland. Part of the dogs in studies I and II were examined at the Kaarina Veterinary Clinic, Finland, and some dogs in study III at the Veterinary Teaching Hospital of the University of Liège, Belgium. This project was made possible by funding provided by Agria Djurförsäkring/Svenska Kennelklubben Forskningsfond, The Finnish Canine Health Research Fund, the Finnish Foundation of Veterinary Research, the Finnish Veterinary Research Foundation, and SEY Animal Welfare Finland, which I am extremely thankful for. I also gratefully acknowledge Nukute Ltd. (Oulu, Finland) for providing the neckband systems for studies I and II. Thank you to all the dogs and their owners that have taken part in this project – I am hugely thankful for your contribution.

I want to express my deepest gratitude to my primary supervisor Docent Minna M. Rajamäki, who has been the most warm, understanding, and supportive team leader one could wish for. Thank you for creating such an inviting atmosphere for research and helping me navigate the at times turbulent seas of combining research, clinical work, and life. I am extremely thankful for the opportunity to take part in this project. To my second supervisor, Docent Liisa Lilja-Maula - this truly would not have been as smooth if it weren't for you. I am so grateful for your insight; you are an inspiration. I also wish to thank my third supervisor, PhD Ninna Koho for all her help and support, especially in the beginning of the project.

I am so grateful to PhD Rowena Packer for accepting the invitation to act as my Opponent. Her work exploring the issues associated with brachycephaly is truly inspiring. I wish to thank the esteemed pre-examiners of this thesis, Professor Sigrid C. Veasey and Professor Eleanor Hawkins, for their time, expertise, and valuable feedback. I am deeply grateful to my thesis committee members, Professor

Tarja Saaresranta and Docent Tarja Pääkkönen, for all their help and support throughout this project.

I owe a lot to my cowriters and extraordinary researchers Professor, MD Sari-Leena Himanen and Docent Mirja Tenhunen from the University of Tampere. Thank you for your expertise, enthusiasm, and friendship. It has been lovely working with you, and I hope to keep collaborating in the future. A heartfelt thank you to the other collaborators and cowriters; Sirkku, Mimma, Saila, Henna, Aline, and Cecil. Thank you to Tiina Heinänen for helping with the study visits at YES. I also want to thank Jouni Junnila for doing an excellent work on the statistical analyses, and for being very patient with all our questions. I am grateful for the support from all the exemplary researchers in our Lung Insight group. I also want to dearly thank Professor Thomas Spillmann, for inspiring me to embark on a career combining research with clinical work, as well as all the practical help throughout the years.

A warm thank you to my superb coworkers and friends at the Kaarina Veterinary Clinic for their interest in and contribution to my research work. I especially want to thank Iris for enabling this project alongside clinical work. Thank you Maria for patiently helping with the collar covers. Much love to my colleagues and dear friends Anna and Mimma, thank you for the support in all aspects of work and life; it means the world to me.

I have been blessed to be surrounded by the most awesome group of friends. Sanni and Suski, thank you for being there every day, through thick and thin. Thank you to Jupe, Jonna, Matias, Eetu, Heini, and all my other lovely friends from Viikki; it has been and continues to be such fun. To all future adventures, cherry tomatoes, and festivals to come! Hannah, you continue to inspire me in both academia and life, thank you for all the help, conversations, and laughter. Jutta, I admire you so much, thank you for sticking by and always having my back. Thank you eepu, for everything. Hanna, Aapo, Anni, Niilo, Elina, Matias, Miisa, and Aatu – thank you for all the warmth. Lilli, Eevi and the rest of ASB N1, thank you for giving me non-veterinary things to think about and do, it's been a blast.

I'm so grateful to Aino, Raimo, Annukka, and Hannu for all the support throughout the years.

Topi and Liisa – I owe you both so much and will never be able to thank you enough.

My amazing family, my lovely parents Heidi and Harri, and the best possible siblings Laura, Aapo, and Roope: thank you for always believing in me, even when I wasn't so sure myself. Your unwavering support truly made all the difference, in this project and in life. My gratitude (and apologies) also go out to Jasper and my darling godson Jouko, for putting up with me and Laura working in their kitchen all these years – the vast majority of this work has been done there in a somewhat non-ergonomic (but fun) fashion.

To Rami, the most wonderful, loving, funny, kind, smart person I've ever had the opportunity of meeting. You make it all worthwhile.

Iida Niinikoski, Turku, April 2024

Abbreviations

AHI	apnea hypopnea index
ANOVA	analysis of variance
BALF	bronchoalveolar lavage fluid
BCS	body condition score
BD	brachycephalic dog
BOAS	brachycephalic obstructive airway syndrome
CCL2	chemokine (C-C motif) ligand 2
CRP	C-reactive protein
CSA	central sleep apnea
CI	confidence interval
CIPF	canine idiopathic pulmonary fibrosis
CPAP	continuous positive airway pressure
EEG	electroencephalography
EMG	electromyography
EOG	electro-oculography
HIF	hypoxia-inducible factor
IL	interleukin
IPF	idiopathic pulmonary fibrosis
MCP-1	monocyte chemoattractant protein 1
NREM	non-rapid eye movement
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
OREI	obstructive respiratory event index
OSA	obstructive sleep apnea
RDI	respiratory disturbance index
REI	respiratory event index
REM	rapid eye movement
RERA	respiratory effort related arousal
SBD	sleep-disordered breathing
SDBI	sleep-disordered breathing index

TNF-α	tumor necrosis factor-alpha
VEGF-A	vascular endothelial growth factor A
WHWT	West Highland White Terrier

Keywords: Brachycephalic obstructive airway syndrome, sleep-disordered breathing, obstructive sleep apnea, low-grade inflammation, proinflammatory mediator

Contents

List of original publications

This thesis is based on the following original publications, referred to in the text by their Roman numerals:

I **Niinikoski, I.**, Himanen, S.-L., Tenhunen, M., Lilja-Maula, L., Rajamäki, M.M. 2023. Description of a novel method for detection of sleep-disordered breathing in brachycephalic dogs. Journal of Veterinary Internal Medicine 37(4): 1475-1481. doi:10.1111/jvim.16783

II **Niinikoski, I**., Himanen, S-L., Tenhunen, M., Aromaa, M., Lilja-Maula, L., Rajamäki, M.M. 2024. Evaluation of risk factors for sleep-disordered breathing in dogs. Journal of Veterinary Internal Medicine 38(2): 1135-1145. doi:10.1111/jvim.17019

III **Niinikoski, I.**, Kouki, S., Koho, N., Aromaa, M., Holopainen, S., Laurila, H.P., Fastrès, A., Clercx, C., Lilja-Maula, L., Rajamäki, M. M. 2022. Evaluation of VEGF-A and CCL2 in dogs with brachycephalic obstructive airway syndrome or canine idiopathic pulmonary fibrosis and in normocephalic dogs. Research in Veterinary Science 152, 557-563. doi:10.1016/j.rvsc.2022.09.022

The materials and methods, results and discussion are based on the original publications. Tables and figures are modified from these studies. The publications are reprinted with the permission of the copyright holders and licensed under the Creative Commons Attribution 4.0 International License.

1 Introduction

Brachycephaly, a shortened and flattened skull produced by decades of selective breeding[1], is associated with numerous welfare issues, including a shortened lifespan[2], in pet dogs. Despite the reduction in the length of the skeletal muzzle observed in brachycephaly[3], there is no decrease in the volume of upper airway soft tissue.[4,5] This leads to a varying degree of upper airway obstruction both during wake and sleep. Brachycephalic obstructive airway syndrome (BOAS) is common in brachycephalic dogs and infrequent in longer snouted normocephalic (mesocephalic or dolicocephalic) dogs.[6] Clinical signs of BOAS include respiratory problems, exercise and heat intolerance, and gastrointestinal issues.[7-11] Severe BOAS can result in cyanosis, syncope, and even death.[10]

Respiration is compromised also during sleep in brachycephalic dogs. Sleep-disordered breathing (SDB), alike human obstructive sleep apnea (OSA), was first described in the brachycephalic English Bulldog in the late 1980's.[7] In canine obstructive SDB[7,12] and human OSA[13] a partial or complete cessation of respiration occurs during sleep as airflow is physically blocked by upper airway collapse and obstruction. Repeated episodes of interruptions in respiration and shallowed breathing lead to hypoxemia, a decreased blood oxygen concentration, and ultimately arousal.[13] After awakening, the abruptly contracting upper airway muscles alleviate the obstruction and normal breathing commences.[14] The cycle continues when sleep resumes. In man, OSA is associated with a myriad of comorbidities, including increased thromboembolic and cardiovascular risk.[15,16] Untreated severe OSA increases mortality[17,18] and results in chronic low-grade inflammation.[19] Low-grade inflammation is an independent risk factor for various chronic disorders[20,21] and increases total mortality.[22]

Research into SDB in dogs has been limited, as current diagnostic methods require extensive equipment, expertise, and a laboratory setting[7,12,23-27], and are hence not feasible in clinical practice. SDB is objectively described in modest groups of brachycephalic English Bulldogs by polysomnography[7,12,23-26] and in three Cavalier King Charles Spaniels by whole-body barometric plethysmography.[27] There is some evidence of a proinflammatory condition in brachycephalic dogs[28-31], although the magnitude of change compared to normocephalic control dogs is not as substantial as in humans with OSA. SDB can be managed, at least to some extent, with conservative medical treatment[24,25,32,33] and surgical alleviation of BOAS.[23,27,34,35]

Knowledge on the presentation, risk factors and treatment of SDB and the comorbid conditions associated with it are scarce. Sleep is critical to life[36,37] and the fragmentation of sleep itself has consequences on the wellbeing of dogs.[38,39]

The purpose of this thesis was to advance knowledge on canine SDB by evaluating the usability of a portable neckband system for detection of SDB in dogs in their home surroundings. The neckband system was used to assess risk factors for SDB in both brachycephalic and normocephalic dogs and to describe the most prevalent owner-perceived signs of SDB in dogs. The inflammatory status of brachycephalic dogs associated with suspected intermittent hypoxemia due to BOAS was investigated.

2 Literature review

This thesis focuses on the diagnostic methods, signs, and risk factors for sleep-disordered breathing (SDB) in dogs, and the proinflammatory condition associated with hypoxemia. To provide an understanding of the health and welfare issues resulting from the fragmentation of sleep due to SDB, the literature review begins with a summary of the current knowledge on sleep in dogs. The literature review then proceeds to compile relevant information on naturally occurring SDB and hypoxemia-related inflammation in dogs, with comparisons to the disease processes in man where appropriate.

2.1 Sleep in dogs

2.1.1 Overview

Sleep is essential to life; sleeplessness ultimately leads to death.[36,37] Sleep can be characterized as a rapidly reversible state of quiescence, often in a stereotypic posture, with greatly reduced responsiveness to external stimuli.[40] Although the fundamental meaning behind sleeping remains an enigma, sleep has a plethora of critical functions to life, presented in Figure 1. Sleep is deeply intertwined with welfare and wellbeing across species: decline in welfare may affect the quality of sleep, and poor sleep quality may negatively influence welfare.

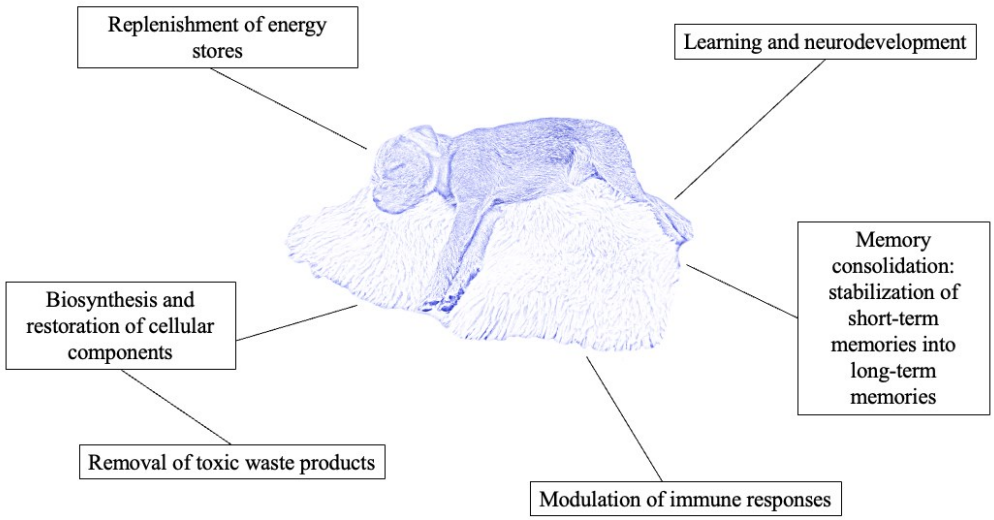


Figure 1 Functions of sleep.[40-42] Picture: Aapo Niinikoski

2.1.2 Regulation of sleep

Dogs alter between wake and sleep states throughout their life. Based on studies in other mammals, regulation between wake and sleep is controlled by specific nuclei and neural circuits in the brain, and the main processes controlling wake and sleep periods are circadian rhythm and homeostatic sleep pressure.[41] Neurons in the brainstem and hypothalamus regulate sleep and wake by releasing different neurotransmitters in different states of vigilance.[42] Wake-promoting cells are silenced in sleep, and reciprocally sleep-promoting cells groups silenced in wake.[42] The timing of sleep, that is, the time of day the dog transitions from wake to sleep, is regulated by the circadian rhythm, while the duration and intensity of sleep are regulated by homeostatic sleep pressure.

The timing of sleep is regulated by an internally generated circadian rhythm. The main coordinator of circadian rhythm is the central pacemaker of the suprachiasmatic nucleus, located in the hypothalamus. The circadian rhythm of approximately 24 hours also regulates numerous other physiological and behavioral processes, such as fluctuation of blood glucose concentration[43] and core body temperature.[44] Although the light-dark cycle is an important cue for synchronization of circadian rhythm, it also prevails in the absence of external

environmental signals.[45] These environmental cues, also known as Zeitgebers, include, among others, social interactions[46] and food availability.[47] Additionally, various peripheral oscillators, located for example in the brain, the heart, and the liver, support the rhythm of the central pacemaker.[48]

The sleep-wake pattern of the domestic dog is largely diurnal: wake period occurs mainly during the day and sleep primarily during the night.[49-51] Other non-domesticated canids, such as grey wolves, do not have diurnal activity patterns, and are instead more active during dawn, dusk, or night-time.[52] The diurnal activity of the pet dog is thought to be a product of extensive domestication over tens of thousands of years[53,54], as the sleep-wake pattern of the dog has adapted to that of humans.

In a laboratory environment, dogs are most active after light onset, but there is variability between individuals and day-to-day rhythm.[49] In contrast, peak activity in pet dogs seems to occur around 7:00 and 19:00.[51] Total sleep time in dogs ranges from 8.4 to 13.5 hours[38,55-57], with nighttime sleep durations reported between 6.1[39] and 7.0 hours[58]. However, besides sleeping at night, sleeping during the afternoon is an important part of the domestic dog's sleep-wake pattern.[58] The daytime sleep bouts can occur between 12:00 and 16:00[49,59] or evenly throughout the day[50], and their combined duration is approximately three hours.[58] Nonetheless, depending on environmental factors, daytime sleep can also be very limited, even to under an hour in a hectic environment.[38,39]

Homeostatic sleep pressure regulates the duration and intensity of sleep, reflected in the length of sleep and neuronal EEG waveform activity.[60] Sleep pressure, or the need for sleep, accumulates in the brain throughout the wake period, until ultimately, the pressure is enough to impose sleep. During sleep, sleep pressure dissipates until low enough for wake state to commence. Although the precise mechanisms behind homeostatic sleep pressure are unknown, sleep-promoting substances, known as somnogens, are present in the cerebrospinal fluid of sleep-deprived animals.[61] Somnogens include, among others, adenosine, interleukin-1 (IL-1), tumor necrosis factor-alpha (TNG-α), prostaglandin D2 and nitric oxide (NO).[60]

The presence of sleep homeostasis is evident in dogs, as the lack of sleep increases total sleep time and proportion of rapid eye movement (REM) sleep.[62] Lack of sleep also affects drowsiness and non-rapid eye movement (NREM) sleep, as decreased alpha activity in drowsiness and NREM and increased slow wave sleep and delta activity in NREM sleep are reported in sleep deprived dogs.[63] Sleep-deprived dogs are more inactive and less alert, eat more and play less than dogs with non-fragmented sleep.[39] It seems nighttime sleep is less fragmented in dogs with very little sleep during the day, allowing for more rest during the night.[39]

Sleep-wake and activity pattern is affected by age. Older dogs sleep altogether more than younger dogs[59], and younger dogs are more active during the day.[44,50,51] Aged dogs exhibit decreased total sleep at night, compensated for by more frequent sleep bouts during the day.[59] However, these daytime sleep bouts are shorter in duration in older dogs compared to younger dogs.[44] More pronounced changes in sleep-wake cycle are prevalent in cognitive dysfunction syndrome common in older dogs, with increased wandering, vocalization, and restlessness during nighttime.[44,64-66]

Sleep-promoting circumstances in dogs include sleep-debt[39,62,63], night-time[49-51], and satiety.[50] Wake-promoting circumstances encompass daytime[39,62,63] and hunger.[50]

2.1.3 Sleep-wake cycle

Sleep in the dog consists of three stages: drowsiness, NREM sleep (sometimes referred to as slow wave sleep), and REM sleep.[55,56,63,67] Sleep stage is characterized by alterations in cortical brain activity, visualized as different waveforms in electroencephalography (EEG). Drowsiness is present in dogs but absent in for example humans.[55,63] During drowsiness, the eyes of the dog can be open or closed and there is little motor activity.[63] Although drowsiness is detectable in EEG recordings by prominent alpha waves[67], in the absence of EEG recording, the distinction between wake and drowsiness is unclear to the viewer. Differences between the four states of vigilance (wake, drowsiness, NREM and REM sleep) in dogs are presented in Table 1. Visual estimates of total sleep time in dogs vary greatly, likely in part due to the difficulty interpreting drowsiness as wake or sleep

without EEG recording. Furthermore, the distinction between drowsiness and other stages may also be ambiguous in evaluation of EEG recordings.[67] Both NREM and REM stages are important for memory consolidation, the stabilization of a new memory into a long-term memory, as well as learning and emotional regulation.

Table 1 Differences in observable and measurable parameters between the four vigilance states in dogs.

EEG, electroencephalography; EMG, electromyography; EOG, electro-oculography; NREM, non-rapid eye movement; REM, rapid eye movement

	Wake	**Drowsiness**	**NREM**	**REM**
Observable movement	Frequent[67]	Slow opening and closing of the eyes possible[55] May be sitting with eyes open[55]		Twitching of eyelids, face, and paws[68]; nose, lips, ears, whiskers and limbs[69] Clonic jerks of shoulders and back[69]
Observable muscle tone	Elevated[63,67]	Low, observable[63,67]	Decreased[67,70]	Muscular atonia[67]
Cardiac function	Highest heart rate in the different vigilance states[71,72]	Heart rate higher than in NREM and REM[72] No difference in heart rate between sleep stages[71]	No difference in heartrate between sleep stages[71]	Irregular[63,67] Lowest heart rate[72] No difference in heart rate between sleep stages[71]
Respiration		Fairly regular[63,67] No difference in respiratory frequency between sleep stages[72]	Relatively regular[63,67] No difference in respiratory frequency between sleep stages[72]	Irregular[63,67] No difference in respiratory frequency between sleep stages[72]
EEG	Increased high-frequency and fast activity[63,67,70] High frequency and low amplitude[62], low voltage[56] Alpha waves: 8.75-12.75 Hz[67] Beta waves: 15-30 Hz[67]	Increased high frequency and fast activity[63,67], similar to wake[70] Alpha waves: 8.75-12.25 Hz[63,67,73] Beta waves: 12.75-30 Hz[63,67]	≥15 µV delta (1-4 Hz) waves[63,67] and/or sleep spindles (duration ≥0.5 s, 12-16 Hz)[63,67,74,75] High voltage slow waves (up to 40 µV)[56,69]	Increased theta (4.25-4.5 and 7-8 Hz) activity[55,63,67,73,75] Fast activity[56,63,67,73], high frequency[62,70] Similar to wake[62]

	Wake	Drowsiness	NREM	REM
EOG	High amplitude and frequency[63,67,70]	Decreased amplitude and frequency[63,67,70]	No or low amplitude activity[63,67,70] Activity at lowest level[55]	Rapid eye movements[63,67,70] Increased activity[62]
EMG	High activity[70] High frequency nuchal activity[62] (O'Donnell et al. 1994)	Reduced but observable activity[70]	Decreased nuchal activity compared to wake[62] Relatively small activity[55]	Low amplitude activity[56], except for muscle twitches[55] Absence of muscle activity[70] Reduced activity compared to wake[62]

Dogs are polyphasic sleepers: sleep cycles are not linked back-to-back to form one long period of sleep like in monophasic adult humans, but sleep is divided into smaller segments throughout the day and night.[63] In one sleep cycle the dog goes through all three sleep stages; drowsiness, NREM and REM. Reports for the average duration of one sleep cycle vary between 20 and 56 minutes (Table 2). It should be noted that the often quoted 20-minute sleep cycle duration is based on observational study[76], and not an EEG recording. This sleep cycle duration is often misconstrued in literature, as it contains a wake phase and is a sleep-wake cycle. Some of the sleep cycle analyses[63,77,78] were performed during daytime sleep, the macrostructure of which differs from nighttime sleep.[79] The variation in reported sleep cycle durations between studies is also likely to result from the polyphasic nature of sleep-wake cycles in dogs, as the sleep pattern in dogs is more fragmented and variable compared to humans.[78] The polyphasic wake-sleep cycle, measured as the interval between two successive wake episodes is, on average, 82 minutes in duration (range 47-127 minutes).[56] This polyphasic sleep-wake cycle contains on average two REM sleep episodes.[56]

Table 2 Summary of literature analyzing sleep cycles in dogs.

Reference	Methodology	n	Length of sleep cycle
Lucas et al. 1977[56]	Invasive polysomnography for 24 hours, intracranial electrodes	6	45 minutes
Wauquier et al. 1979[55]	Invasive polysomnography for 24 hours, intracranial electrodes	7	20 – 30 minutes
Adams and Johnson 1993[76]	Observational: video recordings and visual observation	24	16 minutes
Kis et al. 2014[63]	Noninvasive polysomnography during 3h daytime sleep	15	49 minutes (range 20 – 102 minutes)
Kis et al. 2017[73]	Noninvasive polysomnography during 3h daytime sleep	16	51 minutes after positive experience 56 minutes after negative experience
Reicher et al. 2021[78]	Noninvasive polysomnography during 3h daytime sleep	19	27 – 36 minutes, differing between sleep occasions and sleep cycles

Sleep macrostructure, which encompasses the order, latency to onset, and relative proportions of the three sleep stages, is affected by multiple factors. The relative durations of the different sleep stages differ between nighttime and daytime sleep, as dogs spend more time in NREM and REM stages and less in drowsiness during the night.[79] Pre-sleep activity also has an influence on the relative proportions of REM and NREM stages, as there is more total sleep[63,79], NREM sleep[63,79], and REM sleep after an active day.[79] Learning a novel task affects the electrophysiological activity of both NREM[77,80] and REM sleep[77], seen as changes in frequency of oscillations on EEG. Furthermore, sleeping for at least three hours after learning a novel task improves performance.[77] Emotional experiences preceding sleep affect sleep structure, with negative experiences resulting in faster onset of sleep, an increased proportion of REM sleep and a decreased proportion of both drowsiness and NREM.[73]

Sleep macrostructure is also affected by age.[63,70,81] Puppies spend more time in REM sleep and less time in drowsiness compared to older dogs, with adult proportions of REM and drowsiness reached at around 6 to 8 months of age.[81] Increased NREM during the day and decreased total sleep, NREM and REM sleep, is observed in aging dogs.[59] The occurrence of slow spindles, rhythmic oscillations observed in NREM sleep and positively associated with learning,[80,82,83] declines with age.[74]

Additionally, dogs with more pronounced cognitive dysfunction spend less time in NREM and REM sleep during daytime.[70]

2.1.4 Normal ventilation during wake and sleep

The respiratory system supplies oxygen (O_2) to tissues, removes carbon dioxide (CO_2) from the bloodstream, and maintains the acid-base balance of the body. Ventilation is regulated by a central controller, the respiratory center, within the medulla oblongata and pons of the brain. Central and peripheral sensors transmit information to the respiratory center, which then sends impulses to the effectors for controlling respiration by altering minute ventilation and gas exchange.

The primary central chemoreceptors are located near the medulla and respond to changes in brain extracellular fluid [H^+] concentration, which is directly dependent on arterial partial pressure of CO_2 ($PaCO_2$). A rise in brain extracellular [H^+] concentration leads to increased ventilation. The primary peripheral chemoreceptors are the carotid and aortic bodies, which mainly respond to arterial partial pressure of O_2 (PaO_2) and, to a smaller extent, $PaCO_2$.[84] Information is transmitted to the respiratory center from the carotid and aortic bodies via the glossopharyngeal nerve. Furthermore, stretch receptors in the lungs are sensitive to lung volume and lung irritation, and proprioceptors in the muscles and tendons sensitive to movement. During wake, higher brain centers in the cerebral cortex are also capable of voluntary control of respiration.[85]

During sleep, the primary stimulus for ventilation is $PaCO_2$.[85] During sleep, ventilatory responses to hypoxemia (decreased PaO_2), and hypercapnia (increased $PaCO_2$), are decreased, resulting in a reduced response to alterations in O_2 and CO_2 concentrations.[69] Muscle activation is altered during sleep, mostly in the upper airway: there is reduced activity and response to increased $PaCO_2$. The upper airway is susceptible to collapse, as it largely consists of muscle, fat and connective tissue and lacks bony support. Vascular congestion due to reduced sympathetic vasoconstriction may occur during sleep, leading to narrowing of the upper airway.[85] There is no voluntary control of respiration during sleep.

There is little data on the respiratory parameters of dogs during sleep. There is some evidence for lack of difference in respiratory rate between wake and sleep or between the sleep stages.[72] However, contradicting findings also exist, with slower and steadier respiration during NREM sleep and rapid, shallow, and irregular breathing during REM sleep.[69] Nonetheless, changes in respiratory patterns are also often used to support EEG analysis during sleep stage scoring[63,67], suggestive of differences in ventilation between the phases.

2.2 Sleep-disordered breathing

2.2.1 Classification and definitions

Sleep-disordered breathing (SDB) is an umbrella term for all breathing disorders occurring during sleep.[13,86] In dogs, naturally occurring SDB was first described the brachycephalic English Bulldog[7], and has since been reported in modest groups[26,27] and individuals[32,33] of other brachycephalic breeds as well. Dogs have also been used as an experimental animal model of human obstructive sleep apnea (OSA), where repeated episodes of upper airway obstruction are produced by occlusion of ventilation, usually through a tracheostomy valve.[87-94] This thesis focuses on naturally occurring SDB in dogs.

In man, the categorization of SDB varies slightly depending on source, but the most recent International Classification of Sleep Disorders classifies SDB into OSA, central sleep apnea (CSA), sleep-related hypoventilation and sleep-related hypoxemia.[13] The naturally occurring forms of SDB in dogs[7,12,27], OSA and CSA, with focus on obstructive SDB, will be addressed in this literature review.

Regarding literature in dogs, the term SDB is often used instead of differentiating between the different backgrounds, obstructive or central, of sleep apnea.[7,12,23-27] In both OSA and CSA, episodes of apnea, a complete cessation of airflow, and hypopnea, shallowed breathing, occur. OSA is caused by upper airway collapse during sleep while respiratory effort is present. In contrast to OSA, in CSA there is a disturbance in respiratory control leading to absent inspiratory effort during apnea and hypopnea. Although centrally occurring apneas and hypopneas have

been described in a small population of English Bulldogs[7,24], the majority of reported SDB events in dogs are of obstructive origin.[7,12,23-27] Mixed sleep apnea, a combination of obstructive and central apneas and hypopneas, is also sometimes seen in man[95] and indeed seems to occur in dogs as well. [7,24]

No consensus guidelines for assessment of SDB or the scoring of apneas or hypopneas in dogs exist currently. In humans, an apnea is defined as a ≥90% decrease in airflow, lasting at least 10 seconds.[13] Hypopnea definitions are more mixed, with the current recommendation being a ≥30% decrease in airflow associated with a ≥3% decrease in oxygen saturation or an arousal.[96] Unlike apneas and hypopneas, respiratory effort related arousals (RERAs) are not always included in the summary of SDB. RERAs are sequences of breaths which lead to arousal, but do not meet the criteria for apnea or hypopnea.[86] RERAs are characterized by a duration of at least 10 seconds with increasing respiratory effort or flattening of the inspiratory flow signal.[86] There are few reports of RERAs in dogs.[24,25]

The number of apneas and hypopneas per hour of sleep is summarized as the apnea-hypopnea index (AHI).[13,86] The AHI value can only be calculated in polysomnography studies, where sleep can be distinguished from wakefulness by EEG.[86] Additionally, respiratory disturbance index (RDI) can be calculated as the number of apneas, hypopneas and RERAs per hour of sleep in a polysomnographic study.[86] In non-polysomnographic studies, a respiratory event index (REI) is used to summarize the number of apneas and hypopneas in the estimated hours of sleep. In REI, the periods of likely wakefulness are excluded from the total recording time by analyzing cardiac and respiratory rate, artifacts, and movement.[86]

In adults, OSA is classified into mild, moderate, and severe forms depending on AHI value and an assessment of symptoms, including daytime sleepiness.[12] Mild OSA is characterized by AHI 5–14.9, moderate by AHI 15–29.9, and severe by AHI over 30.[85] Although under five apnea or hypopnea events per hour is normal in adults[85], the threshold between normal and abnormal AHI value is distinctly different in children, where AHI over 1 is abnormal.[97]

2.2.2 Diagnostic methods

The golden standard for diagnostics of SDB is polysomnography, which includes EEG, electro-oculography (EOG), electromyography (EMG), electrocardiography, and monitoring of airflow, respiratory effort, and blood oxygenation.[85] Other monitoring can include the presence of snoring and body position.[85] Polysomnography gives information on the sleep stages the SDB events occur in, which cannot be obtained by other methods.

Polysomnography has been used in dogs, both in sleep studies[63,73,77] and in SDB assessment.[7,12,23-26] However, there are some difficulties regarding polysomnography in dogs. The placement of electrodes for recording of electrical activity takes time and expertise and can induce mild pain. Although painless noninvasive electrode placement is possible[63], there are currently no studies assessing breathing during sleep with this method. The measurement of blood oxygenation can be difficult in dogs, as both the placement and staying in place of the oximeter in unsedated dogs is challenging. Additionally, the reliability of pulse oximetry as screening method for hypoxemia in wake dogs is questionable.[98] Airflow monitoring can also be complicated, as dogs are nasal breathers and may not tolerate the presence of nasal airflow monitors in their nostrils.

Besides polysomnography, whole-body barometric plethysmography has also been used to study SDB in dogs.[27] Whole body barometric plethysmography is a noninvasive, objective method of measuring respiratory function in unsedated dogs. The dog rests in a whole-body barometric plethysmography chamber, and its respiratory efforts induce barometric pressure oscillations proportional to tidal volume.[99-101]

Table 3 summarizes current literature on the diagnostics and key findings of research on sleep-disordered breathing in dogs.

Table 3 Summary of literature analyzing sleep-disordered breathing in dogs.

EEG, electroencephalography; EOG, electro-oculography; EMG, electromyography; NREM, non-rapid eye movement; REM, rapid-eye movement; RERA, respiratory effort-related arousal RDI; respiratory disturbance index; SDB, sleep-disordered breathing; SDBI, sleep-disordered breathing index

	Study design	**Study population**	**Methodology**	**Main outcome measures**	**Key results**
Hendricks et al. 1987[7]	Prospective observational cross-sectional study	Eleven dogs: - Seven English Bulldogs - Four normocephalic control dogs - Sleep study unsuccessful in an additional three dogs	Modified polysomnography, duration 3-7 hours: EEG, inductive plethysmograph respiratory belt, airflow thermistor, oximeter.	SDBI: rate of respiratory events per hour of sleep. Oxygenation Change in airflow Hypersomnolence Sleep latency Arousal threshold	- English Bulldogs had significantly higher SDBI than control dogs - More pronounced desaturation and reduced airflow in REM than NREM - Both obstructive and central apneas present - English Bulldogs exhibited hypersomnolence, decreased sleep latency (fell asleep faster) and elevated arousal threshold (did not easily wake up) compared to control dogs
Hendricks et al. 1991[12]	Prospective observational cross-sectional study	Eight dogs: - Five English Bulldogs - Three normocephalic control dogs	Polysomnography, duration 3-5 hours: EEG, EOG, EMG (diaphragm and sternohyoid), inductive plethysmograph respiratory belt, oximeter, airflow thermistor	EMG activity of diaphragm and sternohyoid muscles Number and duration of SDB events Oxygenation	- SDB events occurred more in REM than NREM sleep - SDB events associated with phasic REM sleep influences, not arousals or response to hypoxia - Suppression of the sternohyoid muscle was greater than the diaphragm

	Study design	Study population	Methodology	Main outcome measures	Key results
Hendricks et al. 1993[23]	Prospective observational cross-sectional study	Thirteen dogs: - Six English Bulldogs - Seven normocephalic control dogs	Modified polysomnography, duration 3-5 hours: EMG, EEG, inductive plethysmograph respiratory belt, oximetry	Rate of respiratory events per hour of REM sleep Total duration of SDB events in minutes Oxygenation	- SDB events occurred in all English Bulldogs and in none of the control dogs - Surgical resection of pharyngeal soft tissue decreased the rate of SDB events from 48 to 0 in one dog - Compensatory upper airway dilator activity occurred in English Bulldogs during wake and NREM
Veasey et al. 1996[26]	Prospective observational cross-sectional study with cross-over design	Eight English Bulldogs - Eight completed a sleep study - Four completed a wake polygraph with two different serotonin antagonists (ritansetron and methysergide)	Modified wake polygraphy, duration unknown: EMG, oximeter, inductive plethysmograph respiratory belt, Daytime modified polysomnography, as in wake polysomnography above, duration 3-7 hours	EMG activity of sternohyoid, geniohyoid and diaphragm Cross-sectional area of upper airway Oxygenation	- Serotonin antagonists caused suppression of upper airway dilator muscles - Less suppression in diaphragm activity - Suppressions in motor activity coincided with oxyhemoglobin desaturation - Inspiratory collapse of retropalatal airway was seen on imaging
Veasey et al. 1999[25]	Randomized controlled, double-blinded study with crossover design	Five English Bulldogs, each completing 16 randomized studies (four placebo, four at each of the three drug dose conditions)	Modified polysomnography, duration 6 hours: EEG, EMG, oximeter, inductive plethysmography respiratory belt, snore sensor, nasal airflow thermistor	RDI: rate of RERAs, apneas and hypopneas per hour of sleep Number and duration of respiratory events Oxygenation Sleep efficacy and architecture	- Predominant respiratory events during NREM were RERAs - Trazodone combined with L-tryptophan dose-dependently decreased fragmentation of sleep, increased efficacy of sleep and amount of slow-wave sleep, and reduced upper airway

	Study design	Study population	Methodology	Main outcome measures	Key results
				Sternohyoid muscle EMG activity	dilator suppression - Substantial improvement in SDB events occurring in NREM sleep, moderate improvements in REM sleep
Veasey et al. 2001[24]	Randomized controlled double-blinded study with crossover design	Three English Bulldogs Each completed 12 double-blinded, randomized studies	Modified polysomnography, duration 6 hours: EEG, EMG, oximeter, inductive plethysmography respiratory belt, snore sensor, nasal airflow thermistor	RDI: rate of RERAs, apneas and hypopneas per hour of sleep Number and duration of respiratory events Oxygenation Sleep efficacy and architecture	- Ondansetron reduced apneas and hypopneas during REM sleep but not during NREM sleep - No effect on time spent in desaturation - Ondansetron had no effect on sleep efficiency or sleep architecture - SDB events during NREM sleep were infrequent: either RERAs or hypopneas with 2% desaturation and arousal - SDB events during REM sleep were mixed: obstructive and central SDBs
Hinchliffe et al. 2019[27]	Retrospective observational cross-sectional study	Three Cavalier King Charles Spaniels (sleep study unsuccessful in an additional two dogs)	Whole-body barometric plethysmography before surgical intervention, duration not reported, combined with owner interviews pre- and post-surgical intervention	Respiratory trace during sleep Owner-perceived signs of SDB: snoring, sleeping position, hypersomnolence, apneic episodes	- All three dogs had abnormal respiratory traces, including apneas, during sleep - Surgical intervention alleviated signs in all dogs according to owners

Both polysomnography and whole-body barometric plethysmography require expertise and extensive equipment, and for the dog to be able to sleep in a laboratory setting. The unfamiliar surroundings of a sleep laboratory pose an issue

with polysomnography studies in humans due to the first-night effect.[102] The first-night effect causes changes in sleep macrostructure between the first and second sleep occasions[102] and can also affect the rate of SDB events. First-night effect occurs also in dogs, where the amount of sleep is greater and time spent awake less during the first sleeping session compared to following sessions.[103] A considerable portion, one third to almost a half, of dogs in sleep studies have had to be excluded due to lack in sufficient sleep stage duration[7,70,78,104] or panting throughout the recording.[27]

Polysomnography and whole-body barometric plethysmography are cumbersome and not easily applicable to everyday clinical work. In humans, various at-home screening methods are widely used for suspected sleep apnea patients[105,106], but no such options are currently available for dogs. This is reflected on the scarce amount of information available on SDB in dogs, and at-home screening methods would offer new insight into SDB in dogs.

Questionnaire screening tools can be used to assess symptoms of SDB in humans, including daytime sleepiness and snoring.[107,108] Questionnaires on owner-perceived signs of breathing disturbances, both awake and during sleep, have been used in dogs.[11,57,109,110] Questionnaires have mostly been utilized regarding assessment of brachycephalic obstructive airway syndrome (BOAS), but some have predominantly concentrated also on sleep.[111] Sleeping with mouth open, in a sitting position or head elevated, sleeping with toy in mouth, apneic episodes, and frequent change of position have been used as indicators of sleep dysfunction.[57,109] Nighttime behavior has also been evaluated in connection to canine cognitive dysfunction[66] and osteoarthritis[112], which may also cause nocturnal restlessness.

In dogs, daytime sleepiness might not be simple for owners to define, as the polyphasic nature of sleep in dogs means that sleeping during the day is typical.[50,63] Also, determining whether the dog is asleep or awake during the drowsiness stage is problematic for the viewer.[63] Owners, particularly the owners of brachycephalic dogs, can have unrealistically good perceptions of the health of their pet[109,113], which can lead to disregard of respiratory signs. Signs of potential sleep-disturbed breathing, such as snoring, can be deemed normal for the brachycephalic

breeds[57,113] or be difficult to distinguish from wake breathing patterns. Hence, there is no validated questionnaire to assess SDB in dogs.

2.2.3 Signs and clinical findings

As discussed above, recognition of signs related to SDB can be difficult for owners. In addition to not observing possible signs, cognitive dissonance is thought to contribute particularly to the ignorance of signs related to brachycephaly.[109] In cognitive dissonance the owner denies the problem, such as difficulty breathing, in their own pet because of psychological uncomfortability, while at the same time being conscious of the issue in larger scale within the breed.[108]

Signs reported in the brachycephalic breeds surmised to result from SDB include increased upper respiratory sounds or snoring[7,9,11,27,29,57,58,110,111,113-116], apneic episodes[7,27,29,32,34,58,110,117], sleeping with chin elevated[27,29,34,58,110,114], sleeping sitting up[27,34,110], sleeping with mouth open[33,34,110], choking sounds[7,34,110], frequent arousals[7,29,33,58,117], hypersomnolence[7,27,32], sleeping with an item, such as a toy, in mouth[58,114], and sleeping very little[34,110,117]. Additionally, restless sleep[58], cyanosis following an apneic episode[32], and gasping while sleeping[33,117] have been reported.

Hypoxemia occurring during episodes of SDB is documented by pulse oximetry in English Bulldogs.[7,12,23-26] Mild hypoxemia, assessed by arterial blood gas analysis, also occurs in wake brachycephalic dogs[7,116,118-120], which also show somewhat higher hemoglobin[116,120,121] and packed cell volumes.[116] It is postulated that the changes in red blood cell parameters are due to hypoxemia-induced increased erythropoietin production.[116] Higher hemoglobin values were also reported in older brachycephalic dogs, possibly suggestive of more severe recurring hypoxemia with ageing.[116] However, no difference in hemoglobin, hematocrit, or hemoglobin O_2 saturation in venous blood samples between brachycephalic and normocephalic dogs was noticed in other studies.[30,118] It should be noted that pulse oximetry may not be a clinically suitable alternative for assessment of oxygenation status, as the sensitivity for detection of hypoxemia is low.[98] Pulse oximetry is, however, the only noninvasive alternative for evaluation of oxygenation during sleep in dogs.
There are indications of cardiovascular disease possibly related to SDB in brachycephalic dogs. In man, the correlation between OSA and thromboembolic

and cardiovascular risk is well established.[15,16] Sleep fragmentation and sleep deprivation is thought to lead to increased sympathetic activation and low-grade inflammation and result in myocardial damage. Although the association between SDB and cardiac arrhythmias in dogs is unknown, in man, OSA is strongly associated with dysrhythmia.[15,16] Hypercoagulability is reported in modest groups of brachycephalic dogs.[121,122] Both higher mean[119] and systemic[116] arterial blood pressures are observed in brachycephalic dogs. Differences in echocardiographic variables, including higher right heart pressures and lower systolic and diastolic ventricular function in brachycephalic dogs compared to normocephalic dogs, are reported.[123] Cardiac troponin 1, found explicitly in cardiac muscle and utilized as a diagnostic and prognostic marker of cardiac disease[124,125], was over reference range in 47.8% of brachycephalic dogs, indicating myocardial damage.[126] Interestingly, many brachycephalic breeds, including English Bulldogs and Boxers, are affected by arrhythmogenic cardiomyopathy[127], which may present as sudden death. Furthermore, atrial structure remodeling is shown to occur in an induced canine model of OSA.[93]

2.2.4 Risk factors

2.2.4.1 Brachycephaly

Brachycephaly, a shortened and flattened skull type achieved through decades of selective breeding[1], is a risk factor for SDB.[7,12,23] Marked differences in breathing during sleep between modest groups of brachycephalic dogs and normocephalic dogs were first reported in the late 1980's.[7] Subsequently, SDB has been reported in brachycephalic dogs in studies assessing breathing during sleep both objectively[12,23-27] and subjectively.[32,33,117]

In brachycephaly, the skeletal muzzle is shortened due to altered growth of the basioccipital and basisphenoid bones.[3] As there is no corresponding soft tissue decrease, a mismatch of tissue in available space occurs.[4,5] Brachycephaly can be characterized by different morphological measurements of the skull, including the craniofacial index[11] and width-to-length ratio.[1] Extremely brachycephalic breeds are considered to include the English Bulldog, French Bulldog and Pug.[128] Numerous other breeds, including the Boston Terrier, Boxer, Bullmastiff, and

Cavalier King Charles Spaniel, are considered brachycephalic, although they show less severe brachycephalic characteristics. However, there is individual variation in morphological measurements within the non-extremely brachycephalic breeds, and evaluation of the brachycephalic status of individuals in these breeds can thus require measurement of morphometric data.[129]

BOAS is prevalent in brachycephalic dogs and rarely reported in normocephalic dogs.[6] In BOAS, the obstruction of the airways occurs due to the soft tissues, including soft palate, tongue, and tonsils, interfering with airflow in the reduced space.[5] Anatomic abnormalities associated with brachycephaly and the resulting BOAS include narrowing of the nostrils[128,130,131], oversized nasal turbinates[99,132], and an elongated soft palate.[99,130] Hypoplastic trachea[99] and macroglossia[133], an enlarged tongue, are also sometimes seen in BOAS. Overcoming the resulting increased airway resistance requires increased respiratory effort and negative intra-airway pressures, which can result in everted tonsils[130], everted laryngeal saccules[130], laryngeal collapse[99,134], and bronchial collapse.[135] These changes further impede airflow and contribute to worsening clinical signs of BOAS.

In brief, signs of BOAS include difficulty breathing, i.e., dyspnea, snoring, stertor and stridor, exercise intolerance, and gastrointestinal issues including regurgitation and vomiting.[11] Severe dyspnea can lead to cyanosis and syncope.[10] Breathing during sleep is compromised.[7,12] Exercise intolerance is common in brachycephalic dogs.[9,11] Additionally, dogs with BOAS are vulnerable to overheating, as the thermoregulatory function of the nose is impeded by obstruction.[8] Changes in sleep macrostructure are also documented in brachycephalic dogs, as brachycephalic dogs sleep more and spend more time in REM than NREM compared to normocephalic dogs.[136]

Severity of BOAS can be graded by assessing breathing and body temperature pre- and post-exercise.[9,100,114] Dogs are assigned to grade 0 (no signs of BOAS), grade 1 (mild signs), grade 2 (moderate signs), or grade 4 (severe signs). The grades can be further divided into two classes: BOAS negative dogs with no or mild signs of BOAS (grades 0 and 1) and BOAS positive dogs with moderate or severe signs of BOAS (grades 2 and 3).

In man, there is evidence for increased anterior facial height and lower hyoid position predisposing to OSA.[137] Other craniofacial findings, including retrognathia and micrognathia[138], overjet[139], and open bite[138] are often associated with OSA. Additionally, in children a major risk factor for SDB is enlarged adenoids and tonsils, and adenotonsillectomy improves the degree of SDB events.[140]

2.2.4.2 Obesity

Associations between obesity and SDB in dogs have not been studied. Obesity is, nonetheless, a risk factor for BOAS.[100,101,129] Obesity deteriorates respiration in brachycephalic dogs, including decreasing minute volume and limiting flow during both inspiration and expiration.[101] Increased tongue fat volume occurs in brachycephalic dogs[133,141,142], which contributes to macroglossia and upper airway obstruction. Obesity worsens respiratory parameters, including tidal volume, also in otherwise healthy normocephalic dogs.[143] Obese dogs have also been shown to have lower PaO_2 compared to dogs of normal weight, indicating diminished lung blood oxygenation.[144] Weight loss improves respiratory function and oxygenation in normocephalic dogs.[144]

In man, obesity is a major risk factor for OSA.[145-147] Particularly excess upper airway fat predisposes to OSA[148,149], and increasing degree of parapharyngeal fat deposition is associated with increasing severity of OSA.[150] Weight loss is an effective treatment form of OSA in humans.[149,151]

Obesity is presumed to lead to pharyngeal collapsibility through impaired neuromuscular and mechanical responses.[152,153] Lung volume is decreased in obesity.[154] Airflow resistance is increased because of the narrowing of the upper airway due to fat deposition.[155] Changes in concentrations of neurohormonal molecules, such as adipokines produced by excess adipose tissue, could contribute to decreased central nervous system activity.[156]

2.2.4.3 Gender and hormonal status

No studies analyzing the associations between SDB and gender or neuter status are available. However, male French Bulldogs are at a higher risk for BOAS than females of the same breed.[100] In numerous studies of dogs with moderate or severe signs of BOAS the males outnumber females, suggestive of higher risk in males[29,110,126,130,157,158], but statistical analyses are lacking.

In man, the prevalence and severity of OSA is considerably higher in males compared to females.[147,159-161] OSA is more common and severe in men even when matched for degree of obesity and age.[162] Apnea duration is higher and oxygen saturation more severe in men compared to women.[163] This gender difference decreases with age with later onset of OSA in women[161], possibly due to postmenopausal hormonal changes.[159] After menopause, progesterone levels decrease, which depress muscle activity of the upper airway dilator muscles.[159,164] Alongside the impact of progesterone, the reasons behind sex differences in OSA risk are suspected to relate to differences in facial[165] and upper airway anatomy.[166]

2.2.4.4 Age

Ageing can worsen signs of BOAS, as secondary changes occur due to the increased pressure gradient needed to overcome upper airway obstruction. These changes, including everted tonsils[130], laryngeal collapse[130], and bronchial collapse[135] cause further respiratory obstruction. Worsening of BOAS signs with age has also been reported by owners.[110] However, when re-evaluated two to three years after initial assessment, the severity of BOAS signs remained fairly constant in a group of healthy young and middle-aged brachycephalic dogs.[115]

In humans, the prevalence of OSA increases with age.[146,161,167] Although studies have suggested a plateau in the prevalence of OSA after the age of 65[168], others have found increasing prevalence until the age of 80.[161] The gender differences presented in the previous paragraph diminish with age.[161] Age-related changes in upper airway collapsibility are, at least in part, attributed to decreasing upper airway reflexes.[169,170]

2.2.4.5 Other risk factors

Other risk factors for SDB possibly relevant in dogs are reviewed in the following section. Discounting Chiari-like syndrome in certain breeds, SDB has not been reported to occur in dogs with these conditions.

In Chiari malformation in man, some or all of cerebellum and brain stem herniate through the foramen magnum into the cervical canal.[171] The condition is associated mainly with CSA.[171-173] A similar developmental abnormality is seen with the Chiari-like syndrome in dogs, prevalent in various small breeds.[174-176] Chiari-like malformation can occur in conjunction with brachycephaly, and it is very common in Cavalier King Charles Spaniels.[175] Chiari-like malformation has also been reported in the extremely brachycephalic Pug.[177] SDB has been described in Cavalier King Charles Spaniels[27], a Pug[33], and a Chihuahua[32], all breeds in which the malformation is prevalent. However, there is no data on whether these dogs with suspected SDB indeed had Chiari-like malformation. Although the distinction between central and obstructive origin of SDB events could not be made with the whole body barometric plethysmography method used by Hinchliffe et al.[27], in humans with Chiari malformation, apneas and hypopneas of both central and obstructive origin are described.[171-173]

Certain sedative medications are associated with apneas and hypopneas in humans. Gabapentin increases the number of apneas and hypopneas during NREM sleep in older men, perhaps through increased upper airway collapsibility due to muscle relaxation.[178] This is relevant in pet dogs, as gabapentin is commonly used in dogs for chronic pain management and, to a lesser extent, epilepsy.[179] Additionally, opioid use is associated with central SDB events in humans[180] due to respiratory rate depression and altered sensitivity to hypoxemia and hypercapnia.[181]

In man, SDB is also described in patients with hypothyroidism[182], acromegaly[183], and chronic kidney disease.[184] Additionally, a specific breathing pattern of centrally originated SDB events, Cheyne-Stokes breathing, is often seen in patients with congestive heart failure.[185]

2.2.5 Pathogenesis of obstructive sleep-disordered breathing

The mechanism behind obstructive apneas and hypopneas is the repeated collapse and obstruction of the upper airways during sleep. The resulting lack of airflow leads to hypoxemia, decreased PaO_2, and hypercapnia, increased $PaCO_2$. Peripheral chemoreceptors are activated and ventilatory effort is increased.[186] Ultimately, hypoxemia leads to an abrupt awakening, and the obstruction of the upper airways is alleviated by the rapidly contracting upper airway muscles during waking.[14] The cycle of collapse and hypoxemia continues when the individual returns to sleep. Repeated arousals lead to fragmentation of sleep and changes in sleep macrostructure. Increased REM sleep, possibly suggestive of REM fragmentation and need for rebound REM sleep, occurs in brachycephalic dogs.[136]

Craniofacial structure, the surrounding tissues of the upper airway, neuromuscular activity of the dilator muscles, and intraluminal negative pressure created by inspiration affect upper airway patency and the size of the airway lumen.[187,188] The canine upper airways consist of the muzzle (nares and nasal cavity), pharynx, larynx, and extrathoracic trachea. In brachycephalic dogs, the size of the pharynx is already narrow due to the skeletal mutation leading to brachycephaly, predisposing the airways to obstruction. During sleep, the activity of the upper airway dilator muscles is reduced, leading to decreased pharyngeal opening. Muscle tone during REM sleep is at its lowest[67], and unsurprisingly, SDB episodes occur mostly in REM sleep in brachycephalic dogs.[12,24] In contrast, RERAs are the most common type of SDB event seen in NREM sleep[24,25], but these are of lesser importance as they seldom lead to desaturation or arousal.[24]

Higher upper airway dilator activity is seen in English Bulldogs compared to normocephalic control dogs.[23] Increased connective tissue and abnormal morphology of muscle fibers are reported in the sternohyoid, an upper airway dilator muscle, of English Bulldogs.[189] The geniohyoid muscle, another upper airway dilator, also showed abnormal morphology but not increased connective tissue.[189] Specifically, the muscle fibers in English Bulldog showed conversion to a faster myosin heavy chain II phenotype[189], suggesting repeated stimulation and contraction during arousals. Fibrosis and increased connective tissue resulting from activity-induced muscle injury in the sternohyoid supports an increased

workload for the dilator muscles.[189] These changes in the morphology of the muscle fibers are likely to worsen collapsibility of the upper airways during sleep.[116] In humans with OSA, disturbances in neuromuscular responses are also seen, as airway obstruction does not lead to the compensatory dilation and elongation of the upper airways to relieve obstruction.[152]

Intrathoracic pressure changes can lead to mechanical trauma, further occluding the airways. Pleural pressures in brachycephalic dogs have not been reported. However, there is evidence for recurring high negative pressures to overcome high inspiratory airflow resistance, as shorter expiratory to inspiratory times ratios[99] and bronchial collapse[135] are reported in brachycephalic dogs.

2.2.6 Pathogenesis of central sleep-disordered breathing

In centrally originated SDB, there is no respiratory effort during apneic and hypopneic episodes.[188] This contrasts with obstructive SDB, where there is ongoing respiratory effort during SDB events. In man, obstructive and central apneas can also overlap resulting in mixed events, which begin with a loss of respiratory effort and continue to an increasing attempt of respiration against a collapsed airway.[188]

Various etiologies with diverse underlying mechanisms lead to CSA in man. In principle, CSA occurs because of brief loss of output from the respiratory center responsible for generating respiratory rhythm.[13,188] There is no transmission of impulse to the effector muscles and consecutively, ventilation does not occur. A defect in respiratory drive or disturbance in the metabolic or neural control of ventilation lead to loss of respiratory output and central apneas and hypopneas.[172,188]

2.2.7 Treatment

2.2.7.1 Surgical

As obstructive SDB is strongly associated with brachycephaly in dogs, surgical treatment of factors contributing to the upper airway obstruction leading to BOAS are likely to alleviate signs of SDB. Numerous surgical procedures are described for

mitigation of clinical signs arising from BOAS.[10,130] This literature review will not go into detail on the different surgical techniques used. However, it should be noted that due to their anatomy, brachycephalic dogs are susceptible to anesthetic complications in both the operative[190] and postoperative[191] period, and surgical alleviation of BOAS is not without risk.

A radical reduction in the amount of SDB events during REM sleep is reported in one English Bulldog after surgical resection of excess pharyngeal soft tissue.[23] In this dog, the rate of SDB events decreased from 48 to 0 events per hour.[23] Owner-reported incidence of apneic episodes during sleep was reduced from 47% (16/34) to 3% (2/62) after multilevel BOAS surgery.[34] Multilevel BOAS surgery alleviated owner-perceived signs of SDB also in a cohort of three Cavalier King Charles Spaniels[27] and in a group of 34 brachycephalic dogs of multiple breeds.[35] However, continuation of SDB signs following multilevel BOAS surgery is also reported.[117]

Briefly, surgical procedures in dogs affected by BOAS include widening the stenotic nares and nasal vestibules[192], removal of nasal conchae[193], shortening and thinning of the soft palate[34], and removal of everted laryngeal saccules and enlarged tonsils.[34] Further procedures, such as laryngoplasty, the trimming of the cuneiform processes, can be performed in dogs with more severe laryngeal collapse.[34]

2.2.7.2 Conservative

In man, conservative, that is, non-surgical, treatment forms of OSA consist primarily of continuous positive airway pressure (CPAP), weight loss, changing sleeping position, and medications.[86]

Response to medical treatment of SDB, aimed at excitation of the upper airway motoneurons and thus increasing the activity of upper airway dilator muscles, has been reported in small groups of brachycephalic dogs.[24,25,32,33]

Serotonin, also known as 5-HT, has a role in the maintenance of upper airway dilator activity.[26] Serotonin antagonists have been shown to reduce upper airway dilator activity, increase upper airway collapse and aggravate desaturation in English Bulldogs in wake.[26] Serotonergic medications, both agonists and

antagonists of serotonin receptors, have been used for treatment of SDB in dogs in clinical studies[24,25] and case reports.[32,33]
Ondansetron, a serotonin receptor antagonist, has been used for treatment of SDB in dogs. Although ondansetron did not fully resolve SBD or affect time spent in desaturation, it reduced the amount of both apneas and hypopneas during REM sleep in a clinical study.[24] Ondansetron has since been successfully used in case studies documenting suspected SDB in a Pug[33] and a Chihuahua.[32] In clinical practice, ondansetron is widely used as an antiemetic, for example to combat nausea associated with vestibular syndrome in dogs.[194]

Trazodone, a mixed agonist and antagonist of serotonin receptors, combined with serotonin precursor L-tryptophan, decreased the amount of SDB events in NREM sleep, but had only moderate improvement on SDB events in REM sleep.[25] Suppression of upper airway dilator muscles and fragmentation of sleep were decreased in English Bulldogs with trazodone and L-tryptophan.[25] However, a common side effect of trazodone is sedation[195], and partly due to this side effect, trazodone is also used to facilitate postsurgical confinement in dogs after orthopedic surgery.[196] This sedative effect and resulting hypersomnolence are likely to limit the usability of trazodone in treatment of SDB in dogs.

A serious, possibly life-threatening side effect of serotonergic medications is serotonin toxicity, in which excessive amounts of serotonin accumulate in the central nervous system.[197] Serotonin toxicity is rare, and usually occurs after treatment with a combination of serotonergic medications or accidental poisoning by overingestion.[197] Signs include hypersalivation, vomiting, tremors, agitation, and hyperthermia.[197] Treatment is supportive and aims at preventing further absorption of the medication and controlling seizure activity and hyperthermia.[197] Brachycephalic dogs have dysfunctional thermoregulation and may thus be more at risk for hyperthermia induced by serotonin toxicosis. Additionally, dogs with a mutation in the ABCB1 gene are more likely to experience toxicosis from ondansetron.[198] Although such side effects are rarely encountered, selecting the right individuals for medical treatment of SDB is crucial.

In humans, CPAP is the gold standard for treatment of OSA. The CPAP device prevents upper airway collapse during sleep by providing continuous positive pressure during inspiration and expiration.[199] It markedly reduces the amount of SDB events, with complete resolution of apneic and hypopneic events in some patients.[200] Subjective evaluations of sleepiness[201] and quality of life[201,202] improve with CPAP treatment. CPAP provided with a pediatric helmet has been used in sedated healthy dogs[203], in dogs recovering from general anesthesia[204,205], in brachycephalic dogs recovering from general anesthesia[206], and in severely ill dogs for treatment of hypoxemic acute respiratory failure[207] and acute cardiogenic pulmonary edema.[208] Although advantageous and relatively well tolerated in sedated and gravely ill dogs, the feasibility of CPAP for treatment of SDB in dogs is questionable. In dogs, familiarization to the use of the device requires great effort.[117] However, CPAP provided by an induction mask and secured with a soft muzzle has been used successfully for years in one Cavalier King Charles Spaniel with marked signs of SDB.[117] The facial conformation of severely brachycephalic dogs might not allow for sufficient and successful fit of the mask[117], and a pediatric helmet could be of more use in these breeds. In humans the adherence to CPAP use is variable[209] and positive response to treatment relies on adequate duration of use each night.[210]

Other potentially usable medication modalities in dogs include intranasal corticosteroids, which can be effective in reducing AHI in some patients with OSA.[211,212]

Although the use of internal and external nasal dilatators[213,214] and surfactant administration via nasal catheter[215] are reported in humans with OSA with varying results, but their usability in dogs is presumed to be low due to issues with administration of these treatments.

Weight loss is indisputable in improving respiratory function in dogs.[144] Weight loss improves lung capacity and oxidative status of healthy dogs.[144] In man, weight loss in OSA treatment has been vastly studied. Weight reduction is effective in decreasing the severity of OSA[216,217] and is recommended for obese and overweight OSA patients.

The collapsibility of the upper airway can be more severe in certain positions due to gravity. Elevation of the head is reported to alleviate signs of suspected SDB in two dogs[32,33], and positional therapy, that is, devices preventing sleeping in a certain position, is effective in some OSA patients.[218] In humans, supine position, i.e., lying on one's back, can exacerbate OSA[219], and positional therapy is recommended for positional OSA.

2.3 Inflammation

2.3.1 Inflammation and inflammatory mediators

Inflammation is a key part of immunity. It is the body's response to tissue injury, aimed at eliminating a triggering stimulus and initiating tissue resolution and repair. Inflammation can be acute and momentary or chronic and sustained. Innate immunity is the first line of defense against nonspecific triggers, while adaptive immunity is acquired through encountering specific stimuli. Stimuli for inflammation include microbes, toxic compounds, damaged cells, and hypoxemia.[220] This literature review focuses on inflammation caused by hypoxia, the lack of oxygen at tissue level, as a result of hypoxemia, an abnormally low concentration of oxygen in blood. In humans, hypoxemia is readily assessed by arterial blood gas analysis or noninvasively by pulse oximetry. However, in non-ventilated dogs pulse oximetry does not appear to be a reliable screening method for hypoxemia, and arterial blood gas analysis or ratio of pulse oximetry saturation to fraction of inspired oxygen might be more valuable alternatives.[98] Although hypoxia and hypoxemia are often used interchangeably in literature, tissue level oxygenation is difficult to evaluate and thus hypoxemia is often a more accurate term.

Inflammatory mediators are part of innate immunity.[221] They control the generation, maintenance, and resolution of the inflammatory response.[220] Inflammatory mediators are molecules produced by the liver or released by inflammatory cells in response to a triggering stimulus.[220] These inflammatory cells include platelets, neutrophils, monocytes, macrophages, and mast cells. Other cell

types, including fibroblasts, smooth muscle cells, and endothelial cells, can also produce inflammatory mediators.

Inflammatory mediators include cytokines, which activate endothelial cells[220], and chemokines, which recruit immune cells to the site of inflammation.[222] Other inflammatory mediators include, among others, vasoactive amines, arachinoid acid derivatives, kinins and polyunsaturated fatty acid derivatives.[220,223] Inflammatory proteins can be categorized into inflammation-promoting proinflammatory mediators and inflammation-neutralizing anti-inflammatory mediators. The exact mechanism of action depends on the particular inflammatory mediator.

Proinflammatory mediators facilitate inflammation by increasing the production of other proinflammatory proteins, activating the production of acute phase proteins including C-reactive protein (CRP), and attracting inflammatory cells to the site of inflammation. They may also act as endogenous pyrogens and induce fever.[221] Proinflammatory mediators include, among others, TNF-α, IL-1, IL-8, vascular endothelial growth factor A (VEGF-A) and chemokine (C–C motif) ligand 2 (CCL2, also known as monocyte chemoattractant protein 1 [MCP-1]).

Anti-inflammatory mediators prohibit inflammation by neutralizing cell stimulation and the synthesis of proinflammatory mediators. The most prevalent anti-inflammatory mediators are IL-4, IL-10, and IL-13.

2.3.2 Assessment of inflammation

Chronic low-grade inflammation is associated with numerous persistent disease processes, such as type 2 diabetes mellitus[224] and SDB.[19] The causality and extent to which low-grade inflammation contributes to the pathogenesis of different disorders is ambiguous. However, in man, chronic low-grade inflammation is recognized as an independent risk factor for a plethora of chronic disorders, including coronary heart disease[20] and neoplasia.[21] Low-grade inflammation also increases total mortality in man.[22] Hence, the presence of low-grade inflammation is relevant.

The classic signs of inflammation are heat, pain, redness, swelling, and loss of function. In low-grade inflammation such visible signs are often not present, and other methods for assessment of inflammation are needed.

Inflammatory mediators increase the production of acute phase proteins. Acute phase proteins, including CRP, serum amyloid A, and haptoglobin, can be assessed from canine serum samples.[225] CRP is currently the most frequently used and commercially widely available inflammatory marker in dogs. The detection limit for commercial measurements is often 10 mg/l[226], and values below this are considered nonsignificant in clinical practice. High-sensitivity CRP is used to measure CRP concentrations below 10 mg/l[227] and can be utilized in detecting low-grade inflammation in various disease processes in dogs.[227-229]

Proinflammatory mediators can be evaluated from different sample types, including serum[230] and plasma[231], tissue biopsies[232], and bronchoalveolar lavage fluid (BALF).[233,234] Laboratory methods for measurement of cytokines and chemokines include bead-based immunoassay[231,235] and enzyme-linked immunosorbent assay.[236] However, the lack of standardization and limited availability of these techniques hinder their use outside research purposes. Additionally, the stability of many proinflammatory mediators does not allow for repeated freeze-thaw cycles or feasible transport to external laboratories.[235,237]

Inflammatory status can also be assessed by evaluation of white blood cells in both blood[238] and BALF samples.[239] Ratios between the different cell lines can also be applied to assess inflammation.[240]

It should be noted that changes in inflammatory status, including CRP values, white blood cell counts and proinflammatory mediator concentrations, are nonspecific to source of inflammation. Such changes also occur in dogs in for example neoplasia[241] and immune-mediated disease.[242] Additionally, exercise[243] and breed[244] can influence results.

2.3.3 Hypoxemia-induced inflammation

Low-grade inflammation associated with SDB in man is assumed to occur due to intermittent hypoxia and hypoxemia caused by the apneic and hypopneic episodes during sleep.[245] Lack of oxygen activates the hypoxia signaling pathway, primarily governed by stabilization of the hypoxia-inducible factor (HIF). The activated HIF complex regulates immune cell functions, including cell motility[246], migration[247], and proliferation.[248] HIF is involved in the upregulation, i.e., increase of gene expression, of numerous cytokines[249] and chemokines.[250]

Hypoxemia-induced inflammation is not always due to disease, but also occurs in healthy dogs. Hypoxemia of high altitude causes increases in inflammatory mediators, including the cytokine VEGF-A[251], which induces vascular growth and increases vascular permeability.[252,253] In man, elevated IL-6, IL-1 receptor antagonist, and CRP concentrations are reported in response to hypoxemia of high altitude.[254]

Table 4 compiles current knowledge of low-grade inflammation in brachycephalic dogs, presumably due to intermittent hypoxemia resulting from upper airway obstruction during wake and sleep. These changes have not been evaluated in connection with assessment of SDB.

Table 4 Summary of literature assessing inflammation in brachycephalic dogs.

– denotes no change, ↑ increase, and ↓ decrease. BD, brachycephalic dog; BOAS, brachycephalic obstructive airway syndrome; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; NO, nitric oxide; TNF, tumor necrosis factor.

* Likely due to postoperatively given corticosteroid medication

Measure of inflammation	**Type of inflammatory mediator**	**In BDs compared to normocephalic control dogs or other specified group**	**In BDs with different severity of BOAS signs**	**Methodology**
CRP	Pro	- [30] - compared to reference values[126] ↑ than reference range in 51,9% of BDs[29]	- [126] - before and after BOAS surgery[157]	Immunoturbidimetric assay[30,126,157]

Measure of inflammation	Type of inflammatory mediator	In BDs compared to normocephalic control dogs or other specified group	In BDs with different severity of BOAS signs	Methodology
Haptoglobin	Pro	- compared to reference range[126]	- [126] ↑ after BOAS surgery*[157]	Colorimetry[126,157]
Eosinophil	Pro	↑ [28] ↓ [30]		
IL-1β	Pro	- [31]		ELISA[31]
IL-6	Pro	- [31]	↓ with moderate signs of BOAS compared to controls[31]	ELISA[31]
IL-10	Anti	↑ [31]		ELISA[31]
IL-13	Anti	↑ [31]	↑ with no signs of BOAS compared to controls[31]	ELISA[31]
IL-17A	Pro	- [31]	↑ with severe signs of BOAS and requiring surgery compared to controls and BDs with no or moderate signs of BOAS[31]	ELISA[31]
Lymphocyte	Pro	↓ [30]		
Monocyte	Pro	↑ [28] - [30]		
Neutrophil	Pro	↑ [28] - [30]	Positive correlation with BOAS severity[28]	
Neutrophil to leucocyte ratio	Pro	↑ [28] ↑ [30]	Positive correlation with BOAS severity[28]	
NO	Anti-inflammatory under normal physiological conditions; proinflammatory in abnormal conditions[255]	↑ [31] ↑ [30]	↑ with severe signs of BOAS and BDs requiring treatment compared to controls[31]	Griess reaction (colorimetry)[31] Chemiluminecence[30]
TNF-α	Pro	↑ [31]		ELISA[31]

The association between low-grade inflammation and OSA is widely documented in humans.[256-259] However, OSA is frequently associated with other comorbidities that induce low-grade inflammation, such as obesity and cardiovascular disease, and this needs to be considered in the interpretation of results. Nonetheless, there are many studies reporting correlation of OSA severity and concentrations of inflammatory mediators, including chemokine CCL2[257,260] and cytokine VEGF-A.[256]

Alongside BOAS and SDB, hypoxemia occurs also in other pathological processes affecting the respiratory system. Canine idiopathic pulmonary fibrosis (CIPF) is a progressive, fibrosing interstitial lung disease.[261,262] CIPF occurs mainly in West Highland White Terriers (WHWTs)[261,262], which is strongly suggestive of a genetic predisposition to the disease. CIPF bears resemblance to idiopathic pulmonary fibrosis (IPF) in humans.[261,263] The etiology of the process is unknown, but microaspiration of gastroduodenal contents is suggested to play a role in the pathogenesis or exacerbation of disease.[264,265] Both CIPF and IPF lead to the abnormal accumulation of collagen in the lung parenchyma[266] and ultimately to end-stage lung disease. The accumulation of collagen prevents gas exchange and results in moderate to severe hypoxemia.[233,263] Inflammatory changes, seen as increases in chemokine CCL2, are reported in both healthy WHWTs in serum[236] and WHWTs with CIPF in serum and BALF.[234,267] In study III, dogs with CIPF were included as a model of chronic hypoxemia in contrast to suspected intermittent mild hypoxemia in brachycephalic dogs.

3 Aims of the thesis

1. To evaluate the usability of a portable neckband system for detection of sleep-disordered breathing in dogs in their home surroundings (I).

2. To assess risk factors for obstructive sleep-disordered breathing in both brachycephalic and normocephalic dogs (II).

3. To describe owner-perceived signs of sleep-disordered breathing in dogs (II).

4. To evaluate the low-grade inflammatory condition of brachycephalic dogs with possible intermittent hypoxemia (III).

4 Materials and methods

4.1 Ethical approval for the study protocols (I-III)

All animals were privately owned pet dogs. Before participation, all owners signed an informed consent form. The study protocols for studies I and II were approved by the Committee of Experimental Animals of Southern Finland (ESAVI/10906/04.10.07/2017, ESAVI/34278/15.11.21/2021) and by the University of Helsinki Viikki Campus Research Ethics Committee (13/2020, 11/2021). For study III, the study protocol was approved by the Committee of Experimental Animals of Southern Finland (ESLH-2008-05403/Ym-23, ESAVI-2010-03587/Ym-23, ESAVI/1005/04.10.03/2011, ESAVI/7383/04.10.07/2013, ESAVI/11519/04.10.07/2014, ESAVI/10906/04.10.07/2017), the Ethical Committee of the University of Liège (no: 1435 and 2245) and by the University of Helsinki Viikki Campus Research Ethics Committee (5B/2008, 1/2014, 13/2020).

4.2 Animals

4.2.1 Studies I and II

The prospective, observational cross-sectional studies I and II were done at the Veterinary Teaching Hospital, University of Helsinki, Finland. A minority of study visits were performed at the Kaarina Veterinary Clinic, Kaarina, Finland. Privately owned dogs were prospectively recruited to participate in these studies. Inclusion and exclusion criteria for studies I and II are presented in Table 5. Dogs with illnesses or medications not directly affecting breathing during sleep were not excluded. Part of the normocephalic dogs (n = 12) and brachycephalic dogs (n = 12) were included in both studies I and II.

Table 5 Inclusion and exclusion criteria for studies I and II.

* Including gabapentin, ondansetron, tricyclic antidepressants

	Study I	**Study II**
Inclusion criteria		
Skull conformation (i.e., brachycephaly or normocephaly)	x	x
Age >1 year	x	x
Weight >9 kg	x	
Weight >4 kg		x
Owner-perceived signs of sleep-disordered breathing		x
Anestrus	x	x
Exclusion criteria		
Pregnancy	x	x
Lactation	x	x
*Medications affecting breathing during sleep**	x	x
Gastroesophageal reflux requiring treatment	x	x

For study I, cooperation with the Finnish French Bulldog Club was done to recruit dogs for sleep recordings. Convenience sampling was used, meaning that dogs living at a convenient geographical proximity to the researchers with owners willing to take part in the study were included. For study II, owners were encouraged to take part by promoting the study in the Finnish Kennel Club magazine and website, and through social media platforms and breed clubs. An online questionnaire for study II was iteratively designed by the authors. The questionnaire was tested with dog owners from a non-veterinary background prior to publication. The questionnaire was managed via the REDCap electronic data capture system hosted at the University of Helsinki.[268,269] The questionnaire was based on prior sleep dysfunction questionnaires for dogs[11,109] and OSA questionnaires for humans.[107] The questionnaire focused on the dog's demographics, medical history, sleeping customs, owner-perceived signs of SDB, owner-perceived sleepiness, and owner-perceived signs of other sleep disorders. The questionnaire is presented in Appendix 1. The owner-perceived signs of SDB were restless sleep, snoring, sleeping sitting up, sleeping with toy in mouth, sleeping with head hanging off the bed, waking up gasping, and apneic episodes during sleep. Dogs for sleep recordings were selected from the questionnaire replies, with emphasis on brachycephalic dogs

and dogs with owner-perceived signs of SDB. Normocephalic dogs without owner-perceived signs of SDB were also selected for sleep recordings.

4.2.2 Study III

The cross-sectional study III was done at the Veterinary Teaching Hospitals of the University of Helsinki, Finland, and the University of Liège, Belgium. Privately owned dogs were recruited. Healthy normocephalic control dogs with BALF samples were examined at the University of Liège, and other dogs at the University of Helsinki. The dogs had been included in other studies before[9,114,115,270], but not in connection to measurement of VEGF-A or CCL2. Inclusion and exclusion criteria for study III are presented in Table 6.

Table 6 Inclusion and exclusion criteria for study III.

BOAS, brachycephalic obstructive airway syndrome; CIPF, canine idiopathic pulmonary fibrosis; WHWT, West Highland White Terrier

	Brachycephalic dogs	**Healthy WHWTs**	**WHWTs with CIPF**	**Normocephalic control dogs**
Inclusion criteria	- English Bulldog or French Bulldog or Pug - Age 1–5 years at initial visit - No history of medical or surgical treatment of BOAS - Owner-reportedly healthy - No signs of disease excluding BOAS - No significant changes on clinical examination	- WHWT - No signs, clinical examination findings or abnormal findings suggestive of CIPF in diagnostic imaging - No hypoxemia	- WHWT - All or most of: compatible history and signs of CIPF, hypoxemia, characteristic findings in diagnostic imaging or post-mortem histopathology	- No signs of illness - Normal physical examination - Normal hematology and serum biochemistry - Not brachycephalic
Exclusion criteria	Corticosteroid or cyclosporine treatment within two weeks of sampling			

4.3 Severity grading of brachycephalic obstructive airway syndrome (I-III)

In all brachycephalic dogs in study III and most brachycephalic dogs in studies I and II, the severity of BOAS was graded either at the study or within six months of

the sleep recording, as presented in Figure 2 and previously described by Liu et al.[100] and Lilja-Maula et al.[9] Grading included the assessment of audible upper respiratory noise and body temperature before and after an exercise tolerance test of either 1000 meters, 6 minutes or 3 minutes, and the assessment respiratory effort, dyspnea, and cyanosis. A body temperature of over 39,3 °C and/or dyspnea, defined as labored breathing with continuous stertor and/or stridor and the use of accessory respiratory muscles, prior to or during the exercise tolerance test resulted in discontinuation of the test and a BOAS grade of 3.

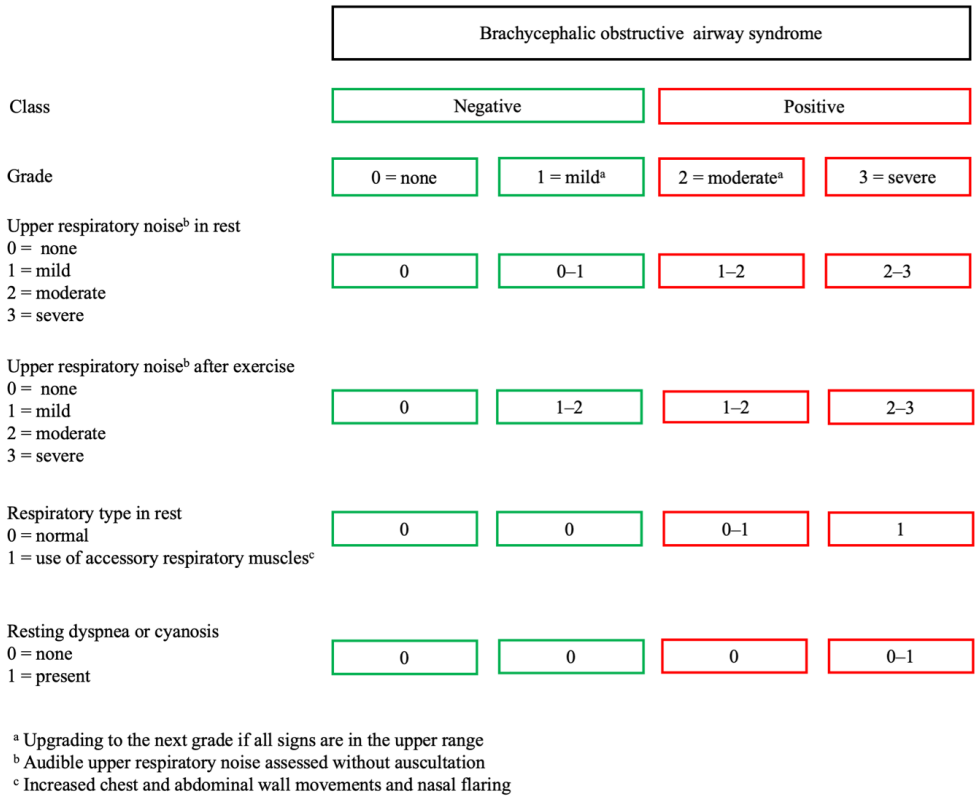


Figure 2 Classification of clinical severity of brachycephalic obstructive airway syndrome, based on Liu et al.[100] and Lilja-Maula et al.[9].

4.4 Study visits

4.4.1 Studies I and II

The majority of dogs in studies I (21/24) and II (46/63) attended a study visit. Due to geographical location or fear of veterinary visits only the at-home sleep recording was performed some dogs (3/24 in study I; 17/63 in study II). During the visit, a physical examination was conducted, and body condition score (BCS) assessed on a scale of 1–9. Blood samples for health verification were obtained, and hematology and basic serum biochemistry were performed. Assessment of BOAS was done for the brachycephalic dogs as described in section 4.3.

4.4.2 Study III

Blood and BALF samples were collecting during a study visit. Samples were stored frozen at – 80 °C until analysis. During the study visit, a physical examination was performed, and BOAS severity grade assessed as described in section 4.3.

For WHWTs and normocephalic control dogs, BALF samples were obtained by bronchoscopy as described previously by Krafft et al.[233] and Heikkilä et al.[263] In brief, one or two portions of sterile saline (1–2 ml/kg of 0.9% NaCl) were introduced via a bronchoscope (Olympus GIF type N30, Fujinon EB-530, Fujifilm) into two different lung lobes. In the brachycephalic breeds, BALF sampling was only performed in the English Bulldogs. BALF samples were collected via a catheter, with portions of 7–8 ml per lung lobe.

To assure compatibility between the differently obtained BALF samples, the percentage of epithelial lining fluid in NaCl was calculated by assessment of urea in BALF samples of all dogs. The epithelial lining fluid percentages of the English Bulldogs corresponded to that of the other dogs (Minna M. Rajamäki, personal communication, January 15th, 2022).

4.5 Assessment of sleep-disordered breathing variables (I, II)

A portable neckband system (Nukute Ltd, Oulu, Finland), developed for OSA diagnostics in humans, was used for one night at the dogs' home surroundings in studies I and II. The device provides results comparable to polysomnography in humans.[271] The neckband system comprises a c-shaped neckband, a pulse oximeter (Berry BM2000D, Shanghai Berry Electronic Technology Co Ltd, Shanghai, China), and a tablet which routes the data and administers instructions on use of the device. The finger probe pulse oximeter could not be used in dogs. Furthermore, pulse oximetry may not be a reliable method of evaluating oxygenation in awake dogs.[98] The neckband includes a piezoelectric microphone for tracheal sounds, an ambient microphone, and a gyroscope for data on position and movement. The neckband comes in four sizes and can be used for neck girths between 25 and 65 cm, and thus the weight limit was set at 9 kg for study I and, after further testing, at 4 kg for study II. A sampling rate of 16 000 Hz was used for recording the audio and the data saved as 2-channel 16-bit integers. Gyroscope data was saved with a sampling rate of 10 Hz as 32-bit integers. Audio was used to derive breathing-related signals from the tracheal sound data and respiratory rate signal.

The owners were instructed to place the neckband on the dog's neck before going to sleep, and to remove it in the morning upon waking. In study II, the neckband was used together with a protective cover, to ensure that the dog could not remove the collar and that it stayed on the dog for the whole duration of the recording. In study I, the neckband was used without a cover. The neckband without and with the protective cover is presented in Figure 3. The recorded time was defined as the duration of recording. To ensure an adequate duration of REM sleep considering that the average duration of a polyphasic sleep-wake cycle in the dog is 82 minutes[56], the minimum duration of recording was specified as two hours. When the device was returned to the investigators, the owner gave oral feedback about potential distractions during recording and usability of the device.

Figure 3 The neckband device without (left) and with the protective cover (right). Images reproduced from studies I and II (supportive material). Photographs: Iida Niinikoski.

An experienced sleep researcher (Sari-Leena Himanen) analyzed the data after transfer to the manufacturer's analysis software. The researcher was blinded to the presence or absence of owner-perceived signs of SDB. As dogs and children have comparably high respiratory rates, children's rules[272] for scoring apneas and hypopneas were used. The tracheal sound signal was utilized to mathematically derive snoring volume and breathing signals. Breathing signal resembles the airflow signal of conventional recordings. The diminishing of a tracheal sound for a minimum of two breathing cycles was considered an apnea. The differentiation between central and obstructive apneas was based on the total cessation of tracheal sounds during central apneas. After obstructive apneas a fast and intense increase in tracheal sound was detected, which was not present during central apneas. Additionally, heart beats were visualized in the breathing channel during central apneas. Central apneas, including physiologic apnea attributed to movement or sighs, were not included in the Obstructive Respiratory Event Index (OREI). Hence, OREI described the number of obstructive apneas and hypopneas per recording time. The presence of snoring was based on the amount of breathing noise. By

listening to each dog's audio signal, an individual detection threshold for snoring was determined. Snore percentage, as time spent snoring of recorded time, was scored manually and ensured by listening. A visual example of the data trace is presented in Figure 4.

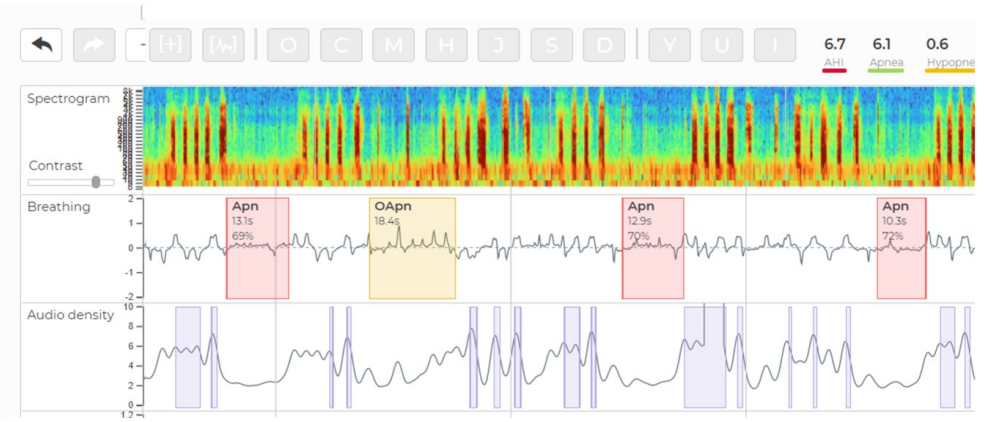


Figure 4 A three-minute epoch of respiratory tracing. Four obstructive apneas are marked as Apn (automatically detected) or OApn (manually scored). The first trace is the spectrogram of the sound signal. The second trace is breathing movements automatically derived from the tracheal sound signal. The third trace is audio density with marked snoring events present. Image reproduced from study I (supportive material).

4.6 Inflammatory mediator analysis (III)

Proinflammatory mediators VEGF-A and CCL2 were analyzed from serum and BALF samples with the canine-specific Luminex multiplex immunoassay (ProcartaPlex, Thermo Fisher Scientific, Vienna, Austria). Duplicate samples were used. Briefly, the sample was added to a mixture of beads pre-coated with analyte-specific capture antibodies. The antibodies bound to the analytes, i.e., VEGF-A or CCL2, and biotinylated detection antibodies specific to the analytes of interest were added. An antibody-antigen sandwich formed. Phycoerythrin-conjugated streptavidin was added, which bound to the biotinylated detection antibodies. The beads were read on a dual-laser flow-based detection system. Bio-Plex 200 (Bio-Rad Laboratories, Hercules, California, USA) was used for reading and Bioplex Manager 6.2 Software for analyzing the samples.

Replicates in each plate and in independent plates were used for evaluation of reproducibility. For the VEGF-A assay, the maximum intra-assay and inter-assay

coefficients of variation were 15% and 12%. The lower limit of quantification was 2.85 pg/ml. For CCL2, the maximum intra-assay and inter-assay coefficients of variation were 15% and 4%. The lower limit of quantification was 13.1 pg/ml. Replicates on each plate were used to calculate correction coefficients between assays from different lots.

4.7 Statistical analysis (I-III)

The statistical analyses were performed using SAS System for Windows version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) (studies II, III), GraphPad Prism for MacIntosh versions 9.3.0 and 10.0.2 (GraphPad Software, San Diego, California, USA) (studies I-III), and IBM SPSS Statistics for Macintosh version 27.0.1 (IBM Corp., Armonk, New York, USA) (study III). *P*-values <0.05 were considered significant.

In study I, the Shapiro-Wilk test was used for normality assessment of continuous data. The Mann-Whitney *U* test was used for analysis of differences in nonparametric OREI values and snore percentages between brachycephalic dogs and normocephalic control dogs, and between dogs aged under and over 5 years. The Student's *t*-test was used for analyzing difference in the normally distributed duration of recording between brachycephalic dogs and normocephalic control dogs. Spearman rank correlation was used for analysis of correlation between OREI value and snore percentage.

In study II, continuous data were evaluated for normality with the Shapiro-Wilk test. The potential risk factors (gender, neuter status, brachycephaly, age, excess weight defined as a BCS of over 5/9, and BOAS class) for high OREI value were evaluated individually with univariable analysis (one-way analysis of variance [ANOVA] or linear regression). Risk factors significant in the univariable analysis were modelled together. To comply with parametric modelling requirements, OREI values were log-transformed. The estimates for differences were backtransformed to original scale for interpretation. The estimates are ratios between the groups and not absolute differences.

In study III, multivariate analysis of variance was used for analysis of differences in serum concentrations of VEGF-A and CCLS at first visit and change between first and second visit. Breed, BOAS class, and interaction between breed and BOAS class were fixed effects in the models. ANOVA models were used to compare the brachycephalic breeds separately against normocephalic control dogs. Here, breed or BOAS severity grade was the only fixed effect. ANOVA was also used for investigation of the effect of change in BOAS severity grade on the change in VEGF-A and CCL2. Change of BOAS severity, defined as constant, deteriorating by one grade, or improving by one grade, was the fixed effect. Additionally, ANOVA was utilized for detection of differences in VEGF-A and CCL2 concentration between WHWTs and normocephalic control dogs in serum, and WHWTs, English Bulldogs, and normocephalic control dogs in BALF samples. Here, group was the sole fixed effect.

In pairwise comparisons, the Tukey-Kramer adjustment was utilized to control for multiplicity, barring BOAS severity grade analyses, where Dunnett's adjustment was used. To comply with normality assumptions, the analyses were performed in log-transformed scale. However, the analyses on change between visits were done in absolute scale. In all analyses, values under the lower limit of detection were replaced with half of the lower limit of quantification.

5 Results

5.1 Studies I and II

5.1.1 Use of the neckband device (I, II)

The owners of dogs participating in studies I and II reported that the neckband device was easy to use. The protective cover developed to ensure the neckband would not fall off the dog's neck during the night prior to study II was well received. However, although all owners (24/24) in study I reported that the neckband did not disrupt the dogs' sleep, one owner (1/63) in study II reported that their dog had difficulty sleeping with the protective cover on. Minor technical issues with the network connection of the device were encountered, which resulted in slightly shorter recording durations for one brachycephalic and one control dog in study I. The length of these recordings was still above the minimal duration of two hours. In study II, technical difficulties resulted in the exclusion of five dogs: in two dogs, the length of recording was insufficient, and in three dogs, an algorithm issue prevented analysis of data.

5.1.2 Study group in studies I and II

The study group of study I included 12 brachycephalic dogs and 12 normocephalic control dogs. Dogs in study I were included in study II, as the owner-perceived signs of SDB were not reported nor risk factors for high OREI evaluated in study I. The study group of study II included altogether sixty-eight dogs, of which sixty-three had sufficient sleep recording data. One Bullmastiff (I, II), one French Bulldog (I, II) and one Pug (II) had had BOAS surgery. Breed distribution of brachycephalic

dogs is shown in Table 7 and normocephalic dogs in Table 8. Demographic data are presented in Table 9.

Table 7 Breed distribution of brachycephalic dogs in studies I and II. Dogs in study I were included in study II.

Brachycephalic breeds	**Study I** ***n*** **= 12**	**Study II** ***n*** **= 28**
French Bulldog	9	10
Cavalier King Charles Spaniel	1	8
Pug		4
Boston Terrier		1
Boxer		1
Bullmastiff	1	1
English Bulldog	1	1
Petit Brabancon		1
Staffordshire Terrier		1

Table 8 Breed distribution of normocephalic dogs in studies I and II. Dogs in study I were included in study II.

Normocephalic breeds	**Study I** ***n*** **= 12**	**Study II** ***n*** **= 35**
Labrador Retriever	3	10
Jack Russell Terrier		3
Parson Jack Russell Terrier		3
Bassett Fauve de Bretagne	2	2
Border Collie		2
Golden Retriever	2	2
Lancashire Heeler		2
Miniature Schnauzer		2
Norwich Terrier		2
Irish Setter	1	1
Kleinspitz		1
Lapponian Herder	1	1
Mixed breed		1
Spanish Water Dog	1	1
Wales Terrier	1	1
Whippet	1	1

Table 9 Demographic data of dogs in studies I and II.

	Brachycephalic dogs in study I (n = 12)	**Control dogs in study I (n = 12)**	**All dogs in study II (n = 63)**
Sex Female Neutered / intact Male Neutered / intact	 5 1 / 4 7 1 / 6	 5 4 / 1 7 2 /5	 28 13/15 35 22/13
Age (years) Median / range	4.4 / 2.0 – 9.0	5.4 / 1.5 – 10.1	5.8 / 1.4 – 12.7
Weight (kg) Median / range	 12.7 / 9.8 – 57.0	 20.0 / 10.4 – 37.8	 12.4 / 4.7 – 57.0

Twenty-one dogs in study I and forty-six dogs in study II attended a study visit. No clinically significant changes in hematology or serum biochemistry were noted. In study I, the median BCS in brachycephalic dogs was 4 (range 4 – 6) and in normocephalic dogs 5 (range 4 – 6). In study II, the median BCS of all dogs was 5 (range 4 – 9). In study II, twelve dogs had excess weight (BCS ≥ 6/9).

Clinical severity of BOAS was graded in nineteen brachycephalic dogs in study II. Ten dogs were classified as BOAS negative: six grade 0 and four grade 1. Nine were BOAS positive: four grade 2 and five grade 3. The BOAS positive dogs were five French Bulldogs, two Pugs, one English Bulldog, and one Cavalier King Charles Spaniel.

In study II, 40% of the owners of normocephalic dogs (14/35) and 67% of owners of brachycephalic dogs (19/28) reported signs of SDB (snoring at least 50% of sleeping time, apneic episodes, or repeatedly sleeping with toy in mouth or sitting up).

5.1.3 Signs of sleep-disordered breathing (I, II)

Brachycephalic dogs snored significantly more than normocephalic dogs in both study I (median, 34.2; range, 2.8-65.7 vs 0.0; 0.0-0.1; Hodges-Lehmann estimator = 34.2, 95% confidence interval [CI] 13.6-60.8; $P < 0.001$) and study II (21.8; 0.0-65.7 vs. 0.0; 0.0-53.2; Hodges-Lehmann estimator = 20.7, 95% CI 12.5-26.2; $P < 0.001$). Snore percentages are presented in Figure 5.

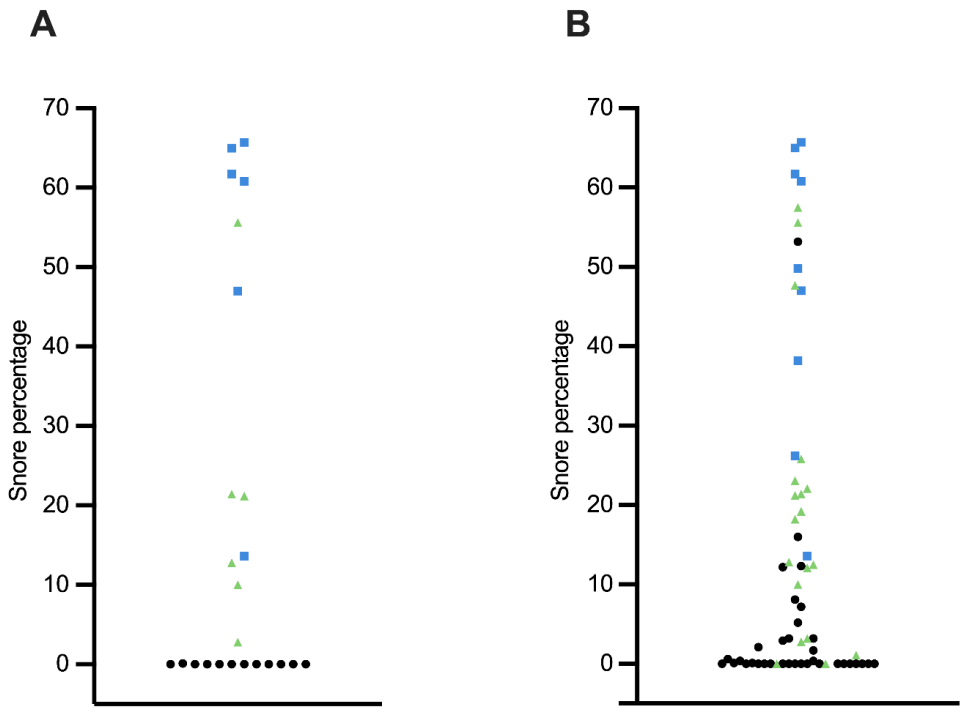


Figure 5 Scatter plot of snore percentage, as time spent snoring of total recording time, in dogs in study I (A) and study II (B). Black circles are normocephalic dogs and green triangles and blue squares are brachycephalic dogs. Blue squares denote dogs with marked brachycephalic obstructive airway syndrome signs (BOAS positive class). Figures reproduced from studies I and II.

In study II, dogs with high OREI values of above 5 had a median snore percentage of 23.1 (range 12.1-60.8) and dogs with OREI under 5 a median of 0.85 (0.0-65.7). In dogs with high OREI values above 5, the most descriptive owner-perceived signs of SDB were restless sleep, snoring, sleeping sitting up, sleeping with toy in mouth and apneic episodes during sleep (Figures 6A-E). An OREI of 5 was chosen as the cutoff value, as OREI under 5 in adults is considered normal.[86]

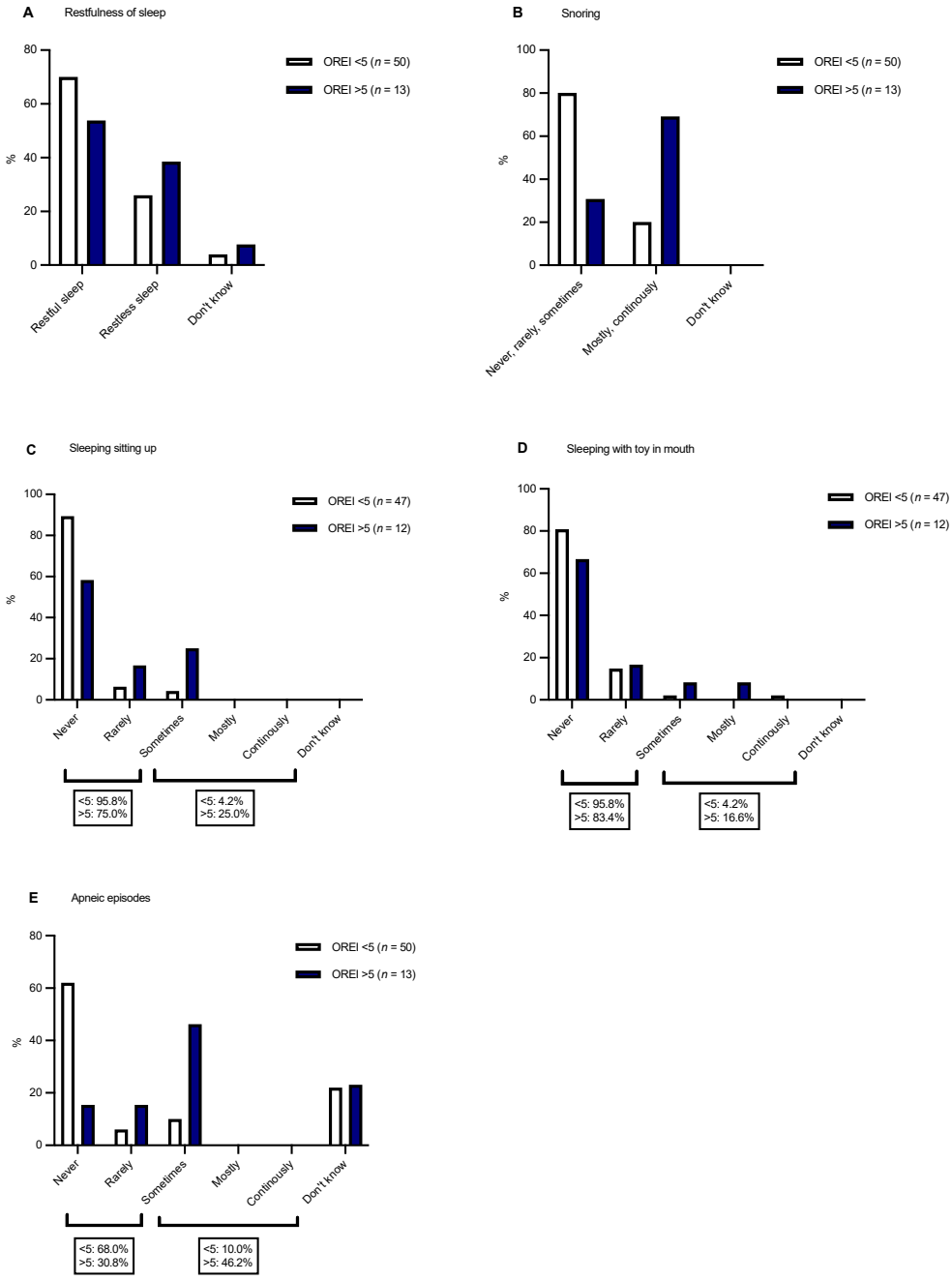


Figure 6 Owner-perception of restfulness of sleep (A), snoring during sleep (B), sleeping sitting up (C), sleeping with toy in mouth (D), and apneic episodes during sleep (E) in dogs with a lower obstructive respiratory event index (OREI) of below 5 and higher OREI of over 5. Figure reproduced from study II.

Occurrence of owner-perceived signs of SDB, including restless sleep, snoring, sleeping sitting up, sleeping with toy in mouth, and apneic episodes during sleep, in normocephalic and brachycephalic dogs, and in BOAS– and BOAS+ dogs are presented in Figure 7A-E.

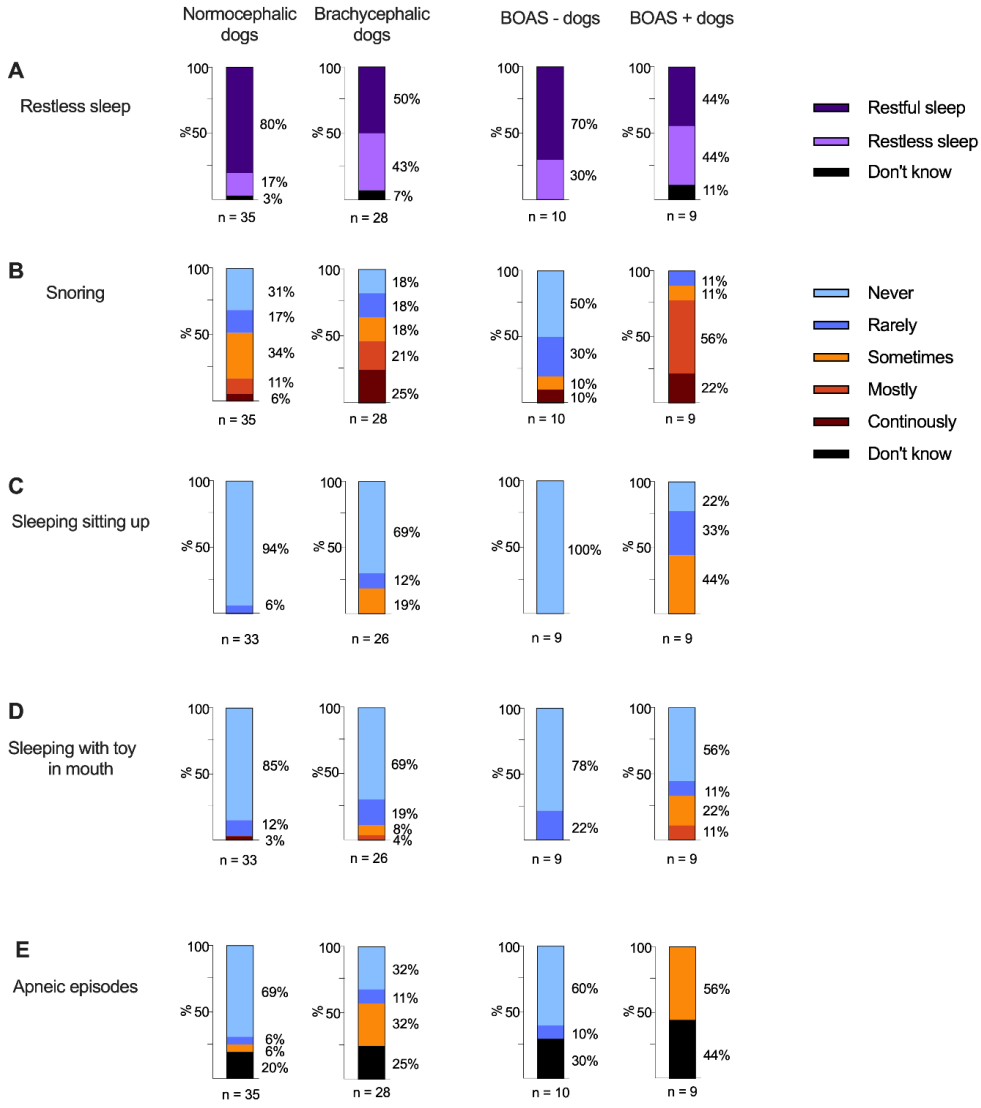


Figure 7 Occurrence of owner-perceived signs of sleep-disordered breathing, including restless sleep (A), snoring (B), sleeping sitting up (C), sleeping with toy in mouth (D), and apneic episodes during sleep (E), in normocephalic dogs and brachycephalic dogs, and in brachycephalic dogs with no or mild signs of brachycephalic obstructive airway syndrome (BOAS –) and brachycephalic dogs with moderate or severe signs of BOAS (BOAS +). Figure reproduced from study II.

5.1.4 Obstructive respiratory event index values (I, II)

The OREI value was significantly higher in brachycephalic dogs than control dogs in both study I (median, 3.8; range, 1.8-15.6 vs 0.6; 0.0-1.9; Hodges-Lehmann estimator for median difference = 3.5, 95% CI 2.2-6.8; $P < 0.001$) and study II (3.8; 0.5-34.6 vs. 0.7; 0.0-6.6; Hodges-Lehmann estimator for median difference = 3.1, 95% CI 1.7-5.4; $P < 0.001$). The OREI values are presented in Figure 8. Central SDB events, excluding physiologic sighs, were not detected.

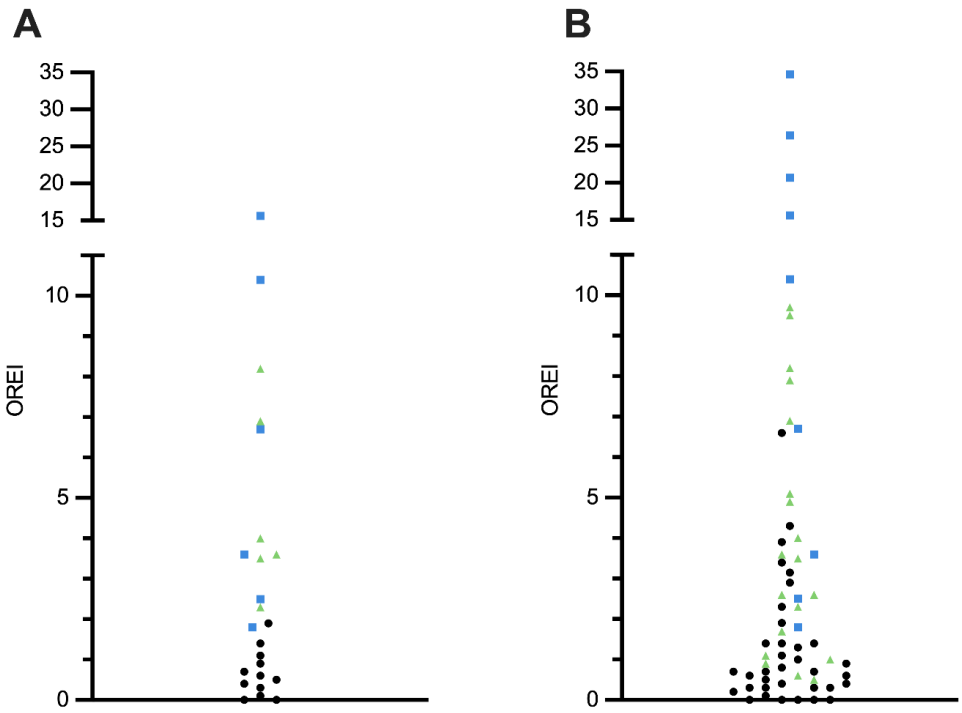


Figure 8 Scatter plot of obstructive respiratory event index (OREI) in dogs in study I (A) and study II (B). Black circles are normocephalic dogs and green triangles and blue squares are brachycephalic dogs. Blue squares denote dogs with marked brachycephalic obstructive airway syndrome signs (BOAS positive class). Figure reproduced from studies I and II.

In study I, a strong positive correlation between OREI value and snore percentage was detected in all dogs ($r_s = 0.79$, $P < 0.001$), but not in brachycephalic dogs ($r_s = 0.15$, $P = 0.65$).

5.1.5 Predisposing factors for sleep-disordered breathing (I, II)

Study II evaluated risk factors for SDB. In all dogs (n = 63), brachycephaly (ratio of the geometric means 5.6, 95% CI 3.2 – 9.9; P < 0.001) was a risk factor for SDB. In dogs with study visits (n = 46), excess weight, defined as BCS of over 5/9, was a risk factor for SDB (3.4, 95% CI 1.8 – 6.7; P < 0.001). This was also evident in brachycephalic dogs with study visits (n = 19, 5.0; 95% CI 1.6-15.9; P = 0.009). Additionally, in brachycephalic dogs with study visits (n = 19), BOAS positive class, that is, moderate or severe signs of BOAS predisposed to SDB (2.5; 95% CI 1.1-5.6; P = .03).

Ageing was not a risk factor for SDB in study II. No difference in OREI value was detected between dogs aged under 5 years and dogs aged over 5 years in study I (median, 2.3; range, 0.0-10.4 vs 1.4; 0.1-15.6; Hodges-Lehmann estimator for median difference = 0.4, 95% CI 3.1-1.4; P = 0.70). In study II, the effect of age was nonsignificant in all dogs (n = 63; P = 0.16) and the subgroups of dogs with study visits (n = 46; P = 0.32) and brachycephalic dogs with study visits (n = 19; P = 0.077). In study II, neither gender, that is, male or female, nor neuter status, that is, neutered or intact, were significant risk factor for SDB. Results are presented in Table 10.

Table 10 Results of univariate analysis of gender (male/female) and neuter status (neutered/intact) for all dogs and all dogs with study visit in study II. The presented results are P-values.

	All dogs (n = 63)	**All dogs with study visit (n = 46)**
Gender	0.97	0.96
Neuter status	0.90	0.61

5.2 Study III

5.2.1 Study group in study III

The study group included 114 brachycephalic dogs, 42 WHWTs (16 with CIPF and 26 healthy), and 39 normocephalic control dogs. The control dogs represented normocephalic breeds other than WHWTs. Demographics are presented in Table 11.

Table 11 Demographics of dogs in study III.

BALF, bronchoalveolar lavage fluid; CIPF, canine idiopathic pulmonary fibrosis; EB, English bulldog; FB, French bulldog; WHWT, West Highland White Terrier

	EB (*n* = 28)	**FB (*n* = 37)**	**Pug (*n* = 49)**	**WHWT CIPF (*n* = 16)**	**WHWT healthy (*n* = 26)**	**Control dogs with**	
						serum (*n* = 25)	**BALF (*n* = 14)**
Gender							
Female	13	23	30	8	14	9	10
Male	15	14	19	8	12	16	4
Age (years) Median / range	3.4 / 2.1 – 5.3	2.5 / 1.2 – 5.6	2.8 / 1.0 – 5.6	12.6 / 9.1 – 14.9	10.0 / 4.3 – 14.0	2.6 / 1.3 – 5.6	6.3 / 1.3 – 11.3

Clinical severity of BOAS was graded in all 114 brachycephalic dogs in study III. Seventy-five dogs were classified as BOAS negative: eleven grade 0 and sixty-four grade 1. Thirty-nine were BOAS positive: twenty-four grade 2 and fifteen grade 3.

A follow-up visit was performed for 54/114 brachycephalic dogs (English Bulldog, n = 8; French Bulldog, n = 16; Pug, n = 30) two to three years after the initial visit. BOAS grade remained constant in 31/54 dogs, deteriorated by one grade in 15/54, and improved by one grade in 8/54 (previously presented in Aromaa et al.[115]).

5.2.2 Serum proinflammatory mediator analysis (III)

English Bulldogs had significantly higher serum VEGF-A concentration than control dogs (median, 7.8; range, 1.4-19.3 vs 3.8; 1.4-11.8; ratio of the geometric means 2.2; 95% CI 1.2-4.0; P = 0.009). No significant difference was detected between the control dogs and either French Bulldogs or Pugs (P = 0.89; P = 0.17).

In all brachycephalic dogs, no difference between BOAS-positive and BOAS-negative class was detected (P = 0.73). There was no significant difference in serum VEGF-A concentration between either WHWT group and control group (CIPF WHWTs vs. control P = 0.08; healthy WHWTs vs. control P = 0.25) nor between the WHWT groups (P = 0.66). Serum VEGF-A results are presented in Figure 9A.

Serum CCL2 concentrations did not differ between controls and brachycephalic dogs (P = 0.12) or between BOAS-positive and -negative classes (P = 0.59). Significantly higher serum CCL2 concentrations were detected in both healthy WHWTs compared to controls (median, 125.2; range, 53.1-524.2 vs 72.1; 41.3-297.6; ratio of the geometric means 0.6; 95% CI 0.4-0.85; P = 0.002) and in WHWTs with CIPF compared to controls (199.8; 104.9-709.4 vs 72.1; 41.3-297.6; 2.6; 95% CI 1.8-3.8; P < 0.001). WHWTs with CIPF had higher serum CCL2 concentration than healthy WHWTs (199.8; 104.9-709.4 vs 125.2; 53.1-524.2; 1.6; 95% CI 1.0-2.3; P = 0.02). Serum CCL2 results are presented in Figure 9B.

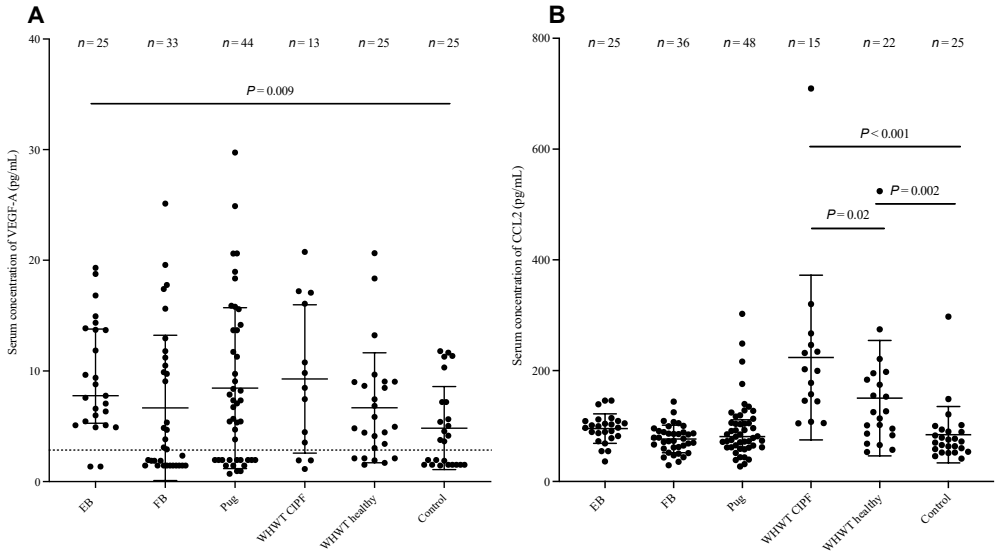


Figure 9 Scatter plot with median and interquartile range of serum concentration of (A) vascular endothelial growth factor A (VEGF-A) and (B) chemokine (C–C motif) ligand 2 (CCL2) in English bulldogs (EB), French bulldogs (FB), Pugs, and West Highland White Terriers (WHWT) affected with canine idiopathic pulmonary fibrosis (CIPF) and healthy WHWTs, and control dogs of other breeds. The dashed line indicates the lower limit of quantification. Significant differences (P < 0.05) compared with healthy dogs are indicated. Figure reproduced from study III.

Neither serum VEGF-A nor CCL2 concentrations did not differ between BOAS severity grades 0–3 (VEGF-A, $P = 0.86$; CCL2, $P = 0.70$).

The concentration of serum VEGF-A significantly decreased in all brachycephalic dogs from first to second visit (median, 5.4; range, 0.7-29.0 vs 3.6; 0.9-20.4; ratio of differences -2.0, 95% CI -3.5--1.1 $P < 0.001$). No significant change in serum CCL2 between visits was noted (P = 0.07). The brachycephalic breeds did not differ in the magnitude of change in VEGF-A concentration (P = 0.97) or CCL2 concentration (P = 0.79) between visits. There was no difference between the BOAS positive and negative classes in the magnitude of change in serum VEGF-A concentration (P = 0.37) or CCL2 concentration (P = 0.89). Change in the BOAS severity grade between visits did not have a significant effect on serum VEGF-A (P = 0.48) or CCL2 (P = 0.43).

5.2.3 Bronchoalveolar lavage fluid proinflammatory mediator analysis (III)

English Bulldogs had significantly lower BALF VEGF-A concentration compared to controls (median, 123.4; range, 9.2-267.7 vs 263.7; 96.4-886.4; ratio of differences 0.5, 95% CI 0.3-0.7 $P < 0.001$). Additionally, significantly lower BALF VEGF-A was noticed in healthy WHWTs compared to controls (104.4; 58.8-167.2 vs 263.7; 96.4-886.4; 0.5, 95% CI 0.3-0.8 $P = 0.007$) and in WHWTs with CIPF compared with controls (137.9; 22.2-243.1 vs 263.7; 96.4-886.4; 0.5, 95% CI 0.3-0.8 $P = 0.006$). BALF VEGF-A concentration did not differ between English Bulldogs and either healthy or CIPF WHWTs (P = 1.0, P =1.0) or within the two WHWT groups ($P = 1.0$). BALF VEGF-A results are presented in Figure 10A.

BALF CCL2 was significantly lower in English Bulldogs compared to both WHWTs with CIPF (median, 123.0; range, 10.4-287.6 vs 1924; 37.7-5084.0; ratio of differences 10.1, 95% CI 3.6-28.4 $P < 0.001$) and healthy WHWTs (123.0; 10.4-287.6 vs 400.2; 133.4-1395.0; 0.2, 95% CI 0.1-0.7 $P = 0.003$), but not compared to controls (P = 0.06). WHWTs with CIPF had significantly higher BALF CCL2 concentrations compared to controls (1924; 37.7-5084.0 vs 224.6; 58.3-2459.0; 4.0, 95% CI 1.5-10.5 $P = 0.01$) but not compared to healthy WHWTs (P = 0.36).

CCL2 concentration did not differ between healthy WHWTs and controls ($P = 0.52$). BALF CCL2 results are presented in Figure 10B.

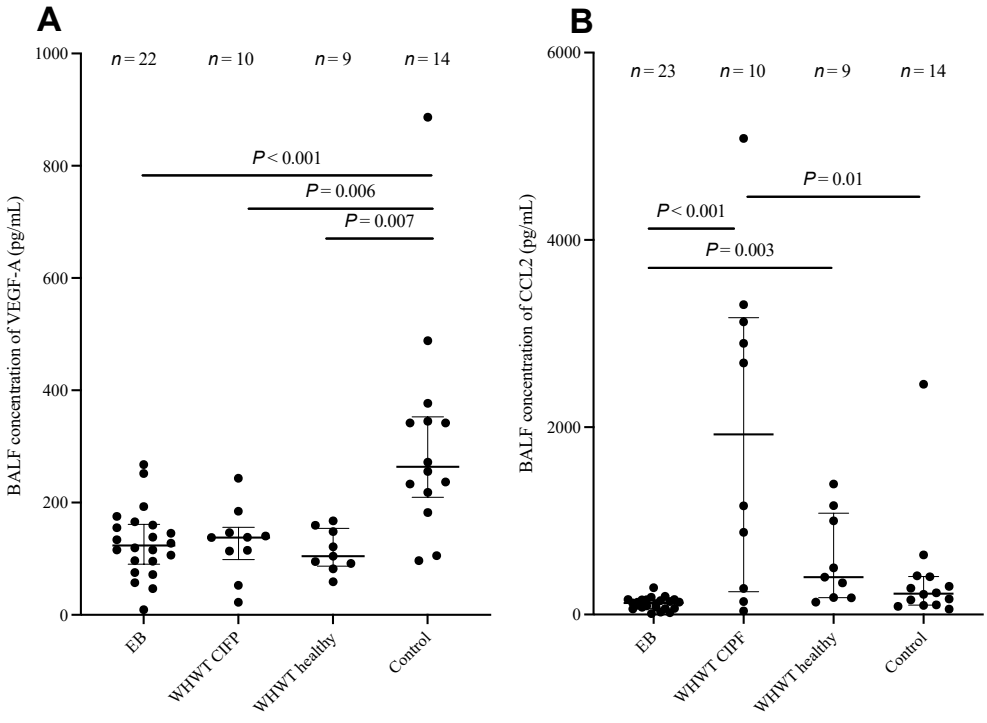


Figure 10 Scatter plot with median and interquartile range of bronchoalveolar lavage fluid (BALF) concentration of (A) vascular endothelial growth factor A (VEGF-A) and (B) chemokine (C–C motif) ligand 2 (CCL2) in English bulldogs (EB), West Highland White Terriers (WHWT) affected with canine idiopathic pulmonary fibrosis (CIPF) and healthy WHWTs, and control dogs of other breeds. Significant differences ($P < 0.05$) are indicated. Figure reproduced from article III.

6 Discussion

The present study introduced a novel neckband system for evaluating SDB in dogs in their home environment. The study demonstrated that brachycephaly, increasing severity of BOAS, and excess weight predispose to SDB, and, for the first time, revealed that SBD also occurs in normocephalic dogs. Evidence of a proinflammatory condition in English Bulldogs, suggestive of hypoxemia-induced inflammation, was found. Possible signs of SDB in dogs included restless sleep, disturbances in sleeping position, and apneic episodes. Although snoring was common in dogs with high rates of obstructive SDB events and in brachycephalic dogs, not all owners recognized snoring as a welfare problem. Brachycephaly generates major welfare problems in dogs, and the future of these breeds should be critically examined. Additionally, obesity is a considerable issue in pet dogs[273] and should be avoided to improve breathing during sleep, especially in brachycephalic dogs.

6.1 Usability of the neckband device (I, II)

In studies I and II, the Nukute neckband system was a feasible device for detection in dogs. The device was simple to use for the owners and tolerated well by the dogs both with and without a protective cover. Although the gold standard for SDB diagnostics is polysomnography[86], portable at-home devices[105,106] present indisputable advantages for SDB screening compared to expensive and arduous polysomnography. Polysomnography, which includes measurement of neural activity, muscle activity, eye movements, heart rhythm and respiratory function, has been used for evaluation of SDB in modest groups English Bulldogs and normocephalic control dogs[7,12,23-26]. Later, noninvasive polysomnography has been extensively used for cognitive studies[63,67,72-74,77,80,82,83,104], but not for evaluation of SDB. Alongside polysomnography, whole-body barometric plethysmography has also been implemented for assessment of SDB in a small group of three dogs.[27]

During whole-body barometric plethysmography, the dog rests in a specialized chamber, and the barometric pressure oscillations produced by breathing patterns are analysed.[27,99-101,128] In the study evaluating SDB, panting prevented data analysis in two of five dogs.[27] Polysomnography and whole-body barometric plethysmography require considerable equipment and, often, for the dog to sleep in a laboratory environment.

The neckband system used in studies I and II enabled the evaluation of breathing during sleep in an entirely new manner in dogs, without the prerequisite of a sleep laboratory or electrode placement. This is important on an individual level, as SDB is, to some extent, treatable with surgical operations, medications, and, presumably, weight loss.[23,24,27,144] Detection of SDB is required for identification of individuals in need of treatment, and the neckband system provides a feasible alternative for this. Nonetheless, polysomnography is the only method of gathering data on sleep macrostructure, as neither whole-body barometric plethysmography nor the Nukute neckband system allow for differentiation between wake and sleep. This especially true in dogs, where drowsiness is a sleep stage often indistinguishable from wake to the viewer.[63]

The duration of the sleep recording was set at a minimum of two hours in both studies I and II. Obstructive apneas and hypopneas in dogs occur mostly in REM stage[12], and a two-hour recording was assumed to be sufficiently long to contain at least one complete polyphasic sleep-wake cycle including REM sleep.[56] The recording was started just before the owner and dog went to sleep in the evening. Although the neckband device does not provide data on sleep stages, active wake is unlikely, as movement during the recording was assessed by gyroscope data. Furthermore, it is established that pet dogs mostly sleep during the night.[49-51] However, sleep in dogs is polyphasic and is divided into various small segments, with time spent awake in-between[63], and thus the recordings are expected to also contain brief wake periods. Although domesticated pet dogs are diurnal[49-51], daytime napping is an important part of the dog's sleep-wake pattern.[49,58] All sleep recordings were performed during night-time, as sleep macrostructure differs between day and night, with more REM sleep at night.[79]

6.2 Study groups (I-III)

In studies I and II there is an overrepresentation of certain breeds due to the convenience sampling method in study I and emphasis of brachycephalic dogs and dogs with owner-perceived signs of SDB for sleep recordings in study II. Extremely brachycephalic French Bulldogs are overrepresented in both study I and study II and less brachycephalic Cavalier King Charles Spaniels are abundant in study II. SDB has been objectively evaluated in brachycephalic dogs[7,27], including Cavalier King Charles Spaniels.[27] We also had an overrepresentation of Labrador Retrievers in study II. One of the aims of study II was to investigate whether excess weight was a risk factor for SDB, and obesity is prevalent in Labrador Retrievers.[273] However, in our study group excess weight was uncommon in Labrador Retrievers, with only one Labrador having a BCS of over 5/9.

The study groups consist of dogs recruited due to their breed and/or the presence of owner-perceived signs of SDB. It is likely owners of dogs with signs of SDB had greater incentive to participate in the questionnaire. Hence, the study group does not depict a random sample representative of the whole population, and estimates of incidence or prevalence cannot be drawn.

Surgical alleviation of BOAS had been performed in two dogs in study I (the Bullmastiff and one French Bulldog) and three dogs in study II (the aforementioned dogs and one Pug). The Bullmastiff had an OREI value of 2.3, the French Bulldog a high value of 6.7, and the Pug an extremely high value of 34.6. The sleep recordings were not performed preoperatively, and thus the effect of surgical intervention cannot be evaluated here. However, it seems surgical treatment of BOAS using traditional surgical techniques (widening of nares, palatoplasty, tonsillectomy) is not a cure for SDB. Individual variation is likely, as the one English Bulldog in Hendricks et al.[23] had significant decrease in amount of SDB events after resection of excess pharyngeal tissue. It is also possible that other surgical techniques offer better outcome regarding SDB, as laser-assisted turbinectomy was part of the surgical protocol in Hinchliffe et al.[27], where all dogs improved after surgery. Indeed, although BOAS surgery often improves the severity of clinical signs of BOAS, not all dogs have a favorable outcome.[10,117] In some severely affected dogs, owners described increased BOAS signs one year after surgery.[10] Not all

components contributing to BOAS, such as advanced laryngeal collapse or hypoplastic trachea, are surgically correctable.

Although the dogs were not age- or weight-matched in studies I-II, there was minimal variation in these parameters. In study III both WHWT groups were markedly older than the other groups, with median age for healthy WHWTs at 10.0 years and CIPF WHWTs 12.6 years, while the median age of Pugs was 2.8 years and French Bulldog 2,5 years. CIPF is a disease which occurs in older WHWTs, and hence the healthy WHWTs were also middle-aged or older to minimize the number of dogs in asymptomatic or early stages of the disease.

6.3 Signs of sleep-disordered breathing (I, II)

In the questionnaire results, we found owner-perceived signs of disturbed breathing during sleep to be more prevalent in dogs with high OREI values above 5. All dogs with very high OREI values of over 15 exhibited owner-perceived apneic episodes during sleep. A cutoff of 5 was used, as 5-15 apneas and/or hypopneas per hour of sleep is classified as mild OSA in adult humans.[13] Over 15 apneas and/or hypopneas per hour is classified as moderate OSA in adults.[13] However, no classifications for dogs exist currently.

Snore percentage, the amount of time spent snoring during the sleep recording, was evaluated in studies I and II. Brachycephalic dogs snored significantly more than normocephalic dogs in both studies. Additionally, dogs with high OREI values above 5 snored more than dogs with lower OREI values under 5. Although snoring was uncommon in normocephalic dogs, some normocephalic dogs in study II snored considerably. The normocephalic dog with the highest snore percentage of 53% was a Norwich Terrier with an OREI value of 4.3; this is discussed later. In study I, snore percentage correlated positively with OREI in all dogs, but not in the brachycephalic dogs. This suggests that snoring does not demonstrate that dog has SDB in brachycephalic dogs, and additional diagnostics are needed for assessment of SDB in these breeds.

The proportion of snoring during sleep has not been previously objectively measured. Snoring is described in studies on SDB utilizing polysomnography[7,12,26] and whole-body plethysmography.[27] Snoring is caused by soft tissue vibration during sleep and is often the result of obstruction due to upper airway property changes.[274] Increased degree of pharyngeal narrowing, but not soft palate length alone, is associated with the severity of owner-reported snoring in brachycephalic dogs.[275]

In study II, snoring most of the time or continuously was frequent in both brachycephalic dogs and dogs with high OREI value above 5. In brachycephalic dogs, the owner-perceived proportion of dogs snoring mostly or continuously was 46%, and in dogs with OREI over 5 this was 60%. Previously reported owner-perceived proportions of snoring in brachycephalic dogs range from 70% to 95%.[34,110,114] The previously presented high percentage is likely affected by the large number of dogs seeking surgical treatment of BOAS in some studies.[34,110] Of note, occasionally owners did not recognize snoring in our study group, as there was a clear inconsistency between objectively measured snoring and owner-perceived snoring. This finding is unlikely to be affected by the neckband device, as the owners did not mention a change in the dog's breathing attributable to the device. Clinical[100] and questionnaire[109,113] studies have previously reported discrepancies between owner-perceived health and clinical findings of BOAS. Snoring during sleep may be considered either normal for the brachycephalic breeds or equivalent to respiratory signs seen in wake animals, instead of an indication of the conformational changes associated with BOAS.[113] Cognitive dissonance may explain the unrealistically good perceptions of brachycephalic dogs' health reported by owners: while appreciative of the issues related to brachycephaly, owners find them psychologically uncomfortable and deny the problems in their own pet.[109]

Restless sleep was described more in brachycephalic dogs and dogs with high OREI value above 5 in study II. We suggest that nighttime restlessness could be a sign of SDB in dogs. Previously, restlessness was reported in 1.8% of young brachycephalic and normocephalic dogs.[58] Although in man daytime sleepiness resulting from recurring arousals and fragmented sleep is an important diagnostic criterion for OSA[86], daytime sleepiness can be difficult to interpret in the dog. Differentiating

between wake and sleep is difficult during the drowsiness stage in dogs, and hence the total amount of sleep during 24 hours is complicated to estimate merely by observing. Furthermore, a considerable amount of sleep naturally occurs during the day in pet dogs, making daytime sleepiness more troublesome to assess. However, it must be noted that other illnesses, such as osteoarthritis[112,276] and canine cognitive dysfunction[65,66], are also recognized to cause nighttime restlessness.

In study II, disturbances in sleeping position, that is, sleeping sitting up or sleeping with a toy in mouth, were more prevalent in brachycephalic dogs and in dogs with high OREI values above 5. Interestingly, sleeping sitting up was reported in two normocephalic dogs, a Norwich Terrier and a Lancashire Heeler. Previously, attempting to sleep sitting up was reported in 24-34 % of brachycephalic dogs presenting for surgical alleviation of BOAS.[34,110] Sleeping sitting up did not occur in the normocephalic control dogs in one study.[110] Sleeping with a toy or other item in mouth occurred seldomly, in 3% of owner-reportedly healthy young brachycephalic dogs.[114] A comparable proportion of owners, 3.7%, reported sleeping with toy in mouth in young normocephalic and brachycephalic dogs.[58] As muscle tone is diminished during REM sleep, disturbances in sleeping position are suspected to reflect the dog's attempt at keeping the airways open during sleep. Obstruction of the upper airways mostly occurs during REM sleep in dogs[12] and airflow may not be as compromised if there is an item keeping the mouth open. Interestingly, positional sleep apnea, where obstructive SDB events occur mostly during sleeping in the supine position, is common in humans with mild OSA.[219]

Apneic episodes, not breathing for multiple seconds during sleep, were reported more in brachycephalic dogs and dogs with high OREI values above 5 in study II. Previously reported owner-perceived proportions of apneic episodes range from 0.5% in a group of young brachycephalic and normocephalic dogs[58] to 47% in a group of brachycephalic dogs presenting for surgical treatment of BOAS.[34] In the latter population, owners reported signs of SDB in only 3% of dogs after surgical alleviation of BOAS[34], which highlights the obstructive origin of these events. In our questionnaire results, ambivalence in choosing an answer to the presence of apneic episodes was noted. A marked proportion, 25% of brachycephalic and 20% of normocephalic dog owners selected "I don't know" to this question. This could be

due to an unclear definition of an apneic episode in the questionnaire or the owner feeling inept at evaluating this, perhaps due to not observing the dog during all sleeping occasions. Nonetheless, such uncertainty was not noted in response to the other questions.

6.4 Obstructive respiratory event index values (I, II)

In studies I and II we showed that the OREI value can be used to summarize obstructive apnea and hypopnea events per recording time in dogs.

Both obstructive[7,12,23-25,27] and central[7,24] apneas and hypopneas occur in dogs. Central apneas occur when there is a lack of respiratory rhythm generated by the respiratory center of the brain.[13] The majority of SDB events reported in dogs are obstructive in origin, and we did not detect central SDB events in studies I and II. Physiologic central apneas related to sighs and movements were removed from the analyses. Morphologically abnormal muscle fibers and increased connective tissue are reported in upper airway dilator muscles of English Bulldogs, suggestive of activity-induced muscle injury due to obstructive SDB or repeated obstruction during wake.[189] In humans, CSA has been associated with Chiari syndrome, where part of the cerebellum and brain stem herniate into the cervical canal.[171-173] However, in patients with Chiari malformation, SDB events of also obstructive origin are described.[171-173] Interestingly, Chiari-like malformation is prominent in multiple brachycephalic breeds, such as the Cavalier King Charles Spaniel[175] and Chihuahua.[174] SDB has been objectively characterized by whole-body plethysmography in a small group of three Cavalier King Charles Spaniels, which responded positively to corrective upper airway surgery according to owners, suggesting SDB events were mostly obstructive in origin.[27] However, whole-body plethysmography was not repeated postoperatively in this study.[27]

The OREI value was significantly higher in brachycephalic dogs compared to normocephalic dogs in both studies I and II. The range of OREI was high in brachycephalic dogs, 1.8 – 15.6 in study I and 0.5 – 34.6 in study II. Variation in normocephalic dogs was markedly less, 0 – 1.9 in study I and 0.0 – 6.6 in study II. Although normocephalic dogs represent various breeds of different sizes, the OREI

values are convergent. It is possible that upper airway conformational changes, not breed or size, affect SDB in dogs.

In study II, a marked proportion of brachycephalic dogs (43%, 12/28) had high OREI values exceeding 5. In normocephalic dogs, OREI values were predominantly under 5 (97%, 34/35) and only 3% (1/35) had an OREI value over 5 (6.6). This normocephalic individual was a Norwich Terrier with a snore percentage of 12.3. The other Norwich Terrier in study II also had a relatively high OREI of 4.3 and a considerably high snore percentage of 53.2. To our knowledge, this is the first time SDB is described in normocephalic dogs. Norwich Terriers are considered mesaticephalic, although they are thought to be in transition to brachycephaly.[277] Norwich Terriers are prone to Upper Airway Syndrome[277,278], where upper airway obstruction is associated with lymphoedema and not BOAS.[279] We suspect that lymphoedema and Upper Airway Syndrome led to obstructive SDB events in the Norwich Terriers in our study group.

The OREI index has not been used in dogs before. Previously, an SDB index has been used in polysomnography screening of SDB in English Bulldogs.[7,12,23] The SDB index is like the apnea hypopnea index (AHI) obtained by polysomnography in humans, used to summarize apneas and hypopneas per hour of sleep.[13,86] The SDB index values in English Bulldogs ranged from 0.5 to 114, with a significantly lower SDB index in control dogs, ranging from 0 to 0.9.[7,12,23] In these studies, the English Bulldogs were more seriously affected by BOAS and SDB compared to our study groups, and this was reflected in the number of SDB events.[7,12,23] However, the SDB index values in the normocephalic control dogs are comparable to the OREI values of most of our normocephalic dogs in our studies.[7,12,23] It should be noted that as the SDB index values are calculated from polysomnography recordings, where wake periods are excluded from the total recording time, the SDB index is considerably more exact than the OREI value.

More research is required to determine cutoff values for OREI between dogs affected and not affected by SDB. In study II, we used a cutoff of 5, similar to adult humans[86]. In children, however, the cutoff between normal and abnormal is 1[272], and a lower cutoff, for example around 2, is possible also in dogs. However, further

information on the morbidities relating to SDB is needed before establishing a cutoff value for at-risk SDB.

6.5 Predisposing factors for sleep-disordered breathing (I, II)

Study II assessed risk factors for SDB. The effects of brachycephaly and ageing on SDB were also investigated in study I. Previously, risk factors for SDB, excluding brachycephaly, have not been explored.

Brachycephaly was a risk factor for SDB (study I, II). The differences in OREI values between brachycephalic and normocephalic dogs are described above. Brachycephalic dogs often suffer from impaired airflow even in wake, as the soft tissues obstruct the upper airways.[5] This also results in increased breathing noise, stridor and stertor, during wake. Previously, SDB events have been quantified in small groups of English Bulldogs[7,12,23-26] and Cavalier King Charles Spaniels.[27] Additionally, owner-perceived signs of SDB have been described in case reports depicting probable SDB[32,33] and reported in questionnaire studies.[9,11,29,34,58,110,113,114,116] In humans, brachycephaly seems to predispose to OSA[280], although contradicting reports exist.[281] Additionally, craniofacial disharmony and mandibular deficiencies are sometimes seen with OSA.[282,283] This craniofacial disharmony is not analogous to brachycephaly in dogs, as it often includes increased anterior facial height and an inferiorly positioned hyoid.[137]

Increasing severity of BOAS was a risk factor for SDB in study II. Brachycephalic dogs with moderate or severe signs of BOAS had higher OREI values than dogs with no or mild signs of BOAS. Although the severity grading of BOAS has been validated only for the English Bulldog, French Bulldog and Pug[284], we performed it identically in other brachycephalic breeds. The impact of increasing BOAS severity on objectively assessed SDB has not been reported previously. As increasing severity of BOAS predisposes to poorer exercise tolerance[9,114] and impaired respiratory function[100,101], the effect on breathing during sleep is unsurprising.

In study II, excess weight, defined as a BCS of over 5/9, was identified as a risk factor for SDB. As an ideal BCS is 4-5/9[285], we defined excess weight as a BCS over

5/9. Over 7/9 is clearly overweight and 9/9 obese.[285] It is possible that the effect of weight would have been greater had the cutoff for excess weight been 7/9 or higher, or if the study group had included more clearly obese dogs. Obesity is a clear risk factor for BOAS[100,101,129], as it limits the amount of airflow per minute.[101] Excess weight is a risk factor for SDB also in normocephalic dogs, and respiratory parameters[143] and oxygenation[144] decrease with obesity in wake dogs. Weight loss has a favorable effect on oxygenation and respiratory function in normocephalic dogs[144] and is recommendable for all dogs with excess weight. Brachycephalic dogs are prone to increased tongue fat which contributes to macroglossia and airflow resistance.[133,141,142] In man, the severity of OSA decreases with declining degree of tongue fat.[149] Alongside excess upper airway fat[148,149], obesity is a considerable risk factor for OSA in man.[145-147] The severity of OSA decreases with weight loss[149,151], and weight loss is recommended for all OSA patients with excess weight.[86] In addition to decreased lung volume[154] and increased airflow resistance[155], impaired neuromuscular and mechanical responses are suggested to lead to increased pharyngeal collapsibility in obese patients.[152,153] Furthermore, changes in adipokine secretion with excess adipose tissue may contribute to the diminished central nervous system activity.[156]

Ageing was not a risk factor for SDB in study II. Furthermore, in study I there was no significant difference in OREI values between dogs aged under and over 5 years. There were several young brachycephalic dogs very severely affected by BOAS and SDB in the study group, which is likely to influence the findings. The signs of BOAS can worsen with age. [110,130,135]Aggravation of BOAS can occur with ageing, as secondary changes including laryngeal collapse[130] and bronchial collapse[135] further obstruct the airways. Impaired respiratory function associated with ageing is reported also by owners.[110] In a recent study, the assessment of BOAS severity in otherwise healthy young and middle-aged brachycephalic dogs was stable when re-evaluated 2-3 years after initial assessment.[115] In humans, OSA prevalence increases with age[146,161,167], possibly due to age-related changes in the collapsibility of the upper airways.[169,170]

In study II, gender and neuter status did not predispose to SDB. Male brachycephalic dogs could be predisposed to more severe signs of BOAS, as males

outnumber females in many previous studies.[29,110,126,130,157,158] Additionally, male French Bulldogs are at a higher risk for BOAS compared to female French Bulldogs.[100] However, there is very limited statistical data on the effect of gender on severity of BOAS. In man the severity and prevalence of OSA in higher in males than females.[147,159-161] This association remains even when matched for obesity and age.[162] There is later onset for OSA in women, and the difference between genders decreases with age.[159] This is suggested to be related to decreasing progesterone levels after menopause.[159,164] Although menopause does not occur in dogs, all sleep recordings in both studies I and II were performed in the anestrus phase in intact female dogs to minimize the effect of sex hormones.

6.6 Proinflammatory condition in brachycephalic dogs (III)

We found higher serum VEGF-A and lower BALF VEGF-A in English Bulldogs compared to control dogs, suggestive of a proinflammatory condition possibly related to intermittent hypoxemia. VEGF-A is a proinflammatory mediator which induces vascular growth and increases vascular permeability both during normal embryonic blood vessel development[252] and under pathologically hypoxemic conditions.[253] VEGF-A is upregulated by HIF-1α in hypoxia.[286] Increased serum VEGF-A concentrations are reported in patients with OSA[230], and serum VEGF-A increases with the severity of OSA and hypoxemia.[256] Hypoxemia of high altitude is known to induce higher serum VEGF-A in healthy dogs.[251] Arterial blood samples were not investigated in study III, but previously mild hypoxemia has been described in English Bulldogs with SDB during sleep by pulse oximetry[7,12,23-26] and in wake brachycephalic dogs of different breeds by arterial blood gas analysis.[116,118,119]

Comparison between English Bulldogs of different BOAS severity grades could not be done due to the small number of dogs in each category, but no differences were seen between BOAS severity grades in all brachycephalic breeds pooled together. Nonetheless, the English Bulldogs may be more affected by SDB than the other brachycephalic breeds. Additionally, the brachycephalic dogs were owner-reportedly healthy, breeding-age dogs with mostly mild or moderate clinical signs

of BOAS, and there might not have been enough variation in BOAS severity to detect differences between groups.

Regardless of breed or severity of BOAS, ageing decreased serum VEGF-A in all brachycephalic dogs. Ageing decreases serum VEGF-A also in humans.[287] The number of English Bulldogs returning for the second visit was low (8/28), which might affect the results. Additionally, BOAS severity remained largely constant in these dogs and signs of BOAS increased in only 15/54 brachycephalic dogs. Thus, the oxidative status of these dogs is likely to have remained similar during both visits.

There was no difference in serum CCL2 between brachycephalic breeds and controls or between BOAS severity grades. The chemokine CCL2, produced by endothelial cells, induces leukocyte migration to inflamed tissue.[288] CCL2 is suspected to advance the progression of cardiovascular disorders related to OSA.[288] The severity of OSA correlates with serum CCL2 concentration, suggesting CCL2 levels indicate degree of hypoxemia.[257,260] The brachycephalic dogs in study III were mostly mildly affected by BOAS, and the length of intermittent hypoxemia in these dogs may have been insufficient for production of CCL2. Ageing did not affect serum CCL2 in brachycephalic dogs.

In BALF, VEGF-A was significantly lower in English Bulldogs compared to control dogs. VEGF-A has not previously been investigated in BALF samples in dogs. This finding may be due to inflammation, as a decrease in the number of alveolar epithelial cells combined with an increase in proteases can lead to degradation of VEGF-A in inflamed tissue.[289,290] No difference between English Bulldogs and control dogs in BALF CCL2 was seen.

6.7 Proinflammatory mediators in canine idiopathic pulmonary fibrosis (III)

The CIPF WHWTs with considerable hypoxemia were included in this study as a reference group for suspected intermittent hypoxemia in brachycephalic dogs.

There was no difference in serum VEGF-A between either WHWT group and control dogs. However, VEGF plays a key role in IPF, as nintedanib, a medication with VEGF receptor antagonism is used for treatment of IPF in humans.[291] Previously, healthy WHWTs without hypoxemia did not have higher serum VEGF-A compared to healthy control dogs of different breeds.[236] However, in this study only 11% of samples were above detection limit, and no comparison to hypoxemic WHWTs with CIPF was performed.[236] In human IPF, both elevated[289,292] and unchanged[293] serum VEGF concentrations have been reported previously. The distinct VEGF-A isoforms seem to be differentially expressed in IPF, leading to a change in their ratios.[289] VEGF-A_{165}b is suggested to be the isoform primarily increased in IPF, and this increase may not be detected as a change in pan-VEGF-A.[289] The immunoassay used in study III detected pan-VEGF-A and not the differential isoforms, and thus this may also be true in our study group. Performing an isoform analysis would have been beneficial in the CIPF WHWTs with marked hypoxemia.

Serum CCL2 was higher in both healthy WHWTs and WHWTs with CIPF, as has been reported before.[234,236,267] Additionally, serum CCL2 was significantly higher in CIPF WHWTs compared to healthy WHWTs. As CCL2 promotes extracellular matrix generation and stimulates collagen secretion[294-296], suggestions of a predisposing role for CCL2 in the development and progression of both CIPF[236] and IPF[297] have been made.

VEGF-A in BALF was lower in both WHWT groups compared with controls, possibly due to the inflammatory process described above. Decreased VEGF-A in BALF in IPF patients is reported.[290,293] Additionally, CCL2 in BALF was significantly higher in WHWTs with CIPF compared to control dogs and English Bulldogs. Increased BALF CCL2 is seen in humans with IPF.[298] Although healthy WHWTs had significantly higher BALF CCL2 compared to English Bulldogs, no significant difference between the two WHWT groups was seen. Previously, higher BALF CCL2 has been reported in CIPF WHWTs compared to healthy WHWTs.[234] The lack of association in our study may be impacted by the low number of BALF samples.

6.8 Study limitations

The main limitations in studies I and II are related to the methodology. The neckband device used in studies I and II is available in four sizes. It can be used in individuals with neck girths between 25 and 65 cm, and thus cannot be used in toy breeds, such as Chihuahuas. Although the majority of dogs (over 70%) attended a study visit in studies I and II, study visits including physical examinations, blood sampling, and body condition scoring, were not performed in all dogs. Comorbid conditions affecting breathing cannot be ruled out in these individuals. The device was used for one night only and recording during multiple nights would have provided more reliable data on breathing during sleep.

We performed BOAS grading similarly in all brachycephalic breeds, although it has only been validated for the extremely brachycephalic English Bulldog, French Bulldog, and Pug.[9,114,284] It is possible that the grading system is not reliable for BOAS severity grading in other breeds, including the less brachycephalic Cavalier King Charles Spaniel.

The study groups in studies I and II are not random representations of the general population, nor was this the goal of the protocols designed to assess the validity of a new method and risk factors in a specific population. Although the prevalence or incidence of SDB in the general Finnish canine population cannot be evaluated based on our results, the results greatly increase knowledge of SDB in both brachycephalic and normocephalic dogs. The sample sizes were large enough for statistically significant findings between groups regarding brachycephaly and excess weight, but it is possible study II is underpowered to distinguish smaller effects, such as the influence of gender, neuter status, and ageing.

Evaluation of SDB in the dogs in study III would have considerably added to the results. However, study III was performed before studies I and II, when the portable neckband system was not yet in use. In study III, the oxidation status of brachycephalic dogs is unknown, as arterial blood sampling was not performed in this group. As this study group represented owner-described healthy young animals, the severely affected animals are likely to be few.

Additionally, in study III BALF samples were only available from English Bulldogs and not the other brachycephalic breeds. It should be noted that serum VEGF-A is influenced by various confounders, such as subclinical neoplasia.[299] As even a short, 10-minute walk increases serum VEGF-A in brachycephalic dogs[300], blood sampling was performed before exercise testing. However, subclinical conditions affecting serum VEGF-A cannot be ruled out despite examining for underlying diseases during the study visits.

6.9 Future research prospects

Sleep is an integral part of wellbeing. Brachycephaly affects the welfare of dogs in a plethora of ways, including disrupting sleep. The future of these breeds should be critically evaluated. The effect of crossbreeding, that is, breeding brachycephalic dogs with normocephalic dogs, on breathing during sleep warrants further investigation. More data is needed to obtain diagnostic criteria for SDB in dogs. Further research into the consequences and comorbidities associated with SDB in dogs is needed to establish cutoff values between normal and abnormal breathing during sleep and to create evidence-based treatment guidelines. SDB is, at least to some extent, treatable with surgical procedures and conservative management, and identifying the individuals in need of treatment is key. The neckband system could be utilized in the evaluation of response to both surgical and medical treatment of SDB, as well as response to weight loss. The relationship between SDB status and low-grade inflammation in dogs should be assessed.

Alongside the neckband system presented here, other at-home devices used in man might be adapted for use in dogs to enhance our understanding of canine sleep and the disease processes both affecting and occurring during sleep.

7 Conclusions

1. Sleep-disordered breathing in dogs can be evaluated with a neckband system in the dog's home environment. The obstructive respiratory event index, which summarizes the number of obstructive apneas and hypopneas per hour of recording, can be utilized in the evaluation of severity of sleep-disordered breathing. The neckband system is easy to use for owners and well accepted by dogs both with and without a protective cover. The size of the neckband precludes its use in breeds with neck girth under 25 cm.

2. Brachycephaly has severe consequences on the welfare of dogs, including disruption of sleep. Brachycephaly, moderate or severe signs of brachycephalic obstructive airway syndrome, and excess weight are risk factors for sleep-disordered breathing in dogs. Obesity should be avoided especially in brachycephalic dogs. Ageing, gender, or neuter status do not predispose to sleep-disordered breathing. Sleep-disordered breathing occurs also in normocephalic dogs.

3. Sleeping sitting up, sleeping with a toy in mouth, apneic episodes during sleep, and restlessness during the night can indicate sleep-disordered breathing in dogs. The percentage of time spent snoring during the recording did not correlate with the rate of obstructive apneas and hypopneas in brachycephalic dogs, suggesting that snoring cannot be used as a sole indicator of sleep-disordered breathing.

4. A proinflammatory condition was found in the brachycephalic English Bulldog, detected as an increased concentration of VEGF-A in serum and decreased concentration in bronchoalveolar lavage fluid. This low-grade

inflammation may be linked to intermittent hypoxemia due to sleep-disordered breathing.

References

1. Koch D, Wiestner T, Balli A, et al. Proposal for a new radiological index to determine skull conformation in the dog. Schweiz Arch Tierheilkd 2012;154:217-220.
2. Teng KT, Brodbelt DC, Pegram C, et al. Life tables of annual life expectancy and mortality for companion dogs in the United Kingdom. Sci Rep 2022;12:6415.
3. Stockard CR. The genetic and endocrinic basis for differences in form and behavior: as elucidated by studies of contrasted pure-line dog breeds and their hybrids. In: Animal Anatomical Memoirs No 19. Philadelphia, USA: The Wistar Institute of Anatomy and Biology; 1941.
4. Harvey CE. Inherited and congenital airway conditions. Journal of Small Animal Practice 1989;30:184-187.
5. Oechtering G. Brachycephalic Syndrome - new information on an old congenital disease. Veterinary Focus 2010;20:2-9.
6. Njikam IN, Huault M, Pirson V, et al. The Influence of Phylogenic Origin on the Occurrence of Brachycephalic Airway Obstruction Syndrome in a Large Retrospective Study. Intern J Appl Res Vet Med 2009;7:138-143.
7. Hendricks JC, Kline LR, Kovalski RJ, et al. The English Bulldog: a natural model of sleep-disordered breathing. Journal of Applied Physiology 1987;63:1344-1350.
8. Davis MS, Cummings SL, Payton ME. Effect of brachycephaly and body condition score on respiratory thermoregulation of healthy dogs. Journal of the American Veterinary Medical Association 2017;251:1160-1165.
9. Lilja-Maula L, Lappalainen AK, Hyytiäinen HK, et al. Comparison of submaximal exercise test results and severity of brachycephalic obstructive airway syndrome in English Bulldogs. The Veterinary Journal 2017;219:22-26.
10. Riecks TW, Birchard SJ, Stephens JA. Surgical correction of brachycephalic syndrome in dogs: 62 cases (1991–2004). Journal of the American Veterinary Medical Association 2007;230:1324-1328.
11. Packer RMA, Hendricks A, Burn CC. Do dog owners perceive the clinical signs related to conformational inherited disorders as 'normal' for the breed? A potential constraint to improving canine welfare. Animal Welfare 2012;21:81-93.
12. Hendricks JC, Kovalski RJ, Kline LR. Phasic Respiratory Muscle Patterns and Sleep-disordered Breathing during Rapid Eye Movement Sleep in the English Bulldog. American Review of Respiratory Disease 1991;144:1112-1120.
13. Medicine AAoS. International Classification of Sleep Disorders, Third edition ed. Darien, Illinois, USA: American Academy of Sleep Medicine; 2014.
14. Berry RB, Gleeson K. Respiratory arousal from sleep: mechanisms and significance. Sleep 2000;20:654-675.
15. Dong J-Y, Zhang Y-H, Qin L-Q. Obstructive sleep apnea and cardiovascular risk: Meta-analysis of prospective cohort studies. Atherosclerosis 2013;229:489-495.
16. Chadda KR, Fazmin IT, Ahmad S, et al. Arrhythmogenic mechanisms of obstructive sleep apnea in heart failure patients. Sleep 2018;41:1-14.

17. Dodds S, Williams LJ, Roguski A, et al. Mortality and morbidity in obstructive sleep apnoea–hypopnoea syndrome: results from a 30-year prospective cohort study. ERJ Open Research 2020;6:57.
18. Jennum P, Tonnesen P, Ibsen R, et al. All-cause mortality from obstructive sleep apnea in male and female patients with and without continuous positive airway pressure treatment: a registry study with 10 years of follow-up. Nature and Science of Sleep 2015;7:43-50.
19. Friedlander AH, Bostrom KI, Tran HA, et al. Severe Sleep Apnea Associated With Increased Systemic Inflammation and Decreased Serum Bilirubin. Journal of Oral and Maxillofacial Surgery 2019;77:2318-2323.
20. Antonopoulos AS, Angelopoulos A, Papanikolaou P, et al. Biomarkers of Vascular Inflammation for Cardiovascular Risk Prognostication: A Meta-Analysis. JACC Cardiovascular Imaging 2022;15:460-471.
21. Harlid S, Myte R, Van Guelpen B. The Metabolic Syndrome, Inflammation, and Colorectal Cancer Risk: An Evaluation of Large Panels of Plasma Protein Markers Using Repeated, Prediagnostic Samples. Mediators of Inflammation 2017;2017:4803156.
22. Bonaccio M, Di Castelnuovo A, Pounis G, et al. A score of low-grade inflammation and risk of mortality: prospective findings from the Moli-sani study. Haematologica 2016;101:1434-1441.
23. Hendricks JC, Petrof BJ, Panckeri K, et al. Upper airway dilating muscle hyperactivity during non-rapid eye movement sleep in English Bulldogs. American Review of Respiratory Disease 1993;148:185-194.
24. Veasey SC, Chachkes J, Fenik P, et al. The effects of ondansetron on sleep-disordered breathing in the English bulldog. Sleep 2001;24:155-160.
25. Veasey SC, Fenik P, Panckeri K, et al. The effects of trazodone with L-tryptophan on sleep-disordered breathing in the English bulldog. American Journal of Respiratory and Critical Care Medicine 1999;160:1659-1667.
26. Veasey SC, Panckeri KA, Hoffmann EA, et al. The effects of serotonin antagonists in an animal model of sleep-disordered breathing. American Journal of Respiratory and Critical Care Medicine 1996;153:776-786.
27. Hinchliffe TA, Liu NC, Ladlow J. Sleep-disordered breathing in the Cavalier King Charles spaniel: A case series. Veterinary Surgery 2019;48:497-504.
28. Facin AC, Uscategui RAR, Maronezi MC, et al. Liver and spleen elastography of dogs affected by brachycephalic obstructive airway syndrome and its correlation with clinical biomarkers. Scientific Reports 2020;10:16156.
29. Gianella P, Caccamo R, Bellino C, et al. Evaluation of metabolic profile and C-reactive protein concentrations in brachycephalic dogs with upper airway obstructive syndrome. Journal of Veterinary Internal Medicine 2019;33:2183-2192.
30. Kampf S, Fenk S, Van Cromvoirt A, et al. Differences in selected blood parameters between brachycephalic and non-brachycephalic dogs. Frontiers in Veterinary Science 2023;10:1166032.
31. Rancan L, Romussi S, Garcia P, et al. Assessment of circulating concentrations of proinflammatory and anti-inflammatory cytokines and nitric oxide in dogs with brachycephalic airway obstruction syndrome. American Journal of Veterinary Research 2013;74:155-160.
32. Kopke MA, Wightman P, Ruaux CG. Obstructive sleep apnea in a Chihuahua successfully managed with ondansetron. Clinical Case Reports 2019;7:872-876.
33. Villedieu E, Robinson W, Shales C. Suspected Obstructive Sleep Apnea Successfully Treated with Ondansetron in a Pug. Journal of the American Animal Hospital Association 2022;58:152-155.

34. Pohl S, Roedler FS, Oechtering GU. How does multilevel upper airway surgery influence the lives of dogs with severe brachycephaly? Results of a structured pre- and postoperative owner questionnaire. The Veterinary Journal 2016;210:39-45.
35. Erjavec V, Vovk T, Svete AN. Evaluation of Oxidative Stress Parameters in Dogs with Brachycephalic Obstructive Airway Syndrome Before and after Surgery. Journal of Veterinary Research 2021;65:201-208.
36. Everson C, Bergmann B, Rechtschaffen A. Sleep Deprivation in the Rat: III. Total Sleep Deprivation. Sleep 1989;12:13-21.
37. Rechtschaffen A, Bergmann B. Sleep Deprivation in the Rat: An Update of the 1989 Paper. Sleep 2002;25:18-24.
38. Owczarczak-Garstecka SC, Burman OHP. Can sleep and resting behaviours be used as indicators of welfare in shelter dogs (Canis lupus familiaris)? PLoS One 2016;11:e0163620.
39. Schork IG, Manzo IA, De Oliveira MRB, et al. The cyclic interaction between daytime behavior and the sleep behavior of laboratory dogs. Scientific Reports 2022;12.
40. Siegel J. Introduction/Defining Sleep. In: Kryger M, Roth T, Goldstein CA, eds. Principles and Practice of Sleep Medicine, 7 ed. Philadelphia, PA, UA: Elsevier; 2022:52-53.
41. Borbély AA, Daan S, Wirz-Justice A, et al. The two-process model of sleep regulation: a reappraisal. Journal of Sleep Research 2016;25:131-143.
42. Scammell TE, Arrigoni E, Lipton JO. Neural Circuitry of Wakefulness and Sleep. Neuron 2017;93:747-765.
43. Shea EK, Hess RS. Assessment of postprandial hyperglycemia and circadian fluctuation of glucose concentrations in diabetic dogs using a flash glucose monitoring system. Journal of Veterinary Internal Medicine 2021;35:843-852.
44. Zanghi BM, Gardner C, Araujo J, et al. Diurnal changes in core body temperature, day/night locomotor activity patterns, and actigraphy-generated behavioral sleep in aged canines with varying levels of cognitive dysfunction. Neurobiology of Sleep and Circadian Rhythms 2016;1:8-18.
45. Gooley JJ, Lu J, Fischer D, et al. A Broad Role for Melanopsin in Nonvisual Photoreception. The Journal of Neuroscience 2003;23:7093-7106.
46. Bloch G, Herzog ED, Levine JD, et al. Socially synchronized circadian oscillators. Proceedings of the Royal Society B: Biological Sciences 2013;280:20130035.
47. Stephan FK. The “Other” Circadian System:Food as a Zeitgeber. Journal of Biological Rhythms 2002;17:283-386.
48. Dibner C, Schibler U, Albrecht U. The Mammalian Circadian Timing System: Organization and Coordination of Central and Peripheral Clocks. Annual Review of Physiology 2010;72:517-549.
49. Tobler I, Sigg H. Long-term motor activity recording of dogs and the effect of sleep deprivation. Experientia 1986;42:987-991.
50. Zanghi BM, Kerr W, de Rivera C, et al. Effect of age and feeding schedule on diurnal rest/activity rhythms in dogs. Journal of Veterinary Behavior 2012;7:339-347.
51. Woods HJ, Li MF, Patel UA, et al. A functional linear modeling approach to sleep–wake cycles in dogs. Scientific Reports 2020;10.
52. Ciucci P, Boitani L, Francisci F, et al. Home range, activity and movements of a wolf pack in central Italy. Journal of Zoology 1997;243:803-819.
53. Druzhkova AS, Thalmann O, Trifonov VA, et al. Ancient DNA Analysis Affirms the Canid from Altai as a Primitive Dog. PLoS One 2013;8:e57754.

54. Wang G-D, Zhai W, Yang H-C, et al. Out of southern East Asia: the natural history of domestic dogs across the world. Cell Research 2016;26:21-33.
55. Wauquier A, Verheyen JL, van den Broeck WA, et al. Visual and computer-based analysis of 24 h sleep-waking patterns in the dog. Electroencephalography and Clinical Neurophysiology 1979;46:33-48.
56. Lucas EA, Powell EW, Murphree OD. Baseline sleep-wake patterns in the pointer dog. Physiology & Behavior 1977;19:285-291.
57. Barker DA, Tovey E, Jeffery A, et al. Owner reported breathing scores, accelerometry and sleep disturbances in brachycephalic and control dogs: A pilot study. Veterinary Record 2021;189:e135.
58. Kinsman R, Owczarczak-Garstecka S, Casey R, et al. Sleep Duration and Behaviours: A Descriptive Analysis of a Cohort of Dogs up to 12 Months of Age. Animals 2020;10:1172.
59. Takeuchi T, Harada E. Age-related changes in sleep-wake rhythm in dog. Behavioural Brain Research 2002;136:193-199.
60. Porkka-Heiskanen T. Sleep homeostasis. Current Opinion in Neurobiology 2013;23:799-805.
61. Ram A, Pandley HP, Matsumura H, et al. CSF levels of prostaglandins, especially the level of prostaglandin D2, are correlated with increasing propensity towards sleep in rats. Brain Research 1997;751:81-89.
62. O'Donnell C, King E, Schwartz A, et al. Effect of sleep deprivation on responses to airway obstruction in the sleeping dog. Journal of Applied Physiology 1994;77:1811-1818.
63. Kis A, Szakadát S, Kovács E, et al. Development of a non-invasive polysomnography technique for dogs (Canis familiaris). Physiology & Behavior 2014;130:149-156.
64. Madari A, Farbakova J, Katina S, et al. Assessment of severity and progression of canine cognitive dysfunction syndrome using the CAnine DEmentia Scale (CADES). Applied Animal Behaviour Science 2015;171:138-145.
65. Mondino A, Khan M, Case B, et al. Activity patterns are associated with fractional lifespan, memory, and gait speed in aged dogs. Scientific Reports 2023;13:2588.
66. Mondino A, Ludwig C, Menchaca C, et al. Development and validation of a sleep questionnaire, SNoRE 3.0, to evaluate sleep in companion dogs. Scientific Reports 2023;13.
67. Gergely A, Kiss O, Reicher V, et al. Reliability of Family Dogs' Sleep Structure Scoring Based on Manual and Automated Sleep Stage Identification. Animals 2020;10:927.
68. Mondino A, Delucchi L, Moeser A, et al. Sleep Disorders in dogs: A Pathophysiological and Clinical Review. Topics in companion animal medicine 2021;43:100516.
69. Phillipson EA, Murphy E, Kozar LF. Regulation of respiration in sleeping dogs. Journal of Applied Physiology 1976;40:688-693.
70. Mondino A, Catanzariti M, Mateos DM, et al. Sleep and cognition in aging dogs. A polysomnographic study. Frontiers in Veterinary Science 2023;10.
71. Varga B, Gergely A, Galambos A, et al. Heart Rate and Heart Rate Variability during Sleep in Family Dogs (Canis familiaris). Moderate Effect of Pre-Sleep Emotions. Animals 2018;8.
72. Bálint A, Eleőd H, Körmendi J, et al. Potential Physiological Parameters to Indicate Inner States in Dogs: The Analysis of ECG, and Respiratory Signal During Different Sleep Phases. Frontiers in Behavioral Neuroscience 2019;13:207.

73. Kis A, Gergely A, Galambos Á, et al. Sleep macrostructure is modulated by positive and negative social experience in adult pet dogs. Proceedings of the Royal Society B, Biological sciences 2017;284:20171883.
74. Iotchev IB, Kis A, Turcsan B, et al. Age-related differences and sexual dimorphism in canine sleep spindles. Scientific Reports 2019;9:10092.
75. Lucas E, Foutz A, Dement W, et al. Sleep cycle organization in narcoleptic and normal dogs. Physiology & Behavior 1979;23:737-743.
76. Adams GJ, Johnson KG. Sleep-wake cycles and other night-time behaviours of the domestic dog Canis familiaris. Applied Animal Behaviour Science 1993;36:233-248.
77. Kis A, Szakadát S, Gácsi M, et al. The interrelated effect of sleep and learning in dogs (Canis familiaris); an EEG and behavioural study. Scientific Reports 2017;7:41873.
78. Reicher V, Kis A, Simor P, et al. Interhemispheric asymmetry during NREM sleep in the dog. Scientific Reports 2021;11:18817.
79. Bunford N, Reicher V, Kis A, et al. Differences in pre-sleep activity and sleep location are associated with variability in daytime/nighttime sleep electrophysiology in the domestic dog. Scientific Reports 2018;8:7109-7110.
80. Iotchev IB, Kis A, Bodizs R, et al. EEG Transients in the Sigma Range During non-REM Sleep Predict Learning in Dogs. Scientific Reports 2017;7:12936.
81. Reicher V, Bunford N, Kis A, et al. Developmental features of sleep electrophysiology in family dogs. Scientific Reports 2021;11:22760.
82. Iotchev IB, Reicher V, Kovacs E, et al. Averaging sleep spindle occurrence in dogs predicts learning performance better than single measures. Scientific Reports 2020;10:22461.
83. Iotchev IB, Szabo D, Kis A, et al. Possible association between spindle frequency and reversal-learning in aged family dogs. Scientific Reports 2020;10:6505.
84. Bowes G, Townsend E, Kozar LF, et al. Effect of carotid body denervation on arousal response to hypoxia in sleeping dogs. Journal of Applied Physiology 1981;51:40-45.
85. Eckert DJ, Roca D, Yim Yeh S, et al. Control of breathing. In: Kryget MH, Avidan AY, Berry RB, eds. Atlas of Clinical Sleep Medicine, 2. ed. Philadelphia, PA: Elsevier; 2010:45-52.
86. Chang JL, Goldberg AN, Alt JA, et al. International Consensus Statement on Obstructive Sleep Apnea. International Forum of Allergy and Rhinology 2023.
87. Kimoff RJ, Brooks D, Horner RL, et al. Ventilatory and Arousal Responses to Hypoxia and Hypercapnia in a Canine Model of Obstructive Sleep Apnea. American Journal of Respiratory and Critical Care Medicine 1997;156:886-894.
88. Kimoff RJ, Hamid Q, Divangahi M, et al. Increased upper airway cytokines and oxidative stress in severe obstructive sleep apnoea. The European Respiratory Journal 2011;38:89-97.
89. Kimoff RJ, Makino H, Horner RL, et al. Canine model of obstructive sleep apnea: model description and preliminary application. Journal of Applied Physiology 1994;76:1810-1817.
90. Brooks D, Horner RL, Floras JS, et al. Baroreflex control of heart rate in a canine model of obstructive sleep apnea. American Journal of Respiratory and Critical Care Medicine 1999;159:1293-1297.
91. Horner RL, Brooks D, Kozar LF, et al. Sleep Architecture in a Canine Model of Obstructive Sleep Apnea. Sleep 1998;21:847-858.

92. Horner RL, Brooks D, Kozar LF, et al. Immediate effects of arousal from sleep on cardiac autonomic outflow in the absence of breathing in dogs. Journal of Applied Physiology 1995;79:151-162.
93. Zhao J, Xu W, Yun F, et al. Chronic obstructive sleep apnea causes atrial remodeling in canines: mechanisms and implications. Basic Research in Cardiology 2014;109:427.
94. Yu L, Li X, Huang B, et al. Atrial Fibrillation in Acute Obstructive Sleep Apnea: Autonomic Nervous Mechanism and Modulation. Journal of the American Heart Association 2017;6.
95. Yang X, Xiao Y, Han B, et al. Implication of mixed sleep apnea events in adult patients with obstructive sleep apnea-hypopnea syndrome. Sleep and Breathing 2019;23:559-565.
96. Berry RB, Abreu AR, Krishnan V, et al. A transition to the American Academy of Sleep Medicine-recommended hypopnea definition in adults: initiatives of the Hypopnea Scoring Rule Task Force. Journal of Clinical Sleep Medicine 2022;18:1419-1425.
97. Roland PS, Rosenfeld RM, Brooks LJ, et al. Clinical practice guideline: Polysomnography for sleep-disordered breathing prior to tonsillectomy in children. Otolaryngology–Head and Neck Surgery 2011;145:S1-15.
98. Farrell KS, Hopper K, Cagle LA, et al. Evaluation of pulse oximetry as a surrogate for PaO2 in awake dogs breathing room air and anesthetized dogs on mechanical ventilation. Journal of Veterinary Emergency and Critical Care 2019;29:622-629.
99. Bernaerts F, Talavera J, Leemans J, et al. Description of original endoscopic findings and respiratory functional assessment using barometric whole-body plethysmography in dogs suffering from brachycephalic airway obstruction syndrome. the Veterinary Journal 2010;183:95-102.
100. Liu N-C, Sargan DR, Adams VJ, et al. Characterisation of Brachycephalic Obstructive Airway Syndrome in French Bulldogs Using Whole-Body Barometric Plethysmography. PLoS One 2015;10:e0130741.
101. Liu NC, Adams VJ, Kalmar L, et al. Whole-Body Barometric Plethysmography Characterizes Upper Airway Obstruction in 3 Brachycephalic Breeds of Dogs. Journal of Veterinary Internal Medicine 2016;30:853-865.
102. Le Bon O, Staner L, Hoffmann G, et al. The first-night effect may last more than one night. Journal of Psychiatric Research 2001;35:165-172.
103. Reicher V, Kis A, Simor P, et al. Repeated afternoon sleep recordings indicate first-night-effect-like adaptation process in family dogs. Journal of Sleep Research 2020;29.
104. Kovács E, Kosztolányi A, Kis A. Rapid eye movement density during REM sleep in dogs (Canis familiaris). Learning & Behavior 2018;46:554-560.
105. Alametsä J, Rauhala E, Huupponen E, et al. Automatic detection of spiking events in EMFi sheet during sleep. Medical Engineering and Physics 2006;28:267-275.
106. Tenhunen M, Elomaa E, Sistonen H, et al. Emfit movement sensor in evaluating nocturnal breathing. Respiratory Physiology and Neurobiology 2013;187:183-189.
107. Chung F, Abdullah HR, Liao P. STOP-Bang Questionnaire: A Practical Approach to Screen for Obstructive Sleep Apnea. Chest 2016;149:631-638.
108. Johns MW. A new method for measuring daytime sleepiness : the epworth sleepiness scale. Sleep 1991;14:540-545.

109. Packer RMA, O'Neill DG, Fletcher F, et al. Great expectations, inconvenient truths, and the paradoxes of the dog-owner relationship for owners of brachycephalic dogs. PloS One 2019;14:e0219918.
110. Roedler FS, Pohl S, Oechtering GU. How does severe brachycephaly affect dog's lives? Results of a structured preoperative owner questionnaire. The Veterinary Journal 2013;198:606-610.
111. Tooley C, Heath SE. Sleep Characteristics in Dogs; Effect on Caregiver-Reported Problem Behaviours. Animals 2022;12.
112. Knazovicky D, Tomas A, Motsinger-Reif A, et al. Initial evaluation of nighttime restlessness in a naturally occurring canine model of osteoarthritis pain. PeerJ 2015;3:e772.
113. Packer RMA, O'Neill DG, Fletcher F, et al. Come for the looks, stay for the personality? A mixed methods investigation of reacquisition and owner recommendation of Bulldogs, French Bulldogs and Pugs. PloS One 2020;15:e0237276.
114. Aromaa M, Lilja-Maula L, Rajamäki MM. Assessment of welfare and brachycephalic obstructive airway syndrome signs in young, breeding age French Bulldogs and Pugs, using owner questionnaire, physical examination and walk tests. Animal Welfare 2019;28:287-298.
115. Aromaa M, Rajamäki MM, Lilja-Maula L. A follow-up study of exercise test results and severity of brachycephalic obstructive airway syndrome signs in brachycephalic dogs. Animal Welfare 2021;30:441-448.
116. Hoareau GL, Jourdan G, Mellema M, et al. Evaluation of Arterial Blood Gases and Arterial Blood Pressures in Brachycephalic Dogs. Journal of Veterinary Internal Medicine 2012;26:897-904.
117. Wiseman W, Rosenblatt A, Kung M. Management of sleep disordered breathing in a dog using using continuous positive airway pressure. Australian Veterinary Practitioner 2021;51:19-25.
118. Canola RAM, Souda MG, Braz JB, et al. Cardiorespiratory evaluation of brachycephalic syndrome in dogs. Pesquisa Veterinaria Brasileira 2018;38:1130-1136.
119. de Melo Dias ML, Morris CFM, Moreti BM, et al. Anatomical, Cardiovascular, and Blood Gas Parameters in Dogs with Brachycephalic Syndrome. Acta Scientiae Veterinariae 2018;44:6.
120. Talbot CT, Zersen KM, Poon B, et al. Arterial blood gas measurements are different for brachycephalic and nonbrachycephalic dogs acclimatized to an altitude of 1,535 meters. American Journal of Veterinary Research 2024:1-7.
121. Crane C, Rozanski E, Abelson AL, et al. Severe brachycephalic obstructive airway syndrome is associated with hypercoagulability in dogs. Journal of Veterinary Diagnostic Investigation 2017;29:570-573.
122. Hoareau GL, Mellema M. Pro-coagulant thromboelastographic features in the bulldog. Journal of Small Animal Practice 2015;56:103-107.
123. Brloznik M, Nemec Svete A, Erjavec V, et al. Echocardiographic analysis of dogs before and after surgical treatment of brachycephalic obstructive airway syndrome. Frontiers in Veterinary Science 2023;10:1148288.
124. Fonfara S, Loureiro J, Swift S, et al. Cardiac troponin I as a marker for severity and prognosis of cardiac disease in dogs. the Veterinary Journal 2010;184:334-339.
125. Kilkenny E, Watson C, Dukes-McEwan J, et al. Evaluation of serum cardiac troponin-I concentrations for diagnosis of infective endocarditis in dogs. Journal of Veterinary Internal Medicine 2021;35:2094-2101.

126. Planellas M, Cuenca R, Tabar M-D, et al. Evaluation of C-reactive protein, Haptoglobin and cardiac troponin 1 levels in brachycephalic dogs with upper airway obstructive syndrome. BMC Veterinary Research 2012;8:152.
127. Holdt SL, Peckens NK, Rosenthal S, et al. Arrhythmogenic right ventricular cardiomyopathy in Bulldogs: Evaluation of clinical and histopathologic features, progression, and outcome in 71 dogs (2004-2016). Journal of Veterinary Cardiology 2022;40:170-183.
128. Liu NC, Troconis EL, Kalmar L, et al. Conformational risk factors of brachycephalic obstructive airway syndrome (BOAS) in pugs, French bulldogs, and bulldogs. PLoS One 2017;12:e0181928.
129. Packer RM, Hendricks A, Tivers MS, et al. Impact of Facial Conformation on Canine Health: Brachycephalic Obstructive Airway Syndrome. PLoS One 2015;10:e0137496.
130. Fasanella FJ, Shivley JM, Wardlaw JL, et al. Brachycephalic airway obstructive syndrome in dogs: 90 cases (1991-2008). Journal of the American Veterinary Medical Association 2010;237:1048-1051.
131. Khoa ND, Phuong NL, Tani K, et al. Computational fluid dynamics comparison of impaired breathing function in French bulldogs with nostril stenosis and an examination of the efficacy of rhinoplasty. Computers in Biology and Medicine 2021;134:104398.
132. Ginn JA, Kumas MSA, McKiernan BC, et al. Nasopharyngeal Turbinates in Brachycephalic Dogs and Cats. Journal of the American Animal Hospital Association 2008;44:243-249.
133. Jones BA, Stanley BJ, Nelson NC. The impact of tongue dimension on air volume in brachycephalic dogs. Veterinary Surgergy 2020;49:512-520.
134. White RN. Surgical management of laryngeal collapse associated with brachycephalic airway obstruction syndrome in dogs. Journal of Small Animal Practice 2012;53:44-50.
135. De Lorenzi D, Bertoncello D, Drigo M. Bronchial abnormalities found in a consecutive series of 40 brachycephalic dogs. Journal of the American Veterinary Medical Association 2009;235:835-840.
136. Iotchev IB, Bognár Z, Tóth K, et al. Sleep-physiological correlates of brachycephaly in dogs. Brain Structure and Function 2023.
137. Agha B, Johal A. Facial phenotype in obstructive sleep apnea-hypopnea syndrome: a systematic review and meta-analysis. Journal of Sleep Research 2017;26:122-131.
138. Lowe AA, Fleetham JA, Adachi S, et al. Cephalometric and computed tomographic predictors of obstructive sleep apnea severity. American Journal of Orthodontics and Dentofacial Ortopedics 1995;107:589-595.
139. Miyao E, Noda A, Miyao M, et al. The Role of Malocclusion in Non-obese Patients with Obstructive Sleep Apnea Syndrome. Internal Medicine 2008;47:1573-1578.
140. Mitchell RB, Kelly J. Outcome of adenotonsillectomy for obstructive sleep apnea in obese and normal-weight children. Otolaryngology–Head and Neck Surgery 2007;137:43-48.
141. Siedenburg JS, Dupre G. Tongue and Upper Airway Dimensions: A Comparative Study between Three Popular Brachycephalic Breeds. Animals 2021;11.
142. Song A, Phillips H, Oliveira CR, et al. CT volumetric analysis permits comparison of tongue size and tongue fat in different canine brachycephalic and mesaticephalic breeds. Veterinary Radiology & Ultrasound 2023.

143. Manens J, Bolognin M, Bernaerts F, et al. Effects of obesity on lung function and airway reactivity in healthy dogs. the Veterinary Journal 2012;193:217-221.
144. Pereira-Neto GB, Brunetto MA, Oba PM, et al. Weight loss improves arterial blood gases and respiratory parameters in obese dogs. Journal of Animal Physiology and Animal Nutrition 2018;102:1743-1748.
145. Peppard PE, Young T, Barnet JH, et al. Increased prevalence of sleep-disordered breathing in adults. American Journal of Epidemiology 2013;177:1006-1014.
146. Tufik S, Santos-Silva R, Taddei JA, et al. Obstructive sleep apnea syndrome in the Sao Paulo Epidemiologic Sleep Study. Sleep Medicine 2010;11:441-446.
147. Young T, Palta M, Dempsey J, et al. The occurrence of sleep-disordered breathing among middle-aged adults. The New England Journal of Medicine 1993;328:1230-1235.
148. Mortimore IL, Marshall I, Wraith PK, et al. Neck and Total Body Fat Deposition in Nonobese and Obese Patients with Sleep Apnea Compared with That in Control Subjects. American Journal of Respiratory and Critical Care Medicine 1998;157:280-283.
149. Wang SH, Keenan BT, Wiemken A, et al. Effect of Weight Loss on Upper Airway Anatomy and the Apnea-Hypopnea Index. The Importance of Tongue Fat. American Journal of Respiratory and Critical Care Medicine 2020;201:718-727.
150. Jang M, Jung T, Cho H, et al. Effect of parapharyngeal fat on dynamic obstruction of upper airway in obstructive sleep apnea patients. Sleep Medicine 2015;16.
151. Carneiro-Barrera A, Diaz-Roman A, Guillen-Riquelme A, et al. Weight loss and lifestyle interventions for obstructive sleep apnoea in adults: Systematic review and meta-analysis. Obesity Reviews 2019;20:750-762.
152. Mezzanotte WS, Tangel DJ, White DP. Waking genioglossal electromyogram in sleep apnea patients versus normal controls (a neuromuscular compensatory mechanism). The Journal of Clinical Investigation 1992;89:1571-1579.
153. Li Y, Lin N, Ye J, et al. Upper airway fat tissue distribution in subjects with obstructive sleep apnea and its effect on retropalatal mechanical loads. Respiratory Care 2012;57:1098-1105.
154. Steier J, Lunt A, Hart N, et al. Observational study of the effect of obesity on lung volumes. Thorax 2014;69:752-759.
155. Davies RJ, Stradling JR. The relationship between neck circumference, radiographic pharyngeal anatomy, and the obstructive sleep apnoea syndrome. European Respiratory Society 1990;3:509-514.
156. Li X, He J. The Association Between Serum/Plasma Leptin Levels and Obstructive Sleep Apnea Syndrome: A Meta-Analysis and Meta-Regression. Fronters in Endocrinology 2021;12:696418.
157. Planellas M, Cuenca R, Tabar M-D, et al. Clinical assessment and C-reactive protein (CRP), haptoglobin (Hp), and cardiac troponin I (cTnI) values of brachycephalic dogs with upper airway obstruction before and after surgery. Canadian Journal of Veterinary Research = Revue Canadienne de Recherche Veterinaire 2015;79:58-63.
158. Poncet CM, Dupre GP, Freiche VG, et al. Prevalence of gastrointestinal tract lesions in 73 brachycephalic dogs with upper respiratory syndrome. Journal of Small Animal Practice 2005;46:273-279.
159. Heinzer R, Marti-Soler H, Marques-Vidal P, et al. Impact of sex and menopausal status on the prevalence, clinical presentation, and comorbidities of sleep-disordered breathing. Sleep Medicine 2018;51:29-36.

160. Heinzer R, Vat S, Marques-Vidal P, et al. Prevalence of sleep-disordered breathing in the general population: the HypnoLaus study. The Lancet Respiratory Medicine 2015;3:310-318.
161. Fietze I, Laharnar N, Obst A, et al. Prevalence and association analysis of obstructive sleep apnea with gender and age differences - Results of SHIP-Trend. Journal of Sleep Research 2019;28:e12770.
162. Subramanian S, Jayaraman G, Majid H, et al. Influence of gender and anthropometric measures on severity of obstructive sleep apnea. Sleep and Breathing 2012;16:1091-1095.
163. Leppanen T, Kulkas A, Duce B, et al. Severity of individual obstruction events is gender dependent in sleep apnea. Sleep and Breathing 2017;21:397-404.
164. Popovic RM, White DP. Upper airway muscle activity in normal women: influence of hormonal status. Journal of Applied Physiology 1998;84:1055-1062.
165. Sutherland K, Lee RWW, Chan TO, et al. Craniofacial Phenotyping in Chinese and Caucasian Patients With Sleep Apnea: Influence of Ethnicity and Sex. Journal of Clinical Sleep Medicine 2018;14:1143-1151.
166. Mohsenin V. Effects of gender on upper airway collapsibility and severity of obstructive sleep apnea. Sleep Medicine 2003;4:523-529.
167. Senaratna CV, Perret JL, Lodge CJ, et al. Prevalence of obstructive sleep apnea in the general population: A systematic review. Sleep Medicine Reviews 2017;34:70-81.
168. Young T, Peppard PE, Gottlieb DJ. Epidemiology of Obstructive Sleep Apnea: A Population Health Perspective. American Journal of Respiratory and Critical Care Medicine 2002;165:1217-1239.
169. Malhotra A, Huang Y, Fogel R, et al. Aging influences on pharyngeal anatomy and physiology: the predisposition to pharyngeal collapse. American Journal of Medicine 2006;119:72 e79-14.
170. Murtolahti S, Crouse UK, Pahkala R, et al. Perception and Respiratory Responses of the Upper Airway Mechanism to Added Resistance With Aging. Laryngoscope Investigative Otolaryngology 2017;2:417-422.
171. Tsara V, Serasli E, Kimiskidis V, et al. Acute respiratory failure and sleep-disordered breathing in Arnold-Chiari malformation. Clinical Neurology and Neurosurgery 2005;107:521-524.
172. Rabec C, Laurent G, Baudouin N, et al. Central sleep apnoea in Arnold-Chiari malformation: evidence of pathophysiological heterogeneity. European Respiratory Journal 1998;12:1482-1485.
173. Tran K, Hukins CA. Obstructive and central sleep apnoea in Arnold-Chiari malformation: resolution following surgical decompression. Sleep and Breathing 2011;15:611-613.
174. Kiviranta AM, Rusbridge C, Laitinen-Vapaavuori O, et al. Syringomyelia and Craniocervical Junction Abnormalities in Chihuahuas. Journal of Veterinary Internal Medicine 2017;31:1771-1781.
175. Knowler SP, Kiviranta AM, McFadyen AK, et al. Craniometric Analysis of the Hindbrain and Craniocervical Junction of Chihuahua, Affenpinscher and Cavalier King Charles Spaniel Dogs With and Without Syringomyelia Secondary to Chiari-Like Malformation. PLoS One 2017;12:e0169898.
176. Sparks CR, Gorney A, Williams K, et al. Investigation of sensory thresholds in Cavalier King Charles Spaniels with and without Chiari-like malformations and syringomyelia. Journal of Veterinary Internal Medicine 2018;32:2021-2028.

177. Dewey CW, Marino DJ, Bailey KS, et al. Foramen magnum decompression with cranioplasty for treatment of caudal occipital malformation syndrome in dogs. Veterinary Surgery 2007;36:406-415.
178. Piovezan RD, Kase C, Moizinho R, et al. Gabapentin acutely increases the apnea-hypopnea index in older men: data from a randomized, double-blind, placebo-controlled study. Journal of Sleep Research 2017;26:166-170.
179. Di Cesare F, Negro V, Ravasio G, et al. Gabapentin: Clinical Use and Pharmacokinetics in Dogs, Cats, and Horses. Animals 2023;13.
180. Walker JM, Farney RF, Rhondeau SM, et al. Chronic Opioid Use is a Risk Factor for the Development of Central Sleep Apnea and Ataxic Breathing. Journal of Clinical Sleep Medicine 2007;3:455-462.
181. Teichtal H, Wang D, Cunnington D, et al. Ventilatory Responses to Hypoxia and
Hypercapnia in Stable Methadone Maintenance Treatment Patients. Chest 2005;128:1339-1347.
182. Thavaraputta S, Dennis JA, Laoveeravat P, et al. Hypothyroidism and Its Association With Sleep Apnea Among Adults in the United States: NHANES 2007-2008. Journal of Clinical Endocrinology & Metabolism 2019;104:4990-4997.
183. Vouzouneraki K, Franklin KA, Forsgren M, et al. Temporal relationship of sleep apnea and acromegaly: a nationwide study. Endocrine 2018;62:456-463.
184. Sim JJ, Rasgon SA, Kujubu DA, et al. Sleep apnea in early and advanced chronic kidney disease: Kaiser Permanente Southern California cohort. Chest 2009;135:710-716.
185. Oldenburg O, Lamp B, Faber L, et al. Sleep-disordered breathing in patients with symptomatic heart failure: a contemporary study of prevalence in and characteristics of 700 patients. European Journal of Heart Failure 2007;9:251-257.
186. Schmidt H, Francis DP, Rauchhaus M, et al. Chemo- and ergoreflexes in health, disease and ageing. International Journal of Cardiology 2005;98:369-378.
187. Rowley JA, Badr MS. Anatomy and physiology of upper airway obstruction. In: Kryger M, Roth T, Goldstein CA, eds. Principles and practice of sleep medicine, 7 ed; Philadelphia, PA, UA: Elsevier; 2022:1170-1183.
188. Macrea M, Katz ES, Malhotra A. Central Sleep Apnea: Definitions, Pathophysiology, Genetics, and Epidemiology. In: Kryger M, Roth T, Goldstein CA, eds. Principles and Practice of Sleep Medicine, 7 ed; Philadelphia, PA, UA: Elsevier; 2022:1144-1153.
189. Petrof BJ, Pack AI, Kelly AM, et al. Pharyngeal myopathy of loaded upper airway in with sleep apnea. Journal of Applied Physiology 1994;76:1746-1752.
190. Doyle CR, Aarnes TK, Ballash GA, et al. Anesthetic risk during subsequent anesthetic events in brachycephalic dogs that have undergone corrective airway surgery: 45 cases (2007–2019). Journal of the American Veterinary Medical Association 2020;257:744-749.
191. Lindsay B, Cook D, Wetzel JM, et al. Brachycephalic airway syndrome: management of post-operative respiratory complications in 248 dogs. Australian Veterinary Journal 2020;98:173-180.
192. Trostel CT, Frankel DJ. Punch Resection Alaplasty Technique in Dogs and Cats With Stenotic Nares: 14 Cases. Journal of the American Animal Hospital Association 2010;46:5-11.
193. Oechtering GU, Pohl S, Schlueter C, et al. A Novel Approach to Brachycephalic Syndrome. 2. Laser-Assisted Turbinectomy (LATE). Veterinary Surgery 2016;45:173-181.

194. Foth S, Meller S, Kenward H, et al. The use of ondansetron for the treatment of nausea in dogs with vestibular syndrome. BMC Veterinary Research 2021;17:222.
195. Jay AR, Krotscheck U, Parsley E, et al. Pharmacokinetics, bioavailability, and hemodynamic effects of trazodone after intravenous and oral administration of a single dose to dogs. American Journal of Veterinary Research 2013;74:1450-1456.
196. Gruen ME, Roe SC, Griffith E, et al. Use of trazodone to facilitate postsurgical confinement in dogs. Journal of the American Veterinary Medical Association 2014;245:296-301.
197. Fitzgerald KT, Bronstein AC. Selective serotonin reuptake inhibitor exposure. Topics in Companion Animal Medicine 2013;28:13-17.
198. Mealey KL, Fidel J. P-glycoprotein mediated drug interactions in animals and humans with cancer. J Vet Intern Med 2015;29:1-6.
199. Sullivan CE, Berthon-Jones M, Issa FG, et al. Reversal of Obstructive Sleep Apnoea By Continuous Positive Airway Pressure Applied Through the Nares. The Lancet 1981;317:862-865.
200. Patil SP, Ayappa IA, Caples SM, et al. Treatment of Adult Obstructive Sleep Apnea with Positive Airway Pressure: An American Academy of Sleep Medicine Clinical Practice Guideline. Journal of Clinical Sleep Medicine 2019;15:335-343.
201. Ponce S, Pastor E, Orosa B, et al. The role of CPAP treatment in elderly patients with moderate obstructive sleep apnoea: a multicentre randomised controlled trial. European Respiratory Journal 2019;54.
202. Batool-Anwar S, Goodwin JL, Kushida CA, et al. Impact of continuous positive airway pressure (CPAP) on quality of life in patients with obstructive sleep apnea (OSA). Journal of Sleep Research 2016;25:731-738.
203. Briganti A, Melanie P, Portela D, et al. Continuous positive airway pressure administered via face mask in tranquilized dogs. Journal of Veterinary Emergency and Critical Care 2010;20:503-508.
204. Stabile M, Lacitignola L, Piemontese MR, et al. Comparison of CPAP and oxygen therapy for treatment of postoperative hypoxaemia in dogs. Journal of Small Animal Practice 2021;62:351-358.
205. Staffieri F, Crovace A, De Monte V, et al. Noninvasive continuous positive airway pressure delivered using a pediatric helmet in dogs recovering from general anesthesia. Journal of Veterinary Emergency and Critical Care 2014;24:578-585.
206. Vicenti C, Otero PE, Briganti A, et al. Respiratory Effects of Continuous Positive Airway Pressure Administered during Recovery from General Anesthesia in Brachycephalic Dogs. Veterinary Sciences 2024;11.
207. Ceccherini G, Lippi I, Citi S, et al. Continuous positive airway pressure (CPAP) provision with a pediatric helmet for treatment of hypoxemic acute respiratory failure in dogs. Journal of Veterinary Emergency and Critical Care 2020;30:41-49.
208. Prete GD, Carotenuto AM, Ciampi V, et al. Continuous positive airway pressure ventilation in dogs with acute cardiogenic pulmonary edema is associated with improved clinical parameters and reduced diuretic and oxygen requirements. Journal of the American Veterinary Medical Association 2023:1-8.
209. Palm A, Midgren B, Theorell-Haglow J, et al. Factors influencing adherence to continuous positive airway pressure treatment in obstructive sleep apnea and mortality associated with treatment failure - a national registry-based cohort study. Sleep Medicine 2018;51:85-91.
210. Antic NA, Catcheside P, Buchan C, et al. The effect of CPAP in normalizing daytime sleepiness, quality of life, and neurocognitive function in patients with moderate to severe OSA. Sleep 2007;34:111-119.

211. Craig TJ, Hanks CD, Fisher LH. How do topical nasal corticosteroids improve sleep and daytime somnolence in allergic rhinitis? Journal of Allergy and Clinical Immunology 2005;116:1264-1266.
212. Kiely JL, Nolan P, W.T. M. Intranasal corticosteroid therapy for obstructive sleep apnoea in patients with co-existing rhinitis. Thorax 2004;59:50-55.
213. Amaro AC, Duarte FH, Jallad RS, et al. The use of nasal dilator strips as a placebo for trials evaluating continuous positive airway pressure. Clinics 2012;67:469-474.
214. Yagihara F, Lorenzi-Filho G, Santos-Silva R. Nasal Dilator Strip is an Effective Placebo Intervention for Severe Obstructive Sleep Apnea. Journal of Clinical Sleep Medicine 2017;13:215-221.
215. Kirkness JP, Madronio M, Stavrinou R, et al. Relationship between surface tension of upper airway lining liquid and upper airway collapsibility during sleep in obstructive sleep apnea hypopnea syndrome. Journal of Applied Physiology 2003;95:1761-1766.
216. Ng SSS, Chan RSM, Woo J, et al. A Randomized Controlled Study to Examine the Effect of a Lifestyle Modification Program in OSA. Chest 2015;148:1193-1203.
217. Desplan M, Mercier J, Sabate M, et al. A comprehensive rehabilitation program improves disease severity in patients with obstructive sleep apnea syndrome: a pilot randomized controlled study. Sleep Medicine 2014;15:906-912.
218. Ravesloot MJL, White D, Heinzer R, et al. Efficacy of the New Generation of Devices for Positional Therapy for Patients With Positional Obstructive Sleep Apnea: A Systematic Review of the Literature and Meta-Analysis. Journal of Clinical Sleep Medicine 2017;13:813-824.
219. Mador JF, Kufel TJ, Magalang UJ, et al. Prevalence of Positional Sleep Apnea in Patients Undergoing Polysomnography. Chest 2005;128:2130-2137.
220. Netea MG, Balkwill F, Chonchol M, et al. A guiding map for inflammation. Nature Immunology 2017;18:826-831.
221. Eckersall PD, Bell R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. the Veterinary Journal 2010;185:23-27.
222. Bonecchi R, Galliera E, Borroni EM, et al. Chemokines and chemokine receptors: an overview. Frontiers in Bioscience 2009; 14(2):540-51..
223. Serhan CN, Yacoubian S, Yang R. Anti-Inflammatory and Proresolving Lipid Mediators. Annual Review of Pathology: Mechanisms of Disease 2008;3:279-312.
224. Bertoni AG, Burke GL, Owusu JA, et al. Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care 2010;33:804-810.
225. Viitanen SJ, Lappalainen AK, Christensen MB, et al. The Utility of Acute-Phase Proteins in the Assessment of Treatment Response in Dogs With Bacterial Pneumonia. Journal of Veterinary Internal Medicine 2017;31:124-133.
226. Ibraimi F, Ekberg B, Kriz D, et al. Preparation of a portable point-of-care in vitro diagnostic system, for quantification of canine C-reactive protein, based on a magnetic two-site immunoassay. Analytical and Bioanalytical Chemistry 2013;405:6001-6007.
227. Hillstrom A, Hagman R, Soder J, et al. Validation and application of a canine-specific automated high-sensitivity C-reactive protein assay. Journal of Veterinary Diagnostic Investigation 2015;27:182-190.
228. Baric Rafaj R, Kules J, Marinculic A, et al. Plasma markers of inflammation and hemostatic and endothelial activity in naturally overweight and obese dogs. BMC Veterinary Research 2017;13:13.

229. Sun J, Qi S, Liu W, et al. Pathophysiological Effects of Pancreatic Sympathetic Denervation in Acute Necrotizing Pancreatitis in Dogs. Pancreas 2015;44:1083–1088.
230. Zhang X-B, Jiang X-T, Cai F-R, et al. Vascular endothelial growth factor levels in patients with obstructive sleep apnea: a meta-analysis. European Archives of Oto-Rhino-Laryngology 2017;274:661-670.
231. Parkitny L, McAuley JH, Kelly PJ, et al. Multiplex Cytokine Concentration Measurement: How Much Do the Medium and Handling Matter? Mediators of Inflammation 2013;2013:890706-890713.
232. Moschetta MG, Maschio LB, Zuccari DAPDC, et al. Prognostic value of vascular endothelial growth factor and hypoxia-inducible factor 1[alpha] in canine malignant mammary tumors. Oncology Reports 2015;33:2345.
233. Krafft E, Heikkilä HP, Jespers P, et al. Serum and Bronchoalveolar Lavage Fluid Endothelin-1 Concentrations as Diagnostic Biomarkers of Canine Idiopathic Pulmonary Fibrosis. Journal of Veterinary Internal Medicine 2011;25:990-996.
234. Roels E, Krafft E, Farnir F, et al. Assessment of CCL2 and CXCL8 chemokines in serum, bronchoalveolar lavage fluid and lung tissue samples from dogs affected with canine idiopathic pulmonary fibrosis. the Veterinary Journal 2015;206:75-82.
235. Binnington B, Sakac D, Yi Q, et al. Stability of 40 cytokines/chemokines in chronically ill patients under different storage conditions. Cytokine 2020;130:155057.
236. Roels E, Krafft E, Antoine N, et al. Evaluation of chemokines CXCL8 and CCL2, serotonin, and vascular endothelial growth factor serum concentrations in healthy dogs from seven breeds with variable predisposition for canine idiopathic pulmonary fibrosis. Research in Veterinary Science 2015;101:57-62.
237. Simpson S, Kaislasuo J, Guller S, et al. Thermal stability of cytokines: A review. Cytokine 2020;125:154829.
238. Geovanini GR, Wang R, Weng J, et al. Elevations in neutrophils with obstructive sleep apnea: The Multi-Ethnic Study of Atherosclerosis (MESA). International Journal of Cardiology 2018;257:318-323.
239. Wang Y, Hu K, Liu K, et al. Obstructive sleep apnea exacerbates airway inflammation in patients with chronic obstructive pulmonary disease. Sleep Medicine 2015;16:1123-1130.
240. Rha M-S, Kim C-H, Yoon J-H, et al. Association between the neutrophil-to-lymphocyte ratio and obstructive sleep apnea: a meta-analysis. Scientific Reports 2020;10:10862.
241. Ishioka K, Suzuki Y, Tajima K, et al. Monocyte Chemoattractant Protein-1 in Dogs Affected with Neoplasia or Inflammation. Journal of Veterinary Medical Science 2013;75:173-177.
242. Indzhova V, Czopowicz M, Kilpatrick S, et al. Signalment and C-reactive protein values in dogs with immune-mediated polyarthritis and steroid responsive meningitis arteritis. Frontiers in Veterinary Science 2023;10:1091318.
243. Yazwinski M, Milizio JG, Wakshlag JJ. Assessment of Serum Myokines and Markers of Inflammation Associated with Exercise in Endurance Racing Sled Dogs. Journal of Veterinary Internal Medicine 2013;27:371-376.
244. Campora C, Freeman KP, Lewis FI, et al. Determination of haematological reference intervals in healthy adult greyhounds. J Small Anim Pract 2011;52:301-309.
245. Zamarrón-Sanz C, Ricoy-Galbaldon J, Gude-Sampedro F, et al. Plasma Levels of Vascular Endothelial Markers in Obstructive Sleep Apnea. Archives of Medical Research 2006;37:552-555.

246. Lin N, Simon MC. Hypoxia-inducible factors: key regulators of myeloid cells during inflammation. Journal of Clinical Investigation 2016;126:3661-3671.
247. Hammami A, Charpentier T, Smans M, et al. IRF-5-Mediated Inflammation Limits CD8+ T Cell Expansion by Inducing HIF-1alpha and Impairing Dendritic Cell Functions during Leishmania Infection. PLoS Pathogens 2015;11:e1004938.
248. Tyrakis PA, Palazon A, Macias D, et al. S-2-hydroxyglutarate regulates CD8(+) T-lymphocyte fate. Nature 2016;540:236-241.
249. Barratt SL, Blythe T, Ourradi K, et al. Effects of hypoxia and hyperoxia on the differential expression of VEGF-A isoforms and receptors in Idiopathic Pulmonary Fibrosis (IPF). Respiratory Research 2018;19:1-5.
250. Mojsilovic-Petrovic J, Callaghan D, Cui H, et al. Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes. Journal of Neuroinflammation 2007;4:12.
251. Glaus TM, Grenacher B, Koch D, et al. High altitude training of dogs results in elevated erythropoietin and endothelin-1 serum levels. Comparative biochemistry and physiology Part A, Molecular & Integrative Physiology 2004;138:355-361.
252. Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435-439.
253. Raja R, Kale S, Thorat D, et al. Hypoxia-driven osteopontin contributes to breast tumor growth through modulation of HIF1alpha-mediated VEGF-dependent angiogenesis. Oncogene 2014;33:2053-2064.
254. Hartmann G, Tschöp M, Fischer R, et al. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. Cytokine 2000;12:246-252.
255. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacology 2007;15:252-259.
256. Gozal D, Lipton AJ, Jones KL. Circulating vascular endothelial growth factor levels in patients with obstructive sleep apnea. Sleep 2002;25:59-65.
257. Kim J, Lee CH, Park CS, et al. Plasma Levels of MCP-1 and Adiponectin in Obstructive Sleep Apnea Syndrome. Archives of Otolaryngology-Head & Neck Surgery 2010;136:896-899.
258. Li Q, Zheng X. Tumor necrosis factor alpha is a promising circulating biomarker for the development of obstructive sleep apnea syndrome: a meta-analysis. Oncotarget 2017;8:27616-27626.
259. Uchiyama T, Itaya-Hironaka A, Yamauchi A, et al. Intermittent Hypoxia Up-Regulates CCL2 , RETN, and TNFα mRNAs in Adipocytes via Down-regulation of miR-452. International Journal of Molecular Sciences 2019;20:1960.
260. Gileles-Hillel A, Alonso-Álvarez ML, Kheirandish-Gozal L, et al. Inflammatory Markers and Obstructive Sleep Apnea in Obese Children: The NANOS Study. Mediators of Inflammation 2014;2014:605280-605289.
261. Corcoran BM, Cobb M, Martin MWS, et al. Chronic pulmonary disease in West Highland white terriers. Veterinary Record 1999;144:611-616.
262. Corcoran BM, King LG, Schwarz T, et al. Further characterisation of the clinical features of chronic pulmonary disease in West Highland white terriers. Veterinary Record 2011;168:355.
263. Heikkilä HP, Lappalainen AK, Day MJ, et al. Clinical, Bronchoscopic, Histopathologic, Diagnostic Imaging, and Arterial Oxygenation Findings in West Highland White Terriers with Idiopathic Pulmonary Fibrosis. Journal of Veterinary Internal Medicine 2011;25:433-439.

264. Kouki S, Viitanen SJ, Koho N, et al. Extraesophageal reflux and reflux aspiration in dogs with respiratory diseases and in healthy dogs. Journal of Veterinary Internal Medicine 2023;37:268-276.
265. Määttä OLM, Laurila HP, Holopainen S, et al. Reflux aspiration in lungs of dogs with respiratory disease and in healthy West Highland White Terriers. Journal of Veterinary Internal Medicine 2018;32:2074-2081.
266. King TE, Jr., Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011;378:1949-1961.
267. Krafft E, Laurila HP, Peters IR, et al. Analysis of gene expression in canine idiopathic pulmonary fibrosis. the Veterinary Journal 2013;198:479-486.
268. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. Journal of Biomedical Informatics 2019;95:103208.
269. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. Journal of Biomedical Informatics 2009;42:377-381.
270. Fastrès A, Roels E, Vangrinsven E, et al. Assessment of the lung microbiota in dogs: influence of the type of breed, living conditions and canine idiopathic pulmonary fibrosis. BMC Microbiology 2020;20.
271. Williams N, Kaarlejärvi J, Oikarinen A, et al. Novel wearable technology to screen for sleep apnea: a pilot study. In: Sleep Medicine 2019;S184-S185.
272. Berry RB, Budhiraja R, Gottlieb DJ, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. Journal of Clinical Sleep Medicine 2012;8:597-619.
273. Lindåse S, Feltenmark T, Krantz M, et al. Overweight in Swedish show dogs–prevalence and association with performance in competition. Acta Veterinaria Scandinavica 2021;63.
274. Balsevicius T, Uloza V, Sakalauskas R, et al. Peculiarities of clinical profile of snoring and mild to moderate obstructive sleep apnea-hypopnea syndrome patients. Sleep and Breathing 2012;16:835-843.
275. Seneviratne M, Kaye BM, Ter Haar G. Prognostic indicators of short-term outcome in dogs undergoing surgery for brachycephalic obstructive airway syndrome. Veterinary Record 2020;187:403.
276. Smith M, Mendl M, Murrell JC. Associations between osteoarthritis and duration and quality of night-time rest in dogs. Applied Animal Behaviour Science 2022;253.
277. Koch DA, Rosaspina M, Wiestner T, et al. Comparative investigations on the upper respiratory tract in Norwich terriers, brachycephalic and mesaticephalic dogs. Schweiz Arch Tierheilkd 2014;156:119-124.
278. Johnson LR, Mayhew PD, Steffey MA, et al. Upper airway obstruction in Norwich Terriers: 16 cases. Journal of Veterinary Internal Medicine 2013;27:1409-1415.
279. Marchant TW, Dietschi E, Rytz U, et al. An ADAMTS3 missense variant is associated with Norwich Terrier upper airway syndrome. PLoS Genetics 2019;15:e1008102.
280. Cakiker B, Hans MG, Graham G, et al. The Relationship Between Craniofacial Morphology and Obstructive Sleep Apnea in Whites and in African-Americans. American Journal of Respiratory and Critical Care Medicine 2001;163:947-950.

281. Daboul A, Kruger M, Ivanonvka T, et al. Do brachycephaly and nose size predict the severity of obstructive sleep apnea (OSA)? A sample-based geometric morphometric analysis of craniofacial variation in relation to OSA syndrome and the role of confounding factors. Journal of Sleep Research 2022:e13801.
282. Neelapu BC, Kharbanda OP, Sardana HK, et al. Craniofacial and upper airway morphology in adult obstructive sleep apnea patients: A systematic review and meta-analysis of cephalometric studies. Sleep Medicine Reviews 2017;31:79-90.
283. Noller MW, Guilleminault C, Gouveia CJ, et al. Mandibular advancement for adult obstructive sleep apnea: A systematic review and meta-analysis. Journal of Cranio-Maxillofacial Surgery 2017;45:2035-2040.
284. Riggs J, Liu NC, Sutton DR, et al. Validation of exercise testing and laryngeal auscultation for grading brachycephalic obstructive airway syndrome in pugs, French bulldogs, and English bulldogs by using whole-body barometric plethysmography. Veterinary Surgery 2019;48:488-496.
285. D.P. L. Development and Validation of a Body Condition Score System for Dogs. Canine Practice 1997;22:10-15.
286. Barratt S, Flower V, Pauling J, et al. VEGF (Vascular Endothelial Growth Factor) and Fibrotic Lung Disease. International Journal of Molecular Sciences 2018;19:1269.
287. Saldías MP, Fernández C, Morgan A, et al. Aged blood factors decrease cellular responses associated with delayed gingival wound repair. PloS One 2017;12:e0184189.
288. Hayashi M, Fujimoto K, Urushibata K, et al. Hypoxia-sensitive molecules may modulate the development of atherosclerosis in sleep apnoea syndrome. Respirology 2006;11:24-31.
289. Barratt SL, Blythe T, Jarrett C, et al. Differential Expression of VEGF-Axxx Isoforms Is Critical for Development of Pulmonary Fibrosis. American Journal of Respiratory and Critical Care Medicine 2017;196:479-493.
290. Koyama S, Sato E, Haniuda M, et al. Decreased Level of Vascular Endothelial Growth Factor in Bronchoalveolar Lavage Fluid of Normal Smokers and Patients with Pulmonary Fibrosis. American Journal of Respiratory and Critical Care Medicine 2002;166:382-385.
291. Lancaster L, Crestani B, Hernandez P, et al. Safety and survival data in patients with idiopathic pulmonary fibrosis treated with nintedanib: pooled data from six clinical trials. BMJ Open Respiratory Research 2019;6:e000397.
292. Ando M, Miyazaki E, Ito T, et al. Significance of Serum Vascular Endothelial Growth Factor Level in Patients with Idiopathic Pulmonary Fibrosis. Lung 2010;188:247-252.
293. Meyer KC, Cardoni A, Xiang Z-Z. Vascular endothelial growth factor in bronchoalveolar lavage from normal subjects and patients with diffuse parenchymal lung disease. The Journal of Laboratory and Clinical Medicine 2000;135:332-338.
294. Gharaee-Kermani M, McCullumsmith RE, Charo IF, et al. CC-chemokine receptor 2 required for bleomycin-induced pulmonary fibrosis. Cytokine 2003;24:266-276.
295. Moore BB, Kolodsick JE, Thannickal VJ, et al. CCR2-Mediated Recruitment of Fibrocytes to the Alveolar Space after Fibrotic Injury. American Journal of Pathology 2005;166:675-684.
296. Inomata M, Kamio K, Azuma A, et al. Pirfenidone inhibits fibrocyte accumulation in the lungs in bleomycin-induced murine pulmonary fibrosis. Respiratory Research 2014;15:16.

297. Gui X, Qiu X, Tian Y, et al. Prognostic value of IFN-gamma, sCD163, CCL2 and CXCL10 involved in acute exacerbation of idiopathic pulmonary fibrosis. International Immunopharmacology 2019;70:208-215.
298. Baran CP, Opalek JM, McMaken S, et al. Important Roles for Macrophage Colony-stimulating Factor, CC Chemokine Ligand 2, and Mononuclear Phagocytes in the Pathogenesis of Pulmonary Fibrosis. American Journal of Respiratory and Critical Care Medicine 2007;176:78-89.
299. de Queiroz FG, Zaidan Dagli LM, Meira AS, et al. Serum vascular endothelial growth factor in dogs with soft tissue sarcomas. Veterinary & Comparative Oncology 2013;11:230-235.
300. Wenk J. Zeitlicher Verlauf von Vascular Endothelial Growth Factor und Erythropoietin nach kurzer physischer Belastung und bei meso- und brachycephalen Hunden. In: Vetsuisse-Fakultät Universität Zürich. Zürich: Universität Zürich; 2004.

Appendix 1. Questionnaire on sleep-disturbed breathing and sleeping habits of Finnish dogs.

Translated from Finnish.

Owner name, number, email:
Name of dog:
Official registered name of dog:
Registration number of dog:
Breed: if mixed, write ”mixed”
Gender: Male / Male neutered / Female / Female sterilized
Date of birth: D-M-Y

Weight (kg):
Presence of other dogs in household: Y / N
    If yes, how many: 2 / 3 / 4 / 5 or more
Underlying diseases: Y / N / I don’t know
    If yes, which of the following:
        Canine cognitive dysfunction / dementia
        Epilepsy
        Separation anxiety
        Hypothyroidism
        Pruritus: food allergy or atopic dermatitis
        Osteoarthritis
        Gastroesophageal reflux
        Renal disease
        Chronic respiratory disease, such as chronic bronchitis, canine idiopathic pulmonary fibrosis, or brachycephalic obstructive airway syndrome
        Obsessive-compulsive disorder
        Diabetes mellitus
        Heart disease
        Other, what:
Signs related to aforementioned disease are: occasional / constant
    If occasional, when are they present?:
Is the dog currently on any medications: Y / N / I don’t know
    If yes, which ones:
        Betablockers, such as atenolol or sotalol
        Antiepileptics, such as phenobarbital, potassium bromide, imepitoin

Separation anxiety medications, such as clomipramine
Bronchodilators, such as theophylline
Thyroid medicators, such as levothyroxine
Corticosteroids, such as prednisolone, methylprednisolone
Medication for urinary incontinence, such as phenylpropanolamine
Diuretics, such as furosemide, torasemide
Osteoarthritis injection bedinvetmab (Librela)
Other, what:

Has the dog been sick in the past 6 months: Y / N / I don't know

If yes, what was the disease / condition and how long did it last? Please also report signs even in the absence of a distinct diagnosis or disease.

Has the dog had surgical operations on his/her upper airways (such as widening of nostrils, shortening of soft palate): Y / N / I don't know

If yes, what and when:

Most common sleeping position:

On its side
On its back
Curled up in a loop
On its tummy

The usual sleeping place:

In the same bed with humans
On the floor in the same room with humans
In a different room to humans

Does your dog usually sleep

Calmly
Restlessly: for example changes places multiple times a night or walks around during the night
I don't know

During the last four weeks, has the dog:

	Never	Rarely	Sometimes	Most of the time	Continuously	I don't know
.. snored in its sleep						
.. changed sleeping places multiple times during the same night						
.. slept sitting up						
.. slept with a toy in its mouth						
.. slept on its back						
.. had loud, raspy breathing during sleep						
.. slept with its head hanging off the bed						
.. woken up from sleep gasping						
.. had apneas during sleep, i.e. not breathing for multiple seconds during sleep						

You can clarify and expand on the dog's sleep and behaviors and their length:
Is the dog markedly sleep and/or tired during the day: Y / N / I don't know

Estimate how likely the dog would fall asleep during the following situations:

	Would never fall asleep	Small likelihood to fall asleep	Moderate likelihood to fall asleep	Large likelihood to fall asleep
You watch television for 30 minutes and the dog is with you				
You are at a friend's house for 30 minutes (no other pets or children present)				
A guest comes to your house and you sit chatting for 30 minutes				
You take the dog for vaccinations at the veterinary clinic and wait for 15 minutes in the waiting room before the appointment				

Does the dog wake up to sudden disturbances, such as loud sounds, immediately during the day when it's sleeping? Y / N / I don't know

During the last four weeks, has the dog:

	Never	A few times	Multiple times	I don't know
.. twitched forcefully in its sleep (*tail wagging, moving its paws and small movements in the muscles around the eye is normal)*				
.. howled, growled, or barked in its sleep (*minor whining is normal)*				
.. suddenly got up in the middle of sleeping and attacked a nearby human or animal				

I allow the researchers to contact me for the second part of the study, the sleep recording: Y / N