Age-Related Sarcopaenia of the Posterior Pharynx in a Cadaveric Population

Dr Nicolas Allyn Fitchat

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand,

In fulfilment of the requirements for the degree of

Masters of Science in Medicine (Internal Medicine) by dissertation

Student number: 601936

Supervisor: Prof. S. Maharaj

24 June 2021

TABLE OF CONTENTS

CANDIDATE'S DECLARATION

I, Nicolas Allyn Fitchat, declare that this thesis is my own work. It is being submitted for the degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

DEDICATION

I dedicate this work to my parents (Amparo and Gyfford), siblings (Kevin and Tracey) and companion (Zara). Thank you for your patience, love and continuous support.

ABSTRACT

Background

Sarcopaenia is the loss of muscle associated with ageing. Dysphagia is a disruption in the correct functionality of the pharyngeal muscles with significant implications.

Objective

To investigate the degree of Sarcopaenia in the posterior pharynx in a cadaveric population and its correlation with age at the time of death and sex as a possible contributing factor leading to the development of dysphagia.

Methods

Retrospective review of data collected from 109 cadavers was analysed, 52 males and 57 females. A total of 86 cadavers met the inclusion criteria, 42 males and 44 females. Mean measurements at four aspects of the posterior laryngopharynx at the level of the inferior pharyngeal constrictor were analysed and correlated with age. These means were further compared in cadavers 65 years and younger, and those older than 65 years. This mean was also compared between the two sexes. A paired student's T test and a one sample T test were used to test for significance.

Results

A positive correlation was found between age and the mean of the muscle thicknesses of the posterior laryngopharynx. A significant difference was found between the groups of cadavers divided between those 65 years and younger and older than 65 years. No significant difference was found between the muscle thickness of the posterior laryngopharynx and sex of the cadaver.

Conclusion

There was a positive correlation between age and the mean muscle thickness of the posterior laryngopharynx. Showing that the muscle is thicker in older cadavers. Furthermore, the muscle thickness is significantly thicker in those more than 65 years than those 65 years and less.

It has been shown by multiple previous studies that have shown that constriction of the pharynx appears to decline with age. Other studies have however shown that the force generated during swallowing in the pharynx does appear to increase with age. This has been postulated to be a compensatory response to the decrease in compliance of the cricopharyngeus muscle, as the upper oesophageal constrictor (UES), due to a replacement of normal muscle and connective by fibroadipose tissue.

As a part of sarcopaenia where there is a complex interplay between atrophy and hypertrophy, hypertrophy appears to be the most prominent feature in the posterior laryngopharynx of the elderly as is demonstrated by a thicker muscle layer in our older individuals. This hypertrophy may be a compensatory effect due to the loss of muscle fibres due to atrophy as has been previously explored by other authors. There appears to be no statistically significant difference in this muscle thickness between sexes.

This hypertrophy may be a compensatory effect due to the loss of muscle fibres due to atrophy as has been previously explored by other authors. This may also be a possible reason to explain the increase in the force of contraction during swallowing to overcome the resistance that a food bolus requires to overcome the resistance within the UES, which has been shown to be less compliant in aged individuals, in order to pass into the proximal oesophagus.

ACKNOWLEDGEMENTS

I would like to offer my sincere gratitude to Dr Shivesh Maharaj for all his guidance and incredible assistance over the past few years. I have thoroughly enjoyed working with you and may we continue to produce volumes of work to contribute to the body of knowledge.

I would also like to thank Dr Brunton Wium and Drs Gritzman and Thatcher Laboratories Inc. who sponsored the histopathological processing that allowed this research to come of being.

Lastly, I offer my gratitude to The School of Anatomical Sciences, University of the Witwatersrand, for the use of the cadaveric specimens.

LIST OF ABBREVIATIONS

UES: Upper (O)Esophageal Sphincter

MRI: Magnetic Resonance Imaging

US: United States

cm: centimetres

Fig.: Figure

H&E Stain: Haematoxylin and Eosin stain

MeSH: Medical Subject Headings

ALUM: Amount of Laryngeal Upward Movement

SMI: Skeletal Muscle Mass Index

vs: versus

µm: microns

H0: Null hypothesis

H1: Alternative hypothesis

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

Ageing successfully is a process of adaptation. Due to advancements in medical care the average life expectancy is increasing. [1] Sarcopaenia has been described as a geriatric syndrome with a global and progressive decline in the muscle mass and strength which limits the functioning of the elderly population. [2]

Age-related atrophy, sarcopaenia, of the swallowing musculature in advancing age has been widely adopted as an explanation for the decline in swallow function. [3] Dysphagia is a disruption in the correct functionality of the pharyngeal muscles and may therefore have both social and physical health implications.[4] With an ever-increasing older population, disorders in swallowing have been shown to have multiple health complications with significant morbidity on the geriatric population.

I will therefore review data collected from the posterior pharynx of a cadaveric sample population in 2016 and 2017 from the School of Anatomical Sciences, University of the Witwatersrand in order to explore age-related sarcopaenia of the posterior pharynx, at a histopathological level, and how it could contribute to dysphagia.

1.1 Background

The ability to eat and drink is a life-sustaining function and is also central to both the quality of life and social activity. Safe swallowing, which is necessary for this to happen, is a complex neuromuscular function involving multiple muscles.[5,6]

Ageing successfully is a process of adaptation.

Due to advancements in medical care the average life expectancy is increasing. It is estimated that the proportion of elderly people is expected to increase from 13% in 2010 to 20% in 2030 in the United States.[2] With this increase in the elderly population it has been shown that in the US alone, the sequelae of sarcopaenia are responsible for nearly $18 billion in healthcare costs.[7] Age-related atrophy, sarcopaenia, of the pharyngeal musculature in advancing age has been widely adopted as an explanation for the decline in swallow function.

Impairments in swallowing occur in all age groups, but it has been found that the age group affected most by swallowing impairments is the elderly. Isolated swallowing impairments have been found in a large proportion of the elderly population. One of the most common sequelae with significant morbidity in dysphagic elderly patients is aspiration resulting in pneumonia.[1] Pneumonitis secondary to aspiration may account for as much as 31.5% of nursing-home acquired pneumonias.[8]

A study by Molfenter et al. compared neck Magnetic Resonance Imaging (MRI) studies in younger and older women and found that there was a decrease in the muscle thickness and an increase in the pharyngeal lumen size with ageing.[9]

1.2 Gross Anatomy of the pharynx

The pharynx is the superior, extended part of the alimentary tract found posterior to the nasal and oral cavities. It extends from the base of the cranium to the inferior border of the cricoid cartilage anteriorly and the inferior border of the C6 vertebra posteriorly. The width of the pharynx ranges between approximately 5cm (at its widest opposite the hyoid bone) and 1.5cm (at its inferior end). The posterior aspect of the pharynx is flat and lies against the prevertebral fascia of the deep cervical fascia.[10]

The interior aspect of the pharynx is divided into three separate parts:

1. Nasopharynx
2. Oropharynx
3. Laryngopharynx or hypopharynx

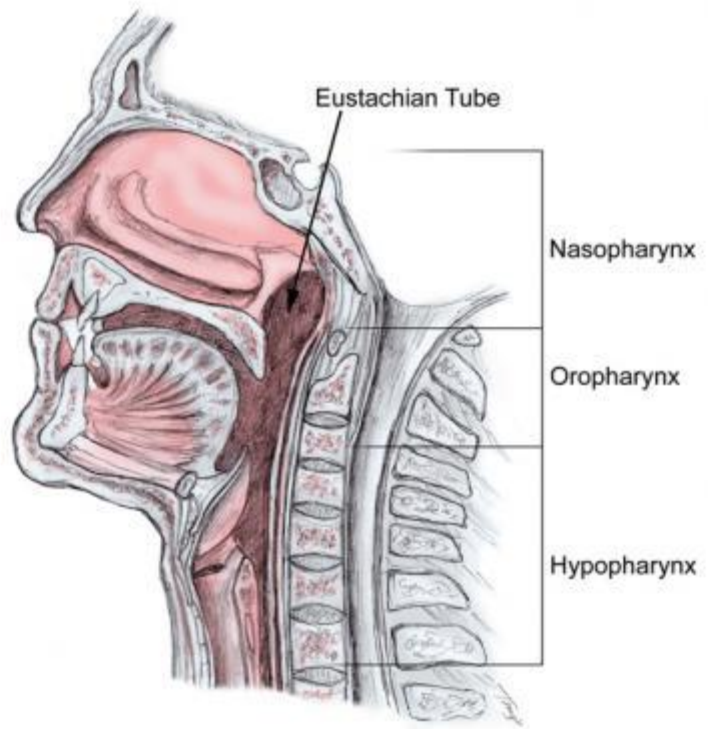


Figure 1 A sagittal section demonstrating the three divisions of the pharynx. [10]

The nasopharynx has a respiratory function is the posterior extension of the nasal cavities as the two nasal choanae open up into it. The oropharynx has a digestive function and extends from the soft palate to the superior border of the epiglottis.

The laryngopharynx lies posterior to the larynx and extends from the superior border of the epiglottis and the pharyngo-epiglottic folds to the inferior border of the cricoid cartilage. At the inferior border of the cricoid cartilage the laryngopharynx narrows and becomes continuous with the oesophagus. The pharyngo-oesophageal junction is the narrowest and least distensile part of the pharynx.

The laryngopharynx, posteriorly, is related to the C4-C6 vertebral bodies. The lateral and posterior walls of the laryngopharynx are formed by the middle and inferior pharyngeal constrictor muscles.

The palatopharyngeus, stylopharyngeus and salpingopharyngeus muscles form the inner longitudinal muscles. The outer circular layer of pharyngeal muscles consists of the three pharyngeal constrictor muscles: superior, middle and inferior. The pharyngeal constrictors have two important fascial linings: the strong, internal pharyngobasilar fascia and the thin, outer buccopharyngeal fascia. The outer buccopharyngeal fascia blends inferiorly with the deep cervical fascia.

All three pharyngeal constrictor muscles are supplied by the pharyngeal plexus of nerves which derives from the pharyngeal branches of the vagus and glossopharyngeal nerves. This plexus lies on the lateral wall of the pharynx, mostly over the middle pharyngeal constrictor. The inferior pharyngeal constrictor also receives some motor fibres from the external and recurrent laryngeal branches of the vagus nerve. [10]

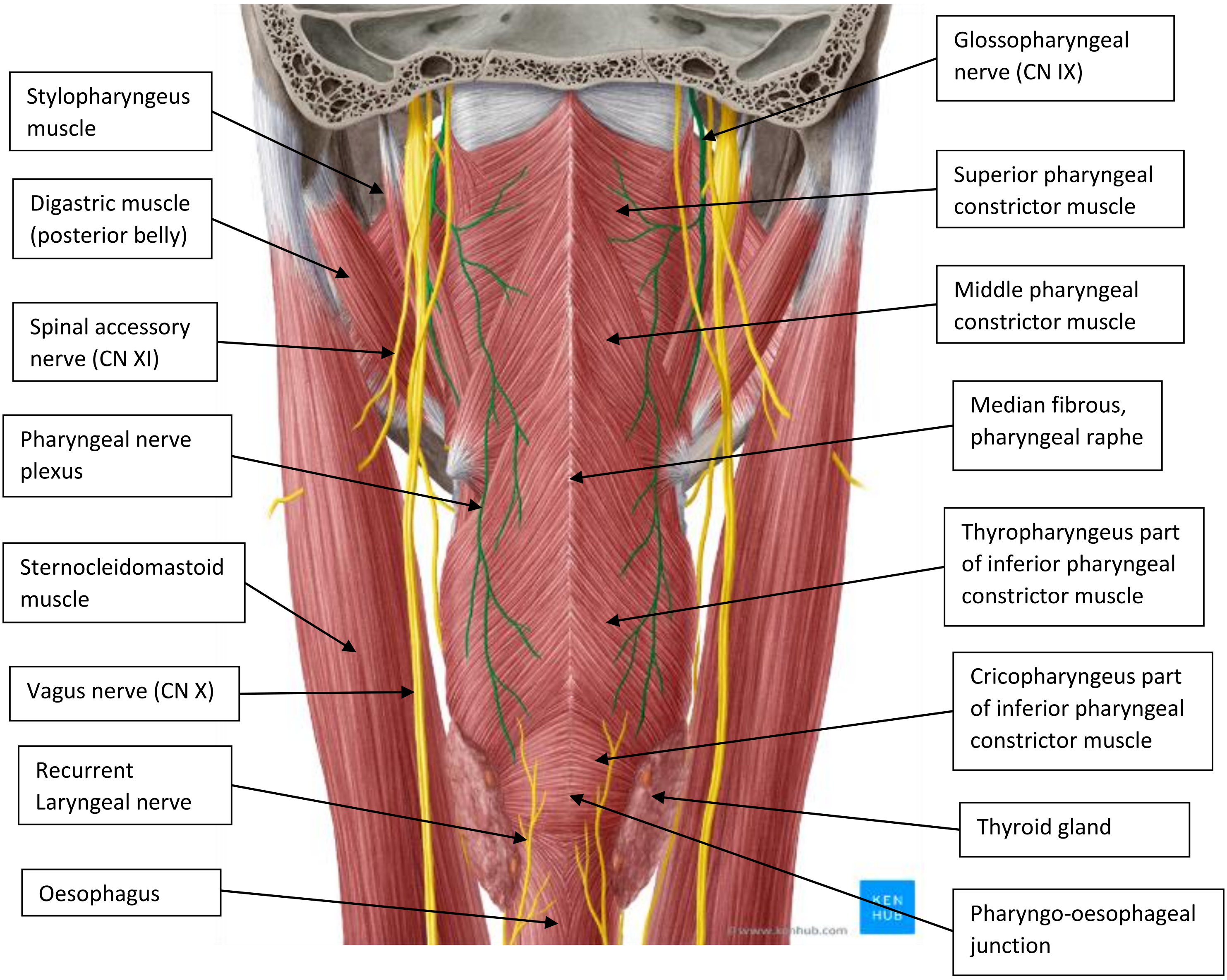


Figure 2 Diagram showing a posterior view of the pharynx, in particular the pharyngeal constrictor muscles and associated structures. (Illustration adapted from Kenhub and produced with permission)[10,12]

1.3 Histology of the Pharynx

The wall of the pharynx is an exception to the remainder of the alimentary tract as it has a muscle layer, the muscularis externa, that comprises entirely of voluntary (skeletal) muscle. Furthermore, the layers of muscle are arranged in such a way that the longitudinal layer of muscle lies internal to the circular layer of muscle. This is contrary to most of the alimentary tract which is composed of smooth muscle with the longitudinal muscle layer found external to the circular muscle layer.[10]

The lowest part of the cricopharyngeus muscle is physiologically referred to as the upper oesophageal sphincter which prevents the entry of air into the oesophagus. This is one of many parts of the digestive tract where the circular smooth muscle layer is thickened to form a sphincter or valve. [13]

The pharynx functions as a passageway for both food and air.[13] The oropharynx and the part of the laryngopharynx involved in deglutition, areas which experience more abrasive force, are lined by non-keratinising stratified squamous epithelium. It lacks both the muscularis mucosa and submucosa. Instead, the epithelium rests on a lamina propria that has a thick layer of longitudinal elastic fibres.[14]

These elastic fibres appear dark on the Haematoxylin and Eosin stain and are found near the muscularis externa. The muscularis externa consists of irregularly arranged skeletal muscle found as both the inner longitudinal fibres and the outer circular constrictor fibres. The outer most layer is the adventitia. It is a thin layer of loose connective tissue. [13]

1.4 Swallowing and the Pharynx

Swallowing, also known as deglutition, is the process by which a bolus of food gets transferred from the mouth through the pharynx and oesophagus and into the stomach. Mastication, the process of chewing in the oral cavity, allows for solid food to be mixed with saliva to form a soft bolus, which is then easier to swallow.[10]

The complex process of deglutition occurs in three stages:

- Stage 1:
  - Voluntary contraction
  - The food bolus is compressed against the palate and pushed into the oropharynx from the mouth (Fig. 1.3A)
  - Movements of the tongue and the soft palate

- Stage 2:
  - Involuntary contraction
  - The soft palate is elevated, which seals the nasopharynx off from the oropharynx and laryngopharynx
  - The pharynx shortens and widens to receive the food bolus as the longitudinal pharyngeal and suprahyoid muscles contract which elevate the larynx (Fig. 1.3B

- Stage 3:
  - Involuntary contraction
  - The three, outer circular, pharyngeal constrictor muscles constrict in a sequential fashion in order to force the bolus of food inferiorly into the oesophagus (Fig. 1.3C)

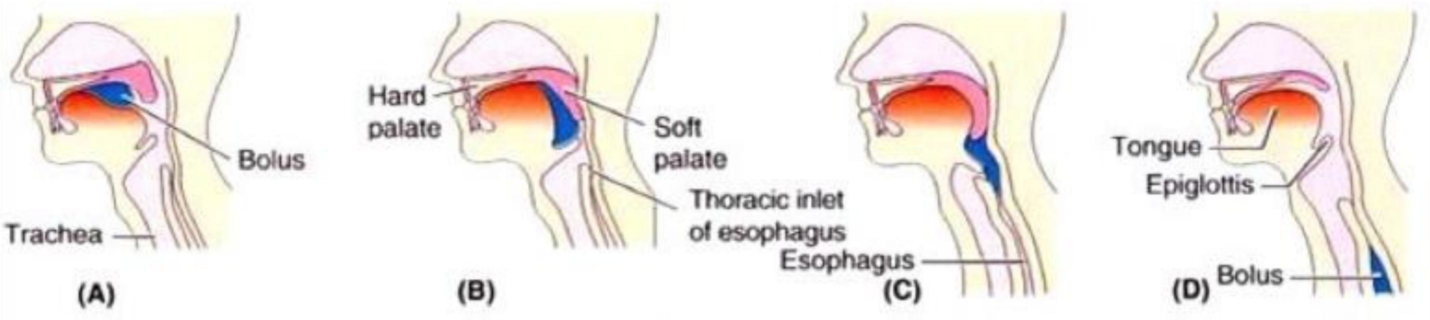


Figure 3 Diagram demonstrating the stages of deglutition. **A.** Bolus compressed against the palate and pushed into the oropharynx. **B.** Soft palate elevated, nasopharynx sealed off, pharynx shortened and widened to receive food bolus. **C.** Sequential contraction of the pharyngeal constrictor muscles moving food into the oesophagus. **D.** Peristaltic contractions of the oesophagus moving the food bolus towards the stomach.[10,15]

CHAPTER 2: Literature Review

2.1 Sarcopaenia

In 1989 Rosenberg first proposed the term **sarcopaenia** as the loss of muscle mass associated with ageing. Epidemiological studies have shown that from 45 years of age a decline in the lean body mass is detectable. This loss in lean body mass has been shown to be 18% in men and 27% in women between the second and the eighth decades of life.[4]

Sarcopaenia has been described as a geriatric syndrome with a global and progressive decline in the muscle mass and strength which limits the functioning of the elderly population. Diz et al. have also further described sarcopaenia to be highly prevalent over the age of 60 years.[2] This decrease in muscle mass due to ageing results from the decrease in both the number of muscle fibres and the size of each muscle fibre. This is different from disuse atrophy which is not due to a decrease in muscle fibre number, but only from the reduction in the muscle fibre size.[4] The annual cost of the consequences of sarcopaenia to the healthcare system in the United States has been estimated to be up $18 billon.[7]

The aetiology of sarcopaenia is complex and involves changes to nutrition, hormones, immunology, physical activity and both the central and peripheral nervous systems. The neuropathic processes are of the most important as they cause alpha-motor neuron degeneration and muscle fibre denervation and thus the loss of motor fibre units. Muscle goes through a continuous cycle of denervation and re-innervation throughout life. Old age seems to impact this process as the denervation outweighs the re-innervation and leads to loss of motor fibre units.

Morphological studies of human muscle and electrophysiological studies have shown that the muscle fibre number remained reasonably constant until the age of 60 years, but declined by 50% from the age of 60 to 80 years.[5] Leese and Hopwood suggest that there is a decrease in muscle fibre density with ageing and that there is greater variability in muscle fibre size in the pharynx and oesophagus with ageing.[16]

Lower levels of muscle mass have been linked to poor health outcomes which include physical disability and death.[1]

Menopause has been shown to play a significant role in the loss of muscle mass in postmenopausal women. Evidence suggests that the hormonal changes, particularly that of the decline in oestrogen, in the menopausal period may negatively affect the health of women.[7] A cross-sectional study by Rolland et al. reported a decline of 0.6% in muscle mass per year after menopause.[17]

Ageing successfully is a process of adaptation. Due to advancements in medical care the average life expectancy is increasing. It is estimated that the proportion of elderly people, being aged 65 years and older, are expected to increase from 13% in 2010 to 20% in 2030 in the United States.[2] With this increase in the elderly population it has been shown that in the US alone, the sequelae of sarcopaenia are responsible for costs to their healthcare system.[7] Age-related atrophy, sarcopaenia, of the swallowing musculature in advancing age has been widely adopted as an explanation for the decline in swallow function. This atrophy has been established in the geniohyoid, tongue and pharyngeal muscles.[3]

A study by Molfenter et al. compared neck MRIs in younger and older women and found that there was a decrease in the muscle thickness and an increase in the pharyngeal lumen size with ageing.[9] Similarly, a study by Aminpour et al. compared the posterior pharyngeal wall thickness using videofluoroscopic studies in younger (<65 years) and older (>65 years) subjects with no complaints of dysphagia. They found that the pharyngeal wall was significantly thinner and constricted to a lesser extent in the older subjects than in the younger ones. [18]

In 2019, Miyahita et al., used videofluoroscopic swallowing studies to explore sarcopaenic dysphagia and its usefulness for diagnosing this condition. They state that sarcopaenia is a major cause for dysphagia in the elderly and is only really observed after aspiration. They looked at participants who visited their clinic with a complaint of a swallowing disorder and compared the area of the pharyngeal cavity and the Amount of Laryngeal Upward Movement (ALUM) between patients who have and have not been diagnosed with sarcopaenia systemically.

Sarcopaenia was diagnosed systemically using the Asian Working Group for Sarcopaenia when both the hand grip strength and the SMI (Skeletal Muscle Mass Index) were below the criteria levels.

- Hand grip strength (<26kg in men and <18kg in women)
- Skeletal Muscle Mass Index (SMI) (<7kg/m^2 in men and <5.7kg/m^2 in women)

In their study they found that the sarcopaenic patients had a larger pharyngeal area which has been associated with pharyngeal contractile dysfunction and pharyngeal residue. Furthermore, they also found that there was decreased ALUM (Amount of Laryngeal Upward Movement) in the sarcopaenic group as compared to the non-sarcopaenic group.[19]

2.2 Swallowing

Adequate pharyngeal strength is crucial for complete constriction of the pharynx behind the tail of the bolus swallowed. Weak pharyngeal constriction causes there to be pharyngeal residue which is then able to be aspirated into the airway. Kendall et al. compared pharyngeal constriction in elderly (over 65 years) non-dysphagic and dysphagic individuals and young (18-62 years) non-dysphagic individuals using videofluoroscopy. They found that the timing of maximal pharyngeal constriction relative to the onset of the swallowing phase of the swallow was significantly delayed in the older group compared to the younger one. At this time of maximal pharyngeal constriction, the pharyngeal area was found to be larger in the older groups rather than the younger one. Furthermore, the bolus transit time in the pharynx was found to be significantly slower in the older group compared to the younger one. However, they concluded that pharyngeal weakness alone is unlikely to be the sole cause of prolonged pharyngeal transit time and other medical problems should be considered.[20]

This delay in the maximal pharyngeal constriction was found to be due to the slowed bolus transit time in the pharynx. These differences between the older and younger groups were found in both the dysphagic and non-dysphagic elderly groups which one can therefore attribute to a normal function of ageing rather than any pathology.[20]

The “Pharyngeal Constriction Ratio” has been developed by Leonard et al. as a measure made on the lateral view of a videofluoroscopy swallow study by calculating the unobliterated area of the pharynx at the time of maximal pharyngeal constriction divided by the area at the time of pharyngeal rest. This is an alternative to the gold standard of pharyngeal manometry for measuring the strength of pharyngeal contraction. Stokely et al. used this method to show a positive relationship between the post-swallow pharyngeal residue and the pharyngeal constriction.[21]

Similarly, Cassiani et al. found a positive correlation between the oral transit time of a high viscosity bolus and the pharyngeal transit time. Both of these times were shown to be affected by the ageing process which further results in a slower pharyngeal clearance time. [6]

Furthermore, Dejaeger et al. looked at the quantitative differences in the oropharyngeal phase of swallowing. They found that there was significantly reduced pharyngeal shortening, being the most important factor, in patients with diffuse pharyngeal retention in their swallows. [22]

Most of the patients with oral-pharyngeal swallowing disorders have been found to be more than 50 years of age. Many of the elderly do not seek medical attention as these are often minor difficulties in swallowing. Normal ageing causes an impairment in both the neural and muscular functions. In a study conducted by Cook et al. it was shown that pharyngeal clearance in the young was found to be nearly complete whereas in the aged the pharyngeal residues ranged between 1 and 13% of the swallowed bolus. They found that pharyngeal post-swallow residual increased significantly with age. Furthermore, they found that the pharyngeal clearance time was also significantly prolonged in the aged group. [23]

2.3 Dysphagia

Impairments in swallowing occur in all age groups, but it has been found that the age group affected most by swallowing impairments is the elderly. Isolated swallowing impairments have been found in a large proportion of the elderly population. One of the most common sequelae with significant morbidity in dysphagic elderly patients is aspiration resulting in pneumonia.[6] Pneumonitis secondary to aspiration may account for as much as 31.5% of nursing-home acquired pneumonias.[8]

The prevalence of dysphagia in the elderly is extremely high, especially when considering that it is often not noticed by some sufferers. This highly prevalent clinical condition is said to affect up to 40% of the population older than 65 years. It was said by Rofes et al. that in the year 2011 more than 30 million European and 16 million older people from the United States would have required specific care for dysphagia.[24]

Serra-Prat et al. looked at the real prevalence of dysphagia in older persons living independently and found that the overall prevalence of dysphagia was 23% and that in persons older than 80 years it was 33%. They also found in the persons older than 80 years that 28% had impaired efficacy of swallow and 18% had impaired safety of swallowing. The prevalence of aspiration in this group was found to be 4.4%. [25] Yang et al. found an overall prevalence of 33.7% in their random sample aged 65 years and older. [26] Dysphagia was found to be present in 44% of admissions to the geriatric ward, contributing largely to health industry costs, with an estimated cost of 547 million dollars per year due to a 40% longer hospital stay. In geriatric patients admitted to hospital, dysphagia has been shown to significantly impact their overall prognosis and mortality. [27]

In patients with a Zenker's Diverticulum as a cause for dysphagia histological findings of the cricopharyngeal muscles showed extensive fibroadipose tissue replacement (more than 50%) and muscle fibre degeneration, resulting in the replacement of normal muscle and normal connective tissue.[28,29,20,31] This fibroadipose tissue replacements leads to compromised elasticity of the cricopharyngeal muscles. It has also been shown to decrease the degree to which the UES (Upper Oesophageal Sphincter) opens which leads to increased intraluminal pressure above the sphincter.

Other findings of patients suffering from Zenker's Diverticulum are of increased fibrosis of the cricopharyngeus muscle.[32,33] This increase in fibrosis further contributes to the increased intraluminal pressure due stiffness and decreased compliance of the muscle.

Nativ-Zeltzer et al., in 2016, used a combination of videofluoroscopic data and high-resolution manometry to explore pressure tomography across the pharynx and proximal oesophagus. They described trans-sphincteric intrabolus pressure across the cricopharyngeus muscle as the upper oesophageal sphincter. They compared these pressures in younger (21-40 years) and older (60-80 years) groups of subjects and found that this trans-sphincteric pressure was greater in the older group of patients. They also found that the older adults showed greater values for pharyngeal contractility compared to the younger ones. They postulate that this greater pharyngeal contractility shows that the natural swallow in older adults utilises more pressure and that this increase in trans-sphincteric pressure may be due to the decreased compliance of the cricopharyngeus muscle.[34]

CHAPTER 3: Material and Methods

3.1 Hypotheses

Main Hypothesis:

- The average muscle thickness of the posterior laryngopharynx will be less in the older (more than 65 years) than in the younger (65 years and younger) cadavers.

Additional Hypothesis:

- The average muscle thickness of the posterior laryngopharynx will have a negative correlation with the age of the cadaver.
- The average muscle thickness of the posterior laryngopharynx will be less in the female than in the male cadavers.

3.2 Aims and Objectives

Aims:

- To compare the average muscle thickness of the posterior laryngopharynx between younger (65 years and less) and older (more than 65 years) cadavers.
- To compare the average muscle thickness of the posterior laryngopharynx with respect to the sex of the cadaver

3.3 Literature Review Search strategy

A literature review was done using PubMed.

Medical Subject Headings (MeSH) search terms used were:

- "Muscles"
- "Pharynx"
- "Atrophy"
- "Deglutition disorders"

In the search strategy the results from the review of the literature found were further categorised into three broad topics:

- Sarcopaenia
- Swallowing
- Dysphagia

3.4 Study Design

This project is based on previous work which investigated why Zenker's Diverticulae occur more on the Left than on the Right side. During this prior study in 2016 and 2017 the age, length, sex and the muscle thickness of the posterior laryngopharynx (in the medial and lateral aspects of the left and right sides) were collected from 110 cadavers.

This study is a retrospective, cross-sectional, analytic study which looked at data collected from the 2016 and 2017 cadaveric population from the School of Anatomical Sciences, University of the Witwatersrand. In this study the cadaveric population has been divided into two sets of subgroups:

- Younger (65 years and less) and Older (more than 65 years)
- Male and Female

3.5 Study Location

This study was conducted at the School of Anatomical Sciences, University of the Witwatersrand, in conjunction with the Department of Otorhinolaryngology and head and Neck Surgery, Charlotte Maxeke Johannesburg Academic Hospital.

3.6 Study Population

The participants of this study was the cadaveric population donated to the School of Anatomical Sciences of the University of the Witwatersrand in the years 2016 and 2017. The cadaveric characteristics relevant to the study were:

- The muscle thickness of the posterior laryngopharynx at the level of the inferior pharyngeal constrictor
- Age
- Sex

3.6.1 Inclusion Criteria

- All cadavers whose posterior laryngopharynx was sampled in 2016 and 2017
- All cadavers where the cadaver's age was recorded
- All cadavers where the cadaver's sex was recorded

3.6.2 Exclusion Criteria

- Any cadaver whose sex was not recorded
- Any cadaver whose age was not recorded

3.7 Data Collection

Three measures were looked at in this study. These measures were: the thickness of the posterior laryngopharynx muscle layer, the age of the cadavers and the sex of the cadavers. These details were extracted from a series of data collected from 110 cadavers over the course of 2016 and 2017.

The characteristics of each cadaver, the age, sex, length and race, were provided by the School of Anatomical Sciences. Each cadaver was identified by the accession number allocated by the School of Anatomical Sciences and was used consistently from 2016 to 2017 to ensure anonymity and accuracy.

Four sagittal sections of the posterior laryngopharynx were sampled using a standardised dissection technique. (Appendix A) These four sections consisted of a medial and lateral aspect of both the right and the left side. (Appendix B) The midline was determined by finding the median fibrous raphe extending inferiorly from the occiput. The medial sections were taken from either side of the midline, approximately 1mm from the midline, and the lateral ones from 3mm lateral to each of the medial sections. The outer adventitial layer was inked for orientation and to allow for better identification of the outer adventitial layer during microscopic examination of the samples.

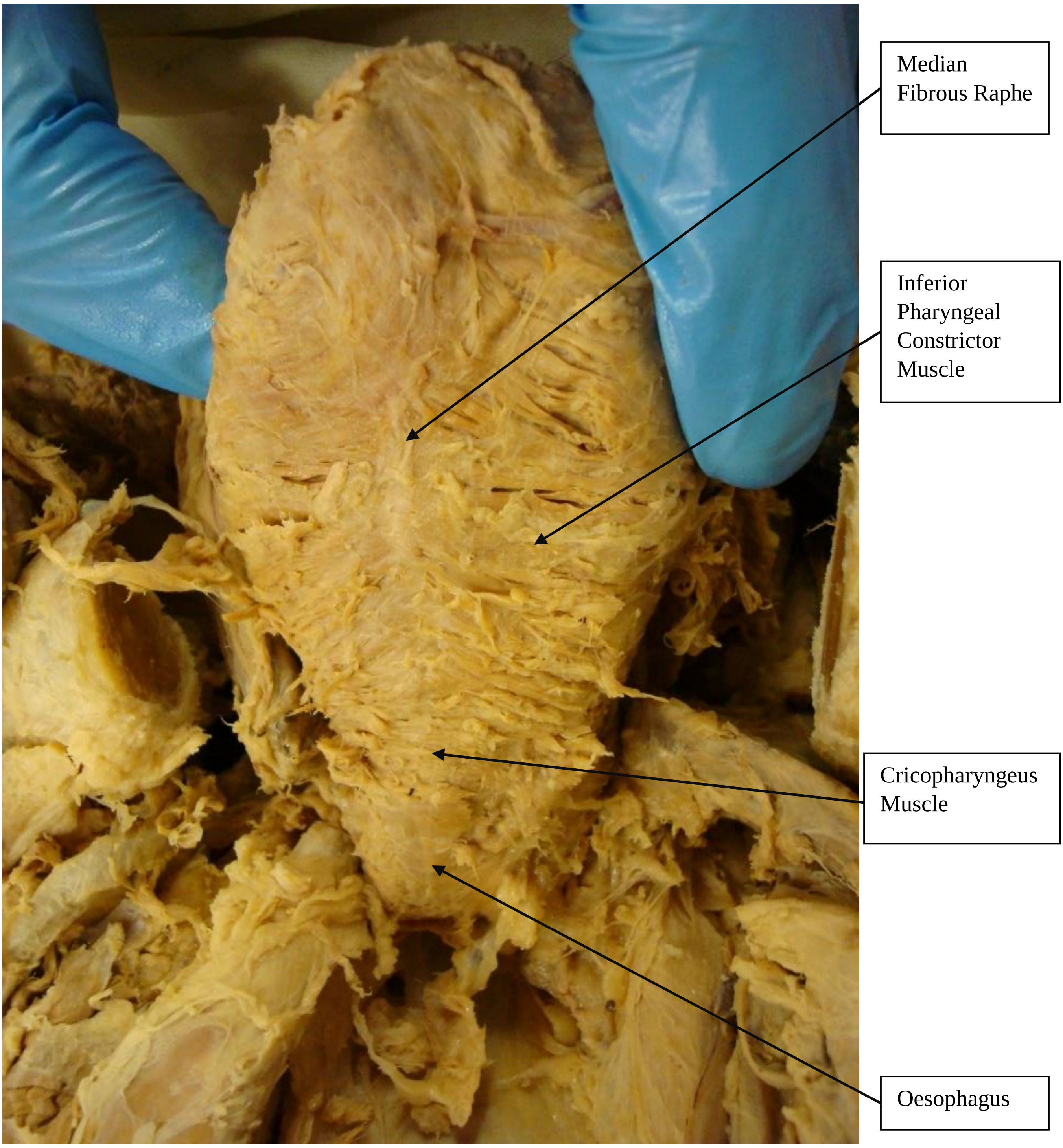


Figure 4 Photograph displaying the macroscopic dissection of the posterior aspect of a cadaveric pharynx

Once these samples were fixed with a formalin agent, embedded in wax and stained with Haematoxylin and Eosin, the muscle layer of the posterior laryngopharynx was measured, using a Leica ICC50 W Microscope. The muscle layer, muscularis externa, for all four samples for each cadaver were measured, to the nearest 1000th of a micron, in their thickest and thinnest aspects. The measurements were recorded according to each individual cadaver's accession number along with the length, age and sex of the cadaver.

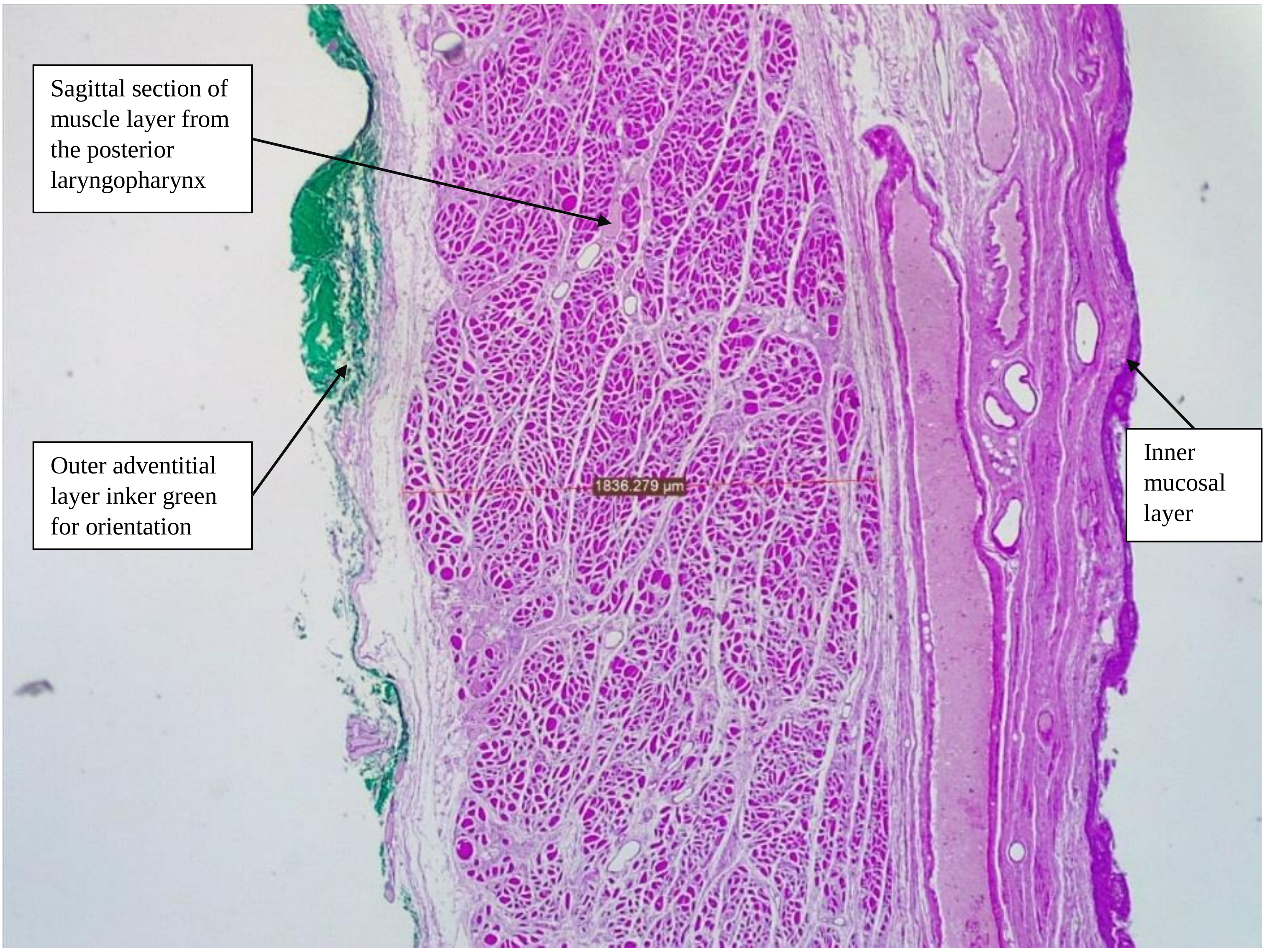


Figure 5 Photograph displaying the microscopic view of the sagittal muscle thickness from the posterior laryngopharynx of a cadaveric sample

The measurements which are pertinent to this study are the measurements of the muscle layer from each of the four sagittal sections and these were analysed and correlated according to the cadaver's age and sex.

The dependent variable:

- The average thickness of the muscle layer of the posterior laryngopharynx

The independent variables:

- Sex of the cadaver
- Age of the cadaver

3.8 Data Record

Data collected was recorded onto a table format on a Microsoft Excel Spreadsheet according to the various categories of data. (Appendix C)

3.9 Data Analysis

- The data collected from the 110 cadavers in 2016 and 2017 was refined by applying the inclusion and exclusion criteria.
- Once refined, the data relevant to this study was entered into a specific data sheet as a Microsoft Excel spreadsheet. (Appendix C)
    - Accession number
    - Age
    - Sex
    - Muscle layer measurement for each of the four sagittal samples
- Data was organised according to each cadaver's accession number to ensure consistency.
- Using Microsoft Excel, the mean of the muscle measurements was calculated and tabulated for each cadaver.
- IBM SPSS Statistics 23 was used for further data analyses.

- p-values of <0.05 were used to reject the below-mentioned null hypotheses.
- The testing of the mean muscle thickness was tested against the separate subgroups:
    - Age:
        - 65 years and younger vs more than 65 years
    - Sex:
        - Male vs Female

To test the mean of the muscle thickness in the younger (65 years and less) vs the older (more than 65 years) cadavers:

- Test: Welch t Test
- Null Hypothesis: the mean muscle thickness is equal in the younger (65 years and less) and older cadavers (more than 65 years).

To test for a correlation between the mean of the muscle thickness and age in the cadavers:

- Test: Paired Samples t test
- Null Hypothesis: the mean muscle thickness of the posterior laryngopharynx has no correlation to the age of the cadaver

To test the mean of the muscle thickness in the female vs the male cadavers:

- Test: Welch t test
- Null Hypothesis: the mean of the muscle thickness in the female individuals is equal to that in the male individuals.

3.10 Ethical Committee Approval

Submission for ethical approval was done directly with the School of Anatomical Sciences, University of the Witwatersrand. An extension of the ethical waiver which has been granted to the School of Anatomical Sciences for the use of the data from the human cadaveric population in 2016 and 2017 was requested and approved. (Appendix D)

As requested by the School of Anatomical Sciences a “Declaration of Amendment to Original Research Project in the Collections of the School of Anatomical Sciences” was signed as the data used in this project was initially collected for the previous study exploring why Zenker’s Diverticulae occur more on the Left than on the Right side. (Appendix E)

Ethics waiver number: W-CJ-140604-01.

3.11 Potential Limitations

As the specimens are from preserved cadavers, there is a known degree of shrinkage of the tissue as a result of the process of preservation. However, it has been shown that tissue which has undergone fixation whilst still intact on the skeleton has an insignificant degree of muscle fibre shrinkage. Therefore, as the Killian’s Dehiscence was fixed intact on the cadaveric skeleton, there shall be insignificant muscle fibre shrinkage.[35]

CHAPTER 4: Results

4.1 Introduction

The correlation between the muscle thickness of the posterior laryngopharynx and age is examined using statistical significance by means of a Paired Sample's t-test. Furthermore, the difference between the muscle thickness in the various age groups was compared using the Independent Samples Test. This same Independent Sample's Test was used to compare the mean muscle thicknesses between the two sexes.

For all of the abovementioned tests, a 5% significance level, p-value of 0.05, was used. A total of 86 cadavers met the inclusion criteria.

All analyses were carried out using the IBM Statistical Package for the Social Sciences Software (SPSS) Statistics version 23.

4.2 Distribution of Cadavers Dissected

In the data sample collected, there were a total of 110 cadavers that were dissected in 2016 and 2017 combined. 58 cadavers in 2016 and 52 cadavers in 2017.

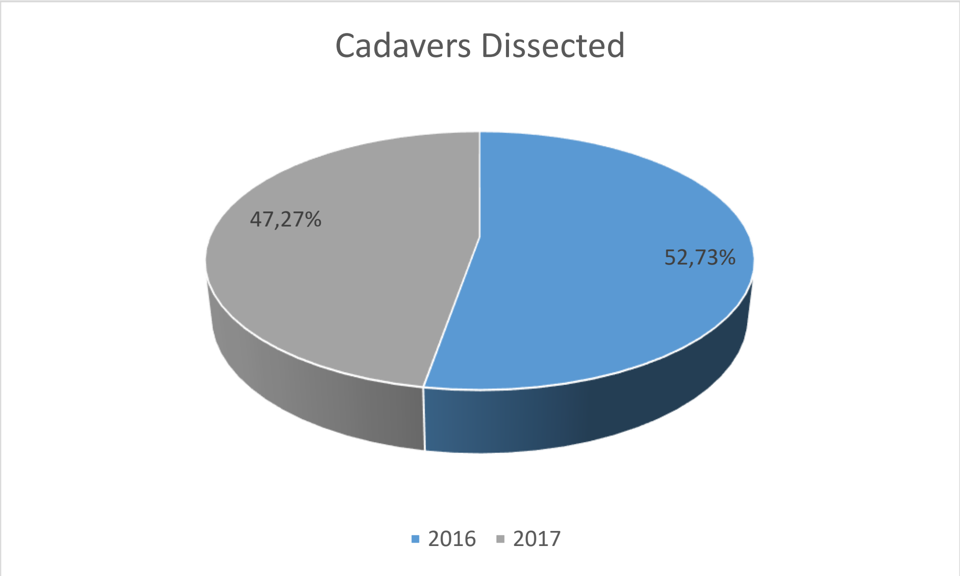


Figure 6 Distribution of Cadavers dissected between 2016 in 2017

Once the inclusion and exclusion criteria were applied, 86 cadavers were eligible to be included in the study. From these 86 cadavers included, 58 cadavers were included from 2016 and the remaining 28 included were from 2017. Twice as many cadavers were dissected in 2016 compared to 2017.

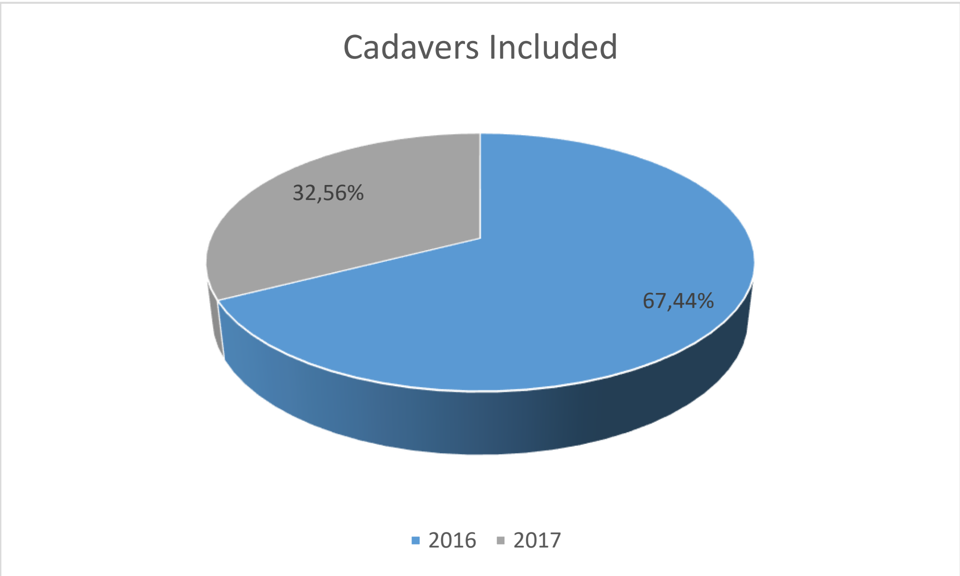


Figure 7 Distribution of Cadavers included in the study from those dissected in 2016 and 2017

4.3 Age of Cadavers

Of the 86 cadavers included in this study, the overall youngest cadaver was 50 years old and the oldest cadaver included was 100 years old. The overall mean age of the 86 cadavers included in the study was 78 years.

With regard to the female cadavers, the youngest cadaver was 51 years and the oldest cadaver was 94 years. The mean age for the female cadavers included was 77.3 years.

With regard to the male cadavers, the youngest cadaver was 50 years and the oldest cadaver was 100 years. The mean age for the female cadavers included was 78.7 years.

Cadavers	Youngest (years)	Oldest (years)	Mean (years)
Male	50	100	78.7
Female	51	94	77.3
Overall	50	100	78

Table 1 Descriptive statistics for the age (years) and sex of the included cadavers

4.4 Sex of Cadavers

In the 86 cadavers, from both 2016 and 2017, that were included in the study, 42 were male and 44 were female.

Of the 42 males, 28 were from 2016 and 14 from 2017. From the females, 30 were from 2016 and 14 from 2017.

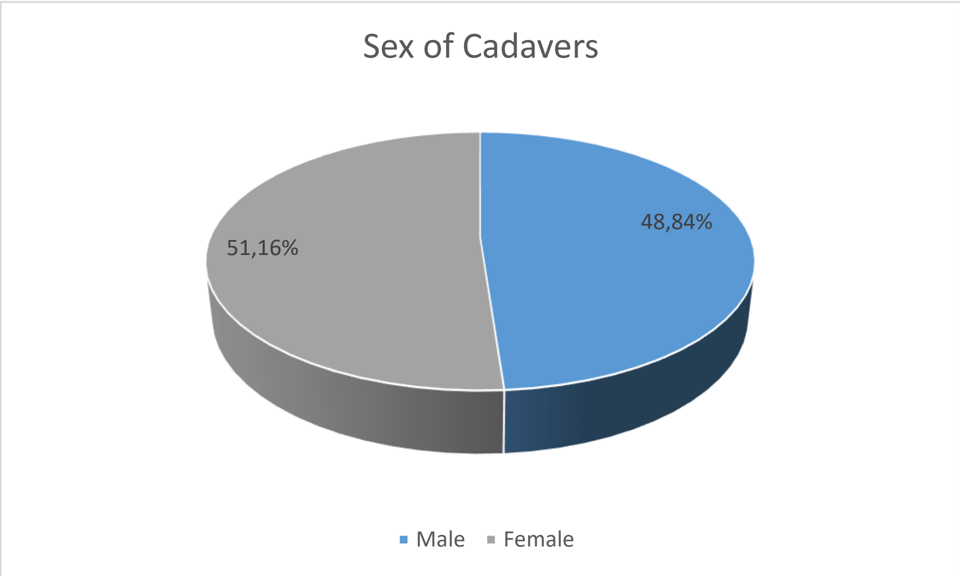


Figure 8 Distribution of the Sex of the Cadavers

4.5 Muscle Thickness

The mean muscle thickness was calculated by finding the mean between the four measurements across the posterior laryngopharynx.

The smallest mean was 1493.72 µm and the largest mean was 5119.64 µm. The mean for the population of cadavers was 2981.34µm with a standard deviation of 789.58µm.

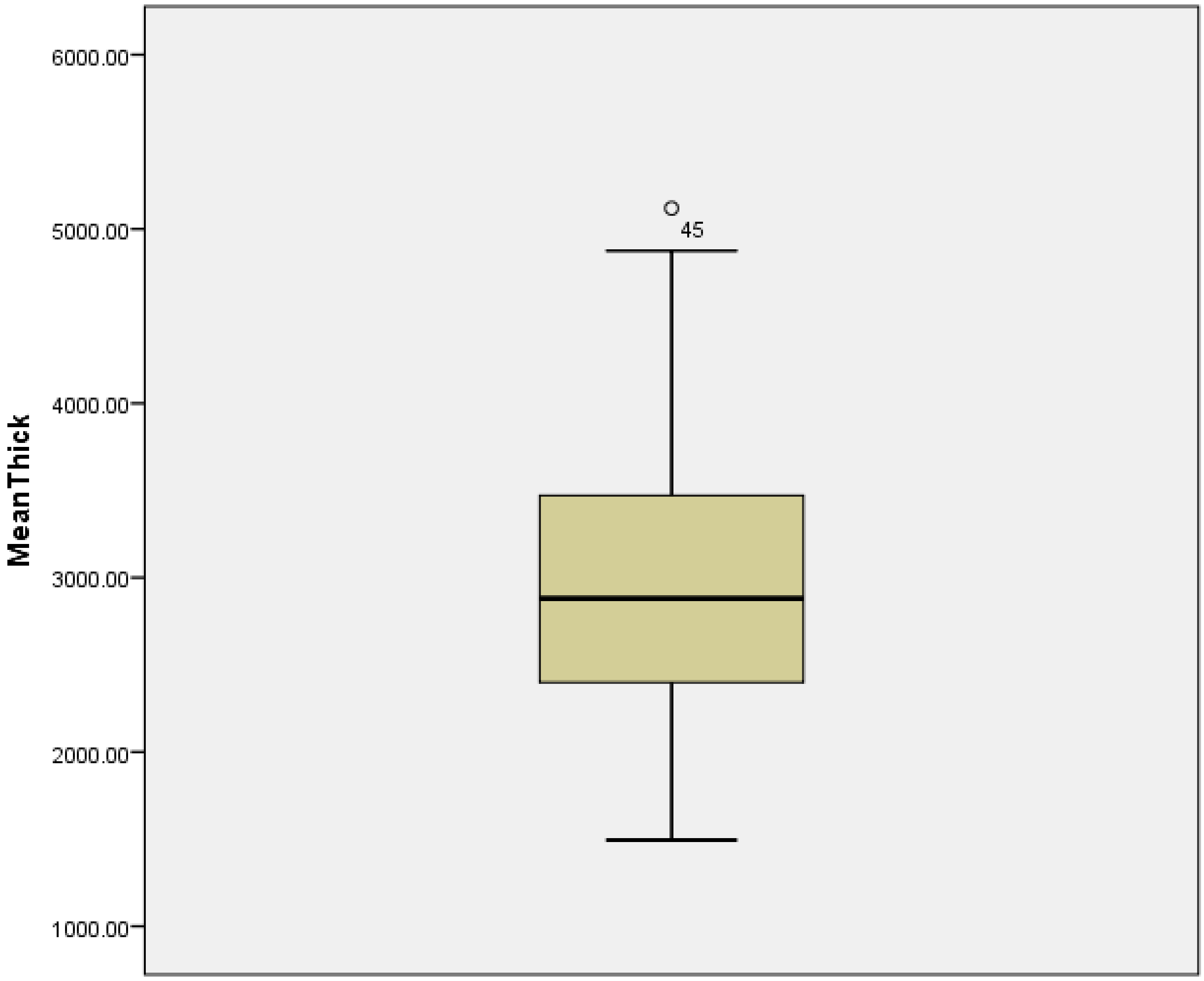


Figure 9 Box plot depicting the distribution of the mean muscle thicknesses (microns) for the posterior laryngopharynx

4.6 Muscle Thickness and Age

The mean muscle thickness for the 86 cadavers was 2981.34 µm with a standard deviation of 789.58 µm. The mean age for the 86 cadavers was 78 years with a standard deviation of 11.47 years.

Using a Paired Samples t test a significant positive correlation was found between the age of the cadavers and the mean muscle thickness. In this test, the null hypothesis (H0) was that there is no correlation between the age of the cadavers and their muscle thickness. The alternative hypothesis (H1) would be that there is a correlation between the age of the cadavers and their muscle thickness.

For this test at a 95% confidence interval there was a p-value of 0.000 which is less than 0.05 (5%) and therefore the null hypothesis is successfully rejected at a significance level of 5%.

In conclusion, there is a statistically significant positive correlation between the muscle thickness and the age of the cadavers at a 5% significance level.

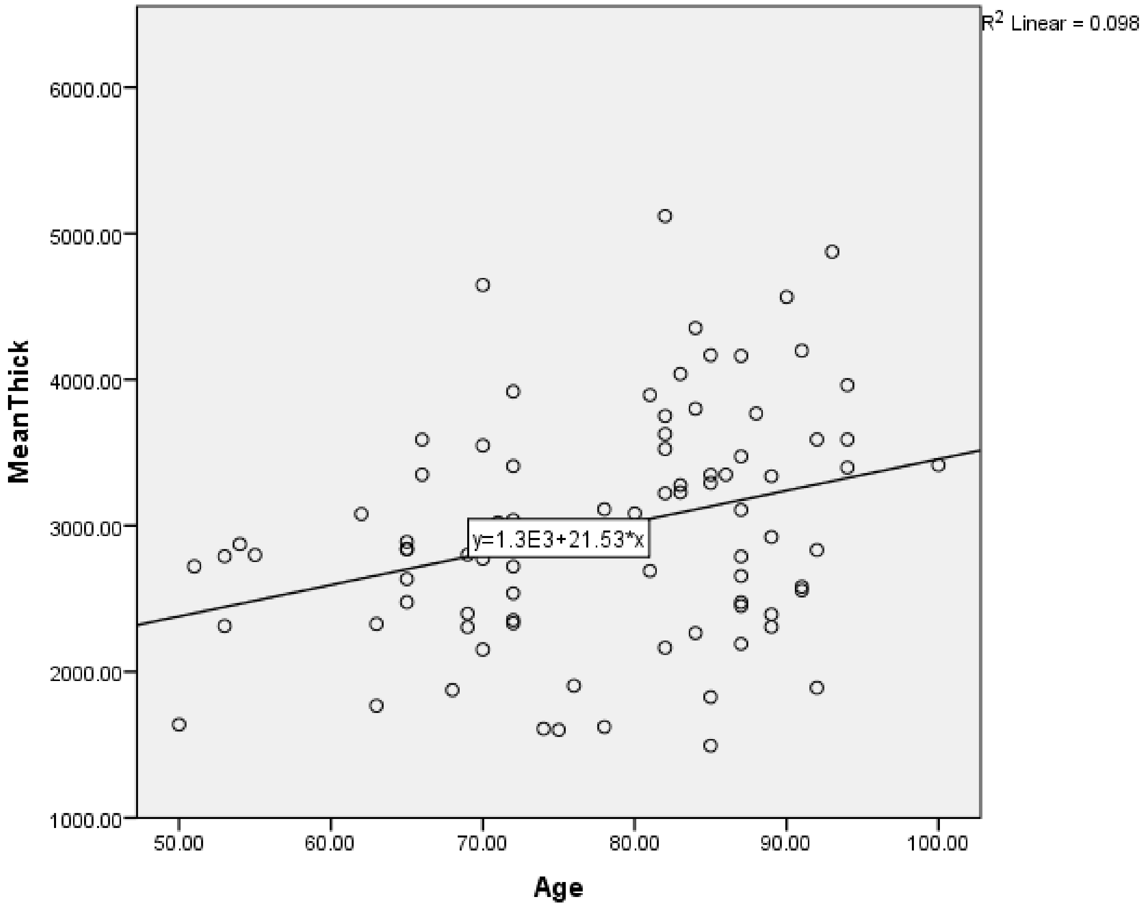


Figure 10 Scatterplot demonstrating the positive correlation between Age (years) and the mean muscle thickness of the posterior laryngopharynx (µm)

4.7 Muscle Thickness compared by Age Groups

<u>65 years and less versus more than 65 years</u>

For this test a Welch t Test was used to compare the muscle thickness between the cadavers that are 65 years and less to those that are more than 65 years.
There were 14 cadavers that were 65 years and less with a mean muscle thickness of 2570.20 µm with a standard deviation of 427.18 µm. There were 72 cadavers that were more than 65 years with a mean muscle thickness of 3061.28 µm with a standard deviation of 820.46 µm.

A Levene's Test for Equality of Variances was first run to compare the distribution of the data within the two different subgroups. For this test, the H0 is that the variance between the two groups is equal and the H1 is that the variance between the two groups is not equal. The Levene's test had a p-value of 0.014, with a significance level of 5%, this means that the null hypothesis of equal variance is successfully rejected and that there is a difference in the variance within the population.

As the population showed unequal variances, a Welch t Test was run to compare the muscle thickness of the posterior laryngopharynx between the two age groups. The H0 for this test was that there would be no difference in the mean muscle thickness between the two age groups and the H1 being that there is a difference between the two groups. For this test with a 95% confidence interval and a p-value of 0.002, the null hypothesis was successfully rejected at a significance level of 5%.

In conclusion, there was a statistically significant difference in the muscle thickness between cadavers 65 years and less and those more than 65 years. The muscle thickness being significantly thicker in those more than 65 years.

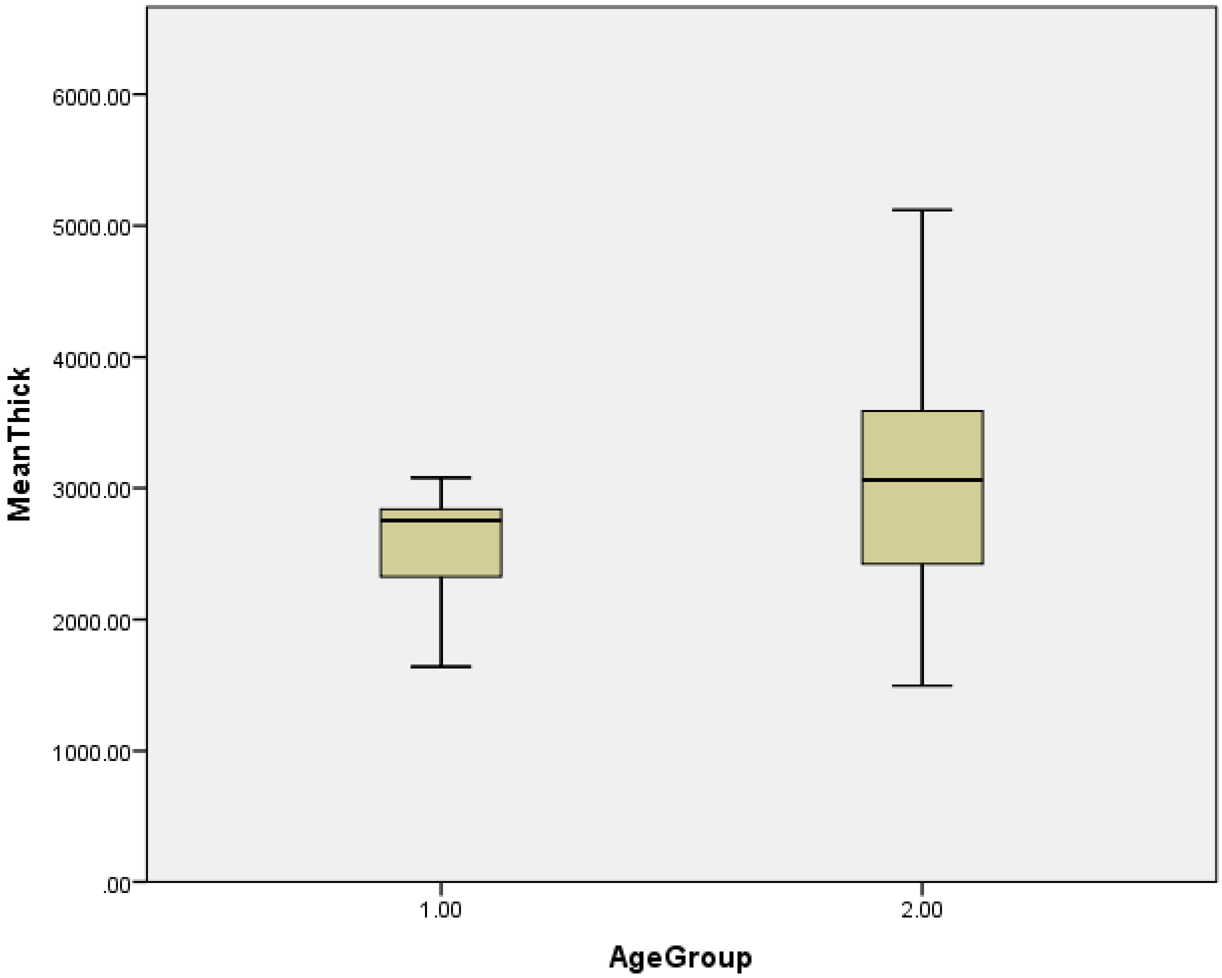


Figure 11 Boxplot displaying the distribution of the mean muscle thickness (µm) in the two groups (**Group 1** being those 65years and less and **Group 2** being those more than 65 years)

4.8 Muscle Thickness and Sex

The muscle thickness of the posterior laryngopharynx was compared between sexes within the population of cadavers. The mean muscle thickness of the posterior laryngopharynx in the male cadavers was compared to the mean thickness of the female cadavers.

Sex	Number of Cadavers	Mean muscle Thickness (µm)	Standard Deviation
Male	42	3074.81	134.28
Female	44	2892.12	105.92

Table 2 A table displaying the number of cadavers for each sex with their respective means and standard deviations

For this test an Independent Samples Test was used to compare the muscle thickness between the male cadavers and the female cadavers.

There were 42 male cadavers with a mean muscle thickness of 3074.81 µm and a standard deviation of 134.28 µm. There were 44 female cadavers with a mean muscle thickness of 2892.12 µm and a standard deviation of 105.92 µm.

A Levene's Test for Equality of Variances was first run to compare the distribution of the data within the two different groups (male and female). For this test, the H0 is that the variance between the two groups is equal and the H1 is that the variance between the two groups is not equal. The Levene's test had a p-value of 0.118, with a significance level of 5%, this means that the null hypothesis of equal variance cannot be rejected and that there is no difference in the variance within the population.

Assuming that the population showed equal variances, an Independent Samples Test was run to compare the muscle thickness of the posterior laryngopharynx between the two groups of sexes. The H0 for this test was that there would be no difference in the mean muscle thickness between the two sexes and the H1 being that there is a difference between the two sexes. For this test with a 95% confidence interval and a p-value of 0.286, the null could not be rejected at a significance level of 5%.

In conclusion, there was no statistically significant difference in the muscle thickness of the posterior laryngopharynx between the male and the female cadavers.

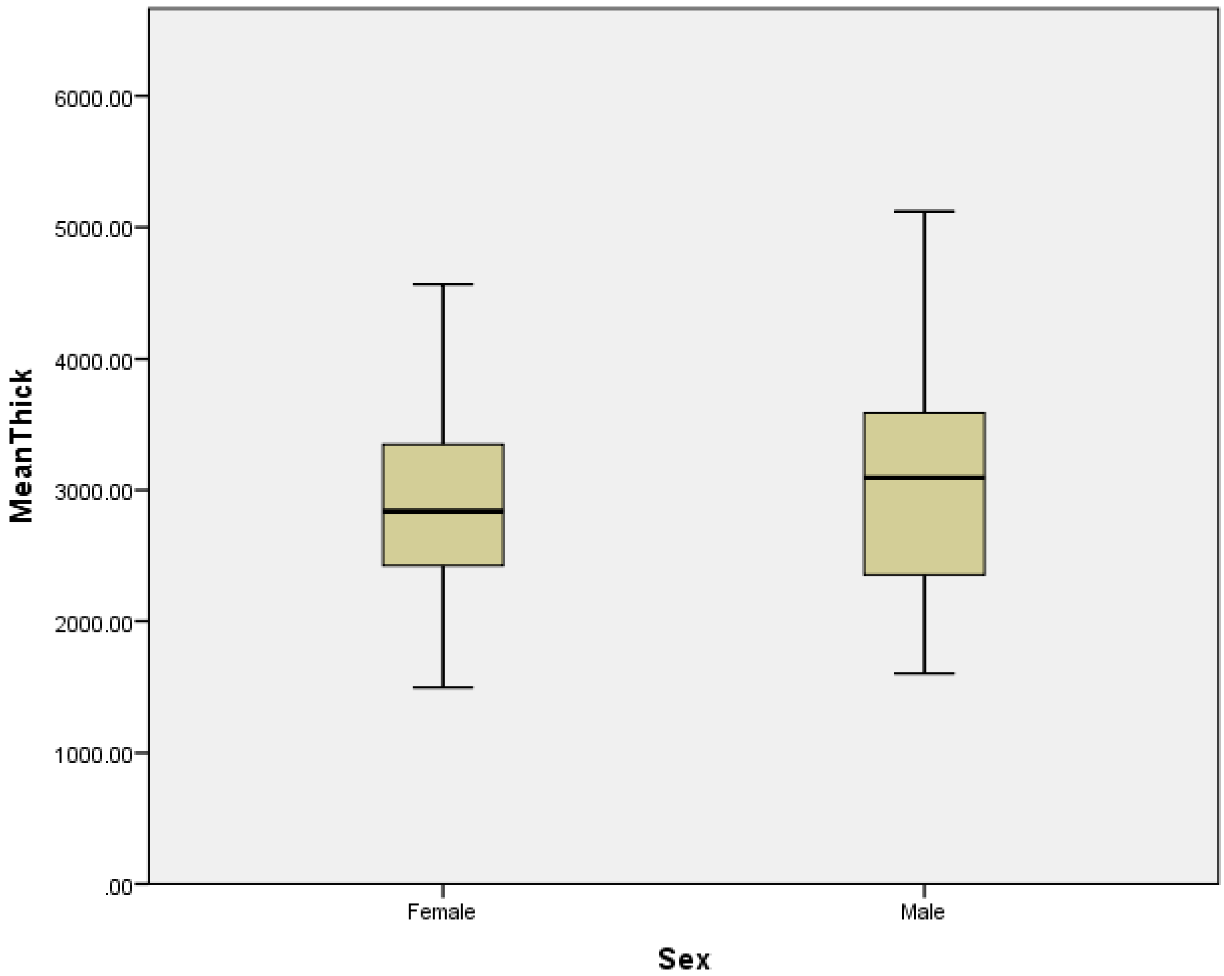


Figure 12 Boxplot displaying the distribution of the mean muscle thickness (μm) in the two sexes of the cadavers (**Female** vs **Male)**

CHAPTER 5: Discussion

This study set out to explore changes in the muscle layer of the posterior pharynx and how this may relate to age at the time of death of cadavers and whether there was any difference in this thickness with regard to the sex of the cadaver. Furthermore, we looked for any differences in this muscle layer thickness between a subset of younger (65 years and less) versus older (more than 65 years) cadavers.

In our study we found that there was a statistically significant positive correlation between age of the cadaver and muscle thickness of the posterior pharynx. It was also found that when comparing the mean muscle thickness between the younger (65 years and less) versus older (more than 65 years) cadavers, we found that the older subgroup had a significantly thicker muscle layer. Our study did not find any statistically significant difference between the thickness of the muscle layer between the sexes of the cadavers.

5.1 Muscle Thickness of the Posterior Laryngopharynx and Age

Our findings are contrary to previously published work, by Molfenter et al. and Aminpour et al., which found that the muscle thickness of the pharynx became thinner with increasing age. (Table 3) [9,18]

There is a substantial amount of data looking at swallowing and comparing it to age in terms of the pharyngeal constriction, bolus transit time, pharyngeal constriction ratio and the pharyngeal clearance time.[18,19,20,21,22]
The general trend found during our study was that the mean muscle thickness of the posterior laryngopharynx became thicker with increasing age.

Molfenter et al. used MR Imaging to analyse the pharynx, at C2, C3 and vallecular level, in a population of females and by looking at how the pharyngeal area and pharyngeal muscle thickness compared between younger and older age groups (20’s, 60’s and 70+ years). They found that there was a statistically significant difference in the muscle thickness of the pharynx, the older being thinner. Our findings could be different to those of Molfenter et al.

as our studies used vastly different modalities to study the muscle thickness. Our study was histopathological in nature and direct visualisation of muscle fibres was possible using a microscope. We also included both female and male cadavers. Molfenter et al. used MR Imagine which is a pixel-based form of imaging. Our study only included the muscle layer and excluded the mucosal and adventitial layers of tissue. It was not described in the study by Molfenter et al. whether the mucosal layer was included in their measurements of the posterior pharynx.[9]

Aminpour et al. looked at differences in the pharynx between normal younger (less than 65 years) and normal older (more than 65 years) individuals using fluoroscopic imaging. Their findings were similar to that of Molfenter et al. where they found a significant difference in the pharyngeal wall thickness between the two groups with the older group having a thinner posterior pharyngeal wall. [18] Our difference in findings could again been possibly explained by the fact that we used different study modalities. Aminpour et al. used fluoroscopic imaging and took their measurement of the posterior pharyngeal wall as a line between the anterior border of the vertebral bodies and the lumen, which would invariably include all layers of tissue, not specifically the muscular layer. They looked at the pharyngeal wall and not specifically the muscle layer unlike our study.

Our study's findings could therefore be different to those found in the studies by Aminpour et al. and Molfenter et al. on the basis that different modalities were used and measurements in the other studies may not have specifically looked at the muscle layer. Differences in the thickness of other tissue planes of the posterior pharynx which were not accounted for could possibly explain our different findings.

Contrary to the studies of Aminpour et al. and Molfenter et al., Leese and Hopwood was a histopathological study which looked at the muscle fibre type and thickness of necropsy patients. They described an increase in muscle fibre size variability, but overall they found that the muscle fibre size increased in the older groups, possibly due to the process of hypertrophy outweighing atrophy. Our study is similar to theirs as they were both studies of a histopathological nature. Our studies are similar as we both found an increase in the measurements of the muscle with an increase in age. However, the differences between our studies are that we looked at the muscle layer thickness and not at individual muscle fibre size.

Leese and Hopwood did not describe how this change in the muscle fibre size may alter the size of the thickness of the muscle layer and whether this change in muscle fibre size correlated with overall muscle layer thickness change. They also found that the density of muscle fibres per unit area were also less in the older group. They explain that this decreased density is from fallout, or atrophy, of muscle fibres with increasing age which is also a process which occurs in limb muscle.[16]

It is notable that in both histopathological studies, Leese and Hopwood and our study, an increase in either the muscle fibre or muscle layer size was found in the older groups. Whereas, in the studies based on imaging, a decrease in the pharyngeal or muscle layer was found in the older groups.

Article	Authors	Year Published	Variables	Number of participants	Findings
Age-Related Changes in Pharyngeal Lumen Size: A Retrospective MRI Analysis	Molfenter et al.	2014	Pharyngeal wall thickness and lumen area at C2, C3 and Vallecular level Young and older women (20's, 60's and 70+ years)	20's years (20 participants) 60's years (20 participants) 70+ years (18 participants) Total= 58 participants	Pharyngeal wall thickness decreased with advancing age. Significant differences between 20's vs 70's and 20's vs 60's. Significant difference between 60's and 70+ at some levels.
Muscle Fibre Typing in the Human Pharyngeal Constrictors and Oesophagus: The Effect of Aging	Leese & Hopwood	1986	Muscle fibre type & thickness (pharyngeal constrictor and oesophagus) Age groups (0-3 years, 12-49 years and >50years) Sex	50 necropsy subjects 0-3years (3 participants) 12-49years (36 participants) >50years (11 participants)	Larger muscle fibre diameter in the older. Density of muscle fibres per unit area significantly less in ageing population. No difference in muscle fibre sizes between sexes.
Pharyngeal wall differences between normal younger and older adults	Aminpour et al.	2011	Younger (less than 65years) Older (older than 65years) Pharyngeal wall thickness at C2 and C3	178 (53 women and 36 men in both older and younger group)	Significant difference pharyngeal wall thickness with older group being thinner than younger group. Older group does not constrict to same extent as younger group.

Table 3 Table comparing articles which investigate the muscle layer and muscle fibre thickness of the posterior pharynx[6,16,1]

5.2 Mean Muscle Thickness of the Posterior Laryngopharynx and Sex

Article	**Authors**	**Mean Pharyngeal Muscle Thickness (µm)**
Age-related sarcopaenia of the posterior pharynx in a cadaveric population (this dissertation)	Nicolas Fitchat	2570µm (65 years and less) 3061µm (>65 years) 2892µm (females) 3075µm (males)
Age-Related Changes in Pharyngeal Lumen Size: A Retrospective MRI Analysis	Molfenter et al.	3872µm (younger/less than 65years) in females 2953µm (older/ more than 65 years) in females
Pharyngeal wall differences between normal younger and older adults	Aminpour et al.	Females only 2500-3700µm (20's years) 2000-3000µm (60's years) 1900-2700µm (70+ years)

Table 4 Table comparing the muscle layer thickness between this study and previous studies by other authors[8,18]

Although our findings with regard to the muscle thickness being thicker in the older compared to the younger population is contrary to the findings in the studies by Molfenter et al. and Aminpour et al., the average muscle thicknesses found are somewhat comparable, particularly in the age groups around 60 years.[8,18] It could be possible that changes in the thickness of the tissue planes other than the muscle layer in the age groups account for the differences in our findings. These other layers of tissue could possibly not have been accounted for in the other studies.

Molfenter et al. who only looked at female participants had an average muscle thickness of 2953µm in cadavers >65years which is similar to our average of 3061µm. Similarly, Aminpour et al. stated that their muscle thicknesses in the 60's age group was between 2000-3000µm. [8,18]

When comparing the muscle thickness of the posterior pharynx between sexes our findings are similar to that of the study by Aminpour et al. where the muscle thickness of the pharynx in the female group was less than the males, but not statistically significant. This lack of statistical significance between sexes was also found by Leese and Hopwood who found no difference between the sexes when looking at muscle fibre size in the pharyngeal constrictor indicating similar usage. (Table 3 and 4) [6,16,18]

5.3 Pharyngeal Constriction, Ageing and Dysphagia

Many authors have both investigated and described decreasing pharyngeal constriction and post-swallow residue with increasing age. (Table 5)
Adequate pharyngeal strength is crucial for complete constriction of the pharynx behind the tail of the bolus swallowed. Weak pharyngeal constriction causes there to be pharyngeal residue which is then able to be aspirated into the airway. [19]

This decrease in pharyngeal constriction has been shown to be associated with residue in the pharynx after swallowing. This in turn has contributed to cases of aspiration and further health complications such as malnutrition and weight loss. Pneumonitis secondary to aspiration may account for as much as 31.5% of nursing-home acquired pneumonias.[7]

A weaker or poorly constricting pharynx could be a contributory factor leading to dysphagia. Impairments in swallowing occur in all age groups, but it has been found that the age group affected most by swallowing impairments is the elderly. Isolated swallowing impairments have been found in a large proportion of the elderly population. [7]

The prevalence of dysphagia in the elderly is extremely high, especially when considering that it is often not noticed by some sufferers. This highly prevalent clinical condition is said to affect up to 40% of the population older than 65 years.

Article	Authors	Year Published	Variables	Number of participants	Findings
Pharyngeal Constriction in Elderly Dysphagic Patients Compared with Young and Elderly Nondysphagic Controls	Kendall & Leonard	2001	Timing of maximal pharyngeal constriction Pharyngeal bolus time Young (18-62years) Elderly (67-83 years)		73% of elderly had incomplete pharyngeal constriction. Poor pharyngeal constriction, suggestive of pharyngeal weakness contributed to 75% of cases of aspiration
The Relationship Between Pharyngeal Constriction and Post-Swallow Residue	Stokely et al.	2015	Pharyngeal constriction based on pharyngeal area Post-Swallow residue	20 healthy young adults 40 patients with suspected neurogenic dysphagia	Decreased pharyngeal constriction results in an increase in the post-swallow residue
Manofluorograhic analysis of swallowing in the elderly	Dejaeger et al.	1994	Elderly subjects (+/- 80 years) vs healthy volunteers Manofluorography to assess the pharyngeal swallow	16 elderly 20 healthy	Decrease in negative pressure from opening of upper oesophageal sphincter. Increase in incomplete relaxations of the sphincter.
Influence of aging on the oral-pharyngeal bolus transit and clearance during swallowing: scintigraphic study	Cook et al.	1994	Healthy aged subjects (55-83years) Young controls (19-377ears) Pharyngeal clearance time Oral and pharyngeal transit time Oral and pharyngeal residues	21 healthy aged 9 young controls	Almost complete pharyngeal clearance in young (<2%) Residue noted between 1-13% of healthy aged subjects

Table 5 A Table comparing the articles describing pharyngeal constriction and clearance and its relation to post-swallow residues[19,20,21,22]

5.4 Pharyngeal Contraction and Age

Some authors have looked at the cricopharyngeus muscle at a histological level to explore the pathophysiology behind the development of Zenker's Diverticulum as a cause for dysphagia. Their findings showed extensive fibroadipose tissue replacement (more than 50%) and muscle fibre degeneration, resulting in the replacement of normal muscle and normal connective tissue. [28,29,20,31] Other findings being the discovery of increased fibrosis of the cricopharyngeus muscle.[32,33] This leads to compromised elasticity and reduced opening or relaxation of the sphincter which leads to increased intraluminal pressure proximal to the cricopharyngeus muscle or the upper oesophaeal sphincter (UES). This further contributes to the increased intraluminal pressure due to due to stiffness and decreased compliance of the muscle.

Trans-sphincteric intrabolus pressure across the cricopharyngeus muscle as the upper oesophageal sphincter was explored in 2016 by Nativ-Zeltzer et al. in their study they used a combination of videofluoroscopic data and high-resolution manometry to explore pressure tomography across the pharynx and proximal oesophagus. They compared these pressures in younger (21-40 years) and older (60-80 years) groups of subjects and found that this trans-sphincteric pressure was greater in the older group of patients. They also found that the older adults showed greater values for pharyngeal contractility compared to the younger ones. They postulate that this greater pharyngeal contractility shows that the natural swallow in older adults utilises more pressure and that this increase in trans-sphincteric pressure may reflect the decreased compliance of the cricopharyngeus muscle.[34]

As a part of rehabilitation for dysphagia, patients are taught to perform the Mendelsohn manoeuvre as a way to compensate for or restore impaired swallowing function.[36,37] This manoeuvre is performed by maintaining suprahyoid contraction at the peak of hyolaryngeal excursion to increase laryngeal displacement and prolong the upper oesophageal sphincter (UES) opening.

The Mendelsohn manoeuvre is performed by asking the participant to feel for their larynx (or Adam's apple) and its upward movement whilst swallowing. During the swallow, when the larynx is at its highest point, the participant is to hold the larynx at this point for at least two seconds. [38]

This manoeuvre has been shown to increase pharyngeal contractile strength by increasing the magnitude and duration of the pharyngeal pressure profile and reducing the peak upper oesophageal contraction pressure and increasing the UES relaxation interval.[39,40] Doeltgen et al. showed that the Mendelsohn Manoeuvre increased the peak pharyngeal pressure and pharyngeal contraction integral and that there was shorter UES opening latency and a longer UES closure latency.[38]

It is notable that an increase in pharyngeal contraction appears to be both a normal part of ageing and is used as a rehabilitative technique to compensate for decreased compliance of the cricopharyngeus muscle and in patients suffering from dysphagia. Our findings of an increase in the muscle thickness within the posterior laryngopharynx with age could explain this increase in the force of contraction within the pharynx found on pressure tomography and the increase in the trans-sphincteric intrabolus pressure across the UES.

CHAPTER 6: Conclusion

Sarcopaenia, loss of muscle mass due to ageing, places its own burden on the healthcare sector as its sequelae are responsible for a large portion of costs to the healthcare sector. With dysphagia being such a prevalent condition in the ever-growing elderly population, it is of considerable benefit to further investigate the complex pathophysiology behind it. In many elderly individuals, dysphagia may be present without the suffer even being aware of it. Along with dysphagia, complications due to incomplete swallowing and post-swallow pharyngeal residue can have detrimental effects on the health of the ageing population and significantly impacts both morbidity and mortality. Impairments in swallowing have been demonstrated in healthy ageing individuals who have no complaints of dysphagia and therefore they are also at risk of complications such as aspiration pneumonia.

It has been shown by multiple previous studies that constriction of the pharynx appears to decline with age. Other studies have, however, shown that the force generated during swallowing in the pharynx does appear to increase with age. This has been postulated to be a compensatory response to the decrease in compliance of the cricopharyngeus muscle, as the upper oesophageal constrictor (UES), due to a replacement of normal muscle and connective by fibroadipose tissue.

Our study has demonstrated at a histopathological level that there are changes to the muscle layer of the posterior laryngopharynx that could be attributed to the normal process of ageing. As a part of sarcopaenia where there is a complex interplay between atrophy and hypertrophy, hypertrophy appears to be the most prominent feature in the posterior laryngopharynx of the elderly as is demonstrated by a thicker muscle layer in our older individuals.

This hypertrophy may be a compensatory effect due to the loss of muscle fibres due to atrophy. This may also be a possible reason to explain the increase in the force of contraction during swallowing to overcome the resistance that a food bolus requires to overcome the resistance within the UES, which has been shown to be less compliant in aged individuals, in order to pass into the proximal oesophagus.

We found no statistical difference in this muscle thickness between sexes.

Further research could be done in this field by looking at the changes of muscle fibre size and the variability thereof in comparison to age and between sexes. There has only been one previous study which looked at muscle fibre size and did not explain how this contributed to the overall size of the muscle layer. Furthermore, investigating the changes in muscle layer thickness in response to rehabilitative techniques would be of enormous benefit to the treatment of dysphagia and the prevention of its various health-related complications.

CHAPTER 7: References

1 Diz, J., Queiroz, B., Tavares, L. and Pereira, L. (2015). Prevalence of sarcopenia among the elderly: findings from broad cross-sectional studies in a range of countries. *Rev. bras. geriatr. gerontol.* [online].18(3):665-678.

2 Molfenter, S., Lenell, C. and Lazarus, C. (2018). Volumetric Changes to the Pharynx in Healthy Aging: Consequence for Pharyngeal Swallow Mechanics and Function. *Dysphagia*, 34(1):129-137.

3 Molfenter, S. and Steele, C. (2014). Use of an Anatomical Scalar to Control for Sex-Based Size Differences in Measures of Hyoid Excursion During Swallowing. *Journal of Speech, Language, and Hearing Research*, 57(3):768-778.

4 Narici, M. and Maffulli, N. (2010). Sarcopenia: characteristics, mechanisms and functional significance. *British Medical Bulletin*, 95(1):139-159.

5 Cassiani, R., Santos, C., Parreira, L. and Dantas, R. (2011). The relationship between the oral and pharyngeal phases of swallowing. *Clinics*, 66(8):1385-1388.

6 Clark, B. and Manini, T. (2010). Functional consequences of sarcopenia and dynapenia in the elderly. *Current Opinion in Clinical Nutrition and Metabolic Care*, 13(3):271-276.

7 Messier, V., Rabasa-Lhoret, R., Barbat-Artigas, S., Elisha, B., Karelis, A. and Aubertin-Leheudre, M. (2011). Menopause and sarcopenia: A potential role for sex hormones. *Maturitas*, 68(4):331-336.

8 Morley, J. (2015). Dysphagia and Aspiration. *Journal of the American Medical Directors Association*, 16(8):631-634.

9 Molfenter, S., Amin, M., Branski, R., Brumm, J., Hagiwara, M., Roof, S. and Lazarus, C. (2015). Age-Related Changes in Pharyngeal Lumen Size: A Retrospective MRI Analysis. *Dysphagia*, 30(3):321-327.

10 Moore K, Agur A, Dalley A. Clinically Orientated Anatomy. 6th ed. Lippincott, Williams and Wilkins; 2010.

11 McGinn J. Oropharynx Biopsy: Overview, Indications, Contraindications [Internet]. Emedicine.medscape.com. 2018 [cited 23 June 2020]. Available from: https://emedicine.medscape.com/article/1520042-overview

12 Glossopharyngeal nerve (Cranial nerve IX) [Internet]. © Kenhub (www.kenhub.com); Illustrator: Yousun Koh [cited 17 August 2020]. Available from: https://www.kenhub.com/en/study/parapharyngeal-space-nerves

13 Ross M, Pawlina W. Histology. 6th ed. Philadelphia [u.a.]: Wolters Kluwer Lippincott Williams & Wilkins; 2011.

14 Pharynx, Esophagus, and Stomach | histology [Internet]. Histology.medicine.umich.edu. 2020 [cited 24 June 2020]. Available from: https://histology.medicine.umich.edu/resources/pharynx-esophagus-stomach

15 [Internet]. S3.amazonaws.com. 2020 [cited 23 July 2020]. Available from: https://s3.amazonaws.com/classconnection/600/flashcards/8885600/png/swallowing-1537C1CD2076B456D21.png

16 Leese, G. and Hopwood, D., 1986. Muscle Fibre Typing in the Human Pharyngeal Constrictors and Oesophagus: The Effect of Ageing. *Cells Tissues Organs*, 127(1):77-80.

17 Rolland, Y., Perry, H., Patrick, P., Banks, W. and Morley, J. (2007). Loss of Appendicular Muscle Mass and Loss of Muscle Strength in Young Postmenopausal Women. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 62(3):330-335.

18 Aminpour, S., Leonard, R., Fuller, S. and Belafsky, P. (2011). Pharyngeal wall Differences between Normal Younger and Older Adults. *Ear, Nose & Throat Journal*, 90(4):E1-E5.

19 Miyashita, T., Kikutani, T., Nagashima, K., Igarashi, K. and Tamura, F., 2020. The effects of sarcopenic dysphagia on the dynamics of swallowing organs observed on videofluoroscopic swallowing studies. *Journal of Oral Rehabilitation*, 47(5):584-590.

20 Kendall, K. and Leonard, R. (2001). Pharyngeal Constriction in Elderly Dysphagic Patients Compared with Young and Elderly Nondysphagic Controls. *Dysphagia*, 16(4):272-278.

21 Stokely, S., Peladeau-Pigeon, M., Leigh, C., Molfenter, S. and Steele, C. (2015). The Relationship Between Pharyngeal Constriction and Post-swallow Residue. *Dysphagia*, 30(3):349-356.

22 Dejaeger, E., Pelemans, W., Ponette, E. and Joosten, E. (1997). Mechanisms Involved in Postdeglutition Retention in the Elderly. *Dysphagia*, 12(2):63-67.

23 Cook, I., Weltman, M., Wallace, K., Shaw, D., McKay, E., Smart, R. and Butler, S. (1994). Influence of aging on oral-pharyngeal bolus transit and clearance during

swallowing: scintigraphic study. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 266(6):G972-G977.

24 Rofes, L., Arreola, V., Almirall, J., Cabré, M., Campins, L., García-Peris, P., Speyer, R. and Clavé, P. (2011). Diagnosis and Management of Oropharyngeal Dysphagia and Its Nutritional and Respiratory Complications in the Elderly. *Gastroenterology Research and Practice,* Vol 2011(2):1-13.

25 Serra-Prat, M., Hinojosa, G., López, D., Juan, M., Fabré, E., Voss, D., Calvo, M., Marta, V., Ribó, L., Palomera, E., Arreola, V. and Clavé, P. (2011). Prevalence of Oropharyngeal Dysphagia and Impaired Safety and Efficacy of Swallow in Independently Living Older Persons. *Journal of the American Geriatrics Society*, 59(1):186-187.

26 Yang, E., Kim, M., Lim, J. and Paik, N. (2013). Oropharyngeal Dysphagia in a Community-Based Elderly Cohort: The Korean Longitudinal Study on Health and Aging. *Journal of Korean Medical Science*, 28(10):1534.

27 Altman, K., Yu, G. and Schaefer, S. (2010). Consequence of Dysphagia in the Hospitalized Patient. *Archives of Otolaryngology–Head & Neck Surgery,* 136(8):784.

28 Law R, Katzka D, Baron T. Zenker's Diverticulum. Clinical Gastroenterology and Hepatology. 2014;12(11):1773-1782.

29 Siddiq M. Pharyngeal pouch (Zenker's diverticulum). Postgraduate Medical Journal. 2001;77(910):506-511.

30 van Overbeek J. Pathogenesis and Methods of Treatment of Zenker's Diverticulum. Annals of Otology, Rhinology & Laryngology. 2003;112(7):583-593.

31 Cook I, Bumbergs P, Cash K, Jamieson G, Shearman D. Structural abnormalities of the cricopharyngeus muscle in patients with pharyngeal (Zenker's) diverticulum. Journal of Gastroenterology and Hepatology. 1992;7(6):556-562.

32 Sen P, Kumar G, Bhattacharyya A. Pharyngeal pouch: associations and complications. European Archives of Oto-Rhino-Laryngology. 2006;263(5):463-468.

33 Ferreira L, Simmons D, Baron T. Zenker's diverticula: pathophysiology, clinical presentation, and flexible endoscopic management. Diseases of the Esophagus. 2008; 21:1-8.

34 Nativ-Zeltzer, N., Logemann, J., Zecker, S. and Kahrilas, P., 2016. Pressure topography metrics for high-resolution pharyngeal-esophageal manofluorography-a normative study of younger and older adults. *Neurogastroenterology & Motility*, 28(5):721-731.

35 Cutts A. Shrinkage of muscle fibres during the fixation of cadaveric tissue. *Journal of Anatomy.* 1988;160:75-78.

36 Kahrilas, P., Logemann, J., Krugler, C. and Flanagan, E., 1991. Volitional augmentation of upper esophageal sphincter opening during swallowing. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 260(3):G450-G456.

37 Kahrilas, P., Logemann, J., Lin, S. and Ergun, G., 1992. Pharyngeal clearance during swallowing: A combined manometric and videofluoroscopic study. *Gastroenterology*, 103(1):128-136.

38 Doeltgen, S., Ong, E., Scholten, I., Cock, C. and Omari, T., 2017. Biomechanical Quantification of Mendelsohn Maneuver and Effortful Swallowing on Pharyngoesophageal Function. *Otolaryngology–Head and Neck Surgery*, 157(5):816-823.

39 Bodén, K., Hallgren, Å. and Witt Hedström, H., 2006. Effects of three different swallow maneuvers analyzed by videomanometry. *Acta Radiologica*, 47(7):628-633.

40 Hoffman, M., Mielens, J., Ciucci, M., Jones, C., Jiang, J. and McCulloch, T., 2012. High-Resolution Manometry of Pharyngeal Swallow Pressure Events Associated with Effortful Swallow and the Mendelsohn Maneuver. *Dysphagia*, 27(3):418-426.

CHAPTER 8: Appendices

8.1 Appendix A: Dissection Protocol for the sampling of the Posterior Laryngopharynx

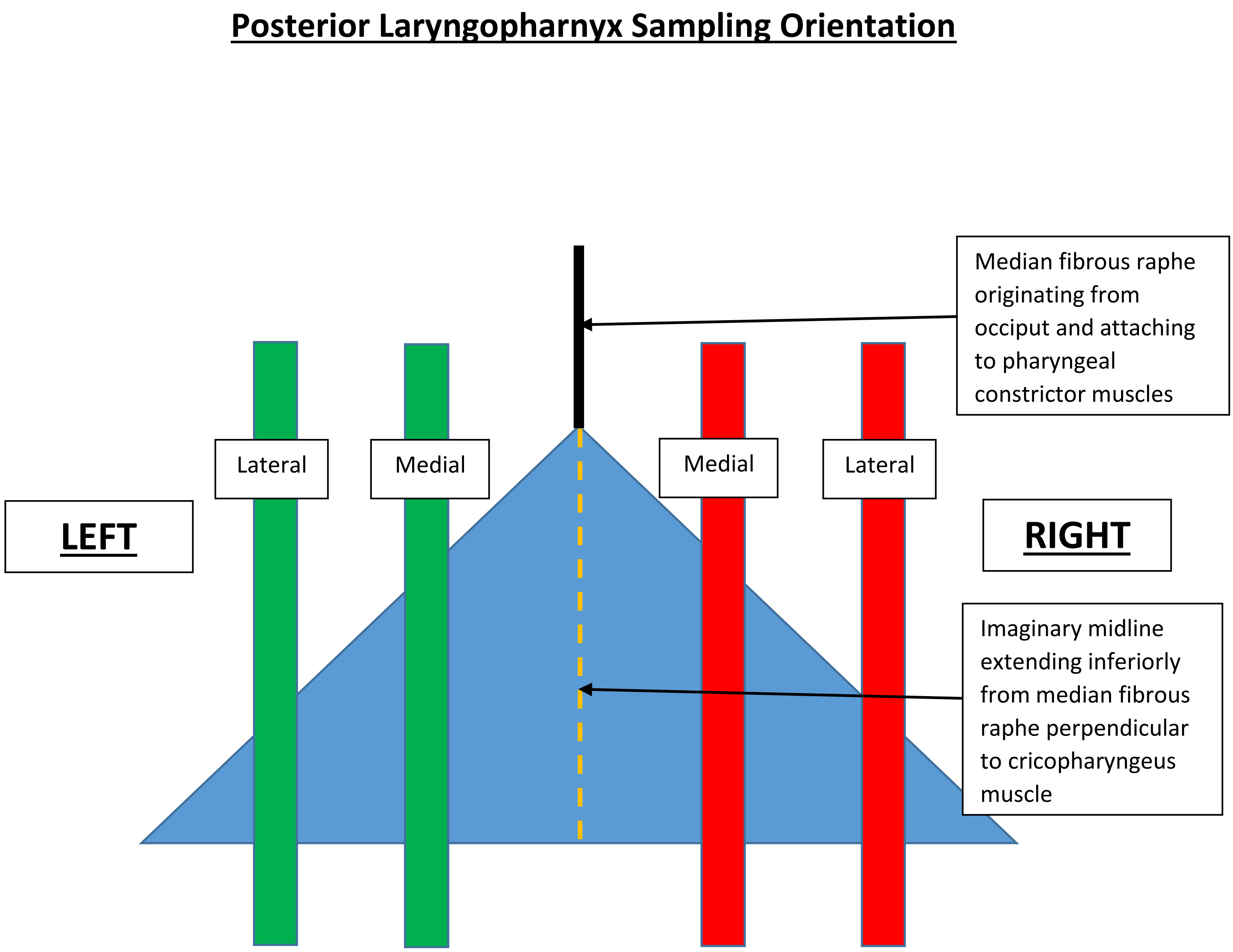
- Place wooden blocks under the cadaver's shoulders to extend the neck and aid the dissection.
- Make the first incision into the skin from the chin inferiorly to the middle of the suprasternal notch.
- The make the next incision into the skin from the acromion prominence of the shoulder medially across the clavicle to the sternum.
- Raise the skin from the anterior-inferior corners of the incision, by blunt dissection, then raise the flap up towards the ear.
- On the raised flap there is a cutaneous muscle called the platysma muscle which is in near proximity to the skin.
- Locate the hyoid bone, then feel for the soft thyrohyoid membrane which is inferior to the hyoid bone.
- Cut through this membrane (the thyrohyoid membrane) in a horizontal plane just below the hyoid bone and thus allowing for mobilisation of both the pharynx and the larynx.
- Pull the larynx anteriorly and free the whole structure by cutting through the neurovascular structures on either side.
    - These neurovascular structures will include the following: the superior laryngeal artery and the superior laryngeal nerve (both the internal and external branches)
- Lateral to this structure, the laryngopharynx, is the thyroid gland which has a very thin true capsule. This capsule is continuous with its stroma as well as the outer false capsule of the pretracheal fascia which binds it firmly to the larynx superiorly and the trachea inferiorly.
    - The thyroid gland has two lobes (left and right) joined by the isthmus which lies anterior to tracheal rings two, three and four. Medial to the lobes of the

thyroid gland, between each thyroid lobe and the pharynx, are the superior thyroid arteries accompanied by the superior thyroid veins.

    - Laterally on each side of the thyroid gland, on the outer aspect, are the inferior thyroid arteries coming from the thyrocervical trunk inferiorly, which originate from the subclavian artery. These inferior thyroid arteries pass behind the carotid sheath which lies lateral to the thyroid gland inferiorly and the pharynx superiorly.

- Posterior to the pharynx, locate the muscular wall of the pharynx formed by the three fan shaped constrictors on each side and a central pharyngeal raphe in the midline.
    - Also note that lining the constrictor muscles is an internal fascial lining called the pharyngobasilar fascia which extends superiorly and attaches to the periosteum of the cranial base defining the limits of the pharyngeal wall in its superior part.
    - The external fascial lining is the buccopharyngeal fascia which inferiorly blends with the pretracheal layer of the deep cervical fascia. Deep to the fascia is the pharyngeal venous plexus.
- Innervating the inferior constrictor muscle are the recurrent laryngeal nerves which both ascend from below and pass through a gap inferior to the inferior pharyngeal constrictor in order to pass superiorly to further supply the larynx.
- One should be able to locate the inferior constrictor muscle lateral to the cricoid cartilage.
    - The cricoid cartilage, being the origin of the inferior constrictor muscle lies inferior to the thyroid cartilage and superior to the first tracheal cartilage ring.
    - The insertion of the inferior constrictor muscle is at the cricopharyngeus muscle which encircles the pharnygoesophageal junction without forming a raphe.
- The central pharyngeal raphe is the imaginary divider of the left and the right side of the inferior pharyngeal constrictor.
    - This raphe is a fibrous band, running vertically in the midline from the basilar part of the occipital bone, to which the superior middle and inferior constrictor muscles insert.

8.2 Appendix B: Posterior Laryngopharynx Sampling Orientation



8.3 Appendix C: Data Collection Sheet

Accession Number	Age (years)	Sex	Left Lateral (μm)	Left Medial (μm)	Right Medial(μm)	Right Lateral (μm)
			Appendix B: Data Collection Sheet			

8.4 Appendix D: Research on cadaveric Material covered under School of anatomical sciences Ethics Waiver ref: W-CJ-140604-1, Human Research Ethics Committee

Human Research Ethics Committee (Medical)

Research Office Secretariat: Faculty of Health Sciences, Phillip Tobias Building, 3rd Floor, Office 301, 29 Princess of Wales Terrace, Parktown, 2193 Tel +27 (0)11-717-1252 /1234/2656/2700
Private Bag 3, Wits 2050, email: zanele.ndlovu@wits.ac.za
Office email: hrec-medical.researchoffice@wits.ac.za
Website: www.wits.ac.za/research/about-our-research/ethics-and-research-integrity/

07 October 2016

TO WHOM IT MAY CONCERN:

This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: Nicolas Fitchat – "Why Do Zenker's Diverticulae occur more on the Left than the Right?" School of Anatomical Sciences (Head: Prof M Steyn - Previously Prof T J M Daly, initial approval 04/06/2014 – recertified 27/01/2016).

Project title: Research on Cadaveric Material covered under School of Anatomical Sciences Ethics Waiver Ref: W-CJ-140604-1 (Prof M Steyn)

Reason: In terms of Chapter 8, sections 62-64 of the National Health Act No 61 of 2003 donated bodies and their tissues may be used for, among other purposes, health, and research. Use of such Material is subject only to permission from the responsible person in the School of Anatomical Sciences – the Head or person designed by the Head.

Professor Peter Cleaton-Jones

Chair: Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
07 OCT 2016
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

Copy - HREC (Medical) Secretariat: Rhulani Mkansi, Zanele Ndlovu.

8.5 Appendix E: Declaration of Amendment to Original Research Project in the Collections of the School of Anatomical Sciences

School of Anatomical Sciences

University of the Witwatersrand, Johannesburg

7 York Rd, Parktown, 2193, South Africa • Tel: +27 11 717 2713 • Fax: +27 11 717 2422 • www.wits.ac.za

Declaration of Amendment to Original Research Project in the Collections of the School of Anatomical Sciences

Researcher name: Dr Nicolas Fitchat

Student/Staff number: 601936

Affiliation/s: Department of Otorhinolaryngology and Head and Neck Surgery

Supervisors/Collaborators: Dr Shivesh Maharaj

Original Project title: Why do Zenker's Diverticulae occur more on the Left than the Right?

Original Date of Approval: 14 October 2016

I, the undersigned, **Dr Nicolas Fitchat** acknowledge that I will not expand the research project approved on the aforementioned date beyond the scope covered in the previously submitted proposal and the amendments motivated below, except under the confirmation of another letter of amendment to the original project as this.

Expanding the research project beyond the scopes of the original proposal and any previous approved letters of amendment without consulting the Chair of the Collections Committee or Head of School, is strictly prohibited.

Amendments and motivation, thereof:

The muscle thicknesses of the posterior pharynx were measured in 110 cadavers in 2016 and 2017. This along with demographic data (Age, Sex and Length) were recorded and tabulated to determine the reason as to Zenker's Diverticulae occur more on the Left than the Right Side, as well as to look for a predisposition in male, longer and older cadavers. The proposed amendment would be to use the same data already collected from this study conducted in 2016 and 2017 in order to explore sarcopaenia of the posterior pharynx in a cadaveric population and how it is related to the cadavers' age and sex. This new study conducted on data already collected from the previous study will be used as part of an MSc (Med) by dissertation. As the data already collected in 2016 and 2017 already has the muscle thicknesses of the posterior pharynx and the sex and age of each cadaver recorded, it would therefore be appropriate and relevant to use the same data collected for this new proposed study.

Signature: Date 25/02/2020
Researcher

Signature: Date
Chair of Collections Committee or Head of School

FOR OFFICE USE ONLY:
Application Reference Number:

Anatomical Sciences

Page **1** of **1**

8.6 Appendix F: Turnitin Digital Receipt

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Nicolas Fitchat
Assignment title:	2021
Submission title:	601936:Age-Related_Sarcopaenia_of_the_Posterior_Pharynx_i...
File name:	of_the_Posterior_Pharynx_in_a_Cadaveric_Population_NFitcha...
File size:	1.86M
Page count:	79
Word count:	12,693
Character count:	66,657
Submission date:	21-Apr-2021 02:40PM (UTC+0200)
Submission ID:	1565571013