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Review Article

Fiber Types in Human Intrinsic Laryngeal Muscles: A Literature Review

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ABSTRACT

The study of muscle fibers allows the composition of muscles and their functional characteristics to be understood in greater detail. In addition, it makes it possible to apply training and rehabilitation programs based on the energy pathways that regulate muscle contraction. Studying muscle fibers is generally associated with the analysis of myosin heavy chains (MHC) which provide information on the functional characteristics and properties of muscles. The objective of this study was to synthesize the available scientific evidence on the distribution of muscle fibers and myosin heavy chain isoforms present in the intrinsic laryngeal muscles of human beings. A systematic review of the literature was carried out and articles found on PubMed, EBSCOHost, and SciELO were analyzed. The findings show the presence of slow-tonic, type I, type II, type IIA, and type IIX/IIB fibers. Additionally, isoforms MHC-I, MHC-IIA, MHC-IIX, MHC-Fetal, MHC-L, and MHC-IIB can be found. In conclusion, intrinsic laryngeal muscles are composed of a combination of slow and fast fibers and MHC isoforms, derived from evolutionary adaptations and changes which have given way, among other things, to the phonetic characteristics of the human voice.

Keywords:
Muscle Fiber Types;
Myosin Heavy Chain;
Laryngeal Muscles;
Phonation

Tipos de fibras de los músculos intrínsecos laríngeos de seres humanos: una revisión de la literatura

RESUMEN

El estudio de las fibras musculares permite comprender con mejor detalle la composición de los músculos y sus características funcionales. Además, facilita la aplicación de programas de entrenamiento y rehabilitación basados en las vías energéticas que regulan la contracción muscular. Su estudio generalmente va unido al análisis de las cadenas pesadas de miosina (MHC), las que informan sobre las características y propiedades funcionales del músculo. El objetivo de este trabajo fue sintetizar la evidencia científica disponible sobre la distribución de fibras musculares y de isoformas de cadenas pesadas de miosina de los músculos intrínsecos de la laringe de seres humanos. Se realizó una revisión sistemática de la literatura mediante el análisis de artículos encontrados en las bases de datos PubMed, EBSCOHost y SciELO. Los hallazgos informan sobre la existencia de fibras tónicas lentas y tipo I, II, IIA y IIX/IIB. Además, se reconoce la presencia de las isoformas MHC-I, MHC-IIA, MHC-IIX, MHC-Fetal, MHC-L y MHC-IIB. En conclusión, los músculos intrínsecos de la laringe presentan una mezcla de fibras y de isoformas de MHC lentas y rápidas, la que obedece a adaptaciones y cambios evolutivos que han permitido, por ejemplo, las características fonatorias que presenta la voz del ser humano.

Palabras clave:
Tipo de Fibras Musculares; Cadena Pesada de Miosina; Músculos Laríngeos; Fonación

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INTRODUCTION

Muscle fibers are the smallest sub-unit found in muscles (Braithwaite & Al Khalili, 2023). They are covered by a layer of tissue called endomysium. A bundle of fibers is called a fascicle, and several fascicles comprise the muscle as such (Carroll, 2007).

Broadly speaking, three types of fibers can be found inside muscles (type I, IIA, and IIX), which differ according to their contractile and metabolic characteristics (Curry et al., 2012). Type I fibers tend to be highly resistant, albeit with low strength. These fibers are designed for sustained activities, with a low to moderate intensity (Lieber et al., 2017). Type IIA fibers are less resistant to fatigue and have more strength compared to type I. Due to their hybrid nature, these fibers are designed for activities with both aerobic and anaerobic components (Karp, 2001). Type IIX fibers have a mainly anaerobic metabolism; hence, they are stronger and less fatigue-resistant than types I and IIA (Vikne et al., 2020).

Technology advances have made it possible to discover a greater variety of fibers than the aforementioned classifications. According to their degree of resistance to fatigue (from highest to lowest), the types of fibers are I, IC, IIC, IIAC, IIA, IIAB, and IIX or IIB (Lieber et al., 2017). All of these types have been observed in humans; however, type IIB fibers are more common in small mammals (Talbot & Maves, 2016).

Actin and myosin filaments can be found inside muscle fibers. The interaction between both filaments results in muscle contraction. In addition, the myosin molecule is comprised of a couple of heavy chains (MHC) and another pair of light chains (MLC) (Sharma et al., 2018). The differential expression of the MHC gene allows the differentiation between slow and fast fibers (Schiaffino & Reggiani, 2011). On a functional level, MHC determines the speed with which the muscle shortens, as well as its resistance, and therefore is the main indicator of the functional properties of the muscle (Pette & Staron, 2000). A fiber can contain multiple MHC isoforms; nevertheless, the muscle's functionality is determined by the most predominant one (Graziotti et al., 2001). Based on their contractile properties, MHC can be divided into fast or slow (Table 1).

Table 1. Myosin heavy chains and muscle fibers in mammals (Pette & Staron, 2000).

Name	Myosin Heavy Chain Nomenclature	Types of fibers they express
Fast	MHC-IIB/IIX	IIB/X, IIBD, IIAB
Fast	MHC-IIA	IIA, IIAB, IC
Fast	MHC_{eom}	Extraocular
Fast	MHC-II	Masticatory
Slow	MHC-Iß	I, IC
Slow	MHC-α	Extraocular, diaphragmatic, and masseter fibers
Slow	MHCI_{ton}	Extraocular and tensor tympani

Abbreviation: MHC, myosin heavy chain.

Generally, healthy muscles present a heterogeneous distribution of fibers and MHC, which allows them to generate the physical capacities required in daily life (strength, power, and resistance) (Talbot & Maves, 2016). Regarding the larynx, the data on fiber distribution reported by different studies have been contradictory. It has been affirmed that the intrinsic laryngeal muscles have adapted to perform fast or high-acceleration tasks, which indicates that they would be conformed predominantly by fast fibers (Cielo et al., 2011). This finding has been confirmed by studies exploring the histoanatomy of laryngeal muscles in small mammals (Schiaffino & Reggiani, 2011). In contrast, studies carried out in humans have revealed a significant predominance of slow MHC in muscles such as the thyroarytenoid (Hoh, 2005).

The degree of variability found among studies has led to confusion and a lack of consensus regarding fiber distribution in laryngeal muscles. In turn, this has negatively impacted the usefulness of this characteristic for speech therapy assessments and interventions and has resulted in contradictions around muscle work, unlike what is done in other areas linked to musculoskeletal rehabilitation (Agten et al., 2021; Hody et al., 2019; Lievens et al., 2020).

Understanding fiber distribution in laryngeal muscles offers multiple benefits. First, it allows us to understand more precisely each muscle's functionality (Hoh, 2005). Secondly, it makes it possible to apply specific workloads to stimulate a particular physical capacity (strength, power, or resistance) (Sandage & Smith, 2017), and lastly, it promotes the use of continuous or interval training specifically adapted to each muscle, which in turn results in muscle performance improvement over time (Johnson & Sandage, 2019).

Considering the benefits of understanding the types of fibers and MHC isoforms in laryngeal muscles, the question behind this research is: What information does current evidence provide on fiber and MHC distribution in human laryngeal muscles?

The objective of this work is to synthesize the scientific evidence available on muscle fiber and myosin heavy chain isoforms distribution found in human intrinsic laryngeal muscles.

METHOD

This systematic literature review was carried out following the guidelines established by the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Page et al., 2021). Therefore, a series of criteria were established, which are detailed below.

Search Strategies

Between March 7 and 13, 2022, a search was conducted on PubMed, EBSCOHost, and SciELO, using the keywords 'cricothyroid', 'posterior cricoarytenoid', 'lateral cricoarytenoid', 'transverse interarytenoid', 'oblique interarytenoid', 'thyroarytenoid', and 'intrinsic laryngeal muscles', in combination with the term 'fiber type', through the Boolean 'and'. Additionally, the term 'myosin heavy chain' was included, combined with 'laryngeal muscles' through the Boolean 'and'. Finally, the Boolean 'not' was used in Pubmed in order to eliminate the MeSH term 'dietary fiber'.

Eligibility Criteria

Experimental and observational studies were included. This comprises clinical trials, as well as quasi-experimental, descriptive, and descriptive-analytical studies. Additionally, no limits were set regarding the date of publication. As for language, articles written in Spanish, English, and Portuguese with an abstract in English and whose content was related to the aim of this research were included. Studies conducted on humans whose ages ranged between 1 and 99 years were also considered. This research exclusively included studies on humans whose laryngeal muscles were undamaged, that is, studies that analyzed muscles explicitly declared as healthy or lacking any morphological or metabolic alterations.

All the articles where the analysis of intrinsic laryngeal muscles (cricothyroid, thyroarytenoid, interarytenoid–oblique and transverse–, and posterior and lateral cricoarytenoid) was performed using morphological, histochemical, biochemical, and immunohistochemical techniques, were selected.

The search process was carried out in English by the author of this review, who selected articles written in the three previously mentioned languages by reviewing their titles and abstracts.

Two independent searches were carried out in total, the first between March 7 and 8, 2022, and the second between March 12 and 13, 2022. Both stages were completed using the previously described strategies. The precision percentage between both searches was 92.9%. An external and independent reviewer with seven years of experience as a professor in the field of voice pathology and rehabilitation was asked to examine the articles in doubt, after which it was agreed to include them.

The results obtained from each search were entered into an Excel spreadsheet, where they were later compared. Subsequently, the author manually eliminated duplicate records and reviewed each article.

Data Extraction

The name of the authors, year of publication, sample (sex, age, and size), type of study, methodology (general procedures), main results, and level of evidence were extracted manually from the selected articles. These data were then organized by year in an Excel spreadsheet, 2019 version.

The levels of evidence proposed by the Joanna Briggs Institute (JBI) (Pearson et al., 2005) were used to assess each paper.

RESULTS

The results are presented below according to search criteria, level of evidence, characteristics of the participants, muscles studied, analysis technique, and types of fibers and MHC of the intrinsic laryngeal muscles.

Search Results

A total of 251 studies were found, which were reduced to 219 after eliminating duplicate records. Subsequently, 56 articles were selected for eligibility based on title and abstract. Of these, 44 were excluded (30 were carried out on animals, two were written in languages other than the three established ones, four studied humans with pathological intrinsic muscles, one was a literature review, and seven had a different objective than the one defined for this study). Thus, 12 studies were in the final selection for analysis. Figure 1 shows a diagram based on PRISMA, detailing the search process and article eligibility.

Level of Evidence

Regarding the level of evidence, 100% of the selected works corresponded to type 4b, observational/descriptive studies (see Table 2).

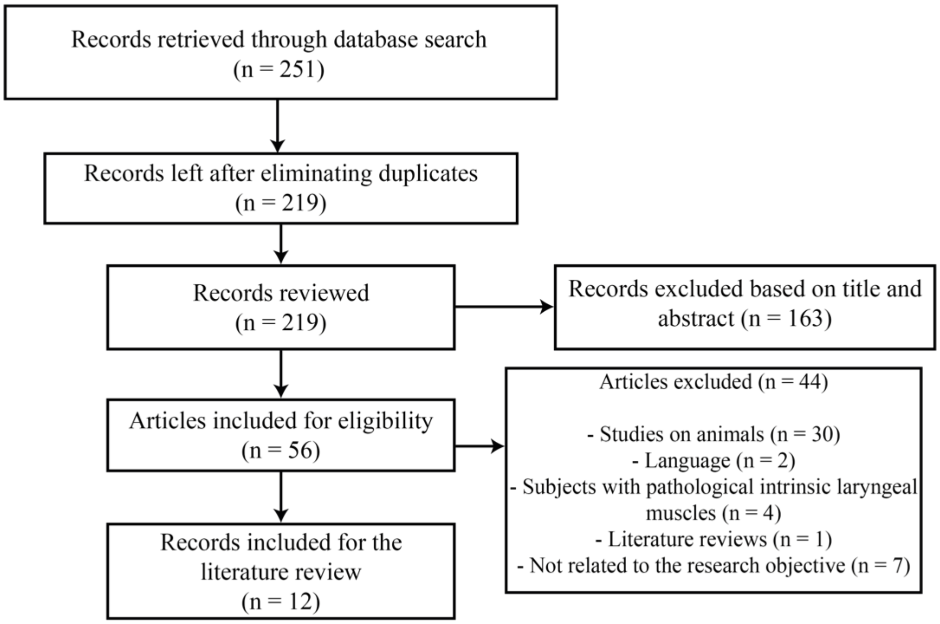

Figure 1. Diagram of the Process of Article Selection.

Characteristics of the Participants

A total of 102 participants were counted (Table 2), whose ages ranged between 7 months and 84 years; however, this data was not provided in one of the studies (Teig et al., 1978). Concerning the sex of the participants, 55.8% were men, and 25.4% were women. Three of the studies did not indicate this information (Li et al., 2004; Périé et al., 2000; Wu et al., 2000).

Of the humans participating in the studies, 50% were living people whose larynxes were removed as part of treatment for laryngeal cancer. The analyzed intrinsic muscles of these people were healthy (Asanau et al., 2011; Claassen & Werner, 1992; Teig et al., 1978; Tellis et al., 2004; Toniolo et al., 2008; Wu et al., 2000). The remaining 50% were deceased people with healthy larynxes, who passed away less than 30 hours before the sample collection (Han et al., 1999; Li et al., 2004; López et al., 2011; Périé et al., 2000; Shiotani et al., 1999; Smerdu & Cvetko, 2013) (see Table 2).

Types of Muscles Studied

Half of the studies analyzed fiber distribution in intrinsic laryngeal muscles (Asanau et al., 2011; Claassen & Werner, 1992; Han et al., 1999; López et al., 2011; Teig et al., 1978; Tellis et al., 2004), while the other half described the MHC isoforms present in these muscles (Li et al., 2004; Périé et al., 2000; Shiotani et al., 1999; Smerdu & Cvetko, 2013; Toniolo et al., 2008; Wu et al., 2000).

With regards to the type of muscle, 83% of the selected articles studied the thyroarytenoid muscle (Claassen & Werner, 1992; Han et al., 1999; Li et al., 2004; López et al., 2011; Périé et al., 2000; Shiotani et al., 1999; Smerdu & Cvetko, 2013; Teig et al., 1978; Toniolo et al., 2008; Wu et al., 2000), 66.7% the posterior crycoarytenoid (Asanau et al., 2011; Claassen & Werner, 1992; Li et al., 2004; Périé et al., 2000; Shiotani et al., 1999; Smerdu & Cvetko, 2013; Toniolo et al., 2008; Wu et al., 2000), 41.7 % analyzed the lateral crycoarytenoid muscle (Claassen & Werner, 1992; Li et al., 2004; Shiotani et al., 1999; Smerdu & Cvetko, 2013; Teig et al., 1978), 50% the interarytenoid (Claassen & Werner, 1992; Shiotani et al., 1999; Smerdu & Cvetko, 2013; Teig et al., 1978; Tellis et al., 2004; Toniolo et al., 2008), and 41.7% studied the crycothyroid muscle (Claassen & Werner, 1992; Shiotani et al., 1999; Smerdu & Cvetko, 2013; Teig et al., 1978; Toniolo et al., 2008) (see Table 2).

Analysis Techniques

All the studies used morphologic, histochemical, biochemical, and immunohistochemical methods to analyze the fibers and MHC isoforms of the intrinsic laryngeal muscles (see Table 2).

Types of Fibers and MHC Found in Laryngeal Muscles

Overall, multiple fiber types were observed in the intrinsic muscles of the larynx, such as types I, II, IIA, and IIX/IIB. Concerning MHC, the isoforms MHC-I, MHC-IIA, MHC-IIX, and MHC-Fetal were found (Périé et al., 2000). Additionally, specific fibers and isoforms were detected, such as MHC-L (Toniolo et al., 2008), MHC-IIB (Smerdu & Cvetko, 2013), and slow-tonic fibers (Han et al., 1999) (see Table 3).

The only abductor of the vocal folds, the posterior cricoarytenoid muscle, showed a significant predominance of type I and IIA fibers (Asanau et al., 2011; Wu et al., 2000), which is similar to the composition of the cricothyroid muscle (Shiotani et al., 1999; Wu et al., 2000). Moreover, it was found that both muscles contain, in a lower proportion, fast isoforms like MHC-IIX, MHC-L (Toniolo et al., 2008), and MHC-IIB (Smerdu & Cvetko, 2013) (see Table 3).

As for the vocal folds adductors, the thyroarytenoid showed a slight predominance of type I and IIA fibers (Claassen & Werner, 1992; López et al., 2011). In addition, slow-tonic fibers (Han et al., 1999) and the isoforms MHC-L (Toniolo et al., 2008), and MHC-IIB (Smerdu & Cvetko, 2013) were observed. On its part, the lateral cricoarytenoid presented a greater proportion of type II fibers (Teig et al., 1978), while type I and IIA were predominant in the interarytenoid (Tellis et al., 2004; Toniolo et al., 2008) (Tabla 3).

Table 2. General characteristics of the studies.

Authors / Year	Research Design / Level of Evidence	Characteristics of the Participants			Main Procedures
		Age (years)*	Sex	Total Samples Analyzed	
Teig et al., 1978	Descriptive/4b	N.I.	7 M	7	Healthy larynxes or larynxes of people with unilateral carcinoma were dissected (the explored muscles were undamaged). The TA, CT, IA, and LCA muscles were examined. Each muscle was cut into eight portions, which were frozen at -85 °C. Subsequently, 16 µm sections were obtained and the type of fiber was analyzed using histochemical techniques.
Claassen & Werner, 1992	Descriptive/4b	M: 45 to 70 W: 39 to 72	7 M 4 W	11	Thirty-six healthy intrinsic muscles from people with laryngeal cancer were examined: TA (9), PCA (11), IA (8), LCA (4), and CT (4). After the total laryngectomy was performed, the muscles were collected and stored at 5 °C for 3 hours. Sections of 10 µm were obtained and their fiber composition was exposed through histochemical techniques.
Han et al., 1999	Descriptive/4b	19 to 70	5 M 2 W	7	Cadaveric samples of healthy larynxes were analyzed. The sample extraction was performed 5 to 12 hours after death. Subsequently, the samples were frozen at -70°C and then separated into 12 groups. The only muscle examined was the TA.
Shiotani et al., 1999	Descriptive/4b	45 to 78	5 M 1 W	6	The larynxes of healthy people whose time of death did not exceed 24 hours were studied. The TA (VOC), LCA, PCA, IA, and CT muscles were examined. Their MHC composition was observed using immunohistochemical tests.
Périé et al., 2000	Descriptive/4b	< 1 (7 months) to 77	N.I.	5	The TA and PCA muscles were excised from undamaged larynxes belonging to people whose date of death was less than 30 hours before the procedure.

					The samples were frozen at -80 °C. The composition of the MHC was observed through electrophoresis.
Wu et al., 2000	Descriptive/4b	60 to 65	N.I.	9	The larynxes used were removed from people with unilateral carcinoma, examining the PCA and TA muscles of the healthy side. The PCA was divided into a horizontal and an oblique segment, and the TA into a medial and a lateral segment. The samples were stored at -20 °C. Subsequently, 40 fibers were microdissected and their MHC composition was examined using histochemical tests.
Li et al., 2004	Descriptive/4b	55 to 75	N.I.	5	The TA, PCA, and LCA muscles of healthy human beings whose date of death did not exceed 24 hours were observed. The samples were stored at -80 °C. Histochemical analysis was carried out to determine the presence of IIX and IIB fibers.
Tellis et al., 2004	Descriptive/4b	$\bar{x}$ = 68	4 M 1 W	5	Healthy IA muscles were excised from people with laryngeal cancer. After laryngectomy, the samples were stored at -70 °C to later section each one into 10 µm portions. The purpose was to determine the type of fiber present in this muscle through histochemical analysis.
Toniolo et al., 2008	Descriptive/4b	55 to 75	11 M 3 W	14	Healthy intrinsic muscles (TA, VOC, CT, IA, and PCA) of people diagnosed with laryngeal cancer were studied. The analysis was carried out using electrophoresis.
Asanau et al., 2011	Descriptive/4b	$\bar{x}$ = 52 to 84	8 M 9 W	17	Healthy PCA muscles of people diagnosed with laryngeal cancer were analyzed. Each muscle was sectioned into a horizontal and a vertical portion. Sections of 10 µm were obtained, which were studied using histochemical and immunohistochemical techniques.
López et al., 2011	Descriptive/4b	M: $\bar{x}$ = 52 W: $\bar{x}$ = 60	6 M 4 W	10	The VOC muscle was obtained from people with healthy larynxes. Subsequently, it was sectioned transversally into 7-mm portions and then into 1-mm segments. The portions were compared with each other using immunohistochemical techniques, to recognize the type of fiber present in the muscle.
Smerdu & Cvetko, 2013	Descriptive/4b	29 to 61	4 M 2 W	6	The CT, PCA, LCA, VOC, and IA muscles were extracted from cadavers without laryngeal lesions, whose date of death did not exceed 24 hours. Immunohistochemical tests were used to determine the expression of their MHC.

Abbreviations: M, men; W, women; N.I, not indicated; µm, micrometer; °C, degree Celsius; mm, millimeter; MHC, myosin heavy chain; CT, cricothyroid; TA, thyroarytenoid; IA, interarytenoid; LCA, lateral cricoarytenoid; PCA, posterior crycoarytenoid; VOC, vocalis portion of the thyroarytenoid.

* Standard deviation is not included in any of the studies.

Table 3. Fiber and myosin heavy chain distribution in intrinsic laryngeal muscles.

A. Types of fibers								
Authors / Year	Muscle							
	TA		VOC	PCA		LCA	IA	CT
Teig et al., 1978	I: 35 ± 11.6 % II: 65 ± 11.6 %		N.S.	I: 67 ± 8.6 % II: 33 ± 8.6 %		I: 40 ± 5.2 % II: 60 ± 5.2 %	I: 46 ± 8.7 % II: 54 ± 8.7 %	I: 47 ± 6.1 % II: 53 ± 6.1 %
Claassen & Werner, 1992	I: 53 % IIA 36 % IIB: 5 %		N.S.	I: 67% IIA: 22% IIB: 5%		I: 42, 51, 55, and 55 %* IIA: 14, 27, 40, and 58 %* IIB: 0, 5, 22 and 31 %*	I: 56 % IIA: 37.5 % IIB: 1 %	I: 31, 39, 52, and 54 %* IIA: 45, 48, 55, and 68 %* IIB: 0, 1, 1, and 6 %*
Han et al., 1999	N.S.		Presents STF[1]. Superior VOC: 40-50 % Inferior VOC: 25-30%	N.S.		N.S.	N.S.	N.S.
Tellis et al., 2004	N.S.		N.S.	N.S.		N.S.	I: 35 % IIA: 45 % IIX: 15 %	N.S.
Asanau et al., 2011	N.S.		N.S.	V: I: 61% II-IIA: 3% IIA: 36%	H: I: 75% I-IIA: 2% IIA: 23%	N.S.	N.S.	N.S.
López et al., 2011	Women: I: 47 % IIA: 24.7 % IIX: 28.2 %	Men: I: 48 % IIA: 25.2 % IIX: 26.7 %	N.S.	N.S.		N.S.	N.S.	N.S.
B. Myosin Heavy Chain Isoforms								
Shiotani et al., 1999	MHC-I: 13.5 ± 5.7 % MHC-IIA: 49.2 ± 6.8 % MHC-IIB: 37.3 ± 11.4 %		MHC-I: 24.7 ± 6.9 % MHC-IIA: 66.7 ± 10.6 % MHC-IIB: 8.6 ± 7.2 %	MHC-I: 36.3 ± 5.5 % MHC-IIA: 53.5 ± 9.2 % MHC-IIB: 10.2 ± 6.0 %		MHC-I: 18.8 ± 7.3 % MHC-IIA: 57.1 ± 6.6 % MHC-IIB: 24.1 ± 8.1 %	MHC-I: 21.5 ± 8.5 % MHC-IIA: 57.9 ± 9.7 % MHC-IIB: 20.6 ± 4.7 %	MHC-I: 34.6 ± 9.1 % MHC-IIA: 61.1 ± 9.7 % MHC-IIB: 4.3 ± 7.5 %
Wu et al., 2000	Medial: MHC-I: 35 % MHC-IIA: 55 % MHC-IIX: 10 %	Lateral: MHC-I: 25 % MHC-IIA: 45 % MHC-IIX: 30 %	N.S.	MHC-I: 40 % MHC-IIA: 45 % MHC-IIX: 10 %		N.S.	N.S.	N.S.

Périé et al., 2000	Predominance of MHC-I and MHC-IIA. To a lesser extent, MHC-IIB† was observed. Presence of MHC-fetal in 7-month-old person.	N.S.	Predominance of MHC-I and MHC-IIA. Lack of MHC-IIB†. Presence of MHC-fetal in 7-month-old person.	N.S.	N.S.	N.S.
Li et al., 2004	MHC-I: 25 % MHC-IIA: 40 % MHC-IIX: 30 %	MHC-I: 25 % MHC-IIA: 35 % MHC-IIX: 40 %	MHC-I: 65 % MHC-IIA: 35 %	MHC-I: 30 % MHC-IIA: 35 % MHC-IIX: 35 %	N.S.	MHC-I 40 %: MHC-IIA: 60 %
Toniolo et al., 2008	MHC-I: 35 % MHC-IIA: 45 % MHC-IIX: 20 %	MHC-I: 35 % MHC-IIA: 25 % MHC-IIX: 30 % MHC-L[2]: 10 %	MHC-I: 40 % MHC-IIA: 30 % MHC-IIX: 25 % MHC-L[2]: 3 %	N.S.	MHC-I: 30 % MHC-IIA: 35 % MHC-IIX: 20 % MHC-L[2]: 10 %	MHC-I: 40 % MHC-IIA: 40 % MHC-IIX: 15 % MHC-L[2]: 5 %
Smerdu & Cvetko, 2013	Presence of MHC-IIB[3]	N.S.	Presence of MHC-IIB[3]	N.S.	N.S.	Presence of MHC-IIB[3]

Abbreviations: TA, thyroarytenoid; LCA, lateral cricoarytenoid; IA, interarytenoid; PCA, posterior cricoarytenoid; CT, cricothyroid; VOC, vocalis; N.S, Not studied; MHC: Myosin Heavy Chain; V, Vertical; H, Horizontal.

* The original research only provided individual values, due to the reduced number of people in which these muscles were studied.

†The research does not provide percentages or exact values regarding the MHC distribution.

[1] STF (slow tonic fibers): The most specialized variant of the regular Type I fibers. They show more resistance and aerobic capacity than the mentioned fibers (Han et al., 1999).

[2] MHC-L: Characterized by an extremely high contractile speed (superior to IIB fibers) (Sciote et al., 2002).

[3] MHC-IIB: A strong and extremely fast type of fiber, with high fatigability (similar to IIB fibers) (Smerdu & Cvetko, 2013).

DISCUSSION

This study aimed to synthesize the scientific evidence available on muscle fiber and MHC isoform distribution found in human intrinsic laryngeal muscles. Our findings indicate the presence of various types of fibers and MHC isoforms that allow the small intrinsic laryngeal muscles to have abductor, adductor, and tensor functions.

The predominant amount of strong and fast fibers (IIX and MHC-IIB) in the thyroarytenoid (López et al., 2011), interarytenoid (Tellis et al., 2004) and lateral cricoarytenoid (Li et al., 2004) is notorious among the studies. It has been proposed that this specific distribution would allow these muscles to respond to intense, short, and high-speed adjustments (Staron, 1997). These functional characteristics are required mainly for the airway protective reflex or activities such as throat-clearing and laughter (Tellis et al., 2004). Similarly, the interarytenoid muscle is composed of faster and more potent MHC isoforms than the ones previously mentioned (Toniolo et al., 2008). This would explain its relevance during tasks that require fast and sudden adjustments such as register shifts during phonation (Chhetri et al., 2014) or airway protection (Fregosi & Ludlow, 2014). Based on this histoanatomical variant, the interarytenoid muscle has been recognized as one of the main vocal fold adductors (Toniolo et al., 2008).

Traditionally, it has been argued that the posterior cricoarytenoid muscle has a significant predominance of type I fibers, which has been explained by its permanent activity during the abduction necessary for breathing (Rammage et al., 2000). This assertion is partly confirmed thanks to the fiber distribution found in the different studies that were analyzed. In this case, slow fibers and MHC-I are activated to maintain abduction (Asanau et al., 2011). However, some results also indicate the presence of MHC-IIB (Shiotani et al., 1999) and fast fibers (Claassen & Werner, 1992) that allow the PCA to abduct the vocal folds during phonation or exercise (Toniolo et al., 2008). Thus, abduction would occur thanks to the muscle's fast component, while its slow and fatigue-resistance fibers are responsible for maintaining this position (Asanau et al., 2011). However, the process of abduction is not exclusively the job of the posterior cricoarytenoid. It has been indicated that fast fibers and MHC found in the cricothyroid allow it to complement this task under special conditions (Mathew et al., 1988; Shiotani et al., 1999; Wu et al., 2000). This is due to the mechanical advantage of this muscle during contraction, which enables the anterior-horizontal movement of the thyroid cartilage and supports vocal fold abduction (Chhetri et al., 2014). This coactivation between the cricothyroid and posterior cricoarytenoid muscles would occur during deep and forced inhalations (for example, during exercise) or when there is some degree of airway occlusion (Toniolo et al., 2008).

The strongest and fastest fibers of the thyroarytenoid are found primarily in its lateral portion (Wu et al., 2000), while its most resistant ones, which are therefore best prepared to tolerate fatigue, are found in its medial portion, called vocalis (Han et al., 1999). It is estimated that the degree of resistance of the medial section of the thyroarytenoid is due specifically to the presence of slow tonic fibers and MHC-I and IIA (Li et al., 2004; Toniolo et al., 2008), which allow muscles to meet the basic phonatory demands in humans (Sanders et al., 1998). When the demand increases and, for example, greater vocal intensity is required, it would be type IIA fibers that are predominantly recruited (Sandage & Smith, 2017). On the other hand, if the vocal task entails ascending on a musical scale using belting, the upper portion of the vocalis would complement its functions with the type I/IIA fibers of the cricothyroid, lateral cricoarytenoid, and interarytenoid (Shiotani et al., 1999; Tellis et al., 2004).

Research on fiber distribution in intrinsic laryngeal muscles has traditionally been carried out on animals. Some texts gather these data to infer human histoanatomy (Andrade & McLoon, 2013). In general, the interarytenoid and lateral cricoarytenoid muscles have a similar distribution among mammals since their basic functions are similar (Schiaffino & Reggiani, 2011). The most significant differences are found in the cricothyroid and thyroarytenoid muscles. Type IIB fibers have been found to make up more than 60% of the cricothyroid muscle in rats and rabbits, and a similar proportion of type IIX fibers has been found in the thyroarytenoid (Rhee et al., 2004; Rhee & Hoh, 2008). In turn, the number of slow fibers in animals is very low; in rats, 0% have been found in the thyroarytenoid muscle and a meager 19% in the cricothyroid muscle (Rhee et al., 2004). The slow-fiber distribution in animals usually fluctuates between 4% and 43%, with the highest proportions being found in baboons (Rhee & Hoh, 2008). It is thought that the higher proportion of hybrid (IIA) and slow fibers in humans can be explained mainly by vocal demand, which has allowed muscle composition to adapt from fast to slow to respond satisfactorily to tasks such as singing, performance, or daily conversations (Rhee & Hoh, 2008; Titze, 2017).

At the clinical level, these results allow professionals to use more precise loads during vocal exercises or phonatory muscle training. This is possible thanks to knowing the different energetic pathways that predominate during voice production, where (according to recent findings) aerobic, oxidative, and mitochondrial activities would prevail (Lin et al., 2017; Nanjundeswaran et al., 2017; Tellis et al., 2011). In this case, type I and IIA fibers and slow chains are the ones that would be activated preferentially to carry out the muscle activity required for phonation (Johnson & Sandage, 2019). Therefore, the process of training these muscles would aim at producing ATP (adenosine triphosphate) aerobically, by prescribing continuous or interval training, as is done with marathon runners (Groennebaek & Vissing, 2017).

This would imply that clinicians know which workloads are adequate to adapt and optimize the function of oxidative fibers in phonatory muscles. Thus, it is appropriate to propose vocal training programs that favor extracting oxygen from muscles, increasing the number and size of mitochondria, and developing capillary density, among other aspects (Johnson & Sandage, 2019; Snell et al., 2020). This entails understanding that each sound used in therapy (its intensity and duration) triggers metabolic responses that could result in pathological overload, maintenance of performance, or adaptation, which is why their application should be reasonable and guided by disciplines such as the physiology of exercise and training sciences (Delprado-Aguirre, 2020; Fuentes Aracena, 2018; Johnson & Sandage, 2019).

The analyzed articles correspond to descriptive studies with an insufficient level of evidence. Moreover, the difficulty to obtain the samples prevents their randomization, and the required preservation times make their treatment difficult over time. For this reason, this type of design is related to the economy and accessibility needed to manage fragile and inaccessible structures, such as muscle fibers (López et al., 2011).

Technological improvements that have been achieved over the years, sample preservation times, and the lack of understanding regarding the vocal use of subjects have led to the distributions differing from each other (Wu et al., 2000). Despite the above, technology development is considered the most sensitive aspect when observing this type of variation. This has been reported about other regions of the human body, where recent analysis techniques have succeeded in revealing new forms of response and adaptation of slow fibers to exercise (Deshmukh et al., 2021). Accordingly, findings of studies carried out between the 1970s and 1990s should be considered a part of the development of this subject, rather than as the most accurate evidence on fiber distribution (Talbot & Maves, 2016).

The limitations of this review include the small number of articles that were selected, the omission of information found in some of the studies such as the participants' age or vocal experience (Li et al., 2004; Périé et al., 2000; Teig et al., 1978; Wu et al., 2000), and the lack of clarity regarding certain results (Claassen & Werner, 1992). Finally, this review was carried out solely by one researcher, which affects the validity of the search and makes it difficult to reduce the probability of selection bias.

It is necessary to carry out narrative and systematic revisions of the processes linked to muscle fibers, such as the bioenergetics of phonatory muscles or vocal exercise prescription according to the physiology of exercise. This will make it possible to propose approaches that allow muscle physiology (based on fibers and their functions) to be applied to vocal rehabilitation and training.

CONCLUSION

According to the available evidence, human intrinsic laryngeal muscles present slow-tonic fibers I, IIA, IIX, and the myosin heavy chain isoforms MHC-I, MHC-IIA, MHC-IIX, MHC-L, and MHC-IIB. Their structure has adapted based on the functions that the larynx has acquired over time. For instance, the thyroarytenoid muscle is highly specialized, which allows it to participate predominantly in voice production; the cricothyroid and posterior cricoarytenoid muscles are composed of fast fibers and isoforms that are activated during breathing, and the histoanatomy of the interarytenoid muscle is specifically designed to protect the airway.

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