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ORIGINAL RESEARCH

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Myopathy of the upper airway in snoring and obstructive sleep apnea

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Abstract

Objective: Previous reports of muscle changes in the upper airways of obstructive sleep apnea (OSA) patients have primarily been attributed to acquired nerve lesions due to snoring vibrations. The aim of this study was to investigate whether alterations reflecting muscle fiber injuries also occur in the upper respiratory tract of snoring and OSA patients and if these changes relate to upper airway dysfunction.

Methods: Muscle changes in biopsies from the soft palate of 20 patients suffering from snoring and OSA were investigated with enzyme, immunohistochemical, and morphometric techniques. Biopsies from eight healthy non-snoring subjects were used as controls. Swallowing dysfunction was assessed with videoradiography.

Results: Fourteen patients had various degrees of swallowing dysfunction. The muscle samples from all the patients showed changes typical for both motor-nerve lesions and muscle fiber injuries. The most common alterations reflecting myopathy were fibers having aggregates and disorganization of cytoskeletal proteins (15.5 ± 10.7%). Other changes were fibers with vacuole-like structures (5.0 ± 4.4%), centrally positioned myonuclei (7.9 ± 4.8%), subsarcolemmal accumulations of nuclei, and various forms and sizes of ring fibers, that is, fibers where the myofilaments were disorganized peripherally (2.8 ± 2.8%).

Conclusion: The results show that muscle changes mirroring both myopathy and neuropathy co-exist in the upper airway of snoring OSA patients. These findings suggest muscle weakness as a contributing factor to the upper airway dysfunction in OSA patients.

KEYWORDS
desmin, muscle pathology, myopathy, obstructive sleep apnea, protein-aggregates

1 | INTRODUCTION

Obstructive sleep apnea (OSA) is a common progressive disorder characterized by snoring, repetitive narrowing of pharyngeal walls, and partial or total collapse of the upper airways during sleep. These events lead to hypoxia, central nervous system arousal, and sleep fragmentation, which can cause adverse effects such as neurocognitive deficits, cardiovascular and metabolic disorders, and other diseases and conditions affecting the quality of life.[1] The pathogenesis behind the nocturnal upper airway obstruction is not fully

understood, but the pathomechanism is likely multifactorial where central and peripheral factors interact.[2] In recent years, several studies have suggested upper airway muscle dysfunction due to traumatic snoring vibrations as a contributing factor for OSA and the occurrence of swallowing dysfunction in these patients.[3–13] Most of these studies have been focused on nerve injuries, and many are clinical in nature, whereas acquired myopathy as a cause for upper airway muscle dysfunction has gained less attention.[14]

An effective response of pharyngeal dilators and tongue muscles is crucial for keeping the respiratory tract open during sleep when the tonic and phasic activity decrease, and this, in turn, depends both on intact feedback from sensory receptors as well as on healthy functional muscles. Our previous reports of neuromuscular abnormalities in the soft palate muscles of OSA patients indicate inefficient upper airway muscle function.[7,8,15–18] This is highlighted by the findings showing a higher degree of neuromuscular abnormalities in the palate muscles of OSA patients with swallowing dysfunction than in patients with normal swallowing function.[7,8] The most common pathology was rounding of muscle fibers, abnormal fiber size variation, fiber type grouping, and increased infiltration of fat and connective tissue, findings that are characteristic of motor-nerve lesions. However, we also found a proportion of muscle fibers with abnormalities indicating muscle fiber injury.[8]

In order to increase our understanding of the pathogenic mechanism in OSA patients, we have performed an in-depth analysis of different pathological features highlighting changes reflecting acquired myopathy in the soft palate. The retropalatal area is of particular interest, as it is reported to be the first site of collapse in a majority of OSA patients during sleep.[19–21]

2 | MATERIALS AND METHODS

The regional Medical Ethical Committee in Umeå, Sweden, approved the study (Dnr-05-130 M). Informed consent was obtained from all participants, and the study was conducted in accordance with the declaration of Helsinki.

2.1 | Patients and controls

Twenty male patients referred for soft palate surgery because of snoring and obstructive sleep apnea were included in the study. Swallowing dysfunction was not included in the criteria for selection of subjects. The mean age was 44 years (range 29–60), and the mean body mass index (BMI) was 28 kg/m^2 (range 24–34). Exclusion criteria were previous palatal surgery, systemic disease, smoking, medications, and drug abuse. For control, eight voluntary males without sleep complaints were recruited, mean age 40 years (range 31–51), mean BMI 24 kg/m^2 (range 22–27). The clinical examinations and exclusion criteria were similar as in patients, with the exception that the exclusion criteria also included habitual snoring and sleep apnea.

2.2 | Sleep apnea recordings

All patients and controls underwent a Type 3 sleep study. The ambulatory overnight sleep apnea recordings (Embletta, Embla Systems) were done using nasal cannula pressure, thoracic and abdominal respiratory effort, recording of snoring sounds, finger oximetry (Nonin Oximeter, Plymouth), and a body-positioning sensor. The recordings were scored according to the American Academy of Sleep Medicine (AASM) recommendations. An apnea was defined as a ≥90% cessation of airflow lasting at least 10 s, while a hypopnea was defined as a 50% reduction in airflow compared with baseline, in combination with an oxygen desaturation of ≥3%.[22]

2.3 | Swallowing examination

Swallowing function was investigated in all patients and voluntary controls using a videoradiographic examination (C-arm, Philips BV 29, field width 23 cm). The examinations were evaluated before palatal surgery at full speed and at slow motion by two investigators blinded to the origin and clinical findings. The Swallowing function was graded as follows: (1) normal function; (2) mild dysfunction in the presence of repeated premature leakage, velar dysfunction, residual, or laryngeal penetration; (3) moderate dysfunction if repeated deviant features in Grade 2 or dysfunction of the upper esophageal sphincter, the epiglottis, or the propagation wave was present; (4) severe dysfunction if the participant aspirated a bolus with aspiration below the vocal cords. For details of method and grading of swallowing dysfunction, see.[7,23]

2.4 | Tissue samples and immunohistochemistry

Samples from 20 uvula and two palatopharyngeus muscles were obtained in connection with the surgical removal of the uvula and distal part of the soft palate. Muscle specimens from voluntary controls were acquired from the corresponding site by punch biopsy technique, except in one case where complete surgical resection of the uvula was performed.

The muscle samples were divided into two parts and oriented for both cross and longitudinal sectioning. One part was immediately oriented and mounted for cross-sectioning in OCT compound (Tissue Tek, Miles) and frozen in liquid propane chilled with liquid nitrogen, while others were fixed before freezing using 4% formaldehyde in 0.1 M phosphate buffer, pH 7.0, for 24 h at 4°C and overnight washing at 4°C in Tyrodes solution containing 10% sucrose. Serial muscle sections, 5 μm thick, were cut at −20° in a Reichert Jung cryostat (Leica) and mounted on glass slides. The muscles sections were immunostained with previously characterized monoclonal (mAb) and polyclonal (pAb) antibodies directed against specific cytoskeletal, basement membrane, and extracellular matrix proteins. The antibodies used are summarized in Table 1. Bound primary Abs were visualized by indirect immunofluorescence using affinity-purified secondary Abs

TABLE 1 Antibodies used for immunohistochemistry

Antibody	Product code	Gene[a]	Host/clone	Dilution	Source
Fibronectin	A0245	FN1	pAb-rabbit	1:5000	1
Desmin	M0760	DES	mAb-mouse/ D33	1:100	1
Desmin	18-0016	DES	mAb-mouse/ ZC18	1:1000	2
Desmin	ab15200	DES	pAb-rabbit	1:2000	3
Dystrophin (C-terminus)	GTX15277	DMD	pAb-rabbit	1:7500	4
Dystrophin (Rod domain)	NCL-DYS1	DMD	mAb-mouse/ Dy4/6D3	1:5	5
Dystrophin (C-terminus)	NCL-DYS2	DMD	mAb-mouse/ Dy8/6C5	1:10	5
Dystrophin (N terminus)	NCL-DYS3	DMD	mAb-mouse/ DY10/12B2	1:10	5
α-actinin	A7732	ACTN2	mAb-mouse/ EA-53	1:500	6
Laminin	PC 128	LAM	pAb-sheep	1:15000	7
Laminin	NCL-Merosin	LAMA2	mAB-mouse/MER3/22B2	1:15000	5

Note: 1. Dako, Sweden; 2. Invitrogen Corporation, CA, USA; 3. Abcam, UK; 4, GeneTex Inc., Taiwan; 5. Novocastra Laboratories Ltd., UK; 6. Sigma-Aldrich Co, UK; 7. Binding site, Inc., USA; 8. Santa Cruz Biotechnology, Inc., UK.

[a]Official gene nomenclature according to OMIM (http://www.ncbi.nlm.nih.gov/omim/).

prepared for multiple labeling and conjugated with fluorochrome with different emission spectra; Fluorescein isothiocyanate (FITC), Rhodamine Red-X (RRX; Jackson ImmunoResearch Laboratories), Alexa fluor 488 and Alexa fluor 647 (Invitrogen). The sections were thereafter washed in PBS for 3 × 5 min and then mounted in Vectashield Mounting Medium (H-1000) or a mounting medium (H-1500) with DAPI (4′,6-diamidino-2-phenylindole) for staining of nuclei (Vector Laboratories). Negative control sections were treated as above, except that the primary Abs were exchanged with non-immune serum. For details of the multi-staining technique, see.[24]

2.5 | Enzyme histochemistry

To demonstrate basic morphology, 7–8 μm thick muscle cross-sections serial to those used for immunohistochemistry were stained with routine hematoxylin–eosin (H&E).

2.6 | Quantification of muscle changes

From each muscle sample, four to five random areas were scanned at ×20 magnification with a fluorescence microscope (Leica DM6000B, Leica Microsystems CMS GmbH), equipped with a digital high-speed fluorescence charge-coupled device (CCD) camera (Leica DFC360 FX). The number of muscle fibers with different types of abnormalities was quantified manually on each photo (Photoshop CS5, version 12.0.4) by two investigators in agreement and blinded to the origin of the samples. The quantitative analysis was based on 20 samples and a total of 8516 muscle fibers from the uvula. The palatopharyngeus muscles were not included in the quantification due to the small sample size.

3 | RESULTS

3.1 | Clinical outcome

All 20 patients snored, and 13 had OSA according to the AASM criteria (mean AHI 16, range 6–84). The swallowing examination showed that seven of the patients had mild and seven moderate swallowing dysfunction. For demographic and clinical data, see Table 2. None of the 10 voluntary controls snored or had OSA, and they all displayed a normal swallowing function.

3.2 | General muscle fiber abnormalities

All muscle samples from the snoring and OSA patients displayed muscle fibers with various morphological and pathological changes reflecting both nerve and muscle injuries (Figure 1). The samples from patients had, compared to controls, a considerably higher presence of muscle fibers with larger variability in size and form and an increased amount of connective tissue as revealed by fibronectin staining. Clusters of both hypo- and hypertrophic fibers and fibers with an irregular or lobulated outer contour were relatively common. Muscle fibers with a weak to absent immunoreaction for desmin and or dystrophin were also frequently observed. Only a few necrotic or angulated formed fibers were present in some of the muscles (<0.1%).

TABLE 2 Demographic and clinical data of 20 male snoring and OSA patients

Patient	Age	BMI	AHI	SpO_2	Snore[a] >5 years	Swallowing function[b]
1	32	30	31	77	Yes	1
2	31	26	1	88	Yes	3
3	57	27	2	86	Yes	3
4	45	30	13	81	Yes	2
5	60	34	47	77	Yes	1
6	43	29	18	74	Yes	1
7	38	31	84	80	Yes	3
8	52	26	26	71	Yes	3
9	57	29	16	76	Yes	2
10	37	29	6	89	Yes	1
11	51	30	2	93	Yes	2
12	41	29	8	85	Yes	1
13	34	27	3	89	Yes	2
14	51	27	13	83	Yes	2
15	54	27	15	88	Yes	3
16	55	24	1	93	Yes	2
17	39	27	29	72	Yes	3
18	31	25	4	90	Yes	3
19	29	33	6	94	Yes	1
20	43	26	2	86	Yes	2
Mean	**44**	**28.3**	**16.4**	**83.6**		**2.1**

Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; SpO_2, peripheral oxygen saturation.
[a]Reported by the subjects and their partners. Snoring was confirmed by overnight registration.
[b]For grading criteria, see Section 2.

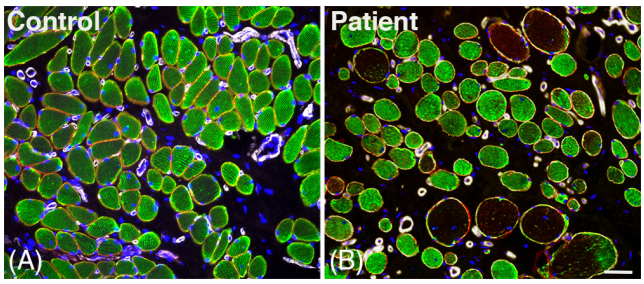

FIGURE 1 Overview of the muscle morphology in a control and a patient. Muscle cross-sections from a control (A) and a patient (B) immunostained for desmin (green color), dystrophin (red color), laminin (white color), and DAPI (blue nuclei). Note the high fiber size variability, high amount of connective tissue, and a large number of fibers unstained for desmin in the patient (B) compared to the control (A). Scale bar = 50 μm

3.3 | Intramyofibrillar alterations

3.3.1 | Protein aggregates

Protein aggregations of desmin and dystrophin were relatively common in muscle fibers of all patients (15.5 ± 10.7%, range 2.8–44.4%) (Figures 2 and 3), while no protein aggregates were observed in the controls. The accumulations of desmin were mainly present as small to large aggregates within the myofibril or as a dens subsarcolemmal rim. In longitudinal sections, the desmin aggregates were commonly found in fibers where the normal desmin striations in the Z-band region of the sarcomere were replaced by a disorganized pattern or by point-like dots (Figure 4). The desmin abnormalities were in some of the fibers present along the entire fiber length, whereas in other fibers, these abnormalities were only observed in certain sections of the fiber (Figure 4F). In the areas where desmin was disorganized, α-actinin was interrupted, deranged or absent at the Z-disk (Figure 4G–I). In longitudinal muscle fibers displaying a normal striation pattern of desmin, small cross-bridges of desmin aggregates spanning over several Z-disks were commonly observed (Figure 4B, inset 1). Moreover, a subgroup of the muscle fibers in the patients containing desmin aggregates also had intramyofibrillar accumulations of dystrophin (3.2 ± 3.9%, range 0–18.1%), a protein normally located between the sarcolemma and the outermost layer of myofilaments. The aggregates of dystrophin and desmin were not always present in the same area of the fiber (Figures 2 and 3).

All muscle fibers in the patient samples showed immunostaining for basement membrane protein laminin α-5 (merosin), although the staining intensity was weak in some of the abnormal fibers.

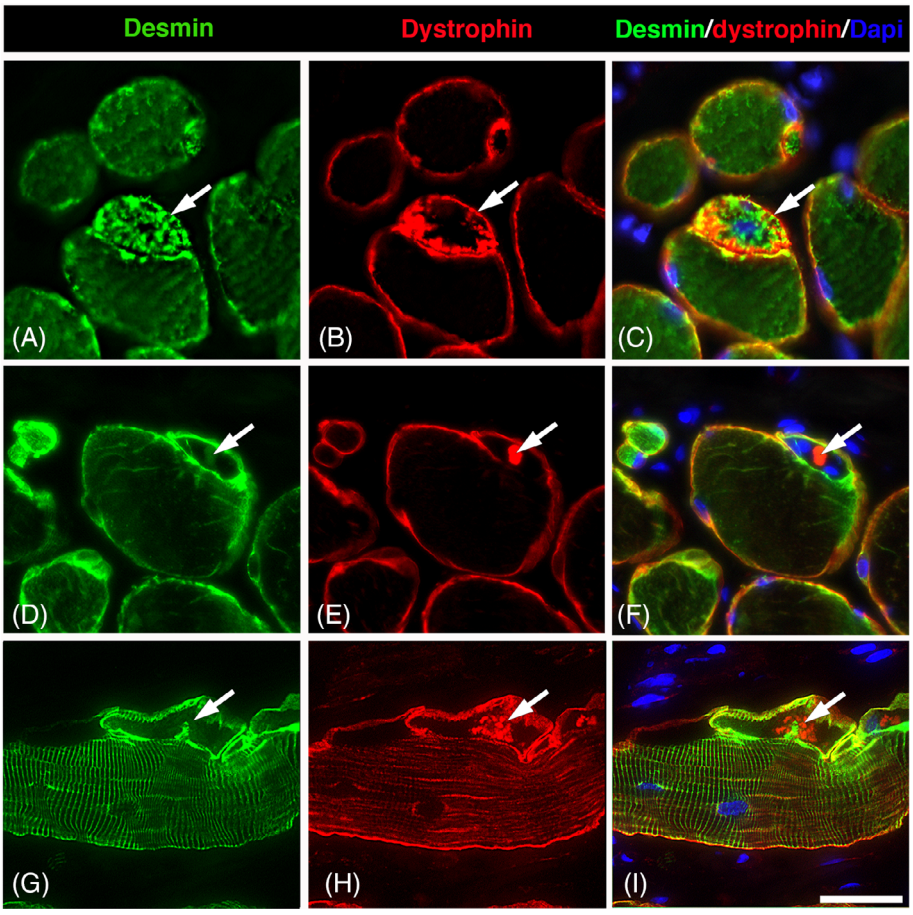

FIGURE 2 Desmin and dystrophin aggregates in muscle fibers of patients. Cross-sections (A–F) and a longitudinal section (G–I) of muscle fibers from patients immunostained for desmin (green), dystrophin (red), and merged staining for desmin dystrophin and DAPI (blue nuclei). Note the accumulation of desmin and subsarcolemmal aggregates of dystrophin in a split fiber (arrows, Figures A–C). Figures (D–E) show dystrophin aggregates in a bulged-out area of fibers lacking internal staining for desmin. Scale bar 50 = μm

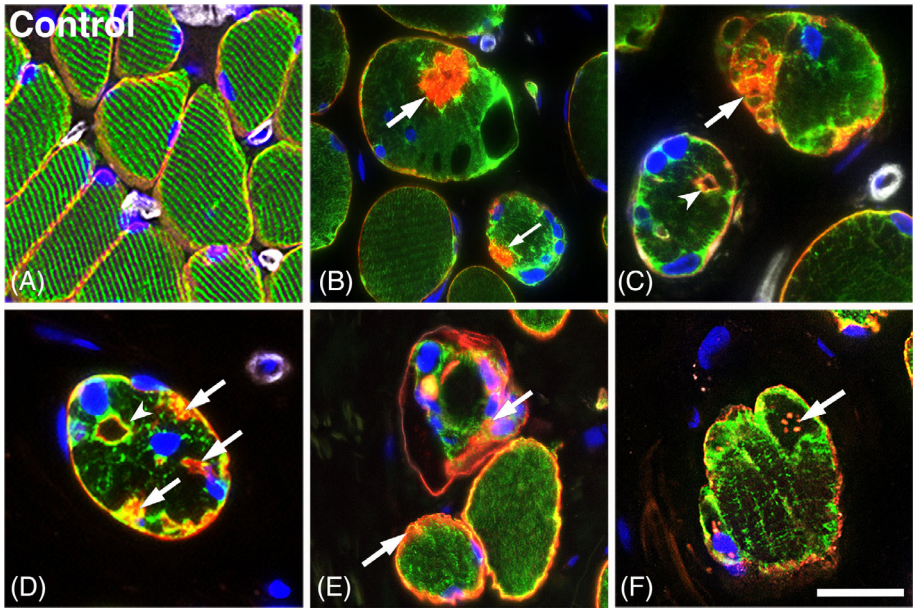

FIGURE 3 Desmin and dystrophin aggregates in soft palate muscles of patients. Muscle cross-section from a control (A) and patients (B–F) showing merged images immunostained for desmin (green), dystrophin (red), laminin (white), and DAPI (blue nuclei). A normal immunostaining pattern in a control is shown in (A). Note the intracellular aggregates of dystrophin and desmin (arrows) both in the cytoplasm and subsarcolemmally beneath the basement membrane (B–D). Also, observe the dens circular staining of desmin and dystrophin around the vacuoles in (C) and (D) (arrowheads). Note also the widespread accumulation of proteins (arrows) in an abnormal fiber (E), and the small-sized dot like aggregates in a fiber with a bulging membrane (F). Scale bar 50 = μm

3.3.2 | Myofibrillar disorganization

Muscle fibers where the myofilaments were disorganized peripherally as a ring of striations perpendicular to the normal orientation were observed in muscle cross-sections of most patients (2.8 ± 2.8%, range 0–5.2%) (Figure 5). These fibers, commonly referred to as ring fibers, were only occasionally observed in the controls (<0.1%). In staining for desmin, the most common immune-reactive pattern showed that

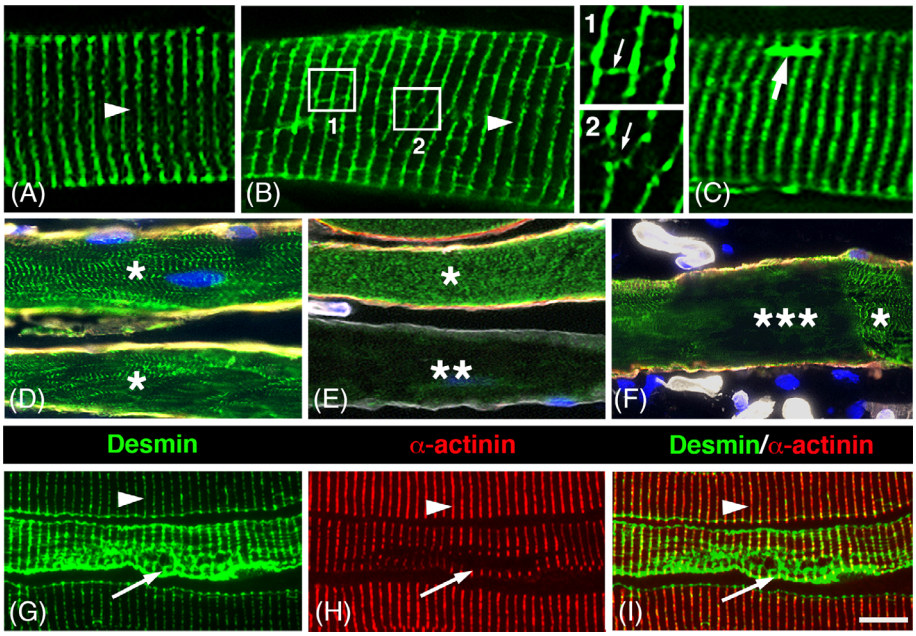

FIGURE 4 Cytoskeletal abnormalities in longitudinally sectioned muscle fibers. Panel (A) shows a control displaying a normal striated immunoreaction for desmin (green) at the Z-disc. Panel (B) show bridges of desmin between the Z-lines (inset 1), remodeling of the sarcomeric Z-lines (inset 2), and desmin streaks across several Z-discs (C) in a patient. Arrowhead in (A) and (B) show normal Z-lines. Panels (D–F) are stained for desmin (green), dystrophin (red), laminin (white), and DAPI (blue). Panel (D) show muscle fibers with disorganized desmin (*), Panel (E) shows a muscle fiber with a segment unstained for both desmin and dystrophin (**) and disorganized desmin (*), while Panel (F) shows a fiber lacking desmin in a segment (***). Panels (G–I) show a longitudinal section stained for desmin, α-actinin, and merged stainings. Note the normal striated pattern for both desmin and α-actinin in the marked area (arrowhead) and the aberrant pattern of these proteins in the area marked with an arrow. The scale bar is 25 μm in Panels (A–C) and 50 μm in Panels (D–I)

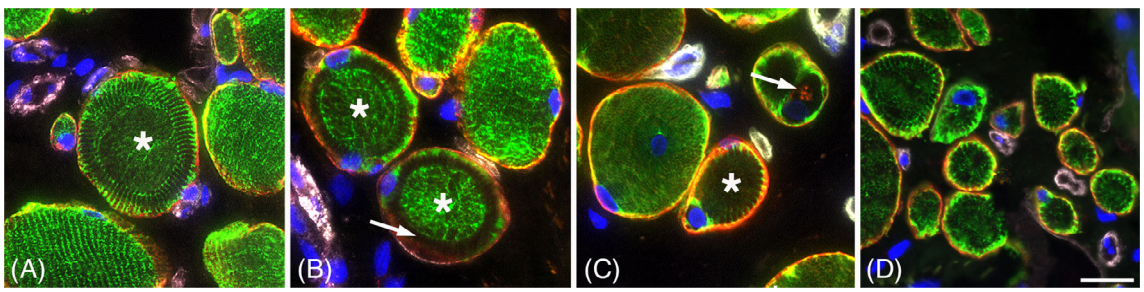

FIGURE 5 Ring fibers in the soft palate muscles of patients. Muscle cross-sections immunostained for desmin (green), dystrophin (red), laminin (white), and DAPI (blue). Muscle fibers where the peripheral myofibrils are dearranged perpendicular to the main body of the fiber forming a ring pattern are shown in Panels (A–D). Large ring fibers (*) of various forms are shown in Panels (A) and (B). In Panel (B), the ring fibers show a weak subsarcolemmal staining for desmin (arrow). Panel (C) shows small-sized ring fibers with weak expression of desmin and a fiber with an accumulation of small dotted dystrophin aggregates (arrow). Clusters of small-sized ring fibers are shown in Panel (D). Scale bar = 25 μm

bundles of myofibrils in the periphery were disoriented at the right angle to the main body of the fiber. In some of the ring fibers, the encircling Z-band striation in the peripheral rearranged myofibrils was distinctly stained for desmin, while in the central parts, desmin was either disorganized or showed a weak to absent immune-reaction. Some other ring fibers showed a distinct staining pattern for desmin in the central region, and apart from a thin band of strong sub-sarcolemmal immune reaction, the longitudinally oriented myofibrils in the periphery were weakly stained. Most of the ring fibers were small sized and grouped in clusters, whereas large-sized ring fibers were only occasionally observed. Various forms and sizes of ring fibers are shown in Figure 5.

Another relatively common abnormality was the presence of fibers displaying multiple trabecular intramyofibrillar septums strongly stained for desmin. The areas between the septa lacked distinct staining for desmin but showed positive staining for myosin and H&E (Figure 6F).

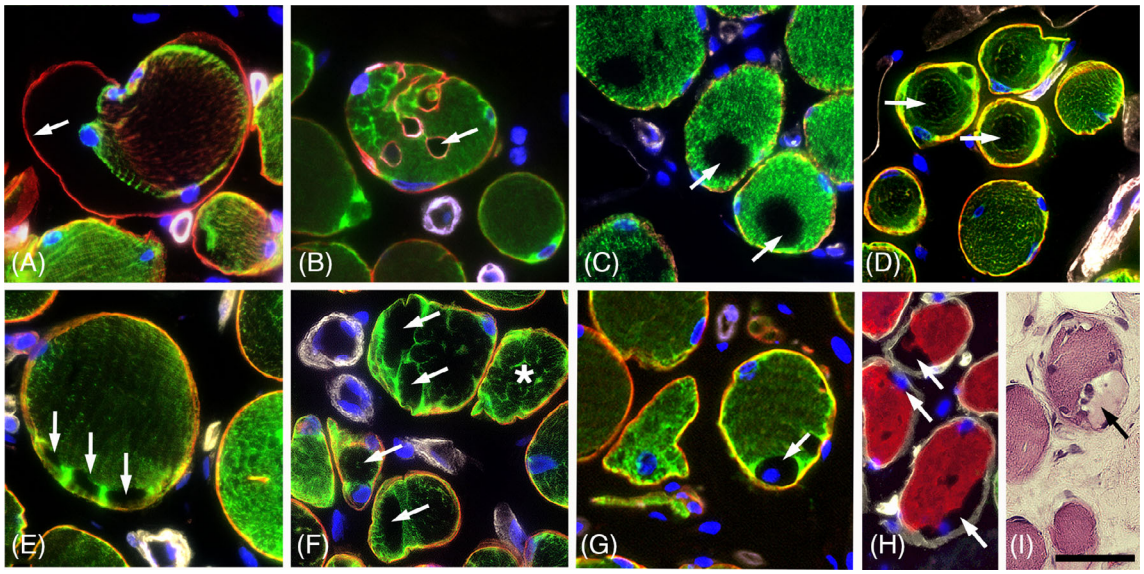

FIGURE 6 Membrane extensions and vacuole-like structures in soft palate muscles of patients. Muscle cross-sections in Panels (A–G) are immune-stained for desmin (green), dystrophin (red), laminin (white), and DAPI (blue). Panel (H) is double-stained for slow MyHC I (red), fast MyHC IIa, and merosin (white), while Panel (I) is stained for H&E. Panel (A) shows an abnormal fiber with an extension of the membrane lacking staining for desmin. Vacuoles with a rim of dystrophin and laminin are shown in Panel (B), and fibers with non-rimmed rubbed-out areas of desmin are shown in (C) and (D) (arrows). Abnormal fibers with vacuole-like structures lacking staining for desmin are shown in Panels (E–G) (arrows). Panel (H) shows a bulged-out area lacking staining for myosin (arrows), but having sarcoplasmic masses as revealed by weak staining for H&E (Panel I, arrows). Scale bar 25 = μm

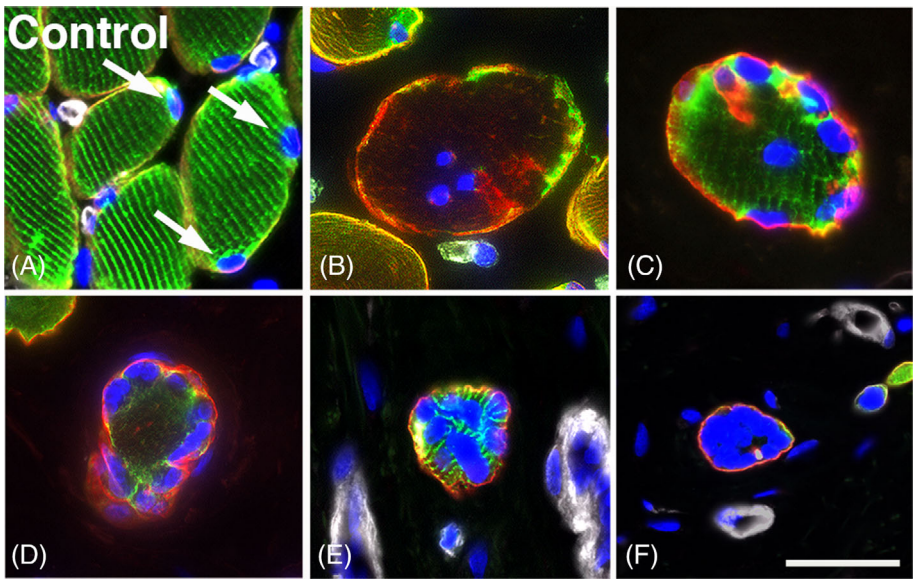

FIGURE 7 Mispositioning and accumulation of nuclei in soft palate muscles of patients. Muscle cross-section from a control (A) and patients (B–F) immune-stained for desmin (green), dystrophin (red), laminin (white), and DAPI (blue). Normally positioned nuclei are shown in Panel (A) (arrows). Panel (B) shows a muscle fiber with several centrally positioned nuclei, while Panel (C) shows a fiber with both a central and high number of subsarcolemmal nuclei. Muscle fibers displaying a high number of subsarcolemmal nuclei are shown in Panels (C) and (D), and fibers containing clumps of nuclei are shown in Panels (E) and (F). Scale bar 50 = μm

3.3.3 | Rimmed and non-rimmed vacuole-like structures

Rimmed and non-rimmed vacuole-like structures of various forms, sizes, and numbers were observed in a subpopulation of muscle fibers in the patients (5.0 ± 4.4%, range 0.4–17.4%), while no such abnormalities were found in the controls. Most of the vacuole-like structures lacked a lining by membrane proteins, while the rimmed vacuoles were lined either by dystrophin or laminin or both (Figure 6). In a subgroup of the fibers, the vacuoles were observed as central circular rubbed-out areas or as single or polymorphic subsarcolemmal circular areas unstained for desmin. Certain of these vacuole-like structures contained one or more myonuclei and or clusters of small dots or even large aggregates of dystrophin and or desmin (Figure 6). In the fibers with peripherally located vacuole-like structures, the basement membrane sometimes showed a slight bulge, as revealed by immunostaining for dystrophin and laminin (Figure 2D–I). These vacuole-like structures lacked internal staining for desmin and myosin

but showed weak staining in H&E (Figure 6H,I). The desmin empty space was surrounded by a rim of desmin and often contained nuclei and clusters of small dots or large aggregates of dystrophin and or desmin (Figures 2C,F, 3F, and 6G).

3.3.4 | Alterations in number and position of myonuclei

Nineteen of the twenty patients had internal nuclei in more than 3% of the fibers, a number considered to be abnormal in limb muscles.[25] Centrally positioned myonuclei were found in 7.9 ± 4.8 (range 1.7–20.9%) of the fibers in patients. The number of internal nuclei ranged mostly from one to three, but muscle fibers containing nuclear clumps were also present (Figure 7). In some small-sized muscle fibers, the nuclear clumps filled up the entire sarcoplasm (Figure 7E,F). Large subsarcolemmal accumulations of nuclei were also present in some fibers. In addition, nuclei, seen as closely packed lined up chains of nuclei in longitudinally sectioned fibers, were occasionally observed. Some nuclei showed a centrally transparent nucleoplasm, while others showed the chromatin material granulated or clumped.

3.4 | Myopathic changes in relation to clinical outcomes

Although all patients had myopthic changes and pharyngeal dysfunction, no statistical relation was found between the proportion of myopathic muscle changes, severity of AHI, and degree of swallowing dysfunction.

4 | DISCUSSION

This study reveals that not only nerve injuries but also muscle fiber damage may contribute to upper airway dysfunction in snoring and OSA patients. The most common myopathic alterations were subsarcolemmal and or sarcoplasmic protein aggregates, myofibrillar and cytoskeletal disorganization, vacuoles, and accumulations and mispositioned myonuclei, all changes typically observed in muscle disorders.[25–29] Since these patients had no general signs or symptoms of any genetic muscle disease, the most likely cause for these alterations is acquired muscle injuries due to traumatic snoring vibrations. This is based on the fact that long-standing exposure to mechanical vibrations in limbs can cause nerve lesions, disturbed cellular cytoarchitecture, vasospasm, reduced blood flow, and reduced muscle strength.[30–32]

Protein aggregation in myofibrillar myopathies is considered to be a disease-specific and disease-significant phenomenon affecting myofiber function by disrupting the sarcomere architecture.[26] Protein misfolding can occur for several reasons, such as mutations in the gene sequence, errors in transcription or translation, dysfunction of chaperones, and ubiquitin proteosome degradation pathways responsible for protein removal,[26,33] or epigenetic changes such as DNA methylation and histone modifications.[34] In addition, hypoxic condition have been shown to form aggregates of sarcoplasmic membrane proteins in animal experiments.[35] Although the cause of the proteins aggregates in the upper airway muscles of snoring and OSA patients is unclear, a defective synthesis or impaired degradation of proteins may be related to traumatic snoring vibrations or hypoxia in the upper airway tissue.

The most common protein aggregations in the palate muscles of patients were small to large sarcoplasmic and subsarcolemmal accumulations of desmin. Desmin is a muscle-specific cytoskeletal protein contributing to structural integrity, force transmission, and load bearing.[36–39] Muscle weakness is a typical sign in disease caused by mutations in genes encoding for desmin.[40,41] The desmin aggregates were generally distributed as large intracellular clumps, clusters of small-sized dots, or as cross-bridges between the Z-lines. Some fibers with desmin abnormalities had a weak or deranged immune-expression of α-actinin. In striated muscles, α-actinin is located together with desmin at the Z-disk, forming a lattice-like structure stabilizing the contractile muscle apparatus. The desmin abnormal fibers often co-expressed protein aggregates of dystrophin, a subsarcolemmal protein linking the intracellular cytoskeleton to the extracellular matrix (Figure 2). The finding of extensively extended dystrophin aggregates in many abnormal fibers is notable since significant accumulations of intramyofibrillar dystrophin have not been reported as a common phenomenon in muscle diseases (Figures 2 and 3). Given the critical structural and regulatory roles of desmin, α-actinin, and dystrophin in myofibrillar organization and muscle contraction, the disorganization of these proteins should affect force generation.

The presence of muscle fibers where desmin was disorganized in a trabecular intramyofibrillar pattern with subsarcolemmal accumulations largely mimic the abnormal mitochondrial distribution in the palate muscles of snoring and OSA patients previously reported by us[15] (Figure 6F). Studies have demonstrated that desmin plays an essential role in the subcellular positioning and function of mitochondria.[42,43] Hence, disorganized desmin might cause an aberrant distribution of mitochondria leading to an uneven intracellular energy production that per se can negatively affect myofibrillar function. Another curious discovery was fibers with a bulge-like extension of the sarcolemmal membrane lacking a normal internal staining pattern for desmin but containing small dotted aggregates of dystrophin (Figures 2 and 3). The drop-formed extension had mostly a subsarcolemmal rim of desmin and weak to moderate internal staining for H&E, suggesting sarcoplasmic masses (Figure 6). Further studies are needed to ascertain the nature and origin of the material in the inclusions.

The finding of palate muscle fibers with rimmed and non-rimmed vacuoles or vacuole-like structures of different sizes and forms in snorers and OSA patients further supports acquired muscle injuries (Figure 6). Intramyofibrillar vacuoles are a morphological hallmark in a wide variety of human skeletal muscle disorders with different etiology.[27,44,45] In congenital muscular dystrophies, vacuoles with

aggregates of subsarcolemmal proteins are common, although the origin of these changes is unknown. Some of the vacuole-like structures showed weak hyaline staining, indicating that they are filled with material.

Another common abnormality in the palate muscles of patients was the relatively high presence of ring fibers (Figure 5). Ring fibers are a non-specific finding in muscle disease and are especially common in muscle dystrophies.[46] Interestingly, these abnormal fibers have also been reported to be frequent in hand muscles of subjects exposed to long-term vibration.[31] The mechanism behind this abnormal myofibrillar formation remains unclear, but it has been suggested that the normal fiber growth pattern is disturbed during myofibrillogenesis, resulting in malformation of the myofibrillar orientation.[47] Given the harmful effects of vibrations, it is likely that the snoring vibrations also interfere with muscle fiber regeneration after injury. Thus, the formation of ring fibers appears to be the result of a de novo myofibrillogenesis rather than a disruption of pre-existing mature myofibrils.

In healthy muscles, myonuclei are normally uniformly distributed in the periphery of the muscle fiber. However, in diseased muscles, the nuclei are often clustered within the center of small-sized muscle cells, and this pattern has been linked to dysfunction, degeneration, and regeneration.[25,48] The presence of atrophied muscle fibers mainly consisting of bags and clumps of myonuclei represents probably a late stage of a degenerative process related to nerve damage rather than muscle fiber injury (Figure 7). When the muscle fibers lose their innervation, they become smaller, and in the final degenerative stage, virtually all sarcoplasm disappears, and the myofiber is reduced to a cluster of nuclei.

In this study, we found no clear relation between the degree of myopathic changes and the severity of sleep apnea (AHI) or degree of swallowing dysfunction. This lack of correlation might be explained by the fact that the myopathic changes only represents a part of the overall pathology including sensory-motor neuropathy.[7] Thus, our findings further reinforce the multifactorial nature of OSA and therefore, the impact of sensory-motor nerve lesions and myopathic changes in the upper airways should be considered together with factors such as input from respiratory centers in the brain, the degree of muscle relaxation during sleep, congenital pharyngeal and jaw anatomy, deposition of fat tissue in the tongue and pharynx, enlarged tonsils, and other co-morbid conditions.

A limitation of this study was the sample size and that the patients were not investigated for gastroesophageal reflux disease (GERD). However, none of the patients reported any symptoms related to GERD.

5 | CONCLUSION

This study adds new data to a growing body of evidence that neuromuscular injuries are a part of the overall pathology in OSA. These changes have the potential to render the upper-airway muscles less capable of generating force, thereby increasing the risk for upper airway dysfunction and collapse during sleep. If snoring causes a vicious circle with muscle and nerve injuries that deteriorate oropharyngeal function, a therapy targeting snoring may potentially break this vicious circle and prevent or reduce symptoms of OSA and swallowing dysfunction.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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