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Standard reference values of the lateral abdominal muscles in maximal inhalation, exhalation and respiratory pause.

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Preface

This thesis is submitted in partial fulfillment of the bachelor's degree in physiotherapy. It is an original, unpublished work of the author Evelien Fleerackers. This research is part of a larger study and is performed in collaboration with the department physiotherapy, speech therapy and medical imaging and radiation therapy. The experimental data reported in chapter three is collected in collaboration with four other paramedical students.

With dedication I have been working on this research from September 2015 to January 2016. This research gave me the opportunity to expand my knowledge and skills in musculoskeletal ultrasound, which is frequently being used in the modern physiotherapy. The process taught me about my interests and ambitions for the development of my future career.

I would therefore firstly like to thank my supervisor Marc Schmitz, for the support and guidance throughout the entire process and also for the inspiration about the topic musculoskeletal ultrasound. I would also like to thank Madelon Pijnenburg for her remarks, advice and availability for questions. Furthermore, I would like to thank my physiotherapy colleague students Megan Kruger and Niels Paters for the motivated team work and for always being willing to help when confronted with problems. Also, I want to thank our colleagues from speech therapy Anne Slegers and Miranda van Bussel for the motivated teamwork during the experimental period. Last but not least I want to thank my family and friends for their moral support.

I hope you will enjoy reading this thesis.

Evelien Fleerackers

December, 2015

Abstract

Background: The purpose of this study was to establish standard reference values of the Transversus abdominis and internal oblique muscles during maximal inhalation, respiratory pause and maximal exhalation in a standing position. Additionally, this study aims to estimate the correlation between those standard reference values and gender, age, length, body weight and BMI.

Method: In this quantitative cross-sectional study 102 healthy participants (43 males, 59 females) within an age of 18-28 years old completed the study. Musculoskeletal ultrasound scanning was used to assess the thickness of the lateral abdominal muscles on the anterolateral part of the belly. To establish standard reference values the median, interquartile range as well as the minimum and maximum were determined. Correlations between muscle thickness and the variables were analyzed statistically.

Results: The pattern of descending muscle thickness in both muscles was noted as maximal exhalation > respiratory pause > maximal inhalation. Males had significantly thicker muscles compared with females. Internal oblique muscle thickness is positive correlated with length and weight, transversus abdominis muscle thickness is positive correlated with weight and BMI.

Conclusion: These findings provide a robust data set of the standard reference values of the lateral abdominal muscles during maximal inhalation, respiratory pause and maximal exhalation in a standing position. This study can be used for comparison to patients with abnormalities of the TrA or IO muscles, secondary to non-specific low back pain. In order to improve the quality of the diagnostic process, the treatment plan and to help determine the effects of the interventions.

Key words: Standard reference values, transversus abdominis, internal oblique, musculoskeletal ultrasound, breathing cycle

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1. Introduction

Low back pain is one of the most common musculoskeletal disorders. It is recognized as a major problem in society, the medical system and as a health problem for individuals (1). It is estimated that 50 to 90% of the Dutch population will experience an episode of low back pain at least once in their lives. Low back pain is also one of the most common conditions treated in the practice of physiotherapists. In the period of 2008 to 2010, approximately 15% of the patients searching for physiotherapeutic care suffered from low back pain (2). In addition, low back pain is a major reason for absences of work, this results in a greater economic problem (1,3). The total costs associated with low back pain in 2007 grew to 3.5 billion euros, approximately 90% are indirect costs such as disability benefits and absenteeism (4).

Low back pain can be classified as non-specific and specific. In specific low back a specific and diagnosable cause for the pain can be found (2). Approximately 90% of the low back pain cases are classified as non-specific. Non-specific low back is defined as non-specific because a definitive diagnosis cannot be achieved (2,5,6). Non-specific low back pain is pain located between the lower ribs and the buttocks, diagnosed by excluding red flags and a lumbosacral radicular syndrome (2).

Functional deficits, such as atrophy and delayed muscle activation, have been reported in the lateral abdominal muscles in individuals with non-specific low back pain. One of the most important roles of the lateral abdominal muscles is to provide spinal stability (7–9). The lateral abdominal muscle group consists of the Transversus Abdominis (TrA) in the inner layer, the Internal Oblique (IO) which forms the middle layer and in the outer layer the External Oblique (EO). The TrA and the posterior part of the IO muscles have the most important role in spinal stability (10,11). For this reason, the focus in this research will be on the TrA and IO muscles. Besides providing spinal stability, the TrA muscle contributes to the continence mechanism, postural control of the lumbopelvic region and to respiration (12). In regards to respiration, the TrA only functions as an accessory muscle. However, the TrA is the first of the abdominal muscles to assist when respiration is challenged or voluntarily activated (13). There is evidence indicating that frequent low back pain is strongly related to disorders of respiration (14,15). According to Smith MD et al. (15), this relation can be explained by physiological limitations of the coordination of postural and respiratory functions of the lateral abdominal muscles.

Musculoskeletal Ultrasound (MSU) is proven to be a reliable and discriminative tool to assess the trunk muscle function in patients with low back pain(7,10,16–18). When based on the mean of two measures of the abdominal muscles, good levels of intra and inter-rater reliability are achieved with experienced and novice raters (7,10,17). Secondly, MSU has been reported as a safe, easy and inexpensive technique to use (19). MSU is being used increasingly as a diagnostic tool among physiotherapists in order to establish a more reliable diagnosis. The International Classification of Functioning, Disability and Health (ICF), published by the World Health Organization, provides both a framework of components as a systematic code for the description of human function and disability

(20). On the level of body function and structures, as an extension of the physical assessment, the physiotherapist can use MSU in order to make the diagnostic process measurable.

According to Teyhen DS et al. (21) MSU is a potential tool to effectively assess the lateral abdominal muscles in the diagnostic process of patients with low back pain. Because frequent low back pain is related to disorders of respiration due to functional deficits of the lateral abdominal muscles, assessment of these muscles in respiratory moments could be helpful to determine the primary cause. Although limited data with standard reference values of the thickness of the lateral abdominal muscles in respiratory moments have been published to facilitate in comparison with patient groups (21). In a recent study of Teyhen DS et al. (21), reference values of the lateral abdominal and lumbar multifidus muscles at rest and while contracted were established in a sample of healthy subjects. They found in males, significantly thicker muscles than females, but the change in thickness during contraction was equivalent. A second research study who established normal reference ranges for abdominal muscles also found that males have significantly thicker muscles compared to females, there was no correlation between muscle size and age (22). Multiple variables should be taken into consideration when comparing muscle thickness. Besides gender, another factor that may influence the interpretation of standard reference values is body mass index (BMI). Positive correlations between muscle thickness and BMI was found by Rankin et al. (22) and Springer et al. (23).

Those normative data sets provide standard reference values of the lateral abdominal muscles at rest and in contracted state. However, these data sets do not provide standard reference values in maximal inhalation, respiratory pause and maximal exhalation. Comparison of the muscle thickness of each lateral abdominal muscle in relation to the others in the three respiratory moments may help to determine whether there is a relative size and consistent order. Those standard reference values of a sample of healthy men and women can be used as a template to compare with a group with low back pain or respiratory disorders. Therefore, the importance of knowledge of those standard reference values for further investigation resulted in the following research questions: "What are the standard reference values of the thickness of the transversus abdominis muscle and the internal oblique muscle during maximal inhalation, respiratory pause and maximal exhalation in a sample of healthy men and women between 18 and 28 years old?" and "What is the correlation between these values and gender, age, length, body weight and BMI ?"

2. Method

2.1 Study design

This experimental cross-sectional research was designed to examine standard reference values in millimeter thickness of the TrA and IO muscles during maximal inhalation, respiratory pause and maximal exhalation. The muscle thickness is determined by the use of musculoskeletal ultrasound. The correlation between these values and gender, age, weight, length and BMI were estimated. The research is part of a larger study and is performed in collaboration with the department physiotherapy, speech therapy and medical imaging and radiation therapy (MBRT) of the Fontys Paramedic University of Applied sciences in Eindhoven.

2.2 Subjects

The aim of this study was to include at least 100 healthy participants between 18 and 28 years of age, recruited at Fontys Paramedic University of Applied sciences. Previous studies that described standard reference values of the thickness of the lateral abdominal muscles included 90, 340 and 123 participants (21,22,24). In order to generalize with other samples, the formula of Tabachnick and Fidell was used to calculate the sample size: $N > 50 + 8m$ (m = the number of independent variables) (25). In this case: $N > 50 + (8 \times 5) = 90$ (N). The participants were approached through social media and verbal recruitment. In addition, an email containing an invitation letter (Appendix I) and the information letter (Appendix II) were sent to all the students of Fontys Paramedic University of Applied sciences. The exclusion criteria of the research are; history of low back complaints (pain lasting more than 24 hours) in the last six months, history of diaphragmatic problems, diagnosed with any respiratory diseases (eg. COPD, cystic fibrosis or pneumonia) or neuromuscular diseases (eg. multiple sclerosis, amyotrophic lateral sclerosis etc.), current pregnancy, a BMI $> 30 \text{ kg/m}^2$ and a history of caesarean sections or any other kind of surgery in the area of the stomach. The information letter contains more criteria for other studies which are not important for this research.

2.3 Data collection and analysis

A physiotherapy student researcher, who did not have earlier experience with MSU, performed musculoskeletal ultrasound scanning. Prior to the study, the researcher was trained in abdominal muscles scanning by their supervisor who is highly educated in MSU. Additionally to the training the researcher practiced for 20 hours to reach a good to excellent reliability (18).

The ultrasound device which was used is a MyLabOne by Esaote (manufactured in Maastricht, the Netherlands in 2010). The muscles were measured in real-time using the ultrasound B-mode technique seeing as the structures were measured in a stationary position. A linear array transducer was used to visualize the thickness of the muscles. All the measurements were taken of the left side of the participant to standardize the protocol. The settings were standardized within a range; frequency: 13.0 MHz, depth: 4-5 cm and gain: 70-80%. Data collection took place from October 19th to November 6th 2015 in room 0.424 and 0.312 at Fontys Paramedic University of Applied Sciences in Eindhoven.

Upon arrival, all participants had time to read the information letter and sign the certificate of consent if they decided to participate in the research. The participant's weight in kilogram (kg) and length in meters (m) were measured and noted on a form together with the gender and age. The form was linked to their participant ID (AMSU1 - AMSU100) which was stored on the MSU device. A brief introduction of ultrasound was given following the standardized instructions of breathing (Appendix III) (26). Subsequently each participant went to the sonographer who was carrying out the measurements.

Participants were placed in a standing position. Although standing is a functional position, measuring the abdominal muscles in a standing position is not often described in the literature (19). To standardize the posture, the shoulder blades and buttocks were placed against the wall. However, the participant needs to be able to reach maximal inhalation and exhalation allowing the participant to deviate slightly from this posture if necessary. The feet were placed in line with the hips and shoes were removed in order to avoid influence on posture. Participants were instructed to look straight forward, thereby the screen of the ultrasound device could not be seen to blind the participant. The left hand of the participant was placed on the right shoulder, as in this position the rater could easily reach the left abdominal muscles.



Figure 1. The transducer is placed between the lowest rib and the superior border of the iliac crest.

Before making contact with the skin ultrasound gel was placed on the transducer. The transducer was placed transversely on the anterolateral part of the belly, halfway between the lowest rib and the iliac crest, perpendicular on the abdominal muscle wall. See figure 1. This is the most investigated and common way for imaging the lateral abdominal muscles (19). Measurements were taken 2 centimeters from the beginning of the TrA. Therefore, the images were taken with the beginning of the TrA on the far left of the image. The student researcher gave a clear instruction when the participant had to inhale and exhale maximally. The first ultrasound image is made in respiratory pause, the second in maximal inhalation and the third in maximal exhalation. This process was repeated two more times which results in a total of 9 images for every participant.

Analyzing the scans in thickness (mm) was done by the same researcher that made the scan. All the images were stored on a USB stick of 16GB. Measures were completed in the software MyLabTM Desk3 version 8 which was installed on a Windows 7 desktop. Values of the muscle thickness of the TrA and IO muscles were obtained by manually measuring the distance between the inside edges of the superior and inferior border of the fascial lines. Measurements were obtained 2 centimeters from the anterior border of the TrA and perpendicular to the probe. See figure 2. The BMI was calculated and together with the weight, length, age, gender and the outcomes of the thickness measurements saved in an SPSS dataset.

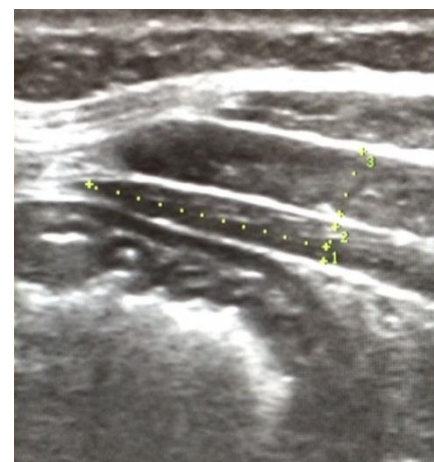


Figure 2. Thickness measurements of the TrA and IO muscles.

2.4 Statistical analysis

All the data was processed and analyzed with SPSS Data editor program version 23. The intraclass correlation coefficient (ICC) was calculated to determine the intra-reliability. An ICC of 0.00-0.20 indicates slight, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 good, 0.81-1.00 excellent reliability (27).

The mean outcomes of the measurements for each muscle were calculated for the three respiratory moments. These outcomes were used to determine descriptive statistics. Scatter plots were made to gain a first insight in the correlation between the data. Then, the Shapiro-Wilk test was used to assess normal distribution. For age, length, weight and BMI the median, interquartile range (IQR), minimum and maximum were determined.

Gender is considered as a nominal variable. The correlation of gender was analyzed through the Mann-Whitney U test. Age, length, weight and BMI are considered as continuous variables. Spearman's correlation was used to analyze the correlation with age, length, weight and BMI on the thickness of the lateral abdominal muscles. Significance for each analysis is achieved when $p < 0.05$. A maximal positive correlation is defined as $r_s = 1$ and a maximal negative correlation as $r_s = -1$. Interpretation of the correlation coefficient (positive or negative) is as follows: 0.00-0.30 indicates a negligible correlation, 0.30-0.50 indicates a low correlation, 0.50-0.70 indicates a moderate correlation, 0.70-0.90 indicates a high correlation and 0.90-1.00 indicates a very high correlation (28).

2.5 Ethical paragraph

The study has been approved by Fontys University of Applied sciences and there are no risks involved in this project. All the subjects were aware that they participated voluntarily and they were allowed to withdraw at any moment. Minimum two days prior to the data collection the participants received the information letter (Appendix II) and the certificate of consent (Appendix IV). The certificate of consent was signed if they fully agreed and handed in prior to the data collection. All the collected data of the participants was encoded and handed over to Fontys Paramedic University of Applied sciences after completing the thesis.

3. Results

One hundred and two participants completed the study (43 males, 59 females). Three participants were excluded from the study after examination. Two participants were excluded because of a BMI > 30 kg/m² and one turned out to have had an appendectomy in history. The participants were between the ages of 18 and 28 with the median age being 21. The demographic data is summarized in table 1.

Table 1. Demographic data of the participants.

Characteristics	Males (n = 43)	Females (n = 59)	Group (n = 102)
Age, y	22 (±3.0) 18-28	21 (±4.0) 18-26	21 (±3.0) 18-28
Length, m	1,83 (±0.07) 1,66-1,96	1,68 (±0.09) 1,47-1,81	1,73 (±0.13) 1,47-1,96
Weight, kg	78 (±14.0) 61,0-114,5	64 (±13.0) 47-80,1	69 (±17.0) 47,0-114,5
BMI, kg/m ²	23 (±3.4) 19,6-29,9	23 (±3.9) 17,5-28,7	23 (±3.7) 17,5-29,9
Median (±IQR) Min-max			

BMI: Body mass index; IQR: Interquartile Range; min: minimum; max: maximum

Intraclass correlation coefficient was calculated to determine the intra-rater reliability of the measurements. The ICC value for the average measures is 0.973 ($p < 0.001$), this represents an excellent intra-rater reliability (ICC= 0.81-1.00) (27).

The median and the IQR were determined for the muscle thickness of both muscles in all three respiratory moments of the total sample. The median of the TrA muscle in respiratory pause was 3,8mm (±2,03), in maximal inhalation the median was 2,87mm (±1,56) and in maximal exhalation 5,43mm (±3,14). The median of the IO muscle in respiratory pause was 10,17mm (±3,59), in maximal inhalation the median was 8,02mm (±3,10) and the median of maximal exhalation was 12,52mm (±4,06).

The TrA muscle thickness in respiratory pause ($p = 0.013$) and maximal exhalation ($p = 0.027$) is larger in males than females. During maximal inhalation the TrA muscle thickness is equivalent in males and females ($p = 0.185$). The thickness of the IO muscle is greater in males than females regarding all three the respiratory moments ($p < 0.001$). The standard reference values for males and females are displayed in table 2. Illustrating the median, IQR as well as the minimum and maximum of the TrA and IO muscles in all three respiratory moments.

Before Spearman's correlation coefficient was calculated, scatter plots were made to make a first analysis of the data. The scatter plots for the correlations between the muscle thickness of the TrA and IO in all respiratory moments and age, length, weight and BMI can be found in appendix VII.

Table 2. Standard references values of males and females of the TrA and IO in breathing pause, maximal inhalation and exhalation.

Muscle	Breathing moment	Males (n = 43)	Females (n = 59)	P
Transversus abdominis	Resp. pause	4,03 ($\pm 1,90$) 1,47-8,47	3,43 ($\pm 2,08$) 1,63-9,90	0.013
	Max. inhalation	3,17 ($\pm 1,40$) 1,22-7,47	2,80 ($\pm 1,83$) 1,27-6,07	0.185
	Max. exhalation	6,20 ($\pm 2,80$) 2,7-11,9	5,16 ($\pm 3,17$) 1,93-13,37	0.027
Internal Oblique	Resp. pause	11,53 ($\pm 3,03$) 4,83-19,9	9,0 ($\pm 3,07$) 3,94-15,47	<0.001
	Max. inhalation	9,5 ($\pm 2,87$) 4,53-13,83	7,13 ($\pm 2,5$) 3,86-15,47	<0.001
	Max. exhalation	14,33 ($\pm 4,17$) 7,37-25,8	11,27 ($\pm 4,03$) 4,22-16,5	<0.001
		Median (IQR)		
		Min-max		

IQR= Interquartile Range; min :minimum; max :maximum

As shown in table 3, no significant correlation is found between age and muscle thickness of the TrA and IO. Length had a negligible positive correlation with muscle thickness of the TrA for respiratory pause ($r_s=0.23$, $p<0.001$) and exhalation ($r_s=0.27$, $p=0.005$). No significant correlation was found with maximal inhalation of the TrA muscle ($p=0.181$). Low positive correlations were found with the thickness of the IO muscle in all three respiratory moments and length ($r_s>0.35$ $p<0.001$).

Weight showed the strongest correlation with muscle thickness on average, although negligible to low positive correlations were found. The correlation between TrA muscle thickness in maximal inhalation is negligible ($r_s=0.23$, $p=0.021$). Low correlations were found between weight and muscle thickness of the TrA in respiratory pause, maximal exhalation and muscle thickness of the IO in all three the respiratory moments ($r_s\geq 0.38$, $p<0.001$).

The correlations between muscle thickness of the TrA in maximal inhalation and muscle thickness of the IO in all three respiratory moments with BMI were negligible. BMI was significantly low correlated to muscle thickness of the TrA in respiratory pause ($r_s=0.36$, $p<0.001$) and maximal exhalation ($r_s=0.35$, $p<0.001$).

Since there is a significant difference in the lateral abdominal muscle thickness in males and females, the muscle thickness of males and females were also correlated separately with age, length, weight and BMI (Appendix VIII). There were no significant correlations with any of the variables in females, except for a negligible positive correlation between BMI and the muscle thickness of the TrA in respiratory pause ($r_s=0.28$, $p=0.035$). Noticeable in males were the significant positive low correlations between BMI and muscle thickness of the TrA in respiratory pause ($r_s=0.51$, $p=0.001$), maximal inhalation ($r_s = 0.49$, $p = 0.001$) and in maximal exhalation ($r_s=0.51$, $p<0.001$). Besides, significant positive low to moderate correlations were found between weight and the muscle thickness of the TrA

in respiratory pause ($r_s=0.49$, $p=0.002$), maximal inhalation ($r_s=0.39$, $p<0.009$) and maximal exhalation ($r_s=0.61$, $p<0.001$).

Table 3. Correlation between the thickness of the abdominal muscles and age, length, weight and BMI of the total group.

Muscle	Breathing moment	Age	Length	Weight	BMI
Transversus abdominis	Resp. pause	0.01 (0.946)	0.23 (<0.001)	0.38 (<0.001)	0.36 (<0.001)
	Max. inhalation	-0.06 (0.512)	0.13 (0.181)	0.23 (0.021)	0.23 (0.019)
	Max. exhalation	0.08 (0.411)	0.27 (0.005)	0.40 (<0.001)	0.35 (<0.001)
Internal Oblique	Resp. pause	0.08 (0.422)	0.35 (<0.001)	0.40 (<0.001)	0.28 (0.004)
	Max. inhalation	0.02 (0.849)	0.40 (<0.001)	0.41 (<0.001)	0.20 (0.046)
	Max. exhalation	0.20 (0.042)	0.43 (<0.001)	0.40 (<0.001)	0.29 (0.003)
Spearman's correlation coefficient (P value)					

4. Discussion

This study has provided standard reference values for the muscle thickness of the TrA and IO in a sample of healthy young males and females. Unlike previous studies with standard reference data sets, these reference data provide values for the muscle thickness of the TrA and IO in a standing position during the respiratory pause, maximal inhalation and maximal exhalation. Additionally, this study aims to estimate the correlation between those standard reference values and gender, age, length, body weight and BMI.

No previous study determined reference values of the lateral abdominal muscles in maximal inhalation and maximal exhalation. Therefore, direct comparisons with previous studies are not possible for the reference values of the muscle thickness in maximal inhalation and maximal exhalation. Both the TrA and IO muscles were found to be thickest in maximal exhalation and thinnest in maximal inhalation, the values of the respiratory pause were in between. This agrees with the findings of Ainscough-Potts et al. (29) who determined muscle thickness at the end of normal exhalation and inhalation in a lying and sitting position.

There are previous studies who determined reference values of the TrA and IO during rest. However, the thickness of the TrA is not distributed evenly so the median was calculated. Therefore, direct comparison with prior studies that used the mean is difficult. But the measurements obtained by this study were still similar to those of Teyhen et al. (21), who determined reference values of the thickness of the TrA and IO muscles in 340 participants. To provide a few comparisons between this study and the study of Teyhen et al. (21): the median reported of the thickness of the TrA in respiratory pause is 4,03mm in males and 3,43mm in females compared with a mean of 3,9mm and 3,3mm by Teyhen et al. (21). The median reported of the thickness of the IO in respiratory pause for males is 11.5mm and in females 9,0mm, compared with 10,4mm and 7,5mm reported by Theyen et al. (21). The values are slightly different between the two studies. This could be explained by the position of the participants. In a supine position, as in the study of Teyhen et al. (21), the TrA muscle is significantly thinner compared to an erect standing position (30). Furthermore, the measurement techniques corresponded closely, except Teyhen et al. (21) measured on three points on the muscle so this will give a better understanding of the overall thickness of the muscles. The medians of the values reported in this study are slightly smaller compared to the values of Springer et al. (23) (n= 32), Rankin et al. (22) (n = 123) and Sugaya et al. (31) (n = 21). Likely attributable to the difference in studied population.

According to the outcomes of this study, muscle thickness of the TrA in respiratory pause and maximal exhalation is significantly larger in males than females. In maximal inhalation the muscle thickness of the TrA is equivalent in males and females. In addition, the muscle thickness of the IO in all three respiratory moments is significantly greater in males than females. Several studies confirm the findings that the lateral abdominal muscle thickness during rest in males are significantly larger compared with females (21,22,32).

We found no correlation between muscle thickness and age. Which could be explained by the small age range we included in this study. However, those results correspond with the results of Rankin et al. (22) who studied a total of 123 subjects with an age range of 21 - 72 years (mean 40.6, \pm SD 14.1). In the total sample negligible correlations between length and TrA muscle thickness were found. Low correlations between length and IO muscle thickness were found. In males and females separately no significant correlation remained. This could be explained by the smaller sample size when taking males and females separately, according to the formula of Tabachnick and Fidell (25) this sample size is too small for analyzing the correlation. Besides, comparison with other studies is not possible because none of the previous studies investigated the correlation with length. In our study we found positive low correlations between weight and TrA and IO muscle thickness. In males and females separately this positive low correlation is confirmed by the TrA muscle in males. These results are in contrast with the study of Rostami et al. (24), who found no significant correlation. This could be explained by the major differences in the average weight and age in the studied populations. According to the results of this study BMI is positive low correlated with the muscle thickness of the TrA. Taking the outcomes of the correlations in males and females separately, only males show significant low to moderate correlations. This is confirmed with the findings of Springer et al. (23), who investigated 32 males and found a positive moderate correlation between BMI and TrA muscle thickness. However, the study of Rostami et al. (24) did not find any significant correlation between BMI and muscle thickness of the TrA muscle. A possible reason for these variations is because BMI cannot distinguish between body fatness, skeletal mass and muscle mass. Comparison of BMI can result in errors in the estimation of body fatness (33).

Strength and limitations

The characteristics of the studied population correspond to the Dutch population beyond 18 years of age. The average height of our participants was 1,83m in males and 1,68 m in females compared to 1,81m and 1,67m. The average weight of our participants was 78 kg in males and 64 kg in females compared to 84 kg and 71 kg according to statistics Netherlands (34). The weight difference can be explained by our young population (18 to 28 years old). Another explanation might be because the vast majority of our participants are recruited at the Fontys Paramedic University, therefore it is likely that they are more aware of their health.

The overall young age and lower weight can explain the fact that those two variables are not normally distributed. The dependent variables, muscle thickness of the TrA in all three respiratory moments, were also not distributed normally. Which could result in a less reliable correlation. The measurements to determine the reference values have an excellent intra-rater reliability. Thereby, we can confirm the results of Theyen et al. that with 20 hours of practice an excellent reliability can be reached (18). One of the primary limitations of this research is the breathing protocol. The participants were asked to inhale and exhale maximally in a way they think is comfortable. However, there are different patterns of breathing. Breathing patterns can be classified according to the expansion of the abdominothoracic region; a superior thoracic expansion, an abdominal and lateral costal expansion and a mix between those types (35). The abdominal and lateral costal expansion is considered as the normal breathing type, it produces an optimal expansion of the rib cage, this influences the TrA and IO muscle thickness

significantly more (35). Besides, the maximal exhalation will become less reliable after several breathing maneuvers due to fatigue (26). It is recommended for further research to aim for a standardized protocol for the maximal breathing moments, in order to objectify maximal breathing moments. Due to the collaboration with other studies a low-end musculoskeletal ultrasound system is used. This is the most common used ultrasound system by the physiotherapist. But as a high-end system measures more accurately, it would be preferable to use in future research when determining reference values. In this research the measurements were obtained in a standing position. Firstly, because until now there were no reference values in a standing position. Secondly, because this is considered as the most functional position. However, measuring in erect standing compared with sway standing results in a significant difference in muscle thickness (30). Therefore, when trying to compare values in those with dysfunctions, it is important to obtain muscle thickness in a similar position.

Clinical relevance

The primary clinical relevance of the determined standard reference values is that the values can be used as a template to compare with patients suffering from low back pain. Most studies establish standard reference values in a contracted state. However, the contribution of the TrA to respiration is functional as well, comparison in the respiratory moments can be useful to detect abnormalities of muscle thickness in respiratory moments. Abnormal muscle thickness values, presumably atrophy, in combination with a physical examination can provide a more specific diagnosis in patients with non-specific low back pain. This makes it possible to create a more specified treatment plan, which can result in a shorter treatment period. In addition, because the norm values are determined in a standing position and later in the rehabilitation process most of the exercises will be in a standing position, those values can easily be used as an evaluative tool during the treatment period. Thus, the quality of the health care of patients suffering from non-specific low back pain will improve and their quality of life will increase. Secondly, work absenteeism will decrease, which will save costs and the economic problem will become smaller.

Recommendations

If this research would be repeated, we have several recommendations to improve the quality. One recommendation is using an high-end MSU device to optimize the imaging quality. Another recommendation is making a more standardized breathing protocol in order to objectify the maximal inhalation and exhalation. In order to determine the breathing pattern, a breathing movement measuring device can be used to measure breathing movements objectively (36,37). Besides, because good reliability is already achieved based on the mean of two measures (7,10,17), to prevent fatigue in the maximal breathing maneuvers we recommend to take two instead of three images in every breathing moment. This will increase the reliability of the values during maximal inhalation and exhalation.

For further research we would recommend to determine standard reference values of the lateral abdominal muscles in patients with low back pain during respiratory moments, for comparison with the standard reference values of this study. Besides, because the TrA and IO muscles are accessory muscles of respiration and since there is evidence indicating that frequent low back pain is strongly

related to disorders of respiration (14,15). Further research is required to assess the lateral abdominal muscle thickness during respiratory moments in patients with respiratory disorders to investigate whether respiratory disorders are related to abnormal values of the lateral abdominal muscles.

5. Conclusion

TrA and IO muscle thickness reference data during the respiratory cycle are reported in a healthy young sample of males and females in a standing position, measured by musculoskeletal ultrasound scanning. In general, males have thicker TrA and IO muscles compared with females. IO muscle thickness is positive correlated with length and weight, TrA muscle thickness is positive correlated with weight and BMI. Overall, this study can be used for comparison to patients with abnormalities of the TrA or IO muscles, secondary to non-specific low back pain. In order to improve the quality of the diagnostic process, the treatment plan and to help determine the effects of the interventions.

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Appendices

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 backgrounds; 2 speech and language therapy students and 3 physiotherapy students. As a multidisciplinary team we are working together to answer several research questions part of 1 investigation; this is why we need you!

The experiment consists of measuring the thickness of your abdominal muscles using ultrasound imaging. This is important because the results will give insight into the use of ultrasound imaging in physiotherapy as well as speech and language therapy. The data will be used to answer multiple research questions and will set the foundation of future research in this topic.

The testing will take place from the 19th of October until the 6th of November at Fontys University of Applied Sciences. We will of course take into consideration your preference for the time and date and a reminder will be emailed one day prior to your meeting. Furthermore, you will be rewarded with a small gift after the experiment.

If you are interested in participating in the experiment, please find attached an information letter with more details. In case of other questions concerning the research, please do not hesitate to contact one of us. We kindly ask you to contact any researcher below if you are willing to participate in order to register for the experiment. (Please include your time and date preference)

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

Kind regards,

Megan Kruger (*English Stream Physiotherapy Student*)

Evelien Fleerackers (*Dutch Stream Physiotherapy Student*)

Niels Paters (*Dutch Stream Physiotherapy Student*)

Miranda van Bussel (*Dutch Stream Speech and language therapy student*)

Anne Slegers (*Dutch Stream Speech and language therapy student*)

Appendix II Information letter

Musculoskeletal Ultrasound of Abdominal Muscles:

Dear Participant,

We would like to thank you for volunteering to participate in our study. We are graduate students from physiotherapy and speech and language 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 or if you have any other questions, please do not hesitate to contact us for more information.

The aim of the project:

The abdominal muscles are important muscles found in front and on the sides of the abdomen (belly area). They play an important role in the prevention of low back pain by keeping the back stable. They also have an important function in our breathing cycle. It is therefore necessary to understand how they work and when they are not working properly.

One method of looking at these muscles is through the use of Musculoskeletal Ultrasound. The aim of this research is to investigate the standard thickness values of the abdominal 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, it is still uncertain how reliable the measurements of this device are. 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.

From a speech and language therapy point of view, the difference in muscle structure between singers (amateur and professional) and non-singers will be assessed using the same ultrasound equipment. Another student will investigate if there is a difference in thickness on the left and right side of the abdominal muscles. The aim for these students is to research the possibility of using ultrasound equipment as a feedback or diagnostic tool in the future.

Who can participate:

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

- Amateur and professional singers (about 15-20 participants)
- Non-singers (about 80 participants)
- No prolonged or diagnosed voice problems (eg. hoarseness)
- No episode of low back complaints (pain lasting more than 24 hours) in the last 6 months
- No asthma or other respiratory diseases (eg. COPD, cystic fibrosis or pneumonia)
- No previous known diaphragmatic problems
- No diagnosed neuromuscular diseases (eg. multiple sclerosis, amyotrophic lateral sclerosis etc.)
- Participant should not be pregnant

- No previous caesarean sections or any surgery in the stomach area
- No 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. 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 abdominal muscles will be imaged using Musculoskeletal Ultrasound. In order to achieve this you will be asked to lift up your shirt to expose your stomach. Next, some gel will be applied to the transducer (see image above) which will then be placed on your lower stomach and on 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 3 participants present in the room 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 for the participant in this project.

The collected data:

All information and data gathered during the project will be handled confidentially and will remain anonymous throughout the investigation. 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 researchers involved that your name, personal details and data will remain confidential.

Finally, it is important to remember that taking part in this research project is completely voluntary and you are free to withdraw whenever you want without explanation. 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,

Megan Kruger (*English Stream Physiotherapy Student*)

Evelien Fleerackers (*Dutch Stream Physiotherapy Student*)

Niels Paters (*Dutch Stream Physiotherapy Student*)

Miranda van Bussel (*Dutch Stream Speech and language therapy student*)

Anne Slegers (*Dutch Stream Speech and language therapy student*)

Appendix III Standardized breathing instructions

During the questionnaire and weight/height measurements the participant has the chance to relax and reach a normal breathing pattern before starting the experiment.

Standardized breathing instructions:

1. Ensure proper posture (upright standing position mentioned in procedure)
2. Head slightly elevated to avoid airway obstruction
3. First achieve normal, relaxed breathing. Breathe in slightly and then exhale until resting point. (Ensure that the participant does not use any effort to reach the resting point and that natural breathing takes place.)
4. Hold 3 seconds.
5. Inhale maximally until no more air is possible and hold 3 seconds. (ensure that the rib cage elevates and that the back extends)
6. Exhale maximally until no more air comes out and hold 3 seconds. (ensure full exhalation when the abdominal muscles are contracted)

Ensure that the patient breathes in a relaxed manner in between the measuring moments. Patient safety comes first!

To be certain that a participant reaches their maximal inhalation/exhalation, the participant is asked to tap their leg when they believe they have reached their maximum. The image is frozen once maximal effort has been reached. The sonographer is allowed to scroll through the last images taken in order to ensure the best quality image. If the researcher has any doubts, he/she can ask the participant to repeat the measurement.

Appendix IV Certificate of Consent

Certificate of Consent

Title of the research: *Musculoskeletal ultrasound of the abdominal muscles*

Statement by the participant:

I have read the above mentioned information 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. In addition I've been informed and am fully aware of possible risks of the research. I consent voluntarily to be a participant in this study. And I know I can quit my participation at any time. I'm aware that my personal details and results will be private and only used for this research.

- 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)

Appendix V Confidentiality statement



Geheimhoudingsverklaring

Naam: Evelien Fleerackers Studentnr: 279066

Titel: Standard reference values of the lateral abdominal muscles in maximal inspiration, expiration and respiratory pause.

Inhoud (omschrijving):

Standard reference values of the transversus abdominis and internal oblique during maximal inspiration, expiration and respiratory pause measured with musculoskeletal ultrasound in a sample of healthy men and women between 18 and 28 years old and the influence of gender, height, weight and BMI.

1. Fontys Paramedische Hogeschool te Eindhoven verbindt zich door ondertekening van deze verklaring, informatie met betrekking tot de verstrekte gegevens en uit onderzoek verkregen resultaten waarvan in het kader van bovengenoemd project praktijkgericht onderzoek kennis wordt genomen en waarvan bekend is of redelijkerwijs begrepen kan worden dat dit als geheim of vertrouwelijk wordt beschouwd, strikt geheim te houden.
2. Tevens geldt deze geheimhoudingsverplichting voor de werknemers van Fontys Paramedische Hogeschool, evenals voor anderen die op enigerlei wijze uit hoofde van hun functie toegang hebben of kennis nemen van bedoelde informatie.
3. Bovenstaande laat onverlet dat de student het project praktijkgericht onderzoek kan uitvoeren volgens geldende voorschriften en regels.

Student:

Naam: Evelien Fleerackers

(handtekening)

Datum: 8/10/2015

PGO-coördinator: voor ontvangst

Begeleider:

Naam: _____

(handtekening)

Datum: / /

Naam: _____

(handtekening)

Datum: / /

Appendix VI Conveyance of rights agreement



Overeenkomst overdracht rechten

OVEREENKOMST

houdende overdracht van rechten en de plicht tot
overdracht/retournering van data, software en andere middelen

Ondergetekenden:

1. de heer/mevrouw Evelien Flerackers [volledige
namen als vermeld in paspoort], woonachtig te 5525AB Duizel [postcode,
woonplaats] aan de Hoef 7 [adres en huisnummer], hierna aan te
duiden als "Student"

en

2. Stichting Fontys h.o.d.n. Fontys Hogescholen, Rachelsmolen 1, 5612 MA Eindhoven, hierna
"Fontys"

CONSIDERANS

- A. Student studeert aan de Fontys Paramedische Hogeschool te Eindhoven en heeft in het kader van zijn/haar studie, al dan niet tezamen met derden en/of in opdracht van derden, (diverse) activiteiten verricht, of zal deze nog verrichten, in het kader van onderzoeken die onder supervisie staan van het lectoraat van Fontys Paramedische Hogeschool. Voornoemde activiteiten zullen hierna worden aangeduid als "**Lectoraat Studieactiviteiten**". Ten tijde van ondertekening van deze verklaring superviseert het lectoraat van Fontys Paramedische Hogeschool in ieder geval de onderzoeken die zijn opgesomd in bijlage 1, maar deze opsomming is niet uitputtend en kan in de toekomst veranderen.
- B. Het is voor Fontys Paramedische Hogeschool van essentieel belang dat (uitwerkingen van) de Lectoraat Studieactiviteiten ongehinderd en zonder enige beperking verder kunnen worden ontwikkeld en toegepast door Fontys Paramedische Hogeschool en/of voor onderwijs van andere studenten kunnen worden gebruikt. Fontys wil in ieder geval – maar niet uitsluitend – (uitwerkingen van) de Lectoraat Studieactiviteiten (i) kunnen delen met en/of over te dragen aan derden, (ii) op eigen naam kunnen publiceren, waarbij Student mogelijk als co-auteur kan worden vernoemd mits dit gezien de omstandigheden redelijk is, (iii) kunnen gebruiken als basis voor nieuwe onderzoeksprojecten.

- C. Als op (uitwerkingen van) Lectoraat Studieactiviteiten intellectuele eigendomsrechten (komen te) rusten en/of daaraan verwante aanspraken van Student, wensende partijen – het onder (B) genoemde in aanmerking nemend – dat Fontys Paramedische Hogeschool de enige rechthebbende ten aanzien van deze rechten en aanspraken is. Student wenst dan ook al zijn/haar huidige en toekomstige intellectuele eigendomsrechten alsook daaraan verwante aanspraken met betrekking tot (uitwerkingen van) de Lectoraat Studieactiviteiten aan Fontys over te dragen, onder de hierna te noemen voorwaarden;
- D. Student wenst voorts de verplichting op zich te nemen – wederom het onder (B) genoemde in aanmerking nemend – om alle door hem/haar in het kader van (uitwerkingen van) de Lectoraat Studieactiviteiten door hem/haar verzamelde data aan Fontys over te dragen en geen kopieën daarvan te bewaren, en eveneens alle in het kader van (uitwerkingen van) de Lectoraat Studieactiviteiten aan hem/haar door Fontys verstrekte eerder verzamelde data, software en/of andere middelen, zoals meet- en testapparatuur, aan Fontys te retourneren zonder kopieën daarvan te bewaren, dit alles onder de hierna te noemen voorwaarden.

KOMEN OVEREEN ALS VOLGT

1. Overdracht intellectuele eigendomsrechten

1.1 Student draagt hierbij over aan Fontys Paramedische Hogeschool al zijn/haar huidige en toekomstige intellectuele eigendomsrechten en aanverwante aanspraken met betrekking tot (uitwerkingen van) de Lectoraat Studieactiviteiten, voor de volledige duur van die rechten.

1.2 Onder intellectuele eigendomsrechten en/of daaraan verwante aanspraken wordt tenminste – maar niet uitsluitend – verstaan het auteursrecht, het databankenrecht, het octrooirecht, het merkenrecht, het handelsnamenrecht, het tekeningen- en modellenrecht, het kwekersrecht, bescherming van knowhow en bescherming tegen oneerlijke mededinging.

1.3 De in 1.1 omschreven overdracht is onbeperkt. Zodoende omvat de overdracht alle aan de overgedragen rechten en aanspraken verbonden bevoegdheden, en geldt de overdracht voor alle landen ter wereld.

1.4 Voor zover enige nationale wetgeving enige nadere medewerking van Student vereist voor de overdracht genoemd onder 1.1, zal Student deze medewerking op eerste verzoek van Fontys Paramedische Hogeschool onmiddellijk en zonder enig voorbehoud verlenen.

1.5 Fontys aanvaardt de in 1.1 omschreven overdracht.

2. *Afstand van persoonlijkheidsrechten*

2.1 Voor zover toegestaan onder artikel 25 Auteurswet, en andere eventueel toepasselijke nationale wetgeving, doet Student afstand van zijn/haar persoonlijkheidsrechten, waaronder begrepen – maar niet uitsluitend – het recht op vermelding van de naam van Student en het recht zich te verzetten tegen wijzigen van (uitwerkingen van) de Lectoraat Studietoepassingen. Indien en voor zover aan Student onder enige nationale wetgeving een beroep toekomt op persoonlijkheidsrechten ondanks het bovenstaande, zal Student zich niet op onredelijke gronden op deze persoonlijkheidsrechten beroepen.

2.2 In afwijking van hetgeen in 2.1 is bepaald kan Fontys Paramedische Hogeschool besluiten de naam van Student wel te vermelden als dit gezien de omvang van zijn/haar bijdrage en werkzaamheden redelijk is.

3. *Vergoeding*

Student gaat ermee akkoord dat hij/zij geen vergoeding voor de in deze verklaring omschreven rechtenoverdracht en -afstand van Fontys zal ontvangen.

4. *Garantie ten aanzien van intellectuele eigendomsrechten*

Student verklaart bevoegd te zijn tot de overdracht en afstand, en verklaart geen licentie(s) tot enigerlei wijze van gebruik van (uitwerkingen van) de Lectoraat Studietoepassingen aan enige derde(n) te hebben verleend of in de toekomst te zullen verlenen. Student vrijwaart Fontys voor iedere aanspraak van derden in dit kader.

5. *Plicht tot overdracht/retournering van data, software en andere middelen*

5.1 Op het moment dat Student niet langer Lectoraat Studietoepassingen verricht en/of niet langer student van Fontys is, verplicht Student zich tot overdracht aan Fontys van alle in het kader van (uitwerkingen van) de Lectoraat Studietoepassingen door hem/haar verzamelde data, in de ruimste zin van het woord, daaronder begrepen – maar niet uitsluitend – onderzoeken en onderzoeksresultaten, tussentijdse notities, documenten, afbeeldingen, tekeningen, modellen, prototypes, specificaties, productiemethoden, procesbeschrijvingen en techniekbeschrijvingen.

5.2 Student garandeert op geen enkele wijze, in welke vorm dan ook, kopieën van de in 5.1 bedoelde data bewaard te hebben.

5.3 Student verplicht zich om aan Fontys te retourneren alle in het kader van de Lectoraat Studietoelagen aan hem/haar door Fontys verstrekte data, software en/of andere middelen, en garandeert op geen enkele wijze, in welke vorm dan ook, kopieën van de verstrekte software en/of andere middelen bewaard te hebben.

5.4 Student gaat ermee akkoord dat indien hij in strijd met de verplichtingen en garanties genoemd onder 5.1 tot en met 5.3 handelt en/of gehandeld blijkt te hebben, (a) hij/zij aansprakelijk is voor alle schade die Fontys hierdoor heeft geleden en/of nog zal lijden, en (b) dat dit als fraude kwalificeert en Fontys daaraan passende sancties mag verbinden. De door Fontys op te leggen sancties kunnen onder meer bestaan uit het niet toekennen van studiepunten, het tijdelijk uitsluiten van Ondergetekende van deelname aan examens, maar ook uit het definitief uitschrijven van Ondergetekende als student van Fontys.

6. Afstand

Student doet afstand van het recht op ontbinding van deze overeenkomst.


7. Overig

7.1 Voor zover deze overeenkomst afwijkt van het studentenstatuut geldt dat deze overeenkomst voor gaat.

7.2 Deze overeenkomst wordt beheerst door Nederlands recht. Alle uit deze verklaring voortvloeiende geschillen zullen worden voorgelegd aan de bevoegde rechter te Amsterdam.

Student:

Naam: Evelien Fleerakkers



(handtekening)

Datum: 19/12/2015

Plaats: Eindhoven

Stichting Fontys

h.o.d.n. Fontys Hogescholen

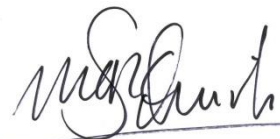
begeleider:

Naam: Marc Schmitz

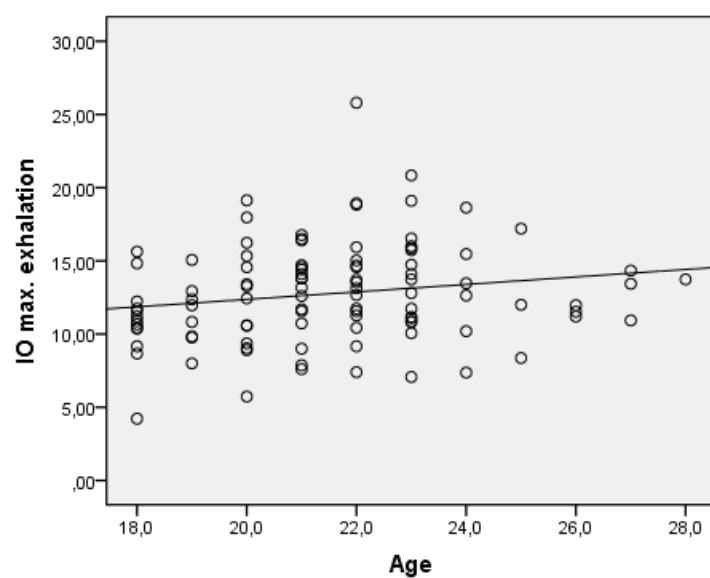
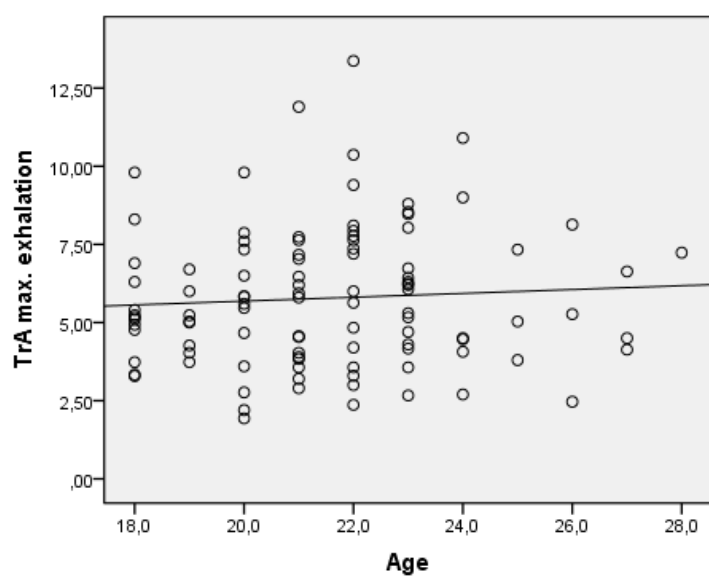
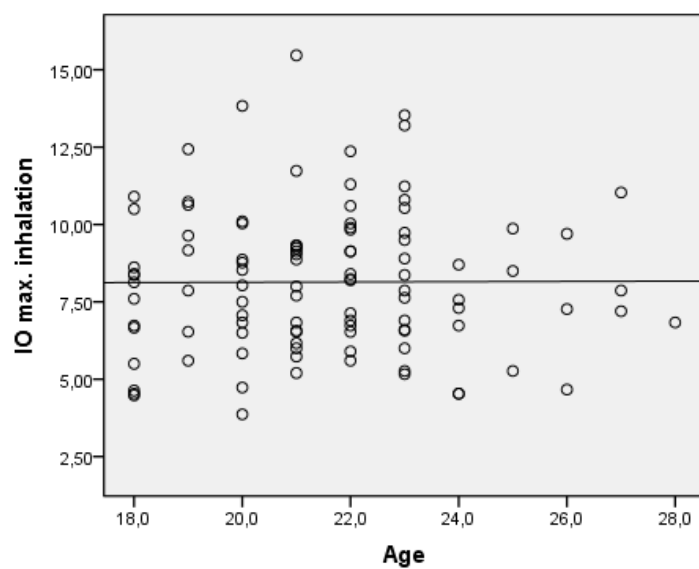
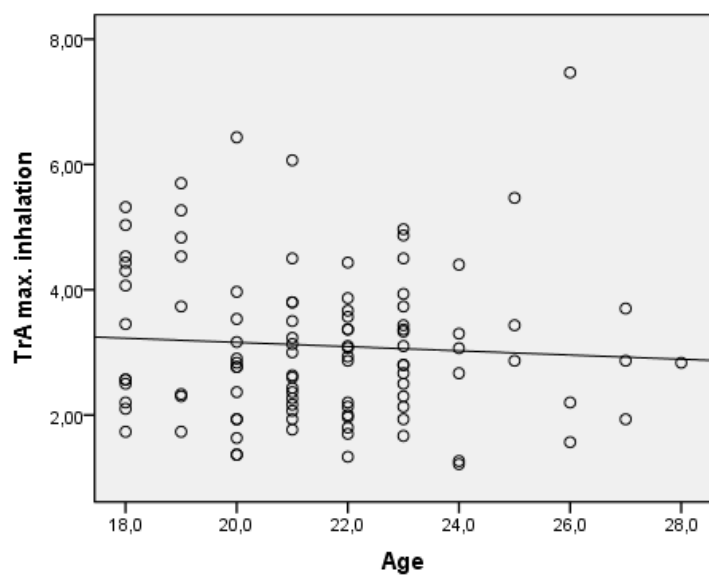
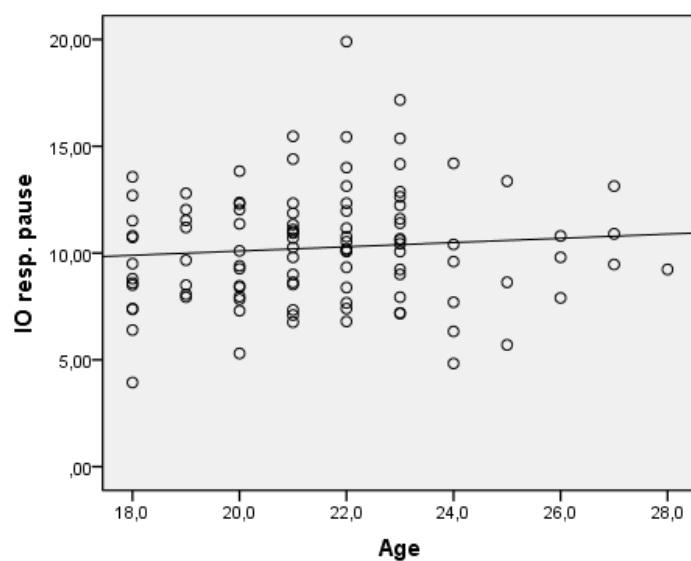
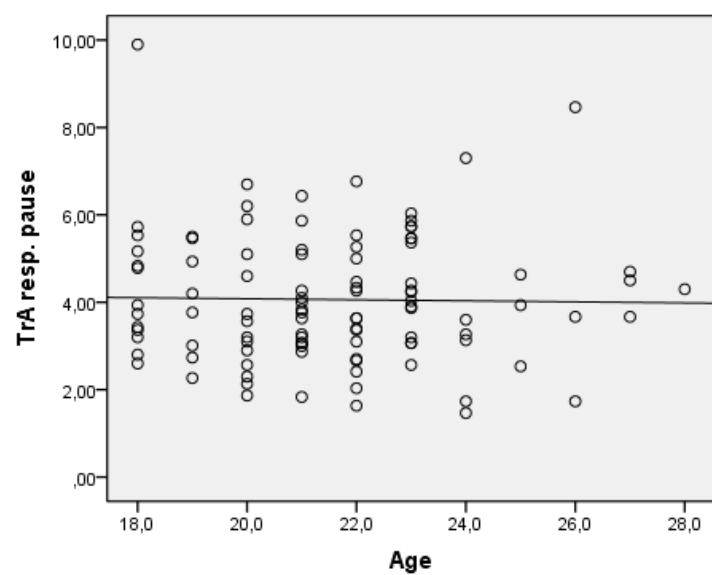
(handtekening)

Datum: 1/1

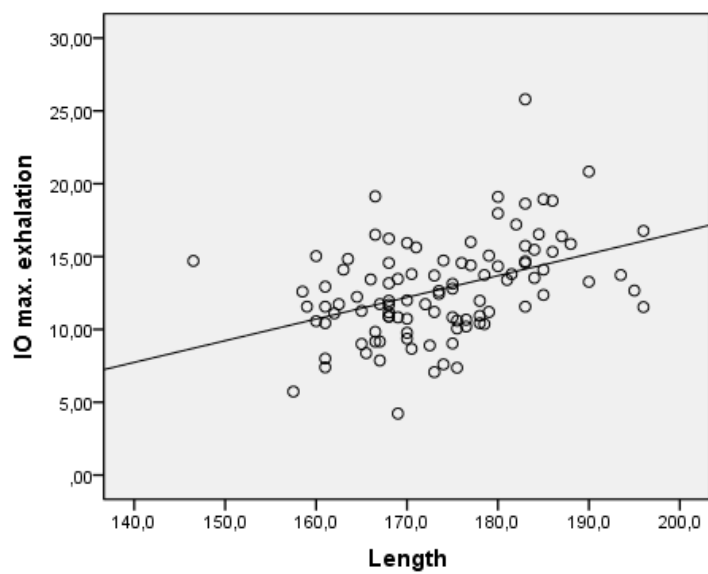
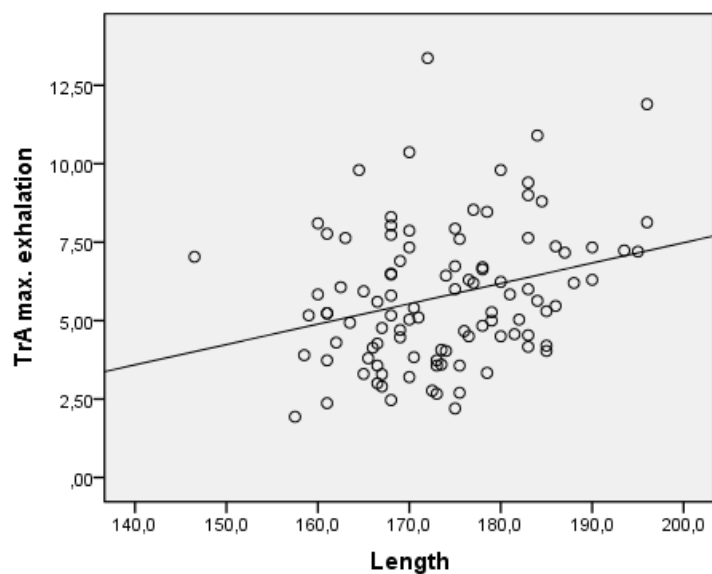
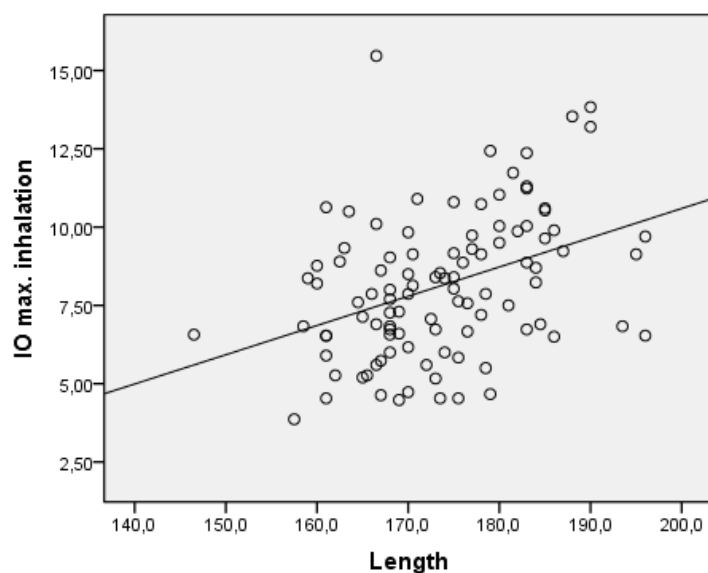
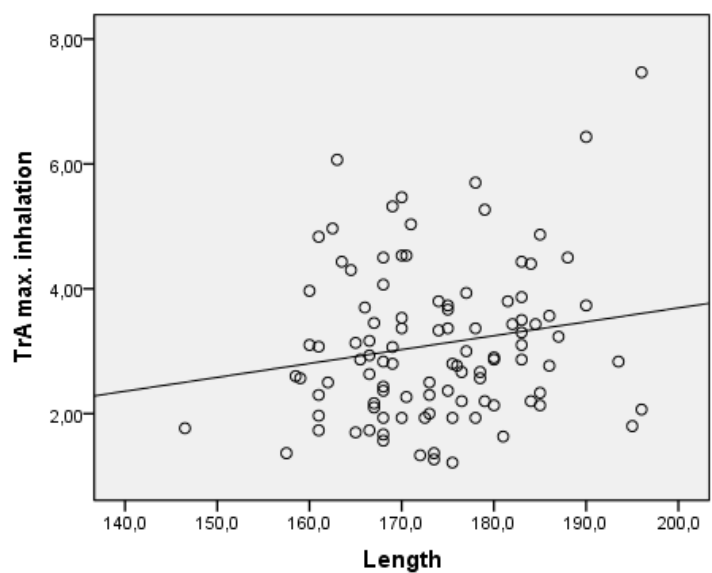
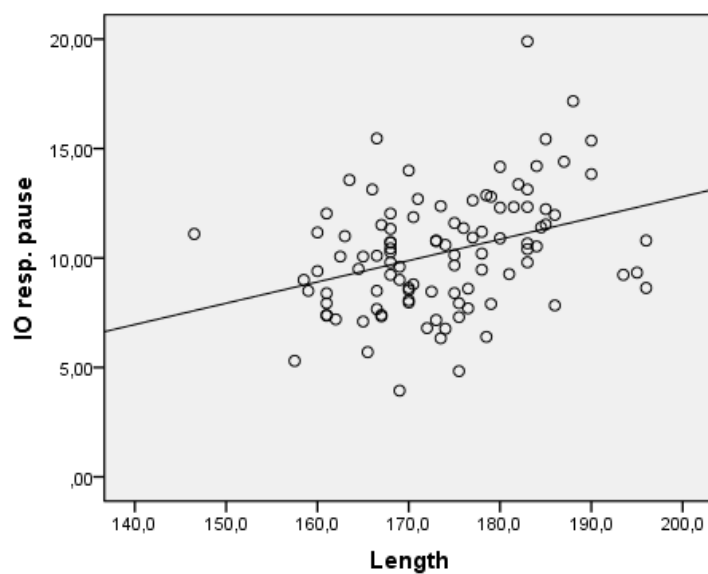
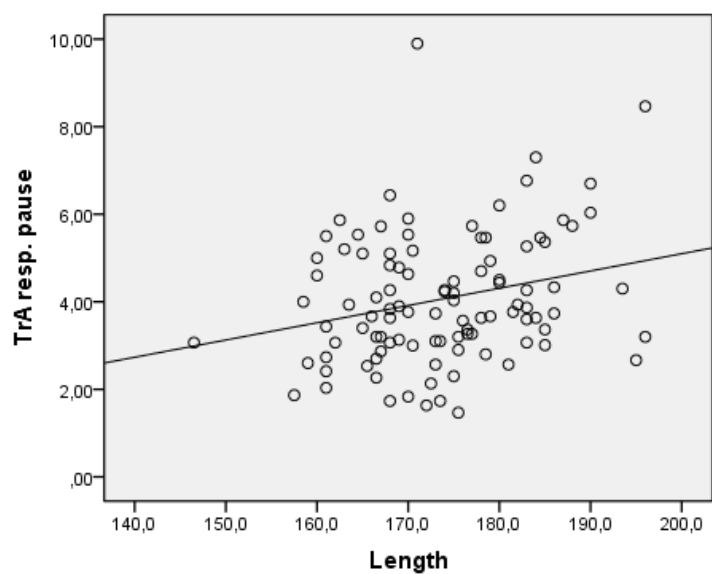
Plaats: _____



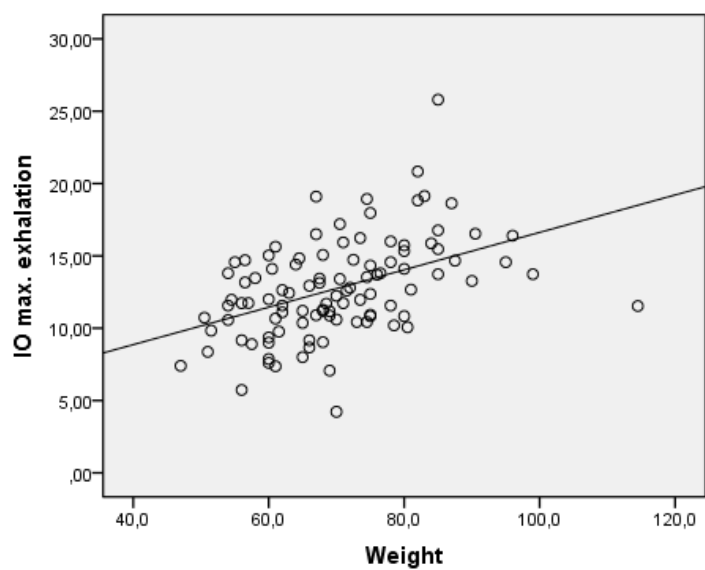
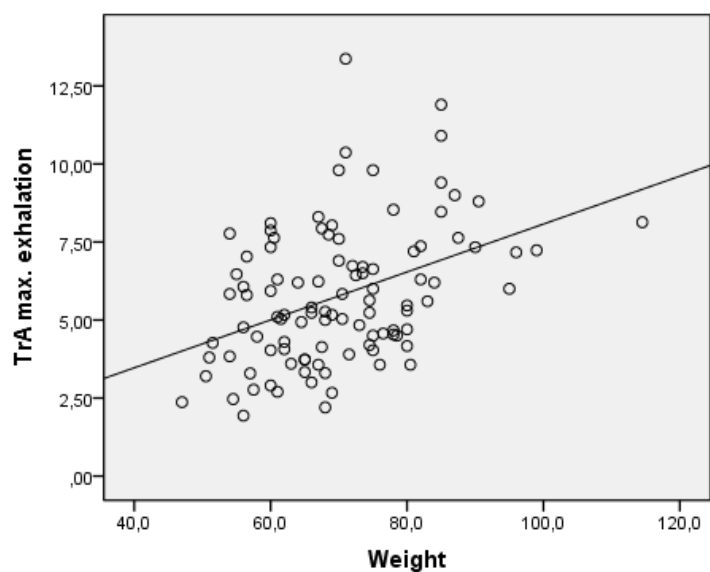
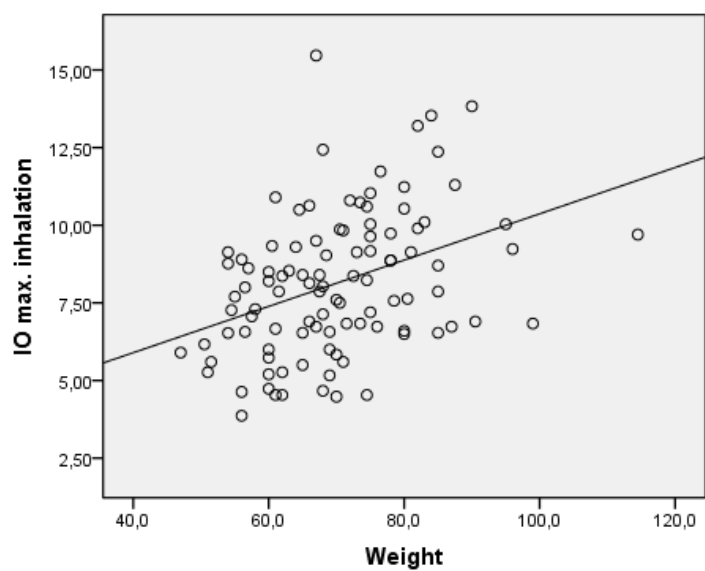
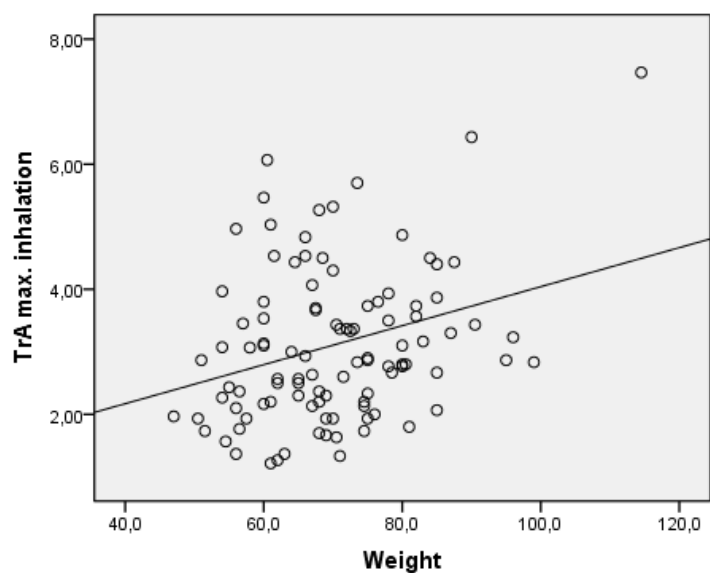
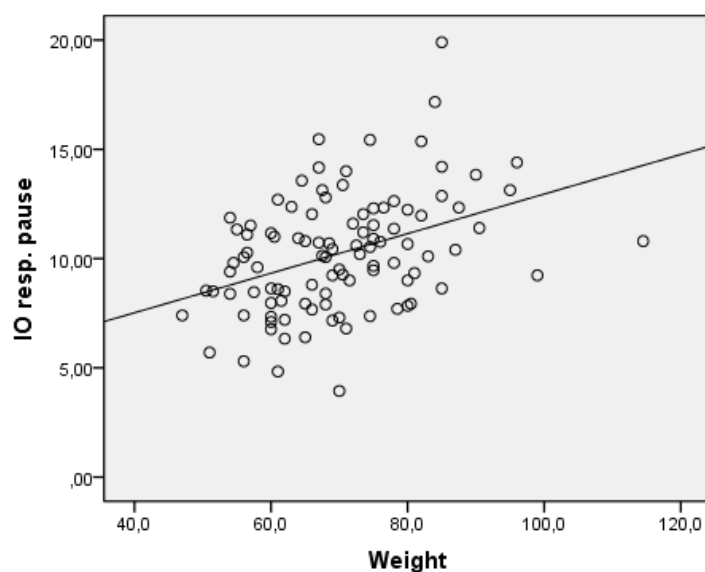
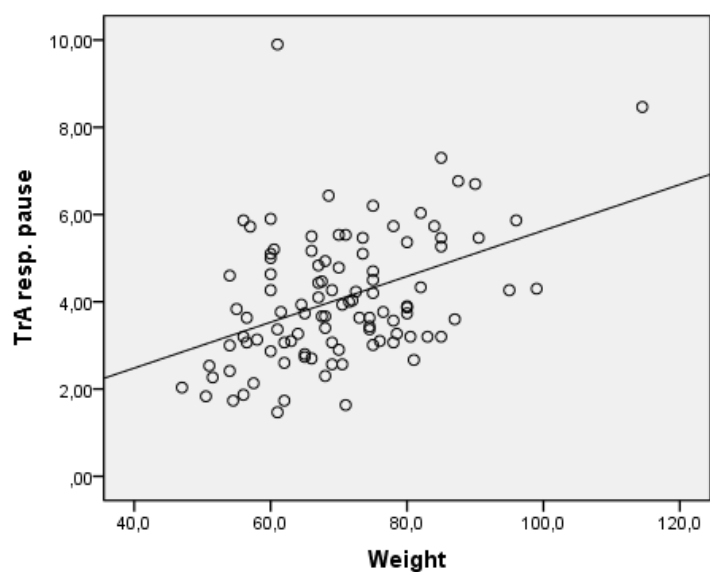
Appendix VII Scatter plots. Correlation age – muscle thickness TrA and IO



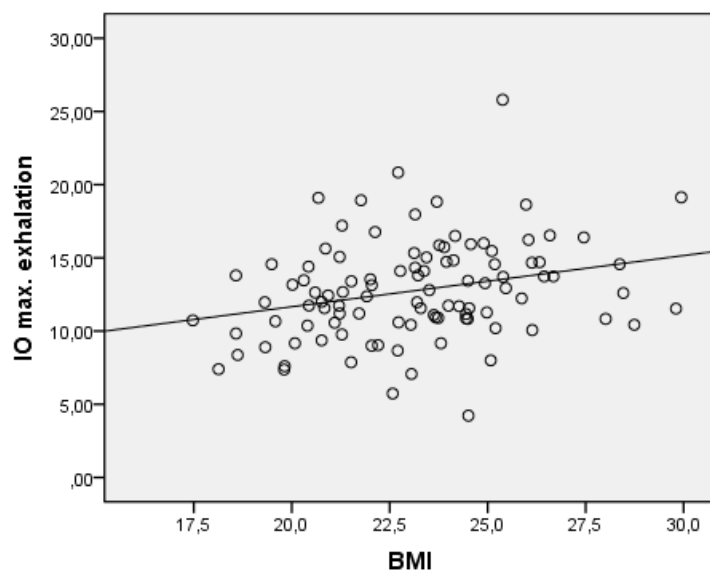
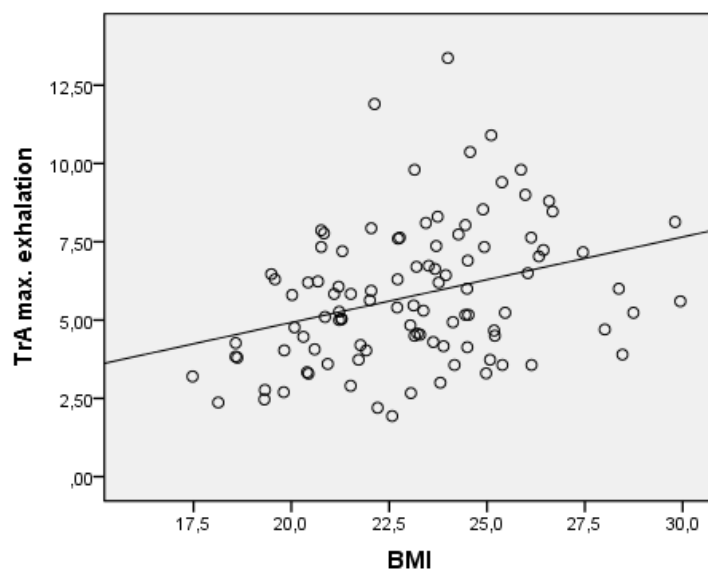
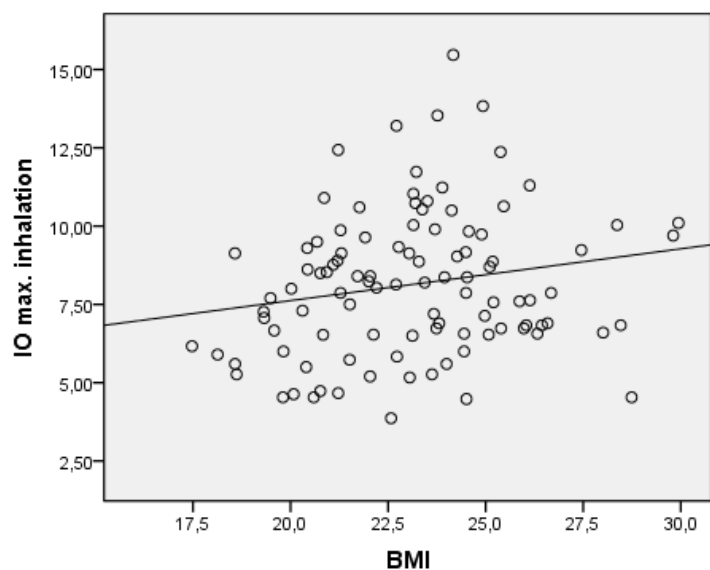
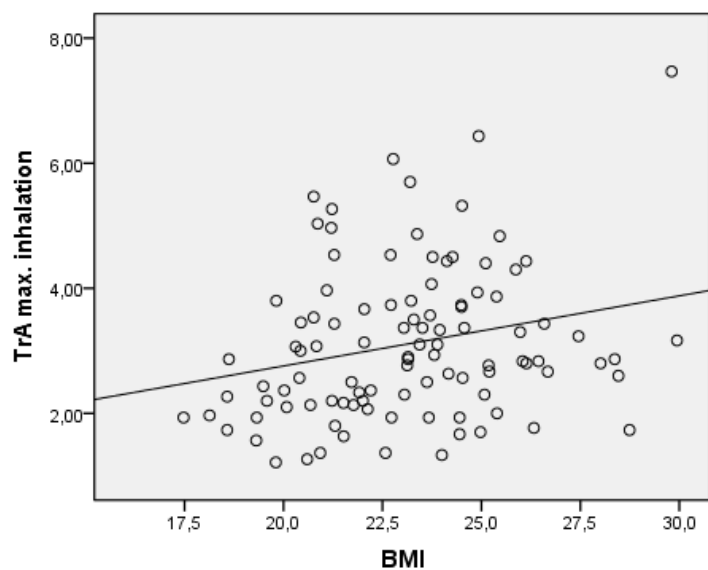
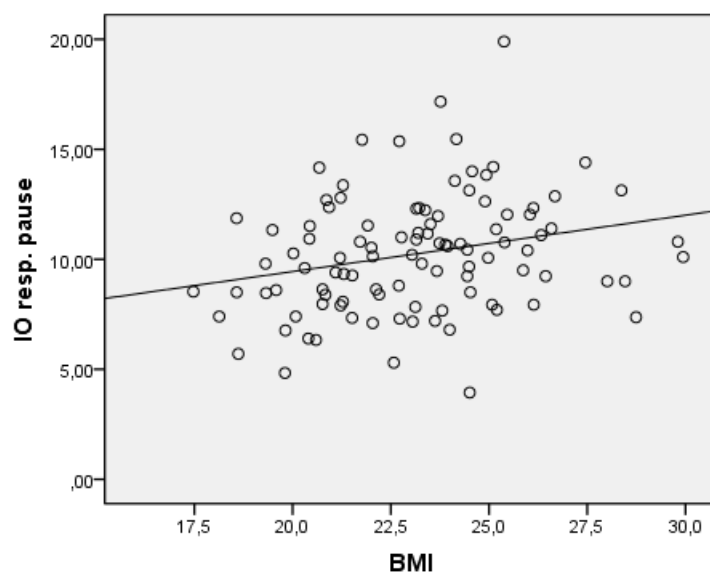
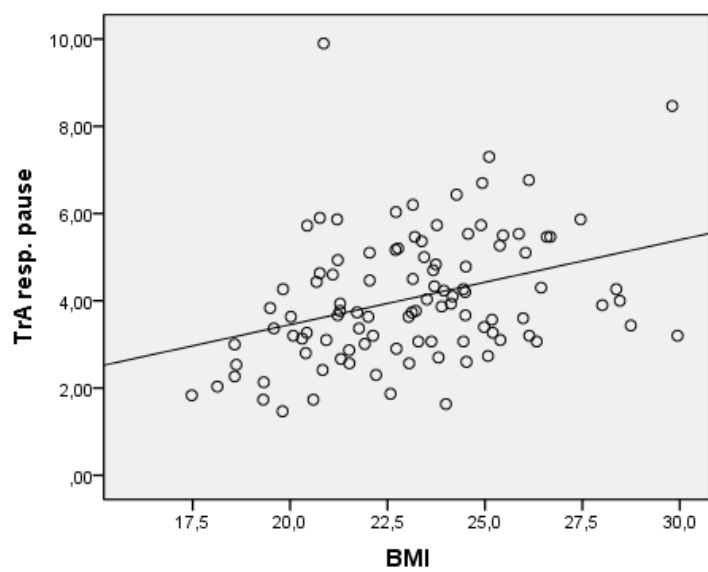
Correlation length – muscle thickness TrA and IO



Correlation weight – muscle thickness TrA and IO



Correlation BMI – muscle thickness TrA and IO



Appendix VIII Correlation muscle thickness and age, length, weight and BMI in females and males separately.

Correlation between the thickness of abdominal muscles and age, length, weight and BMI in Females and Males separately.

Females					
Muscle	Breathing moment	Age	Length	Weight	BMI
Transversus abdominis	Pause	-2.46 (0.610)	-0.32(0.808)	0.23 (0.083)	0.28 (0.035)
	Inhalation	-0.21 (0.117)	0.00 (0.984)	0.05 (0.706)	0.07 (0.584)
	Exhalation	-0.09 (0.492)	0.054 (0.682)	0.23 (0.078)	0.24 (0.074)
Internal Oblique	Pause	-0.10 (0.459)	-0.20 (0.880)	0.06 (0.663)	0.14 (0.301)
	Inhalation	-0.90 (0.517)	0.13 (0.347)	0.09 (0.510)	0.07 (0.610)
	Exhalation	0.13 (0.347)	0.04 (0.765)	0.13 (0.328)	0.15 (0.259)
Males					
Muscle	Breathing moment	Age	Length	Weight	BMI
Transversus abdominis	Pause	0.26 (0.097)	0.26 (0.089)	0.49 (0.002)	0.51 (0.001)
	Inhalation	0.07 (0.673)	0.20 (0.199)	0.39 (0.009)	0.49 (0.001)
	Exhalation	0.17 (0.269)	0.38 (0.013)	0.61 (<0.001)	0.51 (<0.001)
Internal Oblique	Pause	0.07 (0.658)	0.11(0.468)	0.19 (0.225)	0.27 (0.083)
	Inhalation	-0.10 (0.561)	0.18 (0.283)	0.22 (0.164)	0.22 (0.153)
	Exhalation	0.10 (0.542)	0.31 (0.044)	0.40 (0.007)	0.32 (0.036)
Spearman's correlation coefficient (P value)					