Do age, gender, height and fat-free mass index relate to the thickness of the diaphragm in healthy young adults?

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Preface

This study is a quantitative research, performed at the Fontys University of Applied Sciences in Eindhoven. In addition to giving insight into a specific research field, it is also a final project of the graduation year in physiotherapy, aiming to achieve the level of a Physiotherapy Bachelor Program.

Through this 20-week period, I could count on the help and support of many people, whom I would like to thank.

To start off, I would like to thank Marc Schmitz warmly (my tutor throughout this period) for his support, his feedback, his so-called ‘food for thought’ and for his guidance toward a finished product.

Then I would also like to thank Madelon Pijnenburg for taking the time to explain and answer possible questions in statistics, which was more than needed at times, as well as, all the workers in Fontys University who helped organizing and providing the materials and rooms needed to perform this study.

Finally, I’d like to say thank you to my ‘diaphragm team’, Emilie Vallance, Mélanie van Ravels, Miriam ter Stege and Rowaya van der Burgt for working together and keeping the positive and productive attitude. Not to forget my fellow students and friends who supported me throughout the entire period and on whom I could count for constructive feedback.
1. Abstract

BACKGROUND: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death world-wide, representing 6% of the global mortalities in 2012, even though remaining, in some countries, partly undiagnosed. Musculoskeletal ultrasound (MSU) provides an affordable and accessible tool for clinical practice. Reporting diaphragm thickness values in healthy young adults and furthermore, the effects of age, gender, height and fat-free mass index on the diaphragm, this study focuses on standard values and possible correlations, using MSU. This could be of clinical relevance when comparing to findings from COPD-patients, in future clinical practice.

METHODS: 83 healthy young adults, 18 to 28 years of age, participated in this study. The right diaphragm thickness was examined with the use of MSU. Three images were taken for each individual and inserted into SPSS. The Spearman’s rho as well as the Mann Whitney-U were then used as correlation tests for further results.

RESULTS: The results showed no positive, nor negative correlation between the age and diaphragm thickness. Furthermore, a low positive correlation for the height and the fat-free mass index as well as a significant relation with gender, were found.

CONCLUSION: Further research is necessary in order to relate the standard reference values of the diaphragm thickness of healthy young adults to COPD-patients. The found correlations could indicate an implementation of MSU in a COPD specific clinical setting, with the aim of a future comparison of results between healthy and COPD individuals for earlier diagnosis and an increased quality of follow-up sessions.

KEYWORDS: MSU; diaphragm thickness; anthropometric data; clinical practice; COPD
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2. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of death world-wide. In 2012, over 6 million people died from this disease, representing a total amount of 6% of the global deaths.\(^1\) This disease is classified in the Global Initiative on Obstructive Lung Disease (GOLD) into four stages of increasing severity, which range from GOLD I being mild to GOLD IV being severe.\(^2\) The first stages of COPD tend to be the most difficult to diagnose and awareness of early symptoms should be promoted.\(^3\) In many countries, such as Japan, Korea, United States or Sweden, the underdiagnosis of COPD is marked by the difference between the existing prevalence and the rather low one in national surveys, which are often based on self-declared diagnosis.\(^4\) Even though, treatments exist to slow down the progression of the disease, it is not yet possible to be cured.\(^1\) The amount of undiagnosed people shows the importance of early detection of COPD. A study from 2011 written by David Price at al.\(^3\) concluded that early findings of COPD in addition to a diagnosis, maintenance treatment, stopping smoking and exercising may be the best way to control its symptoms as well as the progression and the outcome of the disease.

COPD does not only affect the lungs but also the diaphragm which acts as a respiratory muscle.\(^5\) In fact, the thickness of the cross-sectional area of the diaphragm is directly related to the effects of the disease.\(^10\) Additionally to playing an important role in respiration, the diaphragm is also responsible of the expectoration of waste to clear the airways, controlled by the phrenic nerve.\(^6\) The organ is situated between the thorax and the abdomen. Its outer part consists of muscles and the inner layers of tendons, which gives it its typical dome-shaped structure.\(^7\) During inhalation, the diaphragm flattens, the central tendon moves caudally which increases the space for the lungs to expand.\(^8\) During exhalation, the diaphragm gets back in its dome-shape position and thus decreases the space for the lungs and its content.

Several studies\(^8\)(\(^9\))(\(^10\)) have described a good method of measuring the diaphragm structure and thickness by using musculoskeletal ultrasound (MSU). MSU was primarily used in the radiology department, while physiotherapists used it more for its benefits in tissue repair. MSU is now frequently used in physiotherapy as a diagnostic tool, because of its affordability and the comfort it provides in transporting it everywhere it's needed.\(^11\) One of its many advantages is, that, unlike CT-scans, it is non-invasive and the patient is not exposed to radiation. Furthermore, it is a very direct way of assessing and diagnosing, since the image is captured in real time and feedback for the patient is possible. Visual feedback is another advantage, since the physiotherapist can interact with the patient while teaching to activate a certain target muscle using the screen.\(^11\) The real-time ultrasound consequently not only provides information about injured structures but also about the activity of a muscle, even if it has a small diameter or is situated in a deeper layer.\(^12\)

Different ultrasound modes are available for screening, but knowing the relationship between COPD and the diaphragm, the B-mode (brightness mode) is ideal for investigating its thickness and general structure.\(^9\)(\(^13\)) J. Ueki et al (\(^14\)) confirms the advantages of using the B-mode ultrasonography, while assessing the diaphragm thickness at rest.
While physiotherapists embrace this diagnostic tool (12), easy collectable and measurable anthropometric data in a clinical setting, such as age, gender, height or fat-free mass index (FFMI) could help in observing early changes in diaphragm thickness.

Caskey’s study (15), shows that thickness does not significantly change as the years increase when assessing people between 30 and 80 years of age. However, no research has yet reported about possible thickness changes in young adulthood. Exposure to tobacco smoke and to repeated lung infections during childhood and adolescence may lead to a decreased lung function. (16) Literature indicates that COPD affects the body decades before the first symptoms arise. (16) This functional reduction may, hypothetically, lead to a visible change in diaphragm thickness on MSU.

As for the gender, a study using the B-mode MSU on a minimum of ten people per gender over six decades (20-80 years of age), shows that men tend to have a higher resting diaphragm thickness than women. (8) A reason to investigate even further would be to understand the reasons for a gender difference and if it is still applicable when the age span is reduced, but the sample per gender is increased.

Furthermore, the FFMI of individuals is a predictor of the severity of COPD (17) and thus, is used to forecast mortality in this specific patient group. (18) Based on these facts, Andrea Smargiassi et al. (19), explained in his article that individuals suffering from COPD with a muscle wasting and a decreased fat-free mass had variations in the mass, area and thickness of the diaphragm. Since the atrophy of muscles is a result of an inequality between the synthesis and degradation of proteins because of the deconditioning (20), a reduction of 30% of the predicted body weight is related to a decrease in diaphragm mass. (21)

Unfortunately, no article has yet reported about the relationship between the height and the thickness of the diaphragm. Because height is a very accessible data and often measured as a standard procedure, its effects on the diaphragm thickness can be assessed in a clinical setting. This could enable future comparisons with the diaphragms of COPD-patients with the same height, in order to investigate for an earlier diagnosis.

Hence a future goal for clinical practice would be to compare the diaphragm thickness of healthy people with patients suffering from COPD, when assessed under the above mentioned FFMI, age, gender and height variables.

In order to achieve this level of diagnosing, the primary goal of this research paper is to investigate, on previously measured norm values with MSU, if the diaphragm thickness is related to age, gender, height and FFMI in healthy young adults.
3. Method section

3.1 General information and study design

The aim of this experimental research was to establish, with the help of an ultrasound device, if the thickness of the diaphragm is related to age, gender, height and FFMI. A multidisciplinary team consisting of three physiotherapists, one speech therapist and one MBRT student, performed a quantitative data collection. All of the participants were invited into a practical room at Fontys, University of Applied Sciences, where the examinations were performed on several consecutive days.

3.2 Participants

For choosing the amount of participants, previous literature was first looked at. In Andrea J. Boons’ study (10), the diaphragm thickness of 150 healthy subjects was examined. Additionally, in Michael R. Barias’ study (9), 50 people suffering from COPD and 150 healthy subjects were asked to participate. Hence a research measuring standard reference values should have an amount of approximately 150 participants to be reliable. This amount was tried to be reached, however, it was most probably unrealistic. Since the team consisted of 5 students, who all had their own measurements to take, it was not probable to include so many participants in our time frame if the quality of the research was to be insured. Students from Fontys University of Applied Sciences, studying in the speech and language department, MBRT (radiology and echography department) and the physiotherapy department, as well as external young adults were contacted and informed about this research. They were invited verbally in the corridors as well as by e-mail with, as appendix, the information letter (see appendix I and II). Additionally, a social media event was created, in order to invite as many people as possible with eventually graduated students.

Participants included in this research were:
- healthy young adults
- aged between 18 and 28: Keeping the participant’s age population in a close range excluded a too high variability in diaphragm motion.(22)

The exclusion criteria looked as follows¹:
- 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 creates a force

¹ Additional exclusions in the appendix II (information letter) are not relevant for this research paper.
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.(23)

- **Paralysis and weakness of the diaphragm**: The symptoms of diaphragmatic paralysis or severe weakness of its muscles often remain unnoticed. However, difficulty breathing while lying on the back, headaches during the mornings, difficulties sleeping or tiredness throughout the day could be possible symptoms. In contrary to a normal functioning diaphragm, the abdominal wall moves inward during inspiration.(24)

- **Diagnosed neuromuscular diseases**: Neuromuscular disorders can affect the respiratory system and its function. More precisely, myopathic and neurophatic diseases can target the diaphragm which provoke weakness of its muscles.(25)

- **Pregnancy**: Pregnancy influences the diaphragm for the reason that the womb might take more space in the abdomen. This leads to a reduced space for the diaphragm to enlarge the lungs.(26)

- **Previous diaphragm surgery**: Cardiac, thoracic or abdominal surgeries can have an effect on the adequate functioning of the respiratory muscles. In fact, research states that surgery can be the cause of volume displacements between the abdomen and the thorax. Hence, the natural curvature of the diaphragm is changed which results in a decreased generation in pressure during respiration.(27)

### 3.3 Ethics

During this research, only healthy subjects participated and no one was harmed or put to risks. The procedure was pain-free and as stated before, the ultrasound is a non-invasive procedure. In addition to that, not more than one hour was needed to collect all the necessary data for each participant. This is why it is a non WMO obligated study. All the data was collected in respect to the confidentiality obligations and handled in anonymity. The participants were informed about the correct procedure of the experiment and about what is expected of them. However, everyone was free to leave the experiment at any given time without being forced to have a reason or to warn the assessors beforehand. These details are also mentioned again in the informed consent, which had to be signed by the participant.
3.4 Materials

The materials listed below are the equipment used during the measuring days and provided by the same institution, from the department of physiotherapy and the MBRT.

The ultrasound device needed, was available at the Fontys University of Applied Sciences. This high-end ultrasound machine, called Aloka Prosound Alpha 7 (28) (produced in Germany; year 2012) was used for the measurements. A linear-array transducer of 8-12 MHz was also used, because it provides along with a 2-dimensional steering also focusing, an increased inspection velocity and a higher resolution (29).

Some of the data used for the population description and the correlation variables, such as the height, weight and the fat-free mass index, was collected using a measuring tape (metric) and the TANITA-device (Body composition analyser; Version: BC-420MA) (30). The latter is stored in Fontys University of Applied Sciences and could be reserved for the specific testing days. The secondary equipment used were:
- paper sheets to wipe away the gel after the collection of the data
- screens to put in between the different ultrasound devices to preserve the participants’ privacy
- a big room for a warm and welcoming atmosphere as well as more privacy
- new USB sticks to save the selected ultrasound images since the ultrasound devices weren’t programmed with an anti-virus software.
3.5 Procedure and Protocols

Procedure:

Step 1:
Two participants came in at the same time and one assessor made sure that the information about the experimental part was clear and that no questions were left.
Then the informed consent needed to be signed, which stated that all the information about this study had been read and that they have the right to leave the room at any time (see appendix III).

Step 2:
The collection of the anthropometric data began with filling in a small questionnaire (see appendix IV). After indicating the gender and age, the participants were subsequently asked to take off their shoes and socks and stand straight with their back and heels touching the wall in order to measure their height with a regular measuring tape. To round of the anthropometric data collection, they stepped on the TANITA device which measured the weight and the fat percentage. To be able to calculate the FFMI, the following formula by Y. Schutz (31) was used:
\[ \text{BMI} = \text{FMI} + \text{FFMI} \]
\[ \text{FFMI} = \text{BMI} - \text{FMI} \]

Step 3:
The subjects received a sticker with an ID (DMSU 1, 2, 3, 4 etc). The protocol (depicted in the next sub-heading), was then explained before the start of the measurements.

Step 4:
Each participant was led to an ultrasound device. The data of the diaphragm thickness, was measured by a speech therapy student, in order for the author of this research to remain blinded.
Protocol and standardization:

The researchers had a total amount of 20 practice hours before starting the data collection. This was essential in order to get acquainted with the ultrasound device and to learn depicting an ultrasound image. On the 30th of September, an external physiotherapist specialized in MSU flew over from Italy to teach the basics of ultrasound and to discuss the standardization procedure. The researchers then practiced and gave feedback to each other during the following two weeks, in order to progress in measuring the diaphragm thickness.

The assessment was done, using the B-mode, because it is known for its assessment of echogenicity and thickness as well as its real-time imaging. Additionally, a high frequency mode was used (12 MHz) with the transducer placed between the mid and anterior axillary line, giving an intercostal view (Figure 1). During the examination, the right diaphragm was measured, since it is positioned just over the liver and thus more easily visible than the left side where the spleen offers less space for visualization.

Furthermore, research states that the transducer needs to be held in a perpendicular angle to the skin in order for the echo to be entirely reflected towards the transducer again, hence producing a high quality image. However, in the case of the diaphragm, the moving ribs prevented the assessor to hold the transducer in a 90° angle, as seen during the practice hours. To maintain a high quality of the image, it was important to have full contact of the transducer with the skin. To find out the region where to apply the transducer on the skin, some research has been done. From literature, it seems that a distinct costal segment between the seventh and eighth or eighth and ninth rib is used, as for example in the study of A. Sarwal et al. Nevertheless, since the diaphragm moves cranially and caudally, depending if it is an expiration or an inspiration, a more reliable way for our team to standardize the measurement was with the help of the lung-shadow. Following it, it moved the transducer away from the seventh, eighth and ninth ribs. Furthermore, the spot where the thickness was taken gave a visualization of both the pleural and peritoneal membranes.

Finally, the pictures were taken during the respiratory pause. The diaphragm is mostly at rest during quiet exhalation, which enabled high quality measurements for the standard values. In order to standardize the respiratory pause between the team members, we agreed to:
- first let the participant take a couple of normal breaths
- advise the participant not to focus on the breathing
- after the next inhale, the participant just exhales quietly (not completely) and holds his breath for 3 seconds.

The pictures were then coded and saved on a USB-stick.
Since the speech therapy student performed the ultrasound of the participants, she also measured the thickness on her laptop after the testing period. For that she used the OSIRIX program on her Macbook Pro, which enabled her to measure the diaphragm thickness of the collected images without having to use the ultrasound device.

The position of the participant and the measurement protocols had to be standardized as well. This information can be found in chart 1 and 2 in Appendix V.

3.6 Data Analysis

The anthropometric, as well as the diaphragm thickness measurements were inserted in the Statistical Package for the Social Sciences (SPSS; version 20.20). This program was used to calculate the correlations between the age, gender, height, FFMI and the diaphragm thickness.

Descriptive statistics:
The Shapiro-Wilk test was used to check whether or not the measurements were normally distributed. This test is interpreted by means of two hypothesis. H0 means that the data is normally distributed and H1 that it is not. If the statistical probability (SIG. value=p-value) is lower than 0.05, H0 is rejected and vice versa.
The age and height were normally distributed data, for which the mean and the standard deviation were used. The FFMI, diaphragm thickness, as well as the weight and BMI (solely used to describe the population), were not normally distributed. The median, interquartile range, maximum and minimum were then determined.
The gender was described in percentages of males and females.

Inferential statistics:
Scatter plots and a population pyramid (see Appendix VI) were formed to have a first impression about the relations between the diaphragm thickness and the four variables. (See result section; Figures 5, 6, 7 and 8).
The correlation between the diaphragm thickness and gender, being a categorical (nominal) variable, was calculated with the help of the Mann Whitney-U test. Two outcomes of this test are needed to interpret if gender is in relation with the diaphragm thickness. First, the score is converted in mean ranks between male and female. The higher the rank, the thicker the diaphragm thickness and vice versa. Second, the significance level (asymptomatic sig.) describes if there is a statistical significant difference between male and female. The significance is achieved with p<0.05.

The age, height and FFMI are however considered numerical (continuous) values. The Spearman's correlation test was then used to calculate the relationship between these variables and the diaphragm thickness.
The correlation coefficient of the Spearman’s rho test, is significant at the level of 0.01, which indicates that there is a chance of 1 in a 100 that the same results would have been found if the variables were not related. To interpret the results, the closer it is to 1 (positive correlation; \( r = 1 \)) or \(-1\) (negative correlation; \( r = -1 \)), the higher the correlation between the two variables. In contrast, the closer the result is to 0, the weaker the relationship is. The correlation is negligible from 0.00-0.30; low from 0.30-0.50; moderate from 0.50-0.70; high from 0.70-0.90 and very high from 0.90-1.(34) Here again, the significance is achieved with \( p<0.05 \).

4. Results
A post hoc test was performed before calculating the results, to know the power\% of this study. The mean and the standard deviation of a comparative study was used, namely the one from Andrea J. Boon.(10) The result of the analysis was a study power of 100\% with a total amount of 83 participants.

1. Descriptive statistics
A total of 83 (n=83) healthy young adults were recruited (43.3\% male; 56.6\% female), with one exclusion due to a lack of anthropometric data, because of a leg prosthesis.

The age of the participants ranged from a minimum of 18 years to a maximum of 28 years with a mean ± standard deviation (Std.) of 22.27 ± 2.24. The weight ranged from 46.7 kg to 119 kg with a median ± interquartile range (IQR) of 72.6 ± 18.5. Finally, the BMI had a median ± IQR of 23.6 ± 4.7 and ranged from 17.6 kg/m² to 34.8 kg/m². (See Table 1)

<table>
<thead>
<tr>
<th>Table 1. Population description</th>
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<tr>
<td><strong>Gender</strong></td>
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<td>Gender</td>
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<tr>
<td>Gender</td>
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<tr>
<td><strong>Age</strong></td>
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<tr>
<td><strong>Weight</strong></td>
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<td><strong>BMI</strong></td>
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</table>

Std.= Standard deviation
IQR= Interquartile range
2. Description of the correlation variables:

The following results arose from the Shapiro-Wilk test:
The age (Sig. 0.086) and height (Sig. 0.382) were normally distributed data. Their means ± Std. were 22.27 ± 2.24 and 174.43 ± 9.42 respectively.
The FFMI (Sig. 0.003) and the diaphragm thickness (Sig. 0.003) were, on the contrary, not normally distributed data. Medians and IQR were 17.90 ± 3.79 and 1.449 ± 0.619 respectively. The FFMI ranged from a maximum of 23.20 kg/m² to a minimum of 14 kg/m² and the diaphragm thickness from a maximum of 2.581 mm to a minimum of 0.863 mm.

As already mentioned in Table 1, the gender is expressed in % with 43.3% of the participants being male and 56.6% being female. (See Table 2)

The related histograms can be found in Appendix VII.

<table>
<thead>
<tr>
<th>Table 2. Correlation variables</th>
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<tbody>
<tr>
<td>Shapiro-Wilk test</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Gender</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Height</td>
</tr>
<tr>
<td>FFMI</td>
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<tr>
<td>Diaphragm thickness</td>
</tr>
</tbody>
</table>

Std. = Standard deviation  
IQR = Interquartile range  
Max.-Min. = Maximum-Minimum

3. Correlation results:

The Spearman’s rho test was performed to correlate the age, height and FFMI to the diaphragm thickness.
The age (Sig. 0.793) showed a correlation coefficient of 0.29. The height (Sig. 0.002) and the FFMI (0.000) had a correlation coefficient of 0.335 and 0.444 respectively.

The Mann-Whitney U test was performed to correlate the gender with the diaphragm thickness. The following test showed a mean rank of 55.90 for men and 31.35 for women. The interpretation of the
test arises from the asymptomatic Sig-value, which was 0.000. (See Table 3)
The related scatter-plots and population pyramid can be found in Appendix VI.

Table 3. Correlation results

<table>
<thead>
<tr>
<th></th>
<th>Gender</th>
<th>Age</th>
<th>Height</th>
<th>FFMI</th>
</tr>
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<tbody>
<tr>
<td>Diaphragm</td>
<td></td>
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<tr>
<td>Spearman's rho test</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Correlation coefficient*</td>
<td>0.29</td>
<td>0.335</td>
<td>0.444</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)**</td>
<td>/</td>
<td>0.793</td>
<td>0.002</td>
<td>0.000</td>
</tr>
<tr>
<td>n</td>
<td>83</td>
<td>83</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Mann Whitney-U test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean rank</td>
<td>Male:55.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male:31.35</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mann-Whitney U</td>
<td>345.500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymp. Sig. (2-tailed)**</td>
<td>0.000</td>
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</tbody>
</table>

* the correlation is significant at the level of 0.01
** p-value significant at p<0.05
5. Discussion

The aim of this research is to find a relationship between the age, gender, height, fat-free mass index (FFMI) and the diaphragm thickness. The correlations are based on previously measured standard reference values with the use of MSU. These values correspond to the diaphragm thickness measured during the respiratory pause and in a functional standing position.

5.1 Description of results

The correlation between the age and the diaphragm thickness was negligible (CC=0.29). The scatter plot indicated no positive (nor negative) linear relation, thus the outcome was not as conclusive as initially hypothesized. The population used for this research was between 18 and 28 years of age and no changes in diaphragm thickness have been noticed with the increase of years. A study about skeletal muscle fibers and their classification, from Scott Wayne et al.(35), introduced the effects of aging to the muscle tissue. In fact, there is a loss of muscle fibers (especially type II fibers) as the years increase.(35) This loss and thinning of the muscle fibers could potentially be seen on the diaphragm as well.(8) However, the populations’ age frame in this study was first of all too restricted to see age related muscle wasting. Second, the lack of experience of the author in analyzing ultrasound pictures, could be a reason for overseeing minimal thickness changes, if these were to be present. Additionally, when assessing people with a longer age span, as in C. Caskey’s(15) tomographic study in which the population’s age was between 30 and 80, no relation between diaphragm thickness and age was found either.

As C. Harpers’ study(8) explains, the amount of diaphragm muscle is predicting its thickness, suggesting that a wasting of muscles contributes to the thinning of the diaphragm thickness. Since the FFMI expresses the weight of a mass without adipose tissue (as for example, bones, organs, muscles), its increase or decrease could be related to an equal change in the diaphragm thickness. This measurement is more reliable than the body-mass index (BMI), since it also takes in consideration the degeneration and wasting of the skeletal muscles (and not only fat), which is the leading cause for weight loss in people with COPD.(17) This research, however, found a low correlation between the FFMI and diaphragm thickness (CC=0.444), with a trend-line indicating that only 20.5% of the diaphragm thickness can be explained by this variable.

The FFMI varied from person to person, with a noticeable difference between the maximal male value (23.22 kg/m²) and minimal female value (14.07 kg/m²). This can be explained by the fact that women generally have a lower FFMI than men.(31) However, a possible reason for the weak relation between these two variables could be that all of the subjects participating in this study, were healthy. No abnormal fat-free mass index was measured for any participant, which could be related to an unhealthy eating habit or a systemic deconditioning due to a disease.

Next, the correlation between the diaphragm thickness and the height was low (CC=0.335). The trend line, added in the scatter plot, helped displaying the positive linear relation between these two variables with an indication that only 12.5% of the diaphragm thickness variation could be explained by the height. Although so far, no study has used MSU to compare both variables, F.D. McCools’
study(36) highlighted the fact that the height of an individual is influencing the diaphragm thickness. Knowing that the superior surface of the diaphragm inserts into the pulmonary pleura(37) and that Siahkouhia Marefat’s study(38) states that taller subjects have a higher maximum oxygen consumption and a larger lung volume, it can be hypothesized that the diaphragm muscles are more developed in taller subjects (thus an increased diaphragm thickness). According to McCools’ research, however, height is not the only variable influencing the diaphragm thickness. The weight of an individual significantly affects the muscle mass of the diaphragm.(39) This variable has not been taken in consideration when scanning the participants and could have skewed the correlation results between the height and the diaphragm thickness.

Finally, 36 males and 47 females participated in this study. Consequently, the Mann Whitney-U test resulted in a higher mean rank for men (55.90) than women (31.35). Hence men depicted a thicker diaphragm than women, which was also shown on the pyramidal graph with maximums and minimums of 2.581 mm to 1.103 mm for men and 2.163 mm to 0.863 mm for women, where males and females had a difference of 0.418 mm in maximal thickness and 0.240 mm in minimal thickness. The Mann Whitney-U test reports a significant relationship (asym. Sig. value=0.000) between gender and diaphragm thickness. Caitlin J. Harper’s research(8) using the B-mode ultrasound is also supporting these results, except that her study only displayed a slight difference between men and women. Several factors, influencing the thickness of the diaphragm, could explain the difference between the result of this research and the one from Caitlin J. Harper. First of all, the male participants had a height average of 181.28 cm compared to 169.18 cm for the females, with a difference in maximal height of 16 cm. Moreover, men were also heavier (78.3 kg) than women (66.9 kg) with a difference of 28.4 kg between the two maximal weights. As stated before, height and weight (39)(36) are influencing the diaphragm thickness. This raises the suspicion if gender alone is the reason for this significant correlation. Last but not least, even though not asked in the questionnaire, some of the male participants are regular gym members and lift weights. F.D. McCool’s research(36) compared, among other things, the diaphragm thickness between individuals of the same height and weight, who lift weights with the ones who don’t. The results showed an increased diaphragm thickness within the people who lifted weights. Therefore, these three above mentioned factors were playing a role during the scanning and might have skewed the results, which is why they should be taken into consideration when analyzing the outcome.

5.2 Strengths and limitations

The position of the transducer was difficult to maintain while assessing different participants. The intercostal view, used as a standardized apposition point, gave a narrow aspect of the diaphragm. Moreover, it was dependent on variations of the anatomy of the thorax, which made scanning more difficult. A challenge observed during the data collection, was that it became harder for the examiner to freeze an image with a clearly visible diaphragm in female participants. The reason is that females have a smaller dimension of the rib cage and more inclined ribs than men.(40) Not only was it more complicated to place the transducer at the right apposition point and to keep that position because of the above mentioned external factors, but also the lack of training of the examiners performing the data collection, may have influenced the results. 20 hours of practice, were
required before starting the experimental part of this study. However, musculoskeletal ultrasound and the reliability of its images depend on the skills of the user. This technique requires education and experience. (41) Even though the team standardized the procedure, no current protocols exist on how much experience is needed to perform ultrasound and what essential knowledge is needed in order to conduct reliable measurements.

The FFMI calculated for each participant was based on a formula from Y. Schutz (31), for which the BMI (body mass index) and the FMI (fat mass index) were needed. However, most of the studies including the FFM or the FFMI, used a bioelectrical impedance analysis (BIA) generator, which involves the bodies’ resistance to the electrical impulses and calculates the reactance as well. (31) (18) An empty stomach and a good hydration level are required prior to measuring, since the analysis is based on the conduction of the current by lean mass, mostly composed out of water containing electrolytes, and insulation by fat-tissue. (42) The Tanita-device used for the anthropometric data collection, measured the weight and the fat% but not the FFM, hence the reason why Schutz’s formula was needed. The results may therefore not be as precise as if calculated with the help of the BIA, which may subsequently play a role in the correlation outcome between the FFMI and the diaphragm thickness.

The sample size of 83 participants resulted in a 100% power in the post-hoc analysis. To mention is the fact that this analysis did not take into consideration the kind of study that is performed. This describes the difference between the calculated sample size of 83 participants with the sample sizes of 150 participants used in other studies. (9) (10)

Finally, it was possible to take all three MSU pictures to calculate the mean. Furthermore, the standing position introduces a new way of examining the diaphragm, since most studies using MSU adopt the supine lying position. (8) (9) Reproducing an everyday position, in which many activities of daily life (ADLs) are performed, allowed a more functional observation of its thickness.

5.3 Clinical relevance

This study provides information on the application of MSU on the diaphragm and insight in the relevancy of this procedure in a clinical setting. Since MSU allows repeated measurements and is sensitive to change, it provides an optimal tool for early detection and progression of diseases. (43) The real-time imaging and interactive performance of MSU (43) allows a proper assessment of the structure(s) along with the possibility to explain in images to the patient what the structural or physiological problems might be. Thus, the results based on findings of healthy subjects could enable a valuable comparison for future research including COPD patients. Findings in diaphragmatic thickness changes compared to healthy subjects with the same height or gender, or in addition to a decreased FFMI for example, could encourage a new way of diagnosing COPD while still in early stages.

The standing position is not only of advantage for reproducing a functional position. The erect position promotes the co-activation of diaphragm and accessory inspiratory muscles (scalene, intercostal
muscles), while in the supine lying position the respiration is mainly obtained by the diaphragm. Additionally, supine position reduces the functional residual capacity (FRC) of 10% in healthy and COPD subjects. A standing position during screening could therefore reduce the breathlessness symptoms. In a clinical setting the security and well-being of the patient is important and peaks of dyspnea can be expected, especially if exercises are included in the assessment. The preferred resting position for COPD patients to catch their breath, is the forward leaning position, which reduces the upper thoracic movements and distributes the respiration more evenly between the thorax and the abdomen. Thus, the forward leaning position is more easily and rapidly taken if the patient is already in a standing position than if lying supine.

Finally, introducing MSU in the clinical setting could also increase the quality of the follow-up. Regular musculoskeletal ultrasound combined with a treatment (supervised physical activity, patient education) could allow a more specific monitoring of the progression of the disease and a guided treatment.

5.4 Recommendations

A few recommendations for future reproductions of this study are necessary in order to avoid the above mentioned weaknesses.

It may be of interest to place the transducer between the posterior to mid-axillary line to measure the diaphragm thickness. All of the female participants (except for one) wore their bras, even though they were suggested to wear a bikini top. The strap made it difficult to keep the transducer in place, without it being pushed downwards.

One can imagine that the situation will be the same in a future clinical setting, hence the reason to choose an easier way of scanning. These results could be compared to the ones of this study, to investigate if there may be a difference in thickness measurement between the position of the transducer on the anterior or posterior to mid-axillary line.

Furthermore, the age group in this research was restricted, including only 18 to 28 year olds. It was easiest to find participants fitting this age at the Fontys University of Applied Sciences. However, it inhibits the generalization of the results to other target groups, as for example people with COPD. Chronic obstructive pulmonary disease usually starts around the fourth decade, with in many cases a smoking history. Therefore, in order to highlight the clinical relevance for COPD patients, future research including MSU is necessary. Recommended is to investigate relations or differences of diaphragm thickness between a control group (healthy individuals) and a COPD group, or between smokers and COPD-subjects. For both studies, the age span needs to be increased, in order to be able to include COPD patients. As for the recruitment, external participants can be invited to Fontys, University of Applied Sciences, or the examiner(s) go to a specialized COPD center, as for example the Máxima Medisch Centrum (MMC) in Eindhoven, to perform the examination with portable low-end devices.

Finally, the implementation of MSU in the clinical practice is a strong recommendation of this research. The positive sides of MSU have already been described earlier, as for example the affordability and its non-invasive nature. (11) Callaghan depicts the relevance it has in clinical practice. Concerning
COPD, MSU could prove its efficacy not only in diagnosing but also in screening the evolution of the disease in the long term. MSU is a safer tool of examination, since chest radiographs seem to have a high amount of false positives and negatives, while needle EMG examination of the diaphragm could increase the risks of hyperventilation and a possible pneumothorax in COPD-patients. (9) Portability allows assessments outside of the practice (46), which is advantageous for COPD-patients in severe stages and in need of home care.
6. Conclusion

The objective of this experimental research was to determine correlations based on previously measured standard reference values of the diaphragm thickness. The results showed no positive, nor negative correlation between diaphragm thickness and age. Height and FFMI indicated a low positive correlation, while the gender pointed out a significant correlation. A scope for the future would be to use these relations as a comparison to diaphragm thicknesses of COPD-subjects. Further research is needed to promote earlier diagnosis of COPD and increasing the quality of the follow-up sessions by monitoring, with musculoskeletal ultrasound, the efficacy of the treatment.
7. References

1. World Health Organization. WHO | Chronic obstructive pulmonary disease (COPD). World Health Organization;


16. B.R. Celli, W. MacNee and committee members. Standards of the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper.


28. Providian Medical Equipment. Aloka ProSound Alpha 7 Ultrasound Machine For Sale from Providian Medical.


43. Asdis Kristjansdottir et al. Respiratory Movements of Patients with Severe Chronic Obstructive Lung Disease and Emphysema in Supine and Forward Standing Leaning.


8. Appendices

8.1 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
8.2 Appendix 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 abdominal muscles as well as 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 disuse in patients with COPD.

The aim of this research is to investigate the standard thickness values of these muscles 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 ter Stege, Rowaya van der Burgt, Mélanie van Ravels,
8.3 Appendix 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)
8.4 Appendix IV: Questionnaire

Dear Participant,

You’ve agreed to participate in our research. Of course we’re thankful for your time and effort to help us.

Before we start we would like you to fill in this questionnaire, some measuring will also be needed.

**General questions**

1. Name:

2. Year of birth/ Age:

3. Gender:
   - Male
   - Female

4. Fat-free mass:

5. Height:

6. Weight:
8.5 Appendix V: Standardization Charts

Chart 1
This first chart depicts and explains the standardized position for the participants.

<table>
<thead>
<tr>
<th>Positioning of the participant</th>
<th>Even though some literature used a supine position (8)(9) for examination, in this research the participant is asked to stand back to the wall, which makes it a more functional position.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The heels and the back of the head are touching the wall.</td>
</tr>
<tr>
<td></td>
<td>The socks can be left on, but the shoes are removed, since heels could alter the measurements.</td>
</tr>
<tr>
<td></td>
<td>The feet have to be in line with the hips and in a 90° angle with the wall.</td>
</tr>
<tr>
<td></td>
<td>During the examination the subjects are asked to lift the right arm and rest the hand on the head, in order for the assessor to have more space to move the transducer.</td>
</tr>
<tr>
<td></td>
<td>The head is slightly elevated to ease the breathing.</td>
</tr>
<tr>
<td></td>
<td>The participant has to look straight ahead and is not allowed to speak during the examination.</td>
</tr>
<tr>
<td></td>
<td>For the female participants, it is recommended to wear a bikini top, since the bra-stra? make it more difficult for the assessor to pass with the transducer, especially during exhalation.</td>
</tr>
</tbody>
</table>

Chart 2
This chart contains the settings of the MSU-device and the procedure for the measurements.

| MSU-settings                  | The depth: 4-5
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The gain: 88% with a standard deviation of 5</td>
</tr>
<tr>
<td></td>
<td>The contrast: 18 with a standard deviation of 2.</td>
</tr>
</tbody>
</table>

These were the values that were found to enable higher quality of ultrasound pictures, compared to images taken during practice hours with a lower gain or contrast (see ultrasound image below).

<table>
<thead>
<tr>
<th>Measurement procedure</th>
<th>Freezing the image during the respiratory pause</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The thickness of the diaphragm is measured 5 mm away from the lung-shadow, following the direction of the fibres.</td>
</tr>
<tr>
<td></td>
<td>Three images of the diaphragm thickness are taken (e.g P1, P2, P3 for the first, second and third picture of respiratory pause)</td>
</tr>
<tr>
<td></td>
<td>The mean of the three pictures is calculated in order to increase the reliability of the measurement. These means are then inserted into SPSS and used as final data of the diaphragm thickness.</td>
</tr>
</tbody>
</table>

vi
- P1 stands for the first measurement of the respiratory pause
- 5 mm from the lung shadow (1Dist)
- Perpendicular line to the muscle fibers → thickness of the diaphragm (2Dist)
- Standardized settings: frequency of 12 MHz, gain of 88% and a depth of 5 cm
8.6 Appendix VI: Scatterplots and Population pyramid

Correlation age/ diaphragm thickness

Correlation height/ diaphragm thickness
Correlation FFMI/ diaphragm thickness

Correlation age/ diaphragm thickness
8.7 Appendix VII: Distribution of data

![Histogram of Age Distribution](image1)

- Mean = 22.27
- Std. Dev. = 2.242
- N = 83

![Histogram of Height Distribution](image2)

- Mean = 174.43
- Std. Dev. = 9.416
- N = 83
8.8 Appendix VIII: Confidentiality statement

B8. Confidentiality statement

Name: Naelia Giampellegnini  
Student No.: 2205773

Title:
Do age, gender, height and fat-free mass index relate to the diaphragm thickness in healthy young adults?

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: Naelia Giampellegnini  
Signature: 
Date: 14/07/2015

Coordinator: for receipt

Name: 
Signature: 
Date: 

Supervisor:

Name: Steven Argelius  
Signature: 
Date: 

8.9 Appendix IX: 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 Nadia Cianfaglione
   [full name as stated in passport], residing at 2, 3927 Luxembourg
   [postal code, place of residence] at the 54, Grand-vaanderdage
   [street and house number], hereinafter to be called “Student”

and

2. Fontys Institute trading under the name Fontys University of Applied Sciences,
   Ruchelstonen 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 H/m/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 hereof. 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.
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: [Signature]

Date: [Date]

Place: [Place]

---

**Fontys Institute**
trading under the name Fontys Hogescholen

**Supervisor:**

Name: [Signature]

Date: [Date]

Place: [Place]

---

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]