Fontys University of Applied Sciences

Physiotherapy English Stream

Inter-rater reliability analysis of the diaphragm thickness with musculoskeletal ultrasound imaging, during the breathing cycle

What is the inter-rater reliability amongst students of three different paramedical backgrounds (Physiotherapy, Speech therapy and Radiology-assistant) of MSU when measuring the thickness of the diaphragm during respiratory pause, full inhalation and full exhalation?

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Preface

This thesis was achieved in December 2015 and took place at Fontys University of Applied Sciences in Eindhoven. This study started in September 2015, and was the final procedure of the physiotherapy English stream bachelor program. It taught me about researches as well as it gave me the opportunity to gain knowledge of musculoskeletal ultrasound imaging. This topic was very interesting and made me yearn for learning more about MSU, with perhaps taking a more advanced course in the future.
I want to thank my supervisor Marc Schmitz for his feedback throughout the process of my thesis. In addition, I would like to thank Madelon Pijnenburg for her great classes and help concerning statistics. Lastly, I want to thank Megan Kruger and Nadia Giampellegrini for their peer reviewing and their support during this period.

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Abstract

Introduction

The use of musculoskeletal Ultrasound (MSU) has been increasing amongst physiotherapist. The efficacy of MSU as a biofeedback training tool diaphragm muscle has not yet been evaluated even though it is a muscle known to be vital in contributing to spinal stability. Before assessing the effectiveness of MSU as a biofeedback training tool of the diaphragm, it is essential to investigate the inter-rater reliability to guarantee dependable results. In addition; the influence of the professional background amongst raters while using MSU has not yet been evaluated. Therefore this study will address the following question: What is the inter-rater reliability amongst students of three different paramedical backgrounds (Speech therapist, Physiotherapist and Radiologist-assistant) of MSU when measuring the thickness of the diaphragm during respiratory pause, full inhalation and full exhalation.

Method

A number of 65 healthy participants from Fontys University of Applied Sciences were recruited via email and social media. MSU was performed by three raters of different paramedical background (Radiologist assistant (MBRT), Speech therapist (ST) and Physiotherapist (PT)). The thickness measurements were taken from the right diaphragm in respiratory pause, full inhalation and full exhalation. Inter-rater reliability (defined by ICC) was then calculated amongst the three assessors.

Results

The inter-rater reliability amongst the three raters together was from fair reliability (ICC= 0, 4 - 59) to excellent reliability (ICC=0, 75-1) with values ranging from 0,472 (p <0.000) in full exhalation to 0,659 (p<0.000) in respiratory pause and 0,761 (p<0.000) in full inhalation. When comparing the professional background lowest ICC is found between the PT and MBRT in full exhalation with 0,276 (p<0.001) indicating poor reliability (ICC>0, 4), following by the PT and ST in full exhalation with 0,410 (p<0.002). The highest ICC score was found between the MBRT and SP in full inhalation with a score of 0.760 (p<0.000) indicating excellent reliability (ICC=0, 75-1)

Conclusion and recommendations

The results obtained have confirmed a relatively good inter-rater reliability while measuring the diaphragm thickness using MSU, and without taking into consideration the professional background. However, this research cannot conclude in inter-rater reliability amongst the three professions. Therefore, this study recommends assessing the inter-rater reliability with at least 3 raters per professional background who received a course of MSU from an expert within their field.

Key words

Musculoskeletal ultrasound, inter-rater reliability, diaphragm thickness, speech therapy, radiologist assistant, physical therapy, low back pain

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Introduction

Low back pain is an extremely common problem nowadays; the lifetime prevalence is 80% of the population (1)(2). It is seen to be the primary cause of activity limitation and work absence in the world(3). Low back pain is not a burden only to people suffering from it, it places a high load on the economy; the costs in Europe have been estimated to exceed 12 billion Euros every year(3)(4).Core instability is seen as a possible underlying cause of low back pain.(5) Core stability is defined as " the ability to control the movement and position of the muscles of the central 'core' of the body which are responsible for posture and limb movement(6)." The core consists of the abdominal wall, the lower back, the pelvis and the diaphragm muscles; together they maintain the body's usual position during physical activities(7). Wang X-Q. et al, proved that the effect of core stability exercises is very effective in decreasing low back pain and improving physical function.(8)

The diaphragm is a very complex core muscle and is well-known for its vital importance in the process of respiration by being the primary muscle of inspiration(9). In literature, it is described as the roof of the entire core muscles, with the pelvic floor serving as the base.(5) When training core stability, the diaphragm is often overlooked by physical therapists (10)(11)(9). Nevertheless, it is demonstrated that contraction of the diaphragm increases intra-abdominal pressure, thus complementing to spinal stability(12). O'Sullivan et al described that people with sacroiliac pain have weakened recruitment of the diaphragm and pelvic floor(13). In addition, Kolář.P et al, concluded that patients with low back pain, have an unusual position and a steeper slope of the diaphragm, which possibly takes parts in the etiology of the disorder(14). Diaphragmatic breathing techniques are used to minimize the usage of accessory muscles and to strengthen the diaphragm and improve standing posture(15)(16).Thus, diaphragmatic breathing techniques are an essential part of a core-strengthening program(13).

However, a difficulty encountered while training the inner core is that one easily has the tendency to compensate with the dominant superficial muscles(17)(11). Musculoskeletal ultrasound (MSU) is a device that provides images of the soft tissues in the body by using sound waves (18). It gives the possibility to provide visual biofeedback of muscular contractions and therefore it decreases the compensation mechanism of the dominant superficial muscles(19). In fact, Richardson CA et al, describe that real-time MSU imaging can be used to give visual biofeedback and increase performance and maintenance in the ability to activate the lumbar multifidus muscles in healthy subjects (19) The lumbar multifidus are deep core muscles of the lower back and as well as the diaphragm, dysfunction of these muscles are strongly linked with lower back pain(20). Thus, it is interesting to evaluate the efficacy of MSU as a biofeedback training tool of the active lumbar stabilizing system, more precisely for the diaphragm muscle. MSU is used more frequently in several healthcare professions including physical therapy(21)(22). Physical therapists use therapeutic ultrasound mainly to assist the repair of soft tissue in sports injuries. Nonetheless, recently they increased the utility of the device into a diagnostic tool. MSU can therefore facilitate the screening process as well as assess the patient's muscular volume and test their motor activation; in addition it is utilized to give biofeedback to both the therapist and the patient in rehabilitation(22)(23)(19). In speech therapy the use of MSU is currently uncommon; however Bernhardt MB et al concluded that MSU shows positive results as a visual feedback tool in speech therapy(24). Additionally, Shawker et al described that the use of MSU as a biofeedback tool to correct speech defects is a good addition to the profession(25). The increasing use of MSU in healthcare professions can be explained by its numerous advantages; the evolution of ultrasound technology and increasing accessibility due to lower cost. Furthermore; portable ultrasound machines and real time two dimensional visualization being performed more, without difficulty on the diaphragm at the bed side.(26)

Before being able to evaluate the effectiveness of MSU as a biofeedback training tool of the diaphragm, it is essential to first establish standard reference values and to investigate the inter-rater reliability to guarantee dependable results. B mode imaging in medical terminology is " A two-dimensional diagnostic ultrasound presentation of echo-producing interfaces in a single plane"(27) Harper CJ et al ,used B mode imaging of the diaphragm, to establish norm values of the diaphragm thickening with tidal breathing in healthy subjects(28). Nevertheless, the study obtained the inter-rater reliability by having two examiners without specified background experience; perform MSU on the subjects. As a result it is interesting to assess the inter-rater reliability of the MSU imaging of the diaphragm; highlighting the research being done by three students of different backgrounds such as physiotherapy, speech therapy and radiologist-assistant. Further on, the measurements will be taken at maximal inhalation, maximal exhalation and respiratory pause as it is the most reliable standardized breathing moments and when most significance dissimilarity will be distinguished. Latterly, a possible use of these values on the purpose of biofeedback tool on patients suffering from low back pain could be introduced.

Therefore; this study will address the following research question: What is the inter-rater reliability amongst students of three different paramedical backgrounds (Speech therapist, Physiotherapist and Radiologist assistant) of MSU when measuring the thickness of the diaphragm during respiratory pause, full inhalation and full exhalation?

Method

Study design:

This is an experimental research performed at Fontys University of Applied sciences, Eindhoven. The aim was to test the inter-rater reliability through measuring the diaphragm thickness of the subjects' between three students with the following study background: physiotherapy, speech therapy, radiologist-assistant.

Participants:

Process of selection

Participants were recruited from the English/Dutch stream of physiotherapy, speech and language and radiology- assistant students from Fontys Paramedische Hogeschool. If interested they were contacted via email (see Appendix I), the email included an attachment (see Appendix II) containing further information for the subjects who were interested to be part of the research experiment. In addition, announcement on social media was sent for extra participants and graduated students. Furthermore verbal invitation was given at Fontys University and around Eindhoven. It was followed by the same email as described above for those who were interested.

Inclusion criteria

1. **Healthy young adults**
2. **Ages between 18 and 28 will be selected for this study:** Keeping the participant's age population in a close range, excludes a too high variability in diaphragm motion(29).

Exclusion criteria

1. **Onset of lower back complaints in the last 6 months:** Patients with low back pain can have an abnormal position and a steeper slope of the diaphragm, which influence the diaphragm thickness.(14)
2. **No voice problems or dysphonia**: As the diaphragm is a vital muscle for respiration, the thickness and tension might be influenced if one is suffering from voice disorders. Therefore as the aim is to investigate standard reference values every compromising factor was excluded. (30)
3. **No asthma:** In asthmatic people, the inspiratory muscle function of the diaphragm is reduced because of hyperinflation. In this case, the shortening of the muscles, which create a force momentum, is impaired. Thereby, the load on the inspiratory muscles is increased while the ventilator capacity is decreased, which in time will lead to muscular fatigue(31).
4. **No paralysis and weakness of the diaphragm:** The symptoms of diaphragmatic paralysis or brutal weakness of its muscles often remain overlooked. In contrary to a normal functioning diaphragm, the abdominal wall moves inward during inspiration therefore it leads to a different

diaphragmatic thickness. Therefore if subjects had abdominal pain, dyspnea only with effort or respiratory insufficiency; which are the main symptoms of the disorder they did not take part into the study (32)

5. **No diagnosed neuromuscular diseases:** Neuromuscular disorders can affect the respiratory system and its function. More precisely, myopathic and neurophatic diseases can target the diaphragm which provokes weakness of its muscles(33)
6. **Pregnant' participants:** Pregnancy influences the diaphragm for the reason that the uterus might take more space in the abdomen which will lead to the fact that the diaphragm will have less space to enlarge the lungs(34)
7. **Previous diaphragm surgery:** Cardiac, thoracic or abdominal surgeries can have an effect on the adequate functioning of the respiratory muscles. In fact, surgery can be the cause of volume displacements between the abdomen and the thorax. Therefore, the natural curvature of the diaphragm is changed which results in a decreased generation in pressure during respiration(35)
8. **BMI >30kg/m²:** BMI is affecting the diaphragm motion therefore a BMI <30kg/m2 is inquired for the research(29).

Before the experiment took place, an informed consent was given to the participants. After full understanding of the requirements of the study, the participants signed it to prove their voluntary participation in the research.

Ethical aspects

The participants of the experiment study were not harmed in any way therefore the study is non WMO obligated. Ultrasound is a non-invasive device; therefore there were no risks for the participants being involved in this research experiment. In addition, the researchers had an approval from Fontys University of Applied Sciences to assess norm values and inter-rater reliability of the diaphragm thickness for MSU ultrasound research. After full understanding of the research procedure received by email (see Appendix I,II), the test subjects were requested to sign an informed consent before the testing procedures started. The data collection was handled confidentially and remained anonymous.

Measurement tools:

All measurement tools below were provided by Fontys University of Applied Sciences from the physiotherapy and MBRT department.

1. Musculoskeletal Ultrasound machine

The ultrasound machine ProSound Alpha 7 from Aloka (Germany 2012) was used to scan the diaphragm, with the purpose of measuring its thickness(36). Two-dimensional B-mode ultrasound was chosen to measure diaphragm thickness at the zone of apposition during full inspiration, full expiration and respiratory pause using the intercostal view. The gain 88 (SD: 5), depth range (4-5) and the contrast 18 (SD:

2) were chosen according to the best view of the diaphragm recorded during practices.

The choice of this equipment can be justified by the fact that it provides high-resolution images of muscle movement, thickness measurements of individual muscles and echogenicity and is harmless for participants. B mode was chosen for its real time imaging capacity.(37)

2. *Transducer*

Ultrasonic transducers are essential in the research to send and receive the sound wave signal and to give a real time image of the diaphragm muscle. To measure the thickness of the diaphragm in an intercostal view, a high frequency (11-12 MHz) linear-array transducer was needed.(37)

3. *Gel*

A gel “sonogel”, consisting mostly of water was applied on the participants to scan their diaphragm. The reason for using the gel is to transmit the sound waves from the ultrasound head to the skin without crossing through the air which creates a clear image of the diaphragm for the practitioner.

4. *Remaining equipment and facility :*

- Available double room to create more privacy and a more welcoming environment.
- Tissue paper/baby wipes were used to remove the gel after data collected for hygienic reasons and comfort.
- Dividers between each ultrasound station for the participants privacy.
- New USB sticks as MSU doesn’t have an anti-virus software program.
- Chocolate bar will be handed out as a reward for their participation.

Research procedure:

Participants were invited to Fontys Paramedische Hogeschool via email for a maximal duration of an hour. Time and date of the experiment were communicated beforehand. The participants received information about the procedure of the experiment; after full understanding they will sign an informed consent (Appendix III).

All tasks of the procedure were divided amongst the 5 students that are part of the research experiment. Only required information of the flow for this research experiment will be described below; for the entire group flow: see appendix VI. The testing started after participating in a mini MSU course of the diaphragm followed by 20hours practice of the students involved.

Once the subjects signed the informed consent, the anthropometric data were collected by one student in order to define the group population of the study. Then, the subjects received an ID followed by a verbal explanation of the three different respiration phases (respiratory pause, full inhalation, and full exhalation) which was taught according to a standardized protocol(38) (see appendix VI).

Subsequently, they were asked to stand back against the wall, in socks to cancel any influence of the shoes on the participant's posture. Their feet were positioned perpendicular against the wall and in line with the hips; the right arm of the subjects was elevated with the hand on top of his/her head for a better visualization of the anterior axillary line. Finally, the subjects were asked to look straight forward.

Then, a new patient file was created into the device with the name of the sonographer and standardized' settings were adjusted (linear transducer, frequency, gain, contrast and depth). The MBRT student placed the transducer at the anterior axillary line to obtain a sagittal image at the intercostal space between the seventh and eighth or eighth and ninth ribs(37). Nevertheless, in order to capture an optimal image of the diaphragm, the lung shadow was followed during the procedure, which moved away the transducer from the seventh, eighth and ninth ribs (see image below). Furthermore, it provided a visualization of both the pleural and peritoneal membranes where the thickness was taken from the right diaphragm, seeing as that the left one is more difficult to visualize(37). Each image of the diaphragm were saved and recorded in three phases of the breathing cycle; 1st respiratory pause, 2nd full inhalation and 3rd full exhalation.

After the measurements were taken, the participant moved on to the second station. The same procedures were repeated, however it was now measured by a physiotherapist student and then to the third station by a speech therapist student. Lastly the participants were rewarded for their participation in the research.

The image represents the starting position of the transducer used for this research. As describe above, the transducer moved according to the lung shadow for best measurements of the diaphragm. During full inhalation, as the lungs expand the transducer was moved caudally within the anterior axillary line; contradictory the transducer was moved cranially within the anterior axillary line in full exhalation.

Image has been taken during the testing

Data collection:

Per participant, the diaphragm thickness was measured in each breathing phase, in the following order: respiratory pause, maximal inspiration and maximal expiration. To increase the reliability of the measurements, this procedure was repeated three times on the same participant. Subsequently the mean of the data collected in the three procedures were calculated; in some case due to a missing image or due to a low quality of the image, the mean was taken from two measurements. The data was saved onto a USB stick, for time management and patient's satisfaction the thickness was measured after the research had terminated with the help of the program "MicroDicom" software on windows 8 (39).

To measure the thickness a 5mm line was drawn from the lung shadow following the upper diaphragm fascia, subsequently a second line perpendicular to the 1st was drawn according to the diaphragm' fiber orientation(see Appendix IV for images). The testing was terminated when a maximum of 66 participants was reached; the number was raised to + 6 participants as prevention measure; in case some data were to be excluded. According to the COSMIN checklist, 66 participants is a good sample for inter-rater reliability study(40).

Data analysis:

The mean of the thickness measurements of each assessor were saved in an SPSS dataset using the Statistical Package for the Social Sciences (SPSS) version 20 released in 2011 for windows. Before analyzing the thickness measurements, demographic data were tested for normal distribution with the Shapiro-Wilk test of normalcy ($p<0.05$). Subsequently, the same test was used to analyze if the thickness measurements were normally distributed. To gain a first insight in the correlation of the data; scatter plots were used for each breathing cycle per assessor, and then the non-parametric data were placed into box plots.

Then, the inter-rater reliability was acquired by using intra-class correlation coefficient (ICC): (2,k) , each participant is examined by each assessors (a physiotherapist, a speech therapist and an MBRT/ radiologist assistant); and assessors represents in this research experiment, other assessors of the same carrier field. Reliability was then calculated by taking an average of the three assessors separately(41). The ICC was calculated using absolute agreement and a confidence interval of 95%. In order to interpret the values given by SPSS an ICC classification was used(42):

ICC	Reliability
0,75 - 1	**Excellent**
0,60 – 0,74	**Good**
0,4 - 59	**Fair**
>0,4	**Poor**

Values closer to 1 suggest high reliability amongst the three testers.

Results

A post hoc calculation was made in order to know if the correct amount of participant was chosen for this research experiment. The results of the calculation confirmed 100%power, concluding that the number of participant was sufficient for the research(43).

After the exclusion of one subject due to higher body mass index (BMI) than the inclusion criteria, a number of 65 participants remained (n=65). During data collection, a small amount of images were excluded due to a low quality making it an unreliable measurement, therefore some means were calculated from two images instead of three. There was a gender distribution of 55% (n=36) female and 45% (n=29) male. For further information on demographic data see table 1.

The demographic data were tested for normal distribution with the Shapiro-Wilk test of normalcy ($p<0.05$). The demographic data (Age, weight, height, BMI) were found to be normally distributed (see Appendix VII)

Table1 : Demographic data ; Abbreviations : kilograms(kg);Centimeters(cm)

	Number of participants	Minimum	Maximum	Mean ± Standard Deviation
Age	65	18	28	22,4±2,3
Weight (kg)	65	49,7	115,7	73,1 ±13,2
Height (cm)	65	146	199	174,8±9,7
BMI	65	17,8	31,2	23,8 ±3,2

The data were firstly analyzed with scatter plots, in order to compare the thickness values of the diaphragm found between each assessor (see appendix VIII). Each breathing cycle was analyzed in scatter plots (Respiratory pause (RP), full exhalation (FE), full inhalation (FI)) for Physiotherapist (PT) versus Speech therapist (SP); PT versus Radiologist-assistant (MBRT) and finally SP versus MBRT. Subsequently, the data were tested for normal distribution with the Shapiro-Wilk test of normalcy ($p<0.05$) for each breathing cycle per assessors. The results demonstrated that all the data were not normally distributed beside for RP- MBRT. Therefore, the data is displayed in box plots see figure 1,2,3.

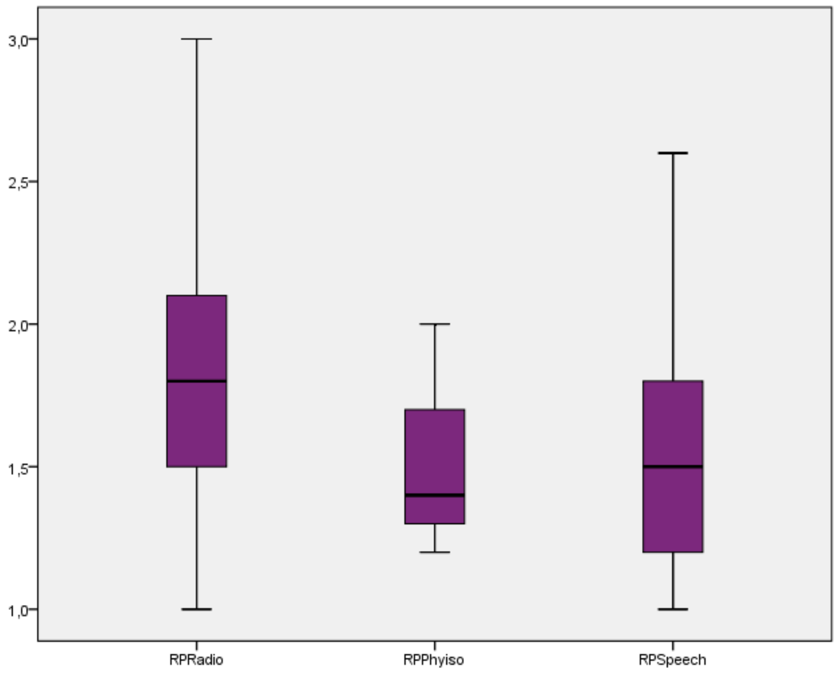


Figure 1 – Respiratory Pause

The data are put into a box plot as they create a visual distribution of the quantitative data.
In this figure, the results of the diaphragm thickness in the breathing cycle of respiratory pause is assessed, for each rater

Key

Bottom line = 25th percentile,
Middle line = median
Top line = 3rd quartile= 75th percentile
Bottom Whiskers = lower data that is not an outlier
Top whiskers = Higher data that is not an outlier
Circles = outliers.
Stars = extreme outliers

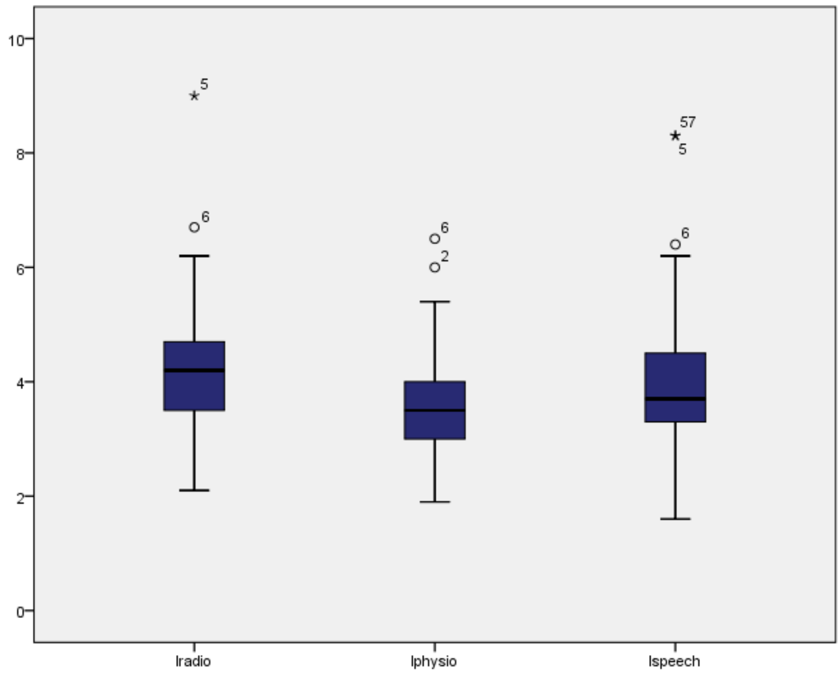


Figure 2 – Full inhalation

In this figure, the results of the diaphragm thickness in the breathing cycle of full inhalation are assessed, for each rater

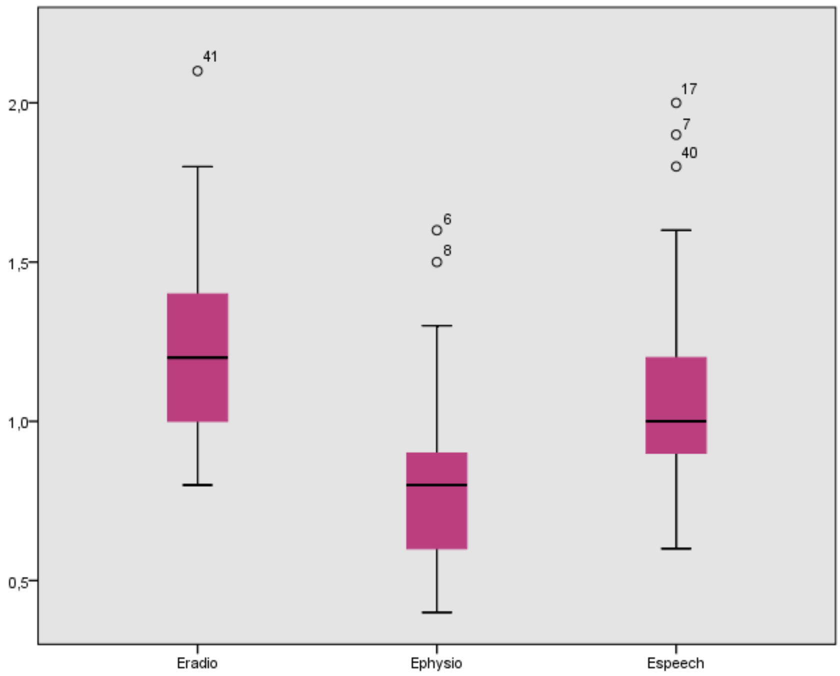


Figure 3 – Full exhalation

In this figure, the results of the diaphragm thickness in the breathing cycle of full exhalation are assessed, for each rater

The intra-class correlation coefficients (ICC) (2,k) were then calculated to establish the inter-rater reliability between the three assessors using the two way random model, absolute agreement and a confidence interval of 95%. Firstly, the ICC of all three raters was assessed together (see table 2). Subsequently, each assessor was compared one by one to see where the biggest difference amongst raters could be found (see table3). The results concluded that the lowest ICC's are during Full exhalation in both tables; more precisely between PT and MBRT.

Table 2- Inter-rater reliability of the diaphragm thickness comparing PT, ST, MBRT together

	Respiratory Pause	Full inhalation	Full exhalation
Significant (p-value)	<0.000	<0.000	<0.000
ICC results	0,659	0,761	0,472

p< 0,05 is seen as statistically significant.

Table 3- Inter-rater reliability of the diaphragm thickness comparing each rater one by one
<u>Abbreviations:</u> PT (Physiotherapist); ST(Speech therapist); MBRT(Radiologist-assistant);VS(Versus).

	Respiratory pause		Full inhalation		Full exhalation	
	ICC results	(p-value)	ICC results	(p-value)	ICC results	(p-value)
PT vs ST	0,551	<0.001	0,660	<0.000	0,410	<0.002
PT vs MBRT	0,417	<0.001	0,593	<0.000	0,276	<0.001
ST vs MBRT	0,670	<0.000	0,760	<0.000	0,478	<0.001

p< 0,05 is seen as statistically significant.

Discussion

The purpose of this study was to assess the inter-rater reliability of the diaphragm thickness with musculoskeletal ultrasound imaging, amongst three students of different paramedical backgrounds, during three phases of the breathing cycle. According to previous research, it was hypothesized that the reliability between the raters, without taking into consideration the professional background, was excellent (ICC >0,75)(26) (28).

However, the results of this research do not confirm the hypothesis. The inter-rater reliability (defined as ICC) of the diaphragm thickness which was assessed between the three raters together, demonstrated a score of 0,472 (p <0.000) in full exhalation. This indicates a fair reliability amongst all raters (ICC= 0, 4 - 59). In addition, during the breathing cycle of respiratory pause, the results showed 0,659 (p<0.000), this signifies a good reliability (ICC=0, 60 - 0, 74). Nevertheless, in full inhalation the ICC is 0,761 (p<0.000) which shows excellent reliability (ICC=0, 75-1). When assessing the reliability of the diaphragm thickness amongst the three raters compared one by one, the results showed values ranging from 0,276 (p<0.001) in full exhalation to 0,760 (p<0.000) in full inhalation. These results indicate values ranging from poor (ICC>0, 4) to excellent reliability (ICC=0, 75-1). More precisely, in full exhalation ICC range from poor (ICC>0, 4) to fair reliability (ICC= 0, 4 - 59) followed by respiratory pause ranging from fair (ICC= 0, 4 - 59) to good (ICC=0, 60 - 0, 74) and lastly, full inhalation ranging from good (ICC=0, 60 - 0, 74) to excellent (ICC=0, 75-1). In summary, by comparing the reliability of the diaphragm thickness from the three raters together and one by one, it can be stated that full exhalation was the least reliable of all breathing cycle phases, followed by respiratory pause and full inhalation. The breathing moment of full exhalation was the most difficult breathing moment to scan for the three raters during the data collection, as when fully exhaled the diaphragm decreases in thickness and moves upward into the chest cavity(37). Therefore, the diaphragm is more prone to be hidden behind the ribs, making it a challenge for the assessors to scan. This could explain the findings of a poor (ICC>0, 4) to fair (ICC= 0, 4 - 59) inter-rater reliability in the moment of full exhalation.

The results found in this article investigate if the study background of each rater could have affected the reliability of the findings. Currently, the reliability of MSU amongst assessors of different professional backgrounds has not been evaluated(44) which explains why this study compared additionally the reliability amongst raters without taking into account the study background. A detailed anatomy base is essential when making use of MSU imaging(45). Hence, it could have been hypothesized that the Physiotherapist (PT) and Radiologist assistant (MBRT) would have the highest reliability score due to the fact that PT's have an increased knowledge in anatomy(46)(47) compared to MBRT and Speech therapist (SP). Furthermore, the MBRT has an increased knowledge in mastering imaging devices compared to PT and SP. The SP has very little knowledge regarding the diaphragm's anatomy and the use of MSU in their curriculum. Nevertheless, the results state that the lowest ICC is found between the PT and MBRT, followed by the PT and ST (see table 2 in result section). In addition, the highest ICC score was found between the MBRT and SP, meaning that the PT had the most different data compared to ST and MBRT. Therefore, one could interpret that the PT of this research had the lowest skill in using MSU to measure the thickness of the diaphragm. However, all raters were students using MSU to measure the thickness of the diaphragm for the first time; they followed the same course of MSU before the experiment. Therefore, each assessor had a uniform training and skills regarding the use of MSU while scanning the diaphragm, thus it can be interpreted that in this case the variation in each rater's study background has no significant difference for this study. It can be argued that that the problem was personal dependent rather than professional dependent; in other words the rater's personal skills could be the reason of the inter-rater reliability findings. Hence, further research with at least three raters per profession, who were each trained by an expert in MSU with an identical professional background, is required in order to confirm the hypothesis stated.

In this study, as described in the method section, B-mode imaging was used to scan the diaphragm's thickness. This choice was made since B-mode offers a better anatomical definition of the muscles and its surrounding structures while simultaneously providing a better panoramic view compared to M-mode(26)(48)(49). In addition, B-mode provides real time feedback for both the patient and the professional, which is of great significance for biofeedback training(26).

In view of the fact that MSU is fairly new when measuring the diaphragm thickness using B-mode imaging, only two articles were found which could be used as comparison to the results of this research, without taking into consideration the professional background of the rater. Boon AJ et al, found an inter-rater reliability between two assessors (defined by ICC) of 0.98 for resting thickness and 0.99 for thickness at end of maximal inspiration indicating in both case an excellent reliability(26). In addition, Harper CJ et al demonstrated an ICC of 0.97 for quiet inspiration and 0.98 for quiet expiration further indicating an excellent reliability (ICC=0, 75-1)(28). Nonetheless, there are a few possible reasons for the dissimilarities found in the inter-rater reliability between these studies compared to this study. Firstly, in both studies the inter-rater reliability was assessed between two examiners with several years of experience in MSU imaging of the diaphragm. This leads to assume that extra training is required to measure the diaphragm thickness. In addition, both studies only had an amount of 12 participants when assessing the inter-rater reliability. According to the COSMIN checklist when investigating inter-rater reliability this number is a poor sample size; therefore it decreases the reliability of their results(40).

Other explanations can be found in the differences of the methodology in each study. For instance, Boon AJ et al, assessed inter-rater reliability while measuring the thickness of the diaphragm only in the breathing cycle of respiratory pause and maximal inspiration(26). According to the results found in this research study, while measuring the diaphragm thickness, full exhalation is the most challenging breathing moment (see table 2, 3 in result section); therefore excluding this breathing moment could have positively benefited their higher score in inter-rater reliability. Moreover, Boon AJ et al did not use a standardized protocol for each breathing moment the subjects had to perform; in fact the examiners decided when the participant was fully inspired as well as in respiratory pause. Furthermore, if the diaphragm became obscured by the lungs, Bonn AJ et al captured the picture before the subject was in his maximal inspiration; thus it is a biased technique and it could have benefited their findings.

Harper CJ et al, assessed inter-rater realibility while measuring the thickness of the diaphgram in quiet inhalation and exhalation, in other words, similar to Boon AJ et al there is no standardized protocol for the breathing moments in their investigation which, once more, benefits their results of high inter-rater reliability(28). Finally, both studies positioned the subjects in supine position in order to provide less overall variability of the diaphragm thickness(50)(26)(28), this may have influenced the results and it could explain the differences between this research's results and theirs.

Strengths and limitations

Executing the measurements in a standing position is a functional method of assessing the diaphragm; currently no study has measured the diaphragm's thickness in this position. Likewise, no study has investigated the difference in inter-rater reliability of a standing position compared to a lying position. In addition, each diaphragmatic breathing protocol as a starting position of lying down with an ultimate goal of performing the breathing technique in a standing position(15)(16). Thus, performing diaphragmatic breathing in daily life is essential; therefore measuring the subjects in a standing posture can be perceived as strength of this research.

The placement of the transducer varied amongst participants caused by different body morphology; it has been demonstrated that the diaphragm's position may vary significantly between subjects(50). For instance women were harder to scan due to their undergarments and their higher general fat percentage compared to men. Although an intercostals view is favored to scan the diaphragm, a posterior sub-costal view could possibly be applied in the upcoming researches to reduce the deviation of the transducer's placement(37).

Considering that this research is analyzing the inter-rater reliability, a fairly high number of participants contributed to the experiment compared to other studies(28)(26). Moreover, a post hoc power analysis has been done which confirmed 100% power, concluding that enough number of participants has been reached for the research increasing the external validity(43)(51). On the other hand, one of the disadvantages is the inclusion of subjects ranging from 18 years old to 28 years old, which reduces the external validity of the other experiment occurring simultaneously; as it includes a small portion of the population(51). This decision was taken due to the fact that keeping the participant's age population in a close range, excludes a too high variability in diaphragm motion(29).

A further strength is that the three assessors participated in a one day course in MSU to become skilled at scanning the diaphragm, followed by an amount of 20 hours practice on random subjects. Özçakar et al concluded that a one day course demonstrates to be effective in the use of MSU imaging(52). However, each assessor of this study had no previous knowledge about the use of MSU; also they followed a uniform training regarding MSU while scanning the diaphragm. Thus, having identical instructions could increase the reliability amongst profession. In addition the diaphragm can be a challenging muscle to scan due to its deep location hidden behind the ribs(37). Therefore, one can say that 20 hours of additional training on random subjects could have positively influenced the validity of the findings. Additionally, having each rater following a course from an expert in MSU within their professional field, could positively affect the variation of the findings amongst professional backgrounds.

In this research a maximum standardized procedure has been used between the three assessors. Firstly, compared to other studies(26)(28), a standardized breathing protocol has been followed during the data collection which increased the reliability of the findings (see appendix V). In addition, the procedures have been standardized for the placement of the transducer on the subjects and for the method to measure the diaphragm thickness using the MicroDicom software (see appendix IV). These measures were taken in order to have the most reliable procedure of the diaphragm scanning and to limit any errors amongst raters. Nevertheless, despite the fact that the subjects followed a standardized breathing protocol during the experiment, it remains a subjective technique to assess if the subjects were fully inhaled, in a respiratory pause or fully exhaled. On the other hand, currently, there is no medical device existing that performs these tasks.

Another limitation of this study is the fact that the subjects were assessed during one whole hour. During this hour, they were requested to repeat many times each breathing moment. The participants demanded several breaks in between each rater's measurement, as they felt out of breath or dizzy. This could have influenced the diaphragm thickness from one assessor to the other due to the participant's fatigue, which could have considerably decreased the reliability of the findings. A recommendation for future studies is to take a minimum of two weeks for the data collection, in which half of the raters the first week, followed by the other half on the second week. In this way, the participant comes twice to the experiment which decreases the overall time spent, as well as the exhaustion the subject can experience.

Lastly, during the measurements of the images, a small number of images were measured from 4 or 6 mm from the lung shadow instead of 5mm. In addition, during the mean calculation some images were missing and several means were calculated from 2 images instead of 3; these errors could have slightly influenced the results found amongst the assessors. These limitations could have been avoided if the study had additional time for the data collection; it would have diminished the pressure amongst raters therefore reducing the speeding up during the scanning to fit in time-wise.

Clinical relevance

This study demonstrates that the reliability between the three raters in general is relatively good, taking in consideration that the diaphragm is a complicated muscle to scan with MSU(37). In addition, the assessors were students with modest experience in the application of MSU. Thus, this research provides essential information for future research and for future use in the assessment of diaphragm dysfunction, as it implies that measuring the diaphragm thickness with MSU is a relatively reliable technique.

As described previously, research has confirmed that the diaphragm plays an essential role in contributing to spinal stability(12)(13)(14). In addition, it has been demonstrated that there is a connection between diaphragm' malfunction and sacroiliac pain(13) along with low back pain (LBP)(14). Therefore, this research also provides a base for future studies to compare the diaphragm thickness in patients with LBP to healthy subjects. Furthermore, it encourages the development of a new rehabilitation approach by using B-mode MSU as a biofeedback tool to control and improve the diaphragm activation in order to enhance the patient's core stability. Nevertheless, additional research continuing the investigation of the diaphragm thickness's inter-rater reliability by implementing the recommendation of this study is necessary. Subsequently, other studies are required to verify whether MSU is a good biofeedback tool, for it to be clinically useful in rehabilitation.

Recommendation for future studies

The use of MSU imaging for the diaphragm thickness is a fairly new method; therefore further research applying the recommendation of this study is required to implement MSU in clinical practice. Three small recommendations have been described previously in this study; to increase the general inter-rater reliability a posterior sub-costal view is suggested to avoid the deviation of the transducer due to diverse body morphology. Moreover, 20 additional training hours on random subjects per rater is necessary, as experience is crucial to master the skills of utilizing MSU. For the subject's well-being throughout the data collection, it is advised to divide the experiment over two weeks.

In addition this study provides a base of essential information for future research and for future use in the assessment of diaphragm dysfunction such as diaphragm thickness in LBP patient. This study recommends future research to verify whether MSU is a good biofeedback tool, for it to be clinically useful in rehabilitation of LBP patients.

Currently, the reliability amongst raters of different professional background has not been evaluated(44). Therefore a major recommendation of this article is to research more precisely whether the professional background influences the findings while using MSU. In order to make it achievable and reliable, the recommendation above should be implemented during the data collection. In addition, a minimum of 3 raters per background, trained by an expert of MSU within their professional field must be assessed. Employing 3 raters per profession will permit to evaluate the inter-rater reliability within their own field along with distinguishing which of the 2 raters obtain similar measurements. Subsequently, the score of inter-rater reliability within each field can be evaluated separately to find the overall ICC for each profession. In addition, another inter-rater reliability test assessing the profession amongst each other must be performed to differentiate which background has most similar measurements compared to the other.

Conclusion

This study addressed the following research question: What is the inter-rater reliability amongst students of three different paramedical backgrounds (Speech therapist, Physiotherapist and Radiologist-assistant) of MSU when measuring the thickness of the diaphragm during respiratory pause, full inhalation and full exhalation?

The results obtained have confirmed a relatively good inter-rater reliability without taking into consideration the professional background with value ranging from fair reliability (ICC= 0, 4 - 59) to excellent reliability (ICC=0, 75-1).

The lowest ICC was found between the PT and MBRT in full exhalation with 0,276 ($p<0.001$) indicating poor reliability (ICC>0, 4), following by the PT and ST in full exhalation with 0,410 ($p<0.002$). The highest ICC score was found between the MBRT and SP in full inhalation with a score of 0.760 ($p<0.000$) indicating excellent reliability (ICC=0, 75-1). However, this research cannot conclude in inter-rater reliability amongst the three professions.

References

1. Hoy D, Brooks P, Blyth F, Buchbinder R. The Epidemiology of low back pain. Best Pract Res Clin Rheumatol. 2010 Dec;24(6):769–81.

2. Vällfors B. Acute, subacute and chronic low back pain: clinical symptoms, absenteeism and working environment. Scand J Rehabil Med Suppl. 1985 Jan;11:1–98.

3. Friedly J, Standaert C, Chan L. Epidemiology of spine care: the back pain dilemma. Phys Med Rehabil Clin N Am. 2010 Dec;21(4):659–77.

4. Bevan S. The Impact of Back Pain on Sickness Absence in Europe. Work Found ,Reports. 2012;1:3–8.

5. Akuthota V, Ferreiro A, Moore T, Fredericson M. Core stability exercise principles. Curr Sports Med Rep. 2008 Feb 1;7(1):39–44.

6. Farlex. Core stability | definition of core stability by Medical dictionary [Internet]. [cited 2015 Oct 1]. Available from: http://medical-dictionary.thefreedictionary.com/core+stability

7. Kibler W Ben, Press J, Sciascia A. The Role of Core Stability in Athletic Function. Sport Med. 2006;36(3):189–98.

8. Wang X-Q, Zheng J-J, Yu Z-W, Bi X, Lou S-J, Liu J, et al. A meta-analysis of core stability exercise versus general exercise for chronic low back pain. PLoS One. 2012 Jan;7(12):e52082.

9. Hodges PW, Butler JE, McKenzie DK, Gandevia SC. Contraction of the human diaphragm during rapid postural adjustments. J Physiol. 1997 Dec 30;505(2):539–48.

10. Boyle KL, Olinick J, Lewis C. The value of blowing up a balloon. N Am J Sports Phys Ther. 2010 Sep;5(3):179–88.

11. Nelson, Nicole MS L. Diaphragmatic Breathing: The Foundation of Core Stability. Strength Cond J. 2012;34(5):34–40.

12. Akuthota V, Nadler SF. Core strengthening. Arch Phys Med Rehabil. Elsevier; 2004 Mar 3;85:86–92.

13. O'Sullivan PB, Beales DJ, Beetham JA, Cripps J, Graf F, Lin IB, et al. Altered motor control strategies in subjects with sacroiliac joint pain during the active straight-leg-raise test. Spine (Phila Pa 1976). 2002 Jan 1;27(1):E1–8.

14. Kolář P, Šulc J, Kynčl M, Šanda J, Čakrt O, Andel R, et al. Postural Function of the Diaphragm in Persons With and Without Chronic Low Back Pain. J Orthop Sport Phys Ther. 2012 Apr;42(4):352–62.

15. Shaw BS SI. Static standing posture and pulmonary function in moderate-persistent asthmatics following aerobic and diaphragmatic breathing training. Pak J Med Sci. 2011;27(3):549–52.

16. Cahalin LP, Braga M, Matsuo Y, Hernandez ED. Efficacy of diaphragmatic breathing in persons with chronic obstructive pulmonary disease: a review of the literature. J Cardiopulm Rehabil. Jan;22(1):7–21.

17. Nerreter T. Training for the deep muscles of the core - Diane Lee and Associates [Internet]. [cited 2015 Sep 16]. Available from: https://www.dianelee.ca/article-training-deep-core-muscles.php

18. Radiology UH. Musculoskeletal Ultrasound, Radiology Imaging Procedures [Internet]. 2014 [cited 2015 Oct 1]. Available from: http://www.uwhealth.org/radiology/musculoskeletal-ultrasound/14050

19. Van K, Hides JA, Richardson CA. The use of real-time ultrasound imaging for biofeedback of lumbar multifidus muscle contraction in healthy subjects. J Orthop Sports Phys Ther. 2006 Dec 1;36(12):920–5.

20. Freeman MD, Woodham MA, Woodham AW. The role of the lumbar multifidus in chronic low back pain: a review. PM R. Elsevier; 2010 Feb 2;2(2):142–6;

21. Potter CL, Cairns MC, Stokes M. Use of ultrasound imaging by physiotherapists: a pilot study to survey use, skills and training. Man Ther. 2012 Feb;17(1):39–46.

22. McKiernan S, Chiarelli P, Warren-Forward H. Diagnostic ultrasound use in physiotherapy, emergency medicine, and anaesthesiology. Radiography. 2010 May;16(2):154–9.

23. Teyhen DS, Miltenberger CE, Deiters HM, Del Toro YM, Pulliam JN, Childs JD, et al. The use of ultrasound imaging of the abdominal drawing-in maneuver in subjects with low back pain. J Orthop Sports Phys Ther. 2005 Jun;35(6):346–55.

24. Bernhardt MB, Bacsfalvi P, Adler-Bock M, Shimizu R, Cheney A, Giesbrecht N, et al. Ultrasound as visual feedback in speech habilitation: exploring consultative use in rural British Columbia, Canada. Clin Linguist Phon. 2008 Feb;22(2):149–62.

25. Shawker TH, Sonies BC. Ultrasound biofeedback for speech training. Instrumentation and preliminary results. Invest Radiol. Jan;20(1):90–3.

26. Boon AJ, Harper CJ, Ghahfarokhi LS, Strommen J a., Watson JC, Sorenson EJ. Two-dimensional ultrasound imaging of the diaphragm: Quantitative values in normal subjects. Muscle and Nerve. 2013;47(6):884–9.

27. Farlex. B-mode | definition of B-mode by Medical dictionary [Internet]. [cited 2015 Sep 16]. Available from: http://medical-dictionary.thefreedictionary.com/B-mode

28. Harper CJ, Shahgholi L, Cieslak K, Hellyer NJ, Strommen JA, Boon AJ. Variability in diaphragm motion during normal breathing, assessed with B-mode ultrasound. J Orthop Sports Phys Ther. 2013 Dec;43(12):927–31.

29. Kantarci F, Mihmanli I, Demirel MK, Harmanci K, Akman C, Aydogan F, et al. Normal diaphragmatic motion and the effects of body composition: determination with M-mode sonography. J Ultrasound Med. 2004;23(2):255–60.

30. Pettersen V, Eggebø TM. The movement of the diaphragm monitored by ultrasound imaging: Preliminary findings of diaphragm movements in classical singing. Logop Phoniatr Vocology. 2010;35(3):105–12.

31. Hill AR. Respiratory muscle function in asthma. J Assoc Acad Minor Phys. 1991 Jan;2(3):100–8.

32. Newsom-Davis J. The Diaphragm and Neuromuscular Disease. Am Rev Respir Dis. American Lung Association; May 14;119(2P2):115–7.

33. Gilchrist JM. Overview of Neuromuscular Disorders Affecting Respiratory Function. Semin Respir Crit Care Med. 2002 Aug 2;23(3):191–200.

34. Lowth DM. Common Problems in Pregnancy. Side Eff pregnancy | Patient. 2013;1:1–48.

35. Siafakas NM, Mitrouska I, Bouros D, Georgopoulos D. Surgery and the respiratory muscles. Thorax. 1999 May 1;54(5):458–65.

36. Toshiba Xario 200 Ultrasound Machine [Internet]. [cited 2015 Sep 23]. Available from: http://www.toshibamedicalsystems.com/products/us/xario200.html

37. Sarwal A, Walker FO, Cartwright MS. Neuromuscular ultrasound for evaluation of the diaphragm. Muscle Nerve. 2013 Mar;47(3):319–29.

38. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005 Aug;26(2):319–38.

39. MicroDicom - Free DICOM viewer and software [Internet]. [cited 2015 Oct 5]. Available from: http://www.microdicom.com

40. Terwee CB. COSMIN checklist with 4-point scale. COSMIN. 2011;6.

41. Shrout PE, Fleiss JL. Intraclass correlations. Uses Assess rater Reliab. 1979;86(2):420–8.

42. Hallgren KA. Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. Tutor Quant Methods Psychol. 2012 Jan;8(1):23–34.

43. Post-hoc Power Calculator [Internet]. Evaluate statistical power of an existing study. 2015 [cited 2015 Nov 25]. Available from: http://clincalc.com/Stats/Power.aspx

44. Karel YHJM, Scholten-Peeters WGM, Thoomes-de Graaf M, Duijn E, Ottenheijm RPG, van den Borne MPJ, et al. Current management and prognostic factors in physiotherapy practice for patients with shoulder pain: design of a prospective cohort study. BMC Musculoskelet Disord. 2013 Jan;14:62.

45. Backhaus M, Burmester GR, Gerber T, Grassi W, Machold KP, Swen W a, et al. Guidelines for musculoskeletal ultrasound in rheumatology. Ann Rheum Dis. 2001;60(7):641–9.

46. PJ O. Physical Therapy - Key Component of the Rehabilitation Team. Int Encycl Rehabil. 2010;1–12.

47. Valenza MC, Castro-Martín E, Valenza G, Guirao-Piñeiro M, De-la-Llave-Rincón AI, Fernández-de-las-Peñas C. Comparison of third-year medical and physical therapy students' knowledge of anatomy using the carpal bone test. J Manipulative Physiol Ther. 2012 Feb;35(2):121–6.

48. Cohn D, Benditt JO, Eveloff S, McCool FD. Diaphragm thickening during inspiration. J Appl Physiol. 1997 Jul;83(1):291–6.

49. Ferrari G, De Filippi G, Elia F, Panero F, Volpicelli G, Aprà F. Diaphragm ultrasound as a new index of discontinuation from mechanical ventilation. Crit Ultrasound J. Springer; 2014 Jan 7;6(1):8.

50. Exhibit E, Roriz D, Abreu I, Soares PB, Alves FC. Ultrasound in the evaluation of diaphragm. 2015;1–16.

51. Dyrvig A-K, Kidholm K, Gerke O, Vondeling H. Checklists for external validity: a systematic review. J Eval Clin Pract. 2014 Dec;20(6):857–64.

52. Ozçakar L, Tok F, Kesikburun S, Palamar D, Erden G, Ulaşli A, et al. Musculoskeletal sonography in physical and rehabilitation medicine: results of the first worldwide survey study. Arch Phys Med Rehabil. 2010 Mar 1;91(2):326–31.

Appendix:

I. Invitation letter

Dear students,

With this email, we would like to invite you to participate in our experiment for our Bachelor thesis. We are 5 students from the following study background; 1 speech therapist, 3 physiotherapists and 1 MBRT (radiography, imaging study) student. As a multidisciplinary team we are working together to collect a maximum of data for our research; this is why we need you!!!

The experiment consists of measuring the thickness of your diaphragm muscle using ultrasound imaging. Once the data has been collected, diverse investigations will take place. For instance; checking the reliability of ultrasound imaging between students from different background or, simply establishing standard value of the muscles mentioned above.

The testing will take place in between the time frames of the 19th of October until the 9th November at Fontys Paramedische Hogeschool in the following rooms: 0.312; 0.424. The exact days and time schedule will be communicated to you once the total number of participants is known. We will of course take into consideration your preferences for the time and dates and you will be rewarded with a small gift after the experiment.

If you want to know more about the experiment you can find attached to this email the information letter. In case of other questions concerning the research, do not hesitate to contact one of us.

We would be thankful if you find time to participate in our study.

Kind regards,

Nadia Giampellegrini; Emilie Vallance;Rowaya van der Burgt, Mélanie van Ravels,Miriam ter Stege,

II. Information letter

Dear Participant,

We would like to thank you for volunteering to participate in our study. We are graduate students from physiotherapy, speech and language therapy as well as MBRT (medical imaging and radiation therapy). This document is to provide you some background information on our project. We kindly ask you to read the information given below in order to make an informed decision about whether or not you would like to be part of our project. If there are terms you are unfamiliar with, please do not hesitate to contact us for more information.

The aim of the project:

Lower back pain and respiratory conditions such as COPD (chronic obstructive pulmonary disease) have a cause-effect relationship with the diaphragm. Weak/atrophied muscles or poor activation can often cause breathing as well as lower back complaints, but poor quality function of the muscles can also be due to disease in patients with COPD.

The aim of this research is to investigate the standard thickness values of the diaphragm and the influence of factors such as gender/height using Musculoskeletal Ultrasound in order to create normal values for reference in the future. This imaging technique is becoming more popular in many medical fields; however, the reliability of this tool is still not clearly assessed, such as inter-rater reliability or test-retest reliability. The goal is therefore to use different approaches to test the diagnostic value of this imaging tool and at the same time collecting the data mentioned above. Furthermore, the differences between 2 different ultrasound system devices will be measured as well to see if this affects the diagnostic process.

Who can participate:

We are looking healthy young adults between the ages of 18-28. The requirements to participate in this study are listed below:

- No voice problems or dysphonia
- No lower back complaints in the last 6 months
- No asthma or other respiratory diseases
- No previous known diaphragmatic problems
- No diagnosed neuromuscular diseases
- No stomach pain/cramps or other stomach problems
- Participant should not be pregnant
- No previous caesarean sections or any surgery in the stomach area
- A BMI >30kg/m²

What is expected of you:

If you choose to participate in this study you will have to sign an informed consent before the beginning of data collection. You will be asked to fill in a small questionnaire about, for example your name and age. Your height and weight will also be measured. You will then move to the next station where you will stand against a wall. Here your diaphragm will be imaged using Musculoskeletal Ultrasound. In order to achieve this you will be asked to lift up your shirt to expose your stomach. For the female participants, we advise to wear a bikini top during the measurements. Next, some gel will be applied to the transducer (see image above) which will then be placed just below your ribs. You will be asked to relax, breathe in fully and breathe out all the way. During these 3 moments of the breathing cycle images will be recorded. We ensure you that as much privacy as possible will be provided throughout the study as there will be numerous participants present at the same time. The entire procedure is expected to last 1 hour.

The study has been approved by Fontys University of Applied Sciences and there are no risks involved in this project.

The collected data:

All information and data gathered during the project will be handled confidentially and will remain anonymous throughout. The results of the study will be presented in 5 written reports as there are 5 thesis projects within this study. These 5 reports are part of a bigger study which will probably be published in a few years. You have the reassurance of all research students involved that your name, personal details and data will remain unidentified.

Finally, it is important to remember that taking part in this research project is completely voluntary and you are free to withdraw if necessary. If you have any concerns or further questions concerning our project, please do not hesitate to contact us!

Thank you for your interest and your time!

Sincerely,

Nadia Giampellegrini, Emilie Vallance, Miriam terStege, Rowaya van der Burgt, Mélanie van Ravels,

III. Informed consent

Statement by the participant:

I have read the above mentioned information in from the information letter, or it has been read to me. I had the time and the opportunity to ask questions about it and all of my questions have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

- Print name of participant:
- Signature of participant:
- Date:
  (Day/month/year)

Statement by the researchers:

We confirm that the participant was given the time and opportunity to ask any related questions about the study, and that all of his/her questions were answered to the best of our ability. We confirm that the individual has not been forced into giving consent, but decided to give it voluntarily.

- Print Name of Researcher:
- Signature of Researcher:
- Date:
  (Day/month/year)

IV Research procedure

Below, you will find three pictures in the following order of the breathing cycle: 1) Respiratory Pause; 2)Full inhalation; 3)full exhalation by respectively the 1)Physiotherapist ; 2) Speech therapist; 3) Radiography. These pictures are from participant n=2 ; the pictures were taken at Fontys University of Applied sciences in room 0.424

Image 1 – Breathing cycle: Respiratory Pause by Physiotherapist

As described in the procedure 5mm is taken from the lung shadow following the diaphragm fascia, then a perpendicular line is drawn to measure the diaphragm thickness.

Type of device: The ProSound Alpha 7 from Aloka

Gain 88 (SD: 5), **Depth range** (4-5) **Contrast** 18 (SD: 2)

Image 2- Full Inhalation by Physiotherapist / Same procedure as below

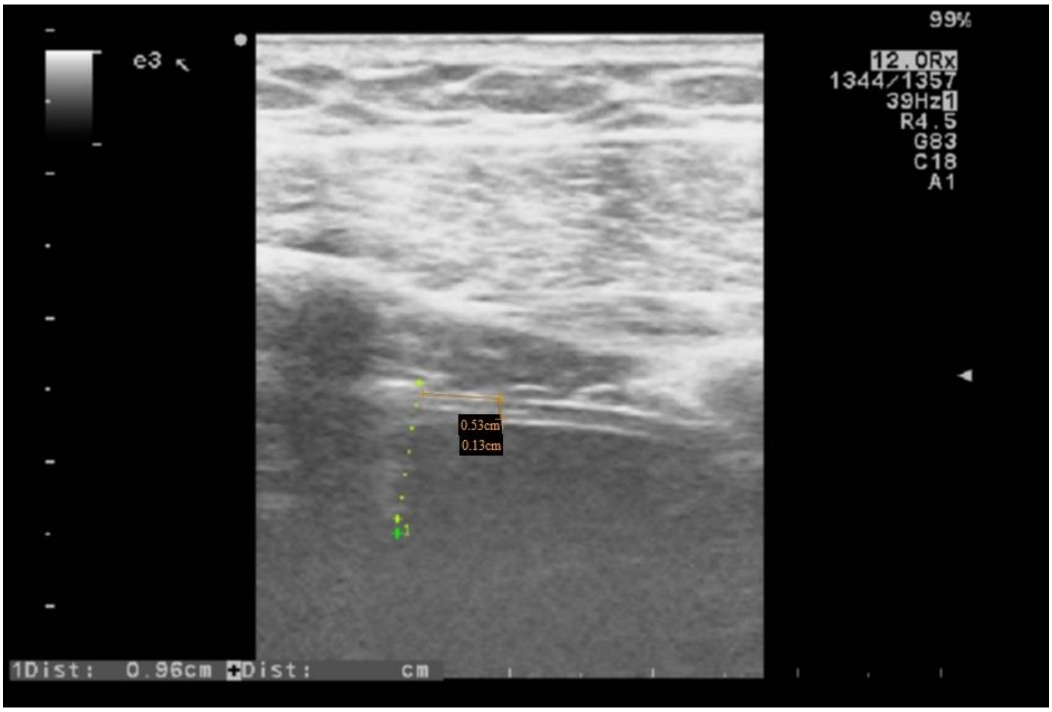


Image 3- Full exhalation by Physiotherapist/

Same procedure as below

Image 4 – Respiratory Pause by Speech therapist

Same procedure as below

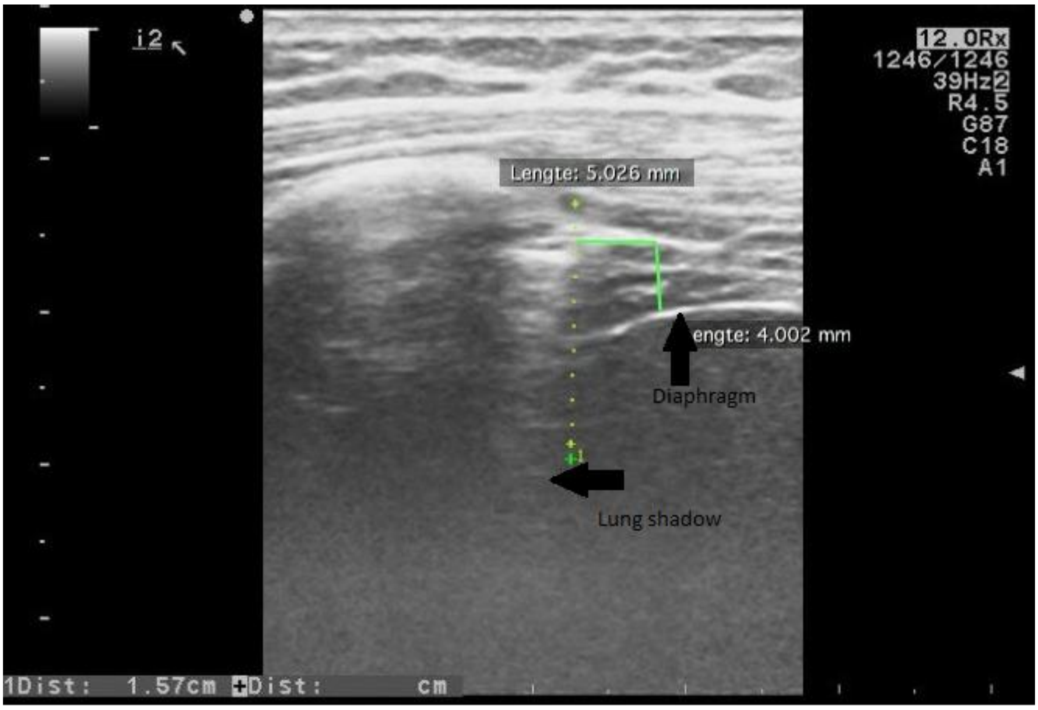


Image 5– Full Inhalation by Speech therapist

Same procedure as below

Image 6 – Full Exhalation by Speech therapist

Same procedure as below

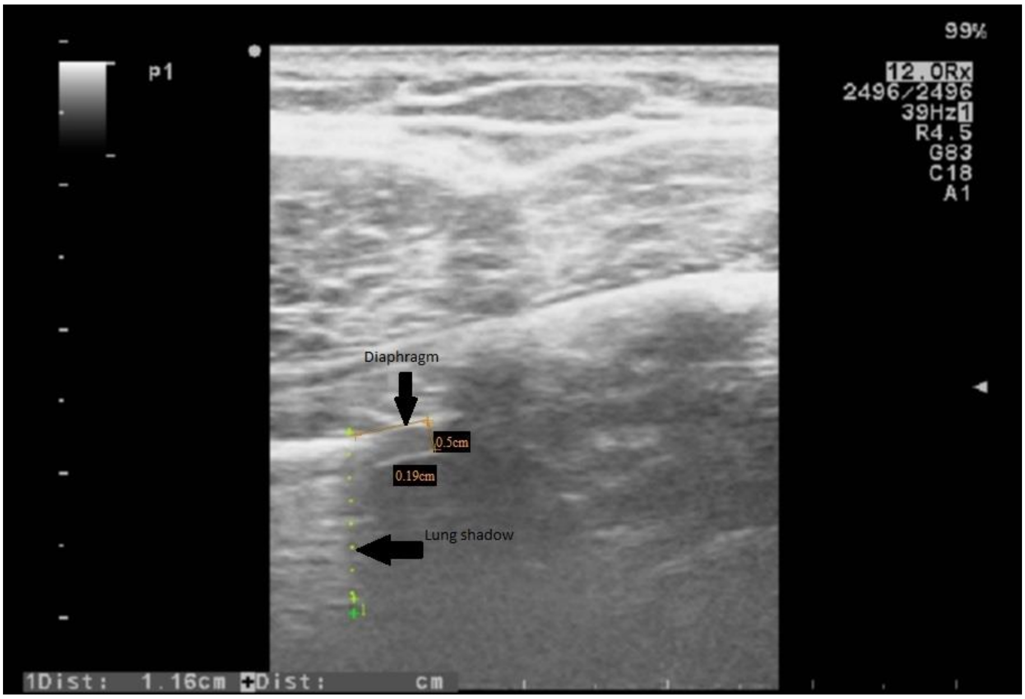


Image 7 – Respiratory Pause by Radiography(MBRT)

Same procedure as below

Image 8 – Full inhalation by MBRT

Same procedure as below

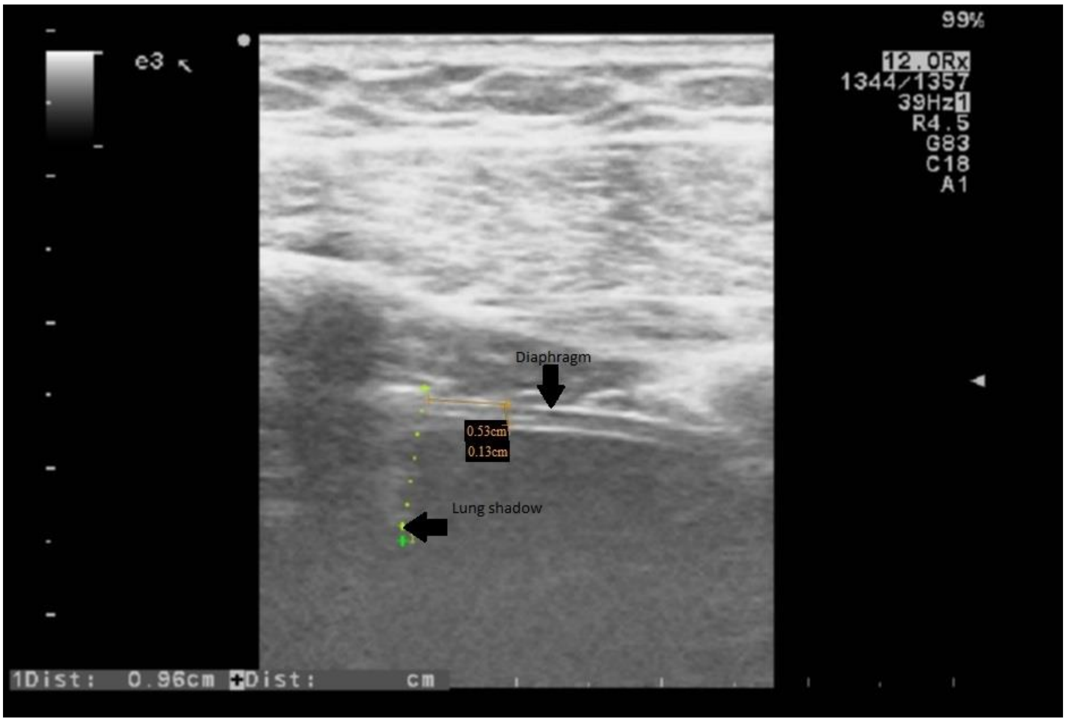


Image 9 – Full exhalation by MBRT

Same procedure as below

V Breathing protocol

Standardized breathing instructions:

One physiotherapist' student will be in charge of giving the instructions and answering all the questions the participant might have.

The step will be as stated below:

1. Have an upright posture against the wall
2. Head slightly elevated
3. Breathe in slightly and then exhale until resting point
4. Hold 3 seconds
5. Inhale maximally until no more air is possible
6. Hold 3 seconds
7. Exhale maximally until no more air comes out
8. Hold 3 seconds.

The participants' safety comes first, if the subjects feel dizzy or out of breath they can take a break and start all over when they feel ready.

The physiotherapist must observe each participant going through the breathing process and ensure that proper breathing is achieved. He/she will look at the following:

1) **Respiratory pause:** The physiotherapist observe the participant's normal breathing pattern without informing him that he/she is observing so that the participants doesn't think about his breathing.
2) **Full inhalation:** The physiotherapist observe the participant's elevation of the ribs, the accessory muscles in the neck and the extension of upper back. The physiotherapist will then encourage the participants to breath in further and further until the maximum is reached.
3) **Full exhalation:** The physiotherapist observe or feel (if needed) the participant's abdominal contraction, and observe if a slight flexion of the trunk is visible

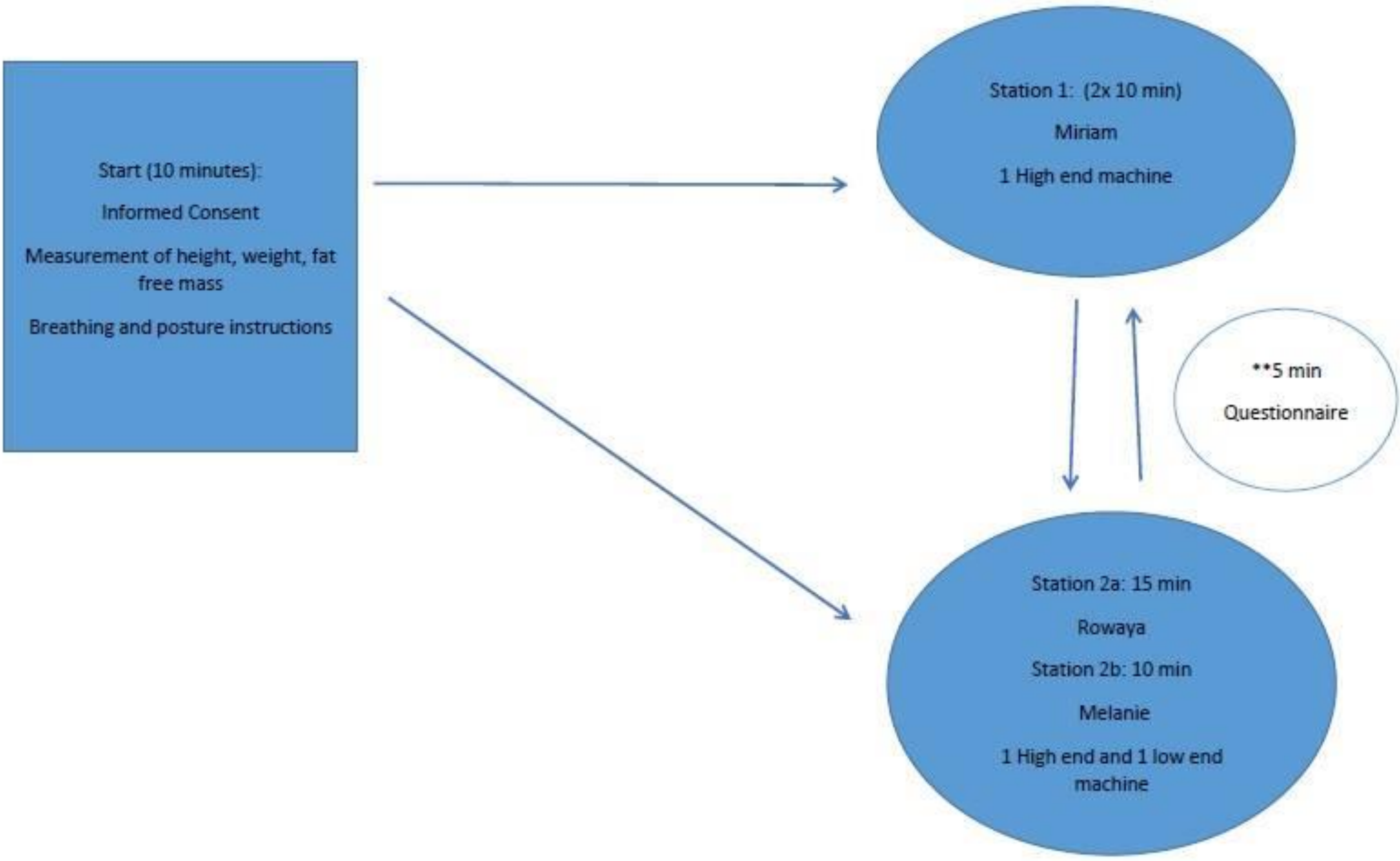
VI Flow of experiment

As mentioned in the plan, 10 students are part of the entire project. Five from which is investigating the diaphragm values. The flow of the experiment is made so that the five students will receive their data needed for their own research.

Flow:

1. Two Participants comes in
2. Informed consent signed and possible questions answered
3. Anthropometric data collection of both participants
4. Respiratory and postural instructions (Respiratory pause, Full inhalation, Full exhalation): see below
5. Participants receive a sticker with an ID (ADMSU 1, 2..3….4ect)
6. Participants receive instructions written to know where to go and a questionnaire (see Appendix IV) to fill in during the 5min waiting time between stations**, the 5min will allow them a small break to recover.
7. First participant goes to : Station **1** (10min) ; *wait 5min**;* Station **2a** (15min); Station **1** (10min);Station **2b**
8. At the same time, the second participant goes to : Station **2a** (15min); Station **1** (10min);*wait 5min**;* Station **2b** ; Station **1**
9. Participants receive a chocolate bar as a gift for their participation and patience
10. **All this will be done within an hour**

Explanations: The flow has been decided within the group so that everyone is able to receive their measurements needed for their own research. Station 1 needs two measurements since test-retest is measured, Station 2a takes 15min since the MBRT student measures with one high-end quality device and one low-end quality. Only two high-end devices are available during the data collection, which is why only 2 stations could be created.



Participant Schedule

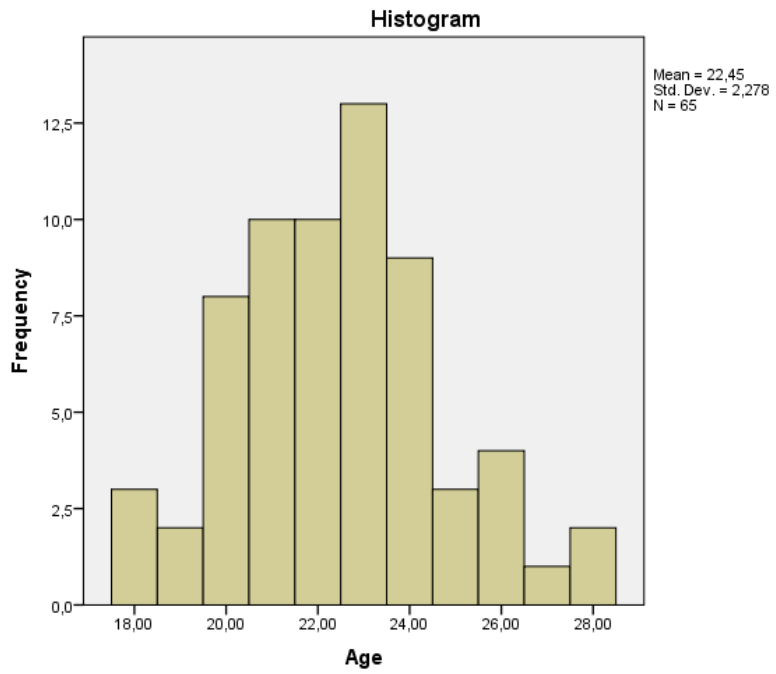
		Date to be confirmed	
Time:		Name participant 1:	Name Participant 2:
8.55	9.50	ADMSU1	ADMSU2
9.40	10.35	ADMSU3	ADMSU4
10.25	11.20	ADMSU5	ADMSU6
11.10	12.05	ADMSU 7	ADMSU8
11.55	12.50	ADMSU 9	ADMSU 10
12.50	13.20	Lunchbreak	
13.20	14.15	ADMSU11	ADMSU12
14.05	15.00	ADMSU13	ADMSU14
14.50	15.45	ADMSU15	ADMSU16
15.35	16.30	ADMSU17	ADMSU18
16.20	17.15	ADMSU19	ADMSU20
17.05	18.00	ADMSU21	ADMSU22
17.50	18.45	ADMSU23	ADMSU24

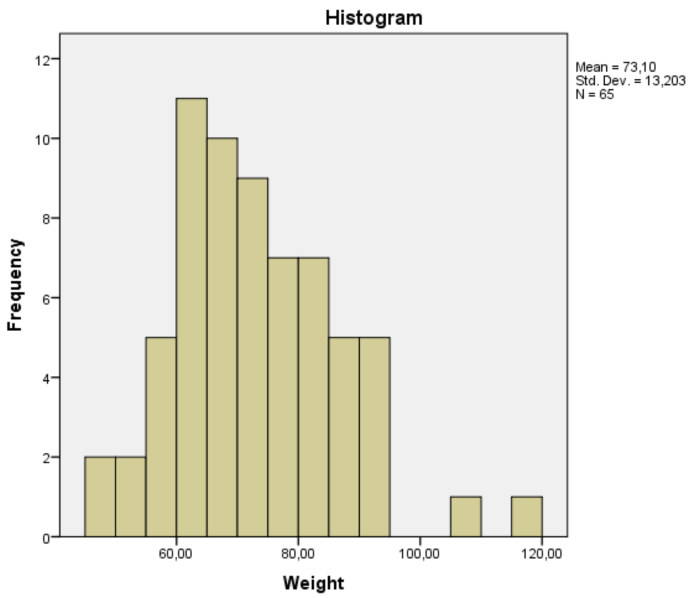
Appendix VII

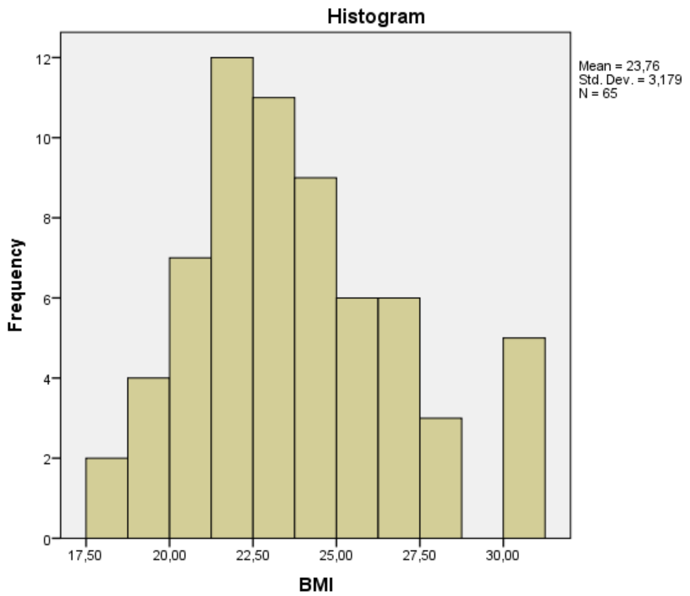
Shapiro-Wilk test of normalcy (p<0.05) for demographic data:

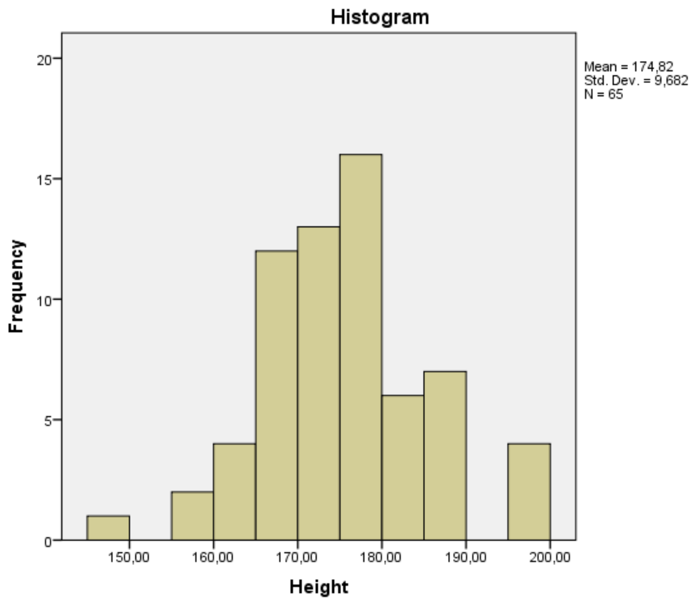
Table 1: Tests of Normality

	SHAPIRO-WILK TEST
Age	0.121
Weight	0.155
Height	0.297
BMI	0.059









Appendix VII

Respiratory pause

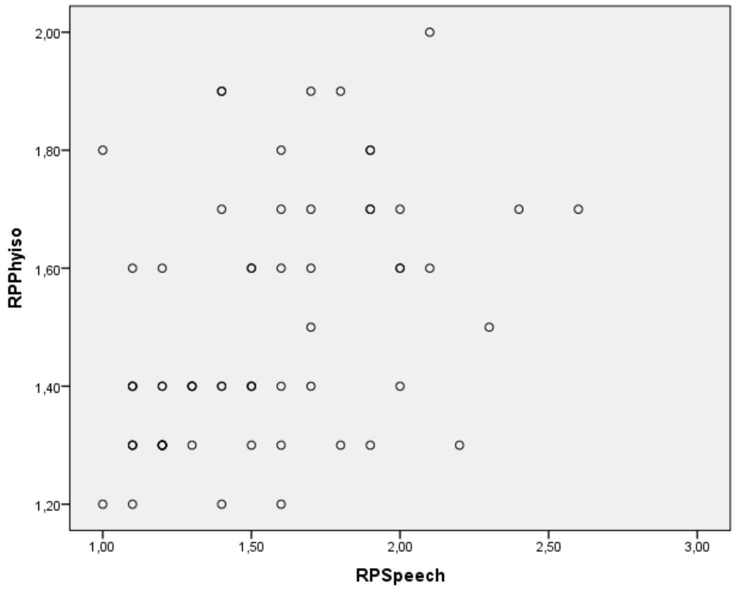


Figure 1:
Scatter plot comparing Physiotherapist (PT) versus (VS) Speech therapist (ST) in the breathing cycle of: Respiratory pause

RPPhysio→ Respiratory Pause Physiotherapist

RPSpeech → Respiratory Pause Speech therapist

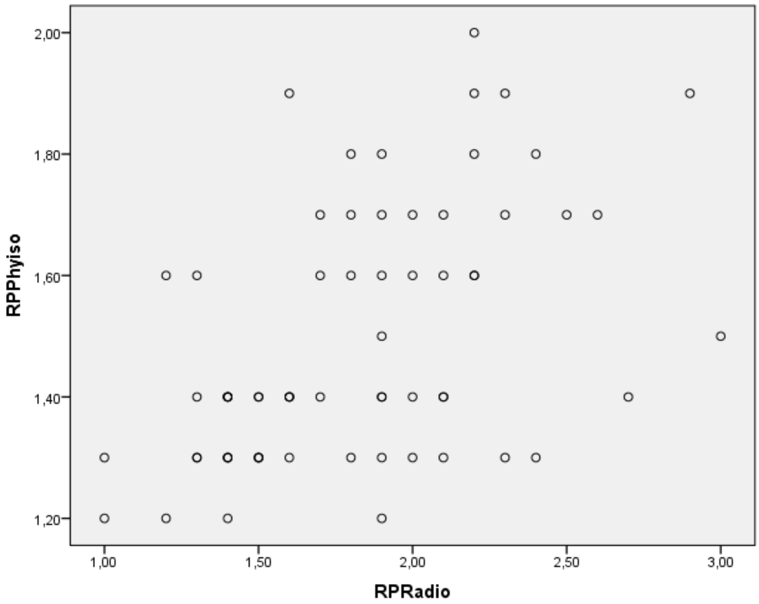


Figure 2:

Scatter plot comparing PT VS Radiologist assistant (MBRT) in the breathing cycle of Respiratory pause

RPRadio → Respiratory Pause Radiologist assistant

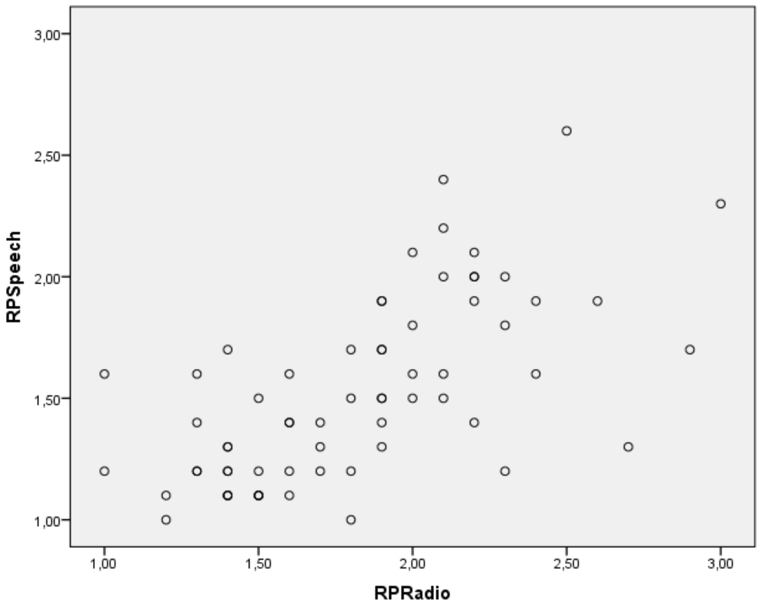


Figure 3

Scatter plot comparing ST vs MBRT in the breathing cycle of Respiratory Pause

Full inhalation

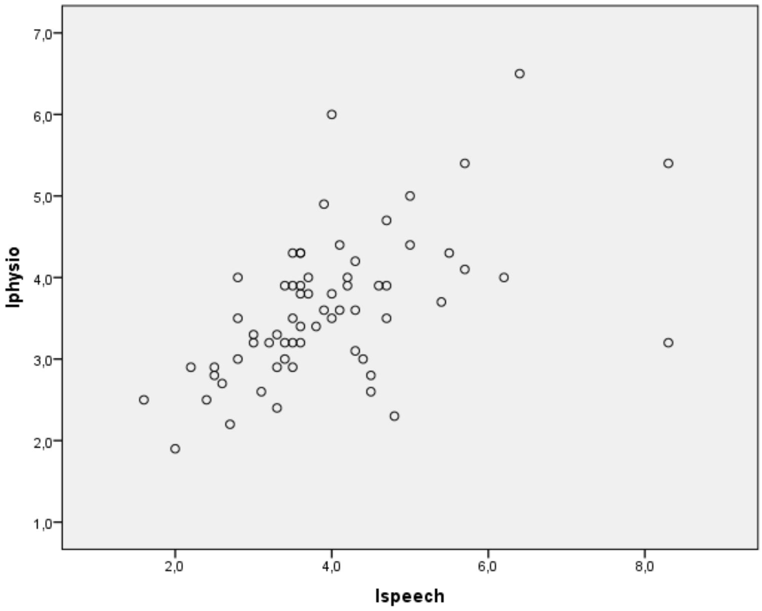


Figure 4

Scatter plot comparing PT VS ST in the breathing cycle of: Full inhalation

Iphysio → Full **I**nhalation Physiotherapist

Ispeech → Full **I**nhalation Speech therapist

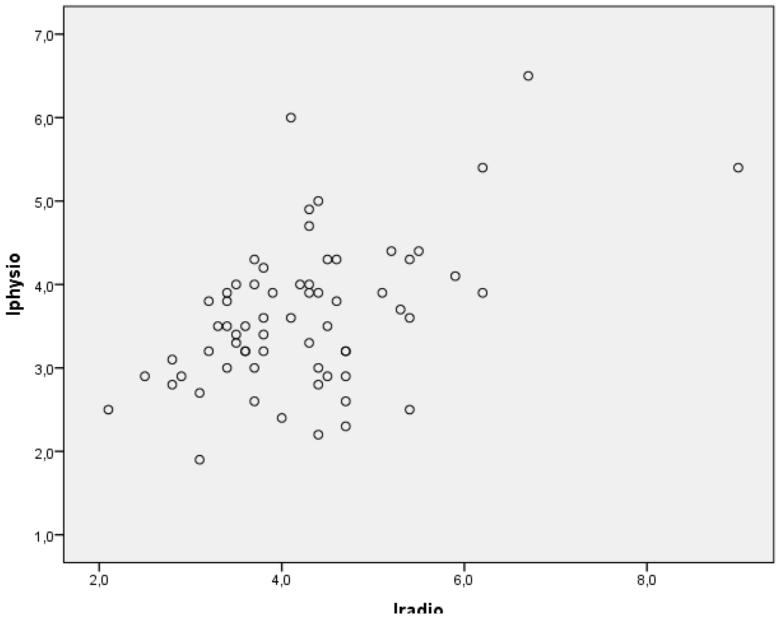


Figure 5

Scatter plot comparing PT VS MBRT in the breathing cycle of full inhalation

Iradio → Full **I**nhalation Radiologist assistant

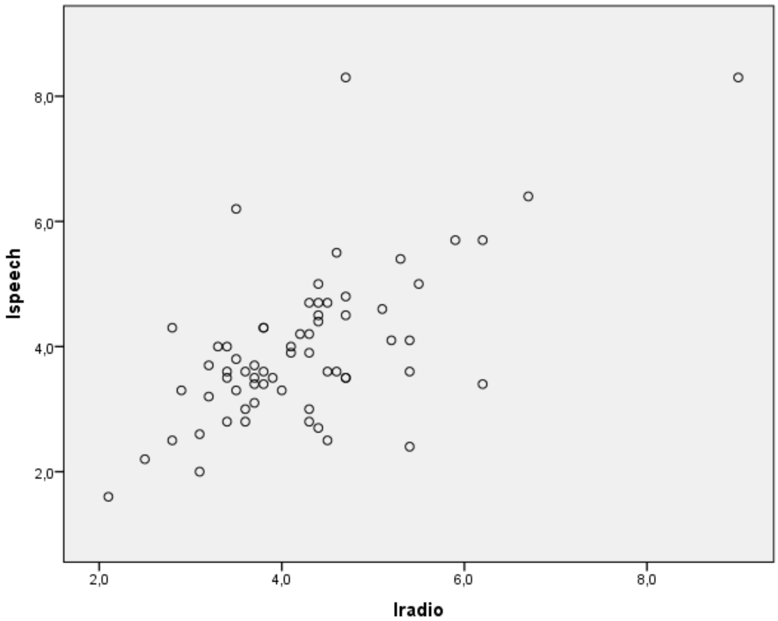


Figure 6

Scatter plot comparing ST VS MBRT in the breathing cycle of: Full inhalation

Full exhalation

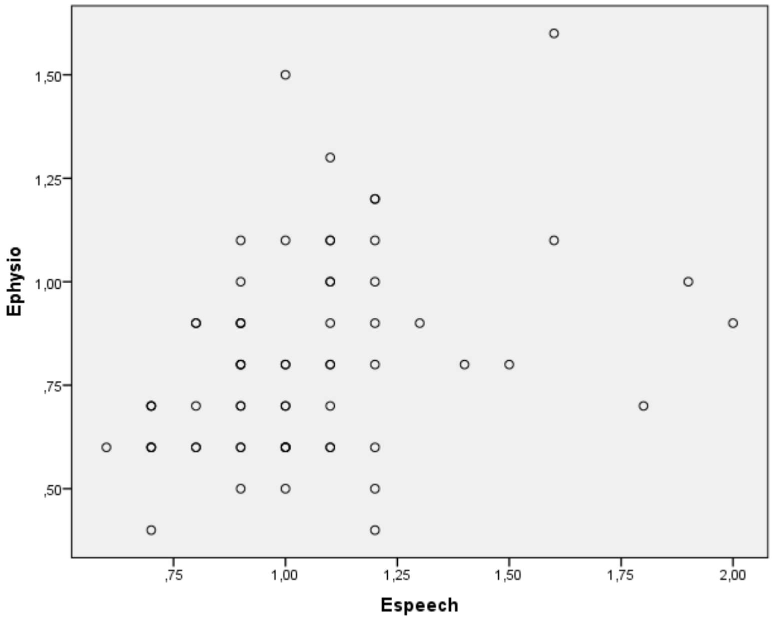


Figure 7

Scatter plot comparing the PT VS ST in the breathing cycle of full exhalation

Ephysio → Full **E**xhalation Physiotherapist

Espeech → Full **E**xhalation Speech therapist

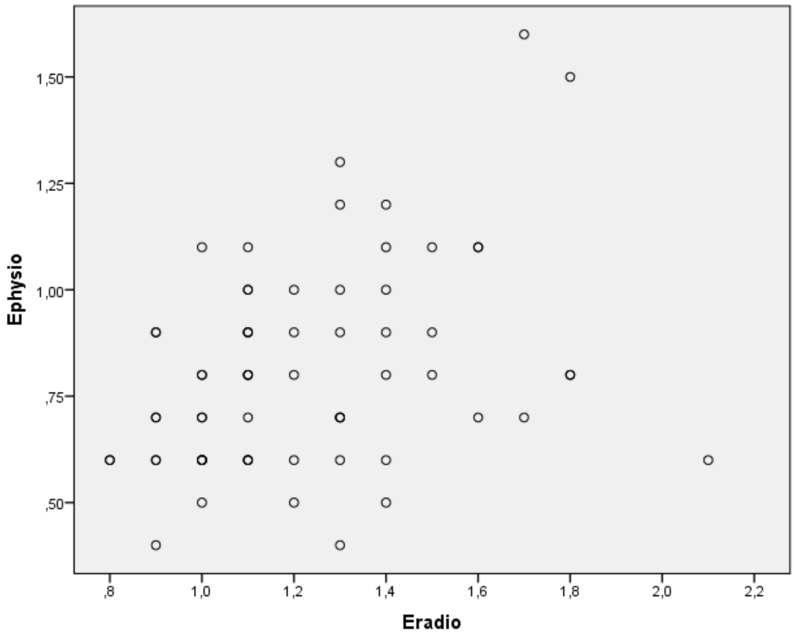


Figure 8

Scatter plot comparing the PT and MBRT in the breathing cycle of Full Exhalation

Eradio → Full **E**xhalation Radiologist assistant

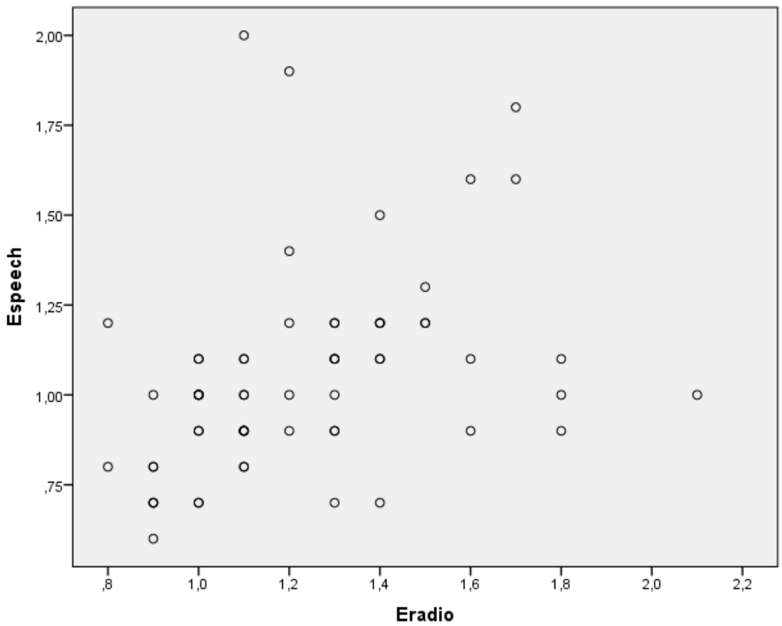


Figure 9

Scatter plot comparing the ST vs MBRT in the breathing cycle of full exhalation

Appendix IX

Fontys Paramedische Hogeschool

B8. Confidentiality statement

Name: Vallance Emilie Student No°: 2199824

Title:

Inter-rater reliability analysis of the diaphregm thikness with musculoskeletal ultrasound imaging during the 3 phases of the breathing cycle

Content (description):

1. By signing this Statement, the Fontys Paramedic University of Applied Sciences in Eindhoven commits itself to keep any information concerning provided data and results obtained on the basis of research of which is taken cognizance as part of the above practical research project and of which it is known or can be reasonably understood that said information is to be considered secret or confidential, in the strictest confidence.
2. This confidentiality requirement also applies to the employees of the Fontys Paramedic University of Applied Sciences, as well as to others who by virtue of their function have access to or have taken cognizance of the aforesaid information in any way.
3. The above notwithstanding, the student will be able to perform the practical research project in accordance with the statutory rules and regulations.

Student:

Name: Vallance Emilie

(signature) Date: 30/11/2015

Supervisor:

Name:

(signature) Date: 30/11/2015

Coordinator: for receipt

Name: ____________

(signature) Date: __/__/____

Appendix X

Fontys Paramedische Hogeschool

B9. Conveyance of Rights Agreement

AGREEMENT

Pertaining to the conveyance of rights and the obligation to
convey/return data, software and other means

The undersigned:

1. Mr/Ms Emilie Vallance
[*full name as stated in passport*], residing at 5623 Ex Eindhoven
[*postal code, place of residence*] at the Raedeckstraat 14
[*street and house number*], hereinafter to be called "**Student**"

and

2. Fontys Institute trading under the name Fontys University of Applied Sciences, Rachelsmolen 1, 5612 MA Eindhoven, hereinafter to be called "**Fontys**"

CONSIDERATION

A. Student is studying at the Fontys Paramedic University of Applied Sciences in Eindhoven and is performing or will perform (various) activities as part of his/her studies, whether or not together with third parties and/or commissioned by third parties, as part of research supervised by the lectureship of Fontys Paramedic University of Applied Sciences. The aforesaid activities will hereinafter be called "**Lectureship Study Activities**". At the time of the signing of this Statement, the Lectureship of Fontys Paramedic University of Applied Sciences supervises in any case the studies listed in Appendix 1, but this list is not an exhaustive one and may change in the future.

B. It is of essential importance to Fontys Paramedic University of Applied Sciences that (the results of) the Lectureship Study Activities can be further developed and applied without any restriction by Fontys Paramedic University of Applied Sciences and/or used for the education of other students. Fontys wishes in any event – but not exclusively – (i) to be able to share with and/or convey to third parties (the results of) the Lectureship Study Activities, (ii) to publish these under its own name, where the Student may be named as co-author providing that this is reasonable under the circumstances, (iii) to be able to use these as a basis for new research projects.

C. In case intellectual ownership rights and/or related claims on the part of Student will be/are attached to (the results of) the Lectureship Study Activities, parties wish – taking into account that which was mentioned under (B) – Fontys Paramedic University of Applied Sciences to be the only claimant with regard to said rights and claims. The Student therefore wishes to convey all his/her current and future intellectual property rights as well as related claims concerning (results of) the Lectureship Study Activities to Fontys, subject to conditions to be specified hereafter;

D. Student furthermore wishes to enter into the obligation – again taking into account that which was mentioned under (B) – to convey all data collected by him/her as part of the (results of) the Lectureship Study Activities to Fontys and not to retain any copies thereof, and also to return all data, software and/or other means previously provided by Fontys as part of (the results of) the Lectureship Study Activities, such as measuring and testing equipment, to Fontys without retaining copies thereof, all the above being subject to conditions to be specified hereafter.

AGREE THE FOLLOWING

1. Conveyance of intellectual property rights

1.1 Student herewith conveys to the Fontys Paramedic University of Applied Sciences all his/her current and future intellectual property rights and related claims concerning (the results of) the Lectureship Study Activities, for the full term of these rights.

1.2 Intellectual property rights and/or related claims are understood to refer to, in any case – but not limited to – copyright, data bank law, patent law, trademark law, trade name law, designs and model rights, plant breeder's rights, the protection of know-how and protection against unfair competition.

1.3 The conveyance described under 1.1 shall be without restriction. As such, the aforesaid conveyance shall include all competences related to the conveyed rights and claims, and said conveyance shall apply to all countries worldwide.

1.4 Insofar as any national law requires any further cooperation on the part of Student for the conveyance mentioned under 1.1, Student will immediately and without reservation lend such cooperation at first request by Fontys Paramedic University of Applied Sciences

1.5 Fontys accepts the conveyance described under 1.1.

2. Waiver of personal rights

2.1 Insofar as permitted under article 25 'Copyright' and any other national laws that may apply, Student waives his/her personal rights, including – but not limited to – the right to mention Student's name and the right to oppose any changes to (the results of) the Lectureship Study Activities. If and insofar as Student can claim personality rights pursuant to any national laws notwithstanding the above, Student will not appeal to said personality rights on unreasonable grounds.

2.2 In deviation from that which was stipulated under 2.1, the Fontys Paramedic University of Applied Sciences may decide to mention the name of Student if this is reasonable in view of the extent of his/her contribution and activities.

3. *Compensation*

Student agrees that he/she will receive no compensation for the conveyance and waiver of rights as described in this Statement.

4. *Guarantee concerning intellectual property rights*

Student declares that he/she is entitled to the aforesaid conveyance and waiver, and declares that he/she has not granted or will grant in future, license(s) for the use of (the results of) the Lectureship Study Activities in any way to any third party/parties. Student indemnifies Fontys from any claims by third parties within this context.

5. *Obligation to convey/return data, software and other means*

5.1 At such a time as Student is no longer performing any Lectureship Study Activities and/or is no longer a student at Fontys, Student is obliged to convey to Fontys all data, in the widest sense of the word, collected by him/her as part of (results of) the Lectureship Study Activities, including – but not limited to – studies and research results, interim notes, documents, images, drawings, models, prototypes, specifications, production methods, process descriptions and technique descriptions.

5.2 Student guarantees not to have kept any copies in any way or form of the data meant under 5.1.

5.3 Student is obliged to return to Fontys all data, software and other means provided to him/her by Fontys as part of the Lectureship Study Activities, and guarantees not to have kept copies in any way or in any form, of the provided software and/or other means.

5.4 Student agrees that if he acts and/or proves to have acted contrary to the obligations mentioned under 5.1 up to and including 5.3, (a) he/she shall be liable for all and any damages incurred or to be incurred by Fontys, and (b) that this will qualify as fraud and that Fontys can apply the appropriate sanctions hereto. The sanctions to be applied by Fontys may consist of, among other things, the denying of study credits, the temporary exclusion of the Undersigned from participation in examinations, but also the definitive removal of the registration of the Undersigned as a student at Fontys.

Fontys Paramedische Hogeschool

6. *Waiver*

Student waives the right to terminate this Agreement.

7. *Further stipulations*

7.1 Insofar as this Agreement deviates from the Student Statute, this Agreement shall prevail.

7.2 This Agreement is subject to Dutch law. All disputes resulting from this statement will be brought before the competent judge in Amsterdam.

Student:

Name: Emilie Vallance

(signature)

Date: 30/11/2015

Place: Eindhoven

Fontys Institute

trading under the name Fontys Hogescholen

Supervisor:

Name:

(signature)

Date: 30/11/2015

Place: EINDHOVEN

I, Ms. M.H. de Waard, sworn translator for the English language registered at the Court in Groningen, the Netherlands, and registered in the Dutch Register of Sworn Translators and Interpreters (Rbtv) under nr. 2202, herewith certify the above to be a true and faithful translation of the attached Dutch document into the English language.

Groningen, 23 May 2012,

[M.H. de Waard]