

Faculteit Geneeskunde en Levenswetenschappen

kinesitherapie

Masterthesis

ultrasound

Eva Schalley Josefine Smeets

PROMOTOR: Prof. dr. Lotte JANSSENS



www.uhasselt.be Universiteit Hasselt Campus Hasselt: Martelarenlaan 42 | 3500 Hasselt Campus Diepenbeek: Agoralaan Gebouw D | 3590 Diepenbeek

master in de revalidatiewetenschappen en de

Thickness of the diaphragm during active straight leg raise: an investigation with

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen

 $\frac{2017}{2018}$



Faculteit Geneeskunde en Levenswetenschappen

master in de revalidatiewetenschappen en de kinesitherapie

Masterthesis

Thickness of the diaphragm during active straight leg raise: an investigation with ultrasound

Eva Schalley

Josefine Smeets

Scriptie ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie, afstudeerrichting revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen

PROMOTOR :

Prof. dr. Lotte JANSSENS

Acknowledgement

First of all, we would like to thank our supervisor Prof. dr. Lotte Janssens for the guidance, support and patience throughout the two year writing process of this Masterthesis. Secondly, we would like to thank the University of Hasselt for providing us five years of knowledge, and all the tools we need to become good physiotherapists. Thirdly we would like to thank Fontys University, to provide us all the equipment needed to perform our research. A special thanks to Frank Musarra, who learned us how to use the Ultrasound measurement device and how to interpret the output. Also, we would like to thank the two students of Fontys University, Laura Janite and Niki Budai, for recruiting the participants of this study.

Research framework

This cross-sectional study was executed, using a central format, in the context of musculoskeletal rehabilitation and was accomplished as a duo master thesis, part two.

In the master thesis part one, 'The role of the diaphragm in trunk stability: a systematic overview of the measurement tools', research was done about different measurement tools to examine the diaphragm during postural tasks. Master thesis part two proceeds on this matter, with focus on ultrasound measurement (US) and active straight leg raise (ASLR) as a postural task.

Two students of Hasselt University executed this cross-sectional study under supervision of promoter Prof. dr. Lotte Janssens. Measurements took place at Fontys University Eindhoven and were taken in April and May 2017 in collaboration with two physiotherapy students of Fontys University. Images of the diaphragm were taken with US during rest and ASLR in healthy subjects. In addition, maximal inspiratory pressure (MIP) was assessed. Both students assessed each subject. Hereafter, data analysis was done separately by each student from September 2017 till March 2018. At last, writing of the master thesis was completed from April till June 2018.

The main goal of this study was to examine the contribution of the diaphragm during a postural task, in particular whether there is a change in thickness of the diaphragm when comparing thickness during rest (supine lying) versus during ASLR. This could be of interest for physiotherapists and researchers who deal with patient populations with disturbed trunk control causing complaints, since the diaphragm could be a contributing factor. Furthermore, inter- and intra-rater reliability of the US measurement, and a possible correlation between MIP and thickness of the diaphragm was investigated.

This duo master thesis, part two was made with similar effort of both students.

Content table

1. Abstract 5
2. Introduction
3. Purpose
3.1 Research question
3.2 Hypothesis
4 Methods 11
4.1 Research design 11
4.2 Research framework 11
4.3 Participants11
4.4 Medical ethics 11
4.5 Experimental set-up 11
4.6 Data reduction17
4.7 Statistical analysis18
5. Results
5.1 Participants
5.2 Inter-rater and intra-rater reliability 19
5.3 Change in diaphragm thickness following ASLR19
5.4 Correlations with MIP21
6. Discussion 23
7. Conclusion
8. Reference list 29
9. Attachments

1. Abstract

Background: The diaphragm is considered as the main inspiratory muscle, which also plays an important role in trunk control during postural tasks. This latter may be disturbed in certain patient populations for example low back pain. Thickening of the diaphragm can be measured directly and non-invasively with ultrasound measurement (US).

Purpose of this research: The main purpose of this study was to investigate whether there is a change in thickness of the diaphragm during a postural task compared to thickness in rest, when investigated with US. In addition, the intra- and inter-rater reliability for this measurement is assessed in healthy subjects and whether there is a correlation between the maximal inspiratory pressure (MIP) and diaphragm thickness.

Methods: Twenty-seven healthy individuals were assessed. Diaphragm thickness was measured with US during rest, ipsilateral and bilateral active straight leg raise (ASLR). Furthermore MIP was assessed.

Results: Inter-rater reliability during rest, ipsilateral and bilateral ASLR was fair to good (ICC=0.458, 0.474, 0.518) and intra-rater reliability was excellent (ICC=0.875, 0.875, 0.889). There was no significant difference in diaphragm thickness between rest and ASLR (p<0.05). There was a correlation between MIP and diaphragm thickness (r> 0.41, p< 0.05).

Discussion and conclusion: The findings in this study suggest that there is no change in thickness of the diaphragm during this specific postural task (ASLR). Also, the reliability of the US measurement could be further improved with a more standardized procedure. Further research is needed to investigate the postural function of the diaphragm with other, more functional postural tasks.

Key words: diaphragm, respiratory muscles, postural function, trunk stability, trunk control, ultrasound, maximal inspiratory pressure, active straight leg raise.

2. Introduction

The diaphragm is considered as the main inspiratory muscle, but also plays an important role in trunk control during postural tasks. When needed, the diaphragm together with other profound trunk controlling muscles such as the multifidus, pelvic floor, and transverse abdominal muscles provide an increased intra-abdominal pressure (IAP). This provides an augmented spinal 'stiffness', which leads to an improved trunk control during postural tasks (Hodges, Eriksson, Shirley, & Gandevia, 2005).

The function of the diaphragm is often disturbed in individuals with low back pain. These individuals show for example limited movement of the anterior medial and costal part of the diaphragm, which results in an asymmetric and less powerful activation of the diaphragm during a postural task. Therefore, spinal and trunk control is reduced through IAP reduction (Kolar et al., 2012). Thus, dysfunction of the diaphragm could be a relevant contributor to this pathology. In addition, considering rehabilitation of this population, inspiratory muscle training can improve proprioceptive use of the trunk muscles during postural tasks and reduce low back pain severity (Janssens et al., 2015). Therefore, it is useful to detect this dysfunction in clinical examination. Yet, there is little direct evidence on how diaphragm contractions contribute to trunk control during a postural task.

Profound structures, such as the diaphragm, can be measured non-invasively with ultrasound measurement (US). Hereby, the thickening of the diaphragm can be measured directly. During respiration, US is proven to be an accurate and reproducible method in measuring thickness of the diaphragm (Cohn, Benditt, Eveloff, & McCool, 1997). In the context of postural control, US has already been used in some patient populations to measure the diaphragm. For example, Terada et al. used US during rest to examine diaphragm contractility in patients with chronic ankle instability (Terada, Kosik, McCann, & Gribble, 2016). Priori et al. analysed chest wall movements in chronic obstructive pulmonary disease, both in supine and seated position. Hereby, US was used to plot the zone of apposition of the diaphragm, and then to calculate the displacement for each position (Priori et al., 2013). Furthermore, O'Sullivan et al. used US to measure diaphragm excursion in patients with sacroiliac joint pain during rest, ASLR and ASLR with manual pelvic compression (O'Sullivan et al., 2002). However, considering the prior, there is a lack of evidence on whether the diaphragm contributes directly to postural control in healthy

subjects and whether it is reliable and valid to measure the contractility of the diaphragm with US during the postural task. As well, there is a lack of knowledge concerning the change in thickness of the diaphragm measured with US during rest versus a postural task.

ASLR is a postural task during which leg and trunk muscles are activated in a certain pattern. Hereby, contralateral forces are produced by cooperation of abdominal and hip muscles to 'stabilize' the sacroiliac joint, which is called 'force closure' (Hu et al., 2012). Through this activation of abdominal muscles there could be an increment of IAP, which coincides with lumbar 'stability', which in turn contributes to trunk control. For example, in pregnancyrelated low back and pelvic pain it could be suggested that an impaired ASLR (a subjective feeling of heaviness of the leg or pain) demonstrates a disturbed load transfer through the sacroiliac joint (de Groot, Pool-Goudzwaard, Spoor, & Snijders, 2008).

Visualisation of the diaphragm using US during ASLR could be a way to examine whether the diaphragm contributes to trunk control during this postural task. There is already evidence of greater postural contribution of the diaphragm during erect postures, when considering standing versus supine (Hellyer et al., 2017a). However, there is little evidence on how the diaphragm contracts in supine during a task to challenge trunk control (e.g. ASLR). Furthermore, in view of the dual role of the diaphragm, it is yet unclear whether there is a correlation between inspiratory muscle force and the diaphragm's contribution to control of the trunk.

The main purpose of this study was to investigate whether there is a change in thickness of the diaphragm during rest (supine lying) versus ASLR, when investigated with US. In addition, the aim was to examine the intra- and inter-rater reliability for this measurement in healthy subjects and whether there is a correlation between the maximal inspiratory pressure (MIP) and the thickness of the diaphragm following ASLR.

3. Purpose

3.1 Research question

The main research question was whether there is a change in thickness of the diaphragm during rest (supine lying) versus unilateral and versus bilateral ASLR, when investigated with US.

Besides, forthcoming questions were whether there is a sufficient inter- and intrarater reliability for this measurement and whether there is a correlation between the MIP and diaphragm thickness during rest and during ASLR.

P: Healthy adolescents aged between 18 and 29 years, with a body mass index (BMI) < 30 kg/m^2 .

I: Measurement of diaphragm thickness in supine lying with US imaging, during rest, unilateral and bilateral ASLR. In addition, measurement of the MIP was performed.

C: /

O: Change in thickness of the diaphragm between rest and ASLR, and its correlation with MIP.

3.2 Hypothesis

The main hypothesis was that there would be a significant increase in thickness of the diaphragm during ASLR when comparing with the thickness during rest, detectable on the US image. In addition, a greater significant increase in thickness was expected when performing a bilateral versus a unilateral ASLR.

Furthermore, it was expected that the US measurement has proper inter-, intra-rater reliability and that the greater the thickening of the diaphragm was between rest and ASLR, the higher the value of MIP would be.

4 Methods

4.1 Research design

This was an observational, cross-sectional study.

4.2 Research framework

This study was in collaboration with two students of Fontys University (Eindhoven, The Netherlands).

4.3 Participants

Participants were recruited at Fontys University in the Netherlands by oral publicity, e-mails and flyers, which started at the first of April until the 10th of May 2017. The data collection took place from the first of May until the 10th of May 2017. Thirty-one healthy persons in the age range of 18-29 were included. Furthermore, participants were excluded according to the following criteria: pregnancy or birth giving in past six months, musculoskeletal pain in lower back or lower extremities in past six months, orthopedic surgery in lumbar spine, pelvis, abdominal or thoracic area in the past year, neurologic disorder affecting lumbopelvic and groin regions, diagnosed neuromuscular diseases (e.g. weakness or paralysis of diaphragm), history of sensory nervous or vestibular system problems, lower limb injuries in the past year, BMI > 30 kg/m² and previously diagnosed lung disorders/illness (e.g. asthma).

4.4 Medical ethics

US imaging is non-invasive, and consequently free from pain and risk.

Preceding the measurements, participants were asked to sign an informed consent form (Attachment 1). This consent contained several informative aspects such as the freedom of the participants to ask questions regarding the research or to withdraw from the research at any time. Furthermore, information about anonymity of the data was given. The US images together with the collected data will be kept at Fontys University for 15 years and erased after the research is finished. *Fontys Ethical Committee* approved the research protocol.

4.5 Experimental set-up

The participant was examined in the same matter for 15 minutes by the first rater and 15 minutes by the second rater. After these two examinations, MIP measurements were taken for approximately 10 minutes.

US MEASUREMENT

In healthy subjects, the thickening of the diaphragm during normal breathing, measured with US, has already been proven highly reliable in supine position. For the inter-rater reliability, the intra-class correlation coefficient (ICC) was 0.97 (95% CI: 0.91-0.99) for inspiratory thickening and 0.98 (95% CI: 0.94-0.99) for expiratory thickening of the diaphragm (Harper et al., 2013). For the intra-rater reliability, the ICC was 0.94 (95% CI: 0.79-0.98) for inspiratory thickening and 0.98 (95% CI: 0.94-0.99) for expiratory thickening of the diaphragm (Harper et al., 2013). US measurements are therapist dependent, but with practice and adherence to criteria for making a proper image, the measurement of diaphragm thickening should be reproducible (Cohn et al., 1997). In healthy subjects, the validity of diaphragm thickening measurements with US is already proven, whereby diaphragm thickening was correlated to inspiratory volume (r= 0.32; p= 0.0001), to diaphragm electromyography (r= 0.32; p= 0.01) and IAP (r= 0.28; p= 0.01) (Goligher et al., 2015).

The US measurements were taken in supine lying position, during three different conditions: rest, ipsilateral ASLR (relative to the side of the US measurement) and bilateral ASLR. The measurements took place on the right side of the body, so the US imaging device (Aloka ProSound Alpha 7, two-dimensional B-mode ultrasound) was to the right of the participant. The participant performed the ipsilateral as well as the bilateral ASLR three times, with every time a resting condition in between. ASLR was the chosen postural task because it was easy to standardize in all participants, and therefore it was easy to obtain objective measurements. Because of the stable position of the trunk in supine, measurements could be taken carefully, and pressure could be exerted on the US probe. This latter would be more difficult during a dynamic or standing task. The height of the leg raise was standardized with cubes left and right to the feet, which gave visual feedback to the participant.



Figure 1. Experimental set up.

Prior to the measurement, the participant was provided information as followed: "You will be asked to undress your upper body up to underwear and lay down on the treatment table in supine position. Then, the measurements of the diaphragm will be taken at rest and at every turn after a 5 second hold period during the active straight leg raise test. In total you will be asked to lift your right leg three times and lifting both legs also three times given a period of rest in between. Do you have any questions?".

The treatment table was adjustable in height and head position. The participant's head was slightly lifted with a pillow and flexed to approximately 45 degrees in order to be able to see his own feet. The arms were resting at the sides of the body during testing and the heels were slightly apart, medial to the cubes (each 10 cm, indicating the lifting height). To ensure correct execution of the ASLR, the rater passively lifted the leg straight 10 cm off the table and said: "This position you will be asked to reach". The participant was asked to breathe in and out normally with the mouth kept open. After a normal exhalation, the participant was asked to stop breathing, keep the mouth open, and lift the right leg (for the ipsilateral ASLR) or both legs (for the bilateral ASLR) 10 cm off the table with the knee joint(s) kept extended. When reached, the participant had to hold the leg(s) there for five seconds without an extra inhalation. The US picture was taken immediately after this five seconds. Followed by the

command, the participant returned to the starting position and had to rest for a minute before the next measurement took place. To ensure the quality of the testing, the participants were given the following commands: "Open your mouth, breath in normally, breath out, stop breathing and lift the leg", followed by "hold", and followed by "lower to starting position".

In total, 13 US pictures were taken per participant, per rater, each before (rest) and during the unilateral/bilateral ASLR, and one at the very end. In total, this provided 7 pictures at rest and 6 active pictures (Table 1).

First rater	Condition	Second rater	Condition
Trial		Trial	
1	Rest	1	Rest
2	First ipsilateral ASLR*	2	First ipsilateral ASLR*
3	Rest	3	Rest
4	Second ipsilateral ASLR*	4	Second ipsilateral ASLR*
5	Rest	5	Rest
6	Third ipsilateral ASLR*	6	Third ipsilateral ASLR*
7	Rest	7	Rest
8	First bilateral ASLR*	8	First bilateral ASLR*
9	Rest	9	Rest
10	Second bilateral ASLR*	10	Second bilateral ASLR*
11	Rest	11	Rest
12	Third Bilateral ASLR*	12	Third Bilateral ASLR*
13	Rest	13	Rest

Table 1 Experimental conditions for diaphragm US measurement

*ASLR= active straight leg raise

When taking the US picture of the diaphragm, the probe was placed on the anterior axillary line in an intercostal space, parallel with the fiber direction of the diaphragm. The probe was directed medially, cephalad, and dorsally, so that the US beam reached nearly perpendicularly the posterior part of the vault of the right diaphragm (Ayoub et al., 1997). Proximal (left side Figure 2), the diaphragm is attached to the lung. The ideal image

consisted of two ribs on each side of the picture, with a small part of the lung shade and the diaphragm in between.





MIP MEASUREMENT

A test that measures inspiratory muscle strength was required to relate the respiratory and the postural function the diaphragm. For this purpose, the MIP measurement was used because it estimates inspiratory muscle force (Jalan et al., 2015).

In healthy subjects, one practice session is needed to become a reliable measurement and at least five measurements are needed to become 3 reproducible values (Smeltzer & Lavietes, 1999). There could be day to day fluctuations of 10% (Volianitis, McConnell, & Jones, 2001). However, it is a reliable measurement tool for inspiratory pressure (Romer & McConnell, 2004). In addition, the between-day reliability improves when the measurements are preceded by an inspiratory muscle warm-up. This could provide a MIP improvement up to 21%. Five to six attempts are needed to reach the maximal MIP (Lomax & McConnell, 2009). Furthermore, the ICC coefficient for intra-rater and inter-rater reliability was respectively 0.96 and 0.92 (Jalan et al., 2015). This is highly reliable, because values above 0.80 are considered highly reliable (Fleiss, J.L., 1986).

For the measurement the participant was seated on a chair, with the spine straight without back support and the feet rested on the ground. A nose clip was placed on the nose to prevent loss of air through the nose (Figure 3). Then, the participant was instructed as followed: "First, you exhale as far as you can, until you can't exhale any further. Take the MIP device (POWERbreathe International Ltd., type KH1, Warwickshire, UK) and put the mouthpiece firmly into the mouth. Next, you will be asked to inhale as powerful and fast as possible". During the performance the researcher supported the device with one hand and the back of the participant's neck with the other hand.



Figure 3. Positioning MIP measurement.

This procedure was repeated at least five times, until there were three values (including the maximal value) that differed less than 5 cm H_2O (Black & Hyatt, 1969). Between each performance, a break of one minute was held to prevent that potential muscle fatigue would bias the result. When there was one outlier value that exceeded the three values that differed less than 5 cm H_2O , this maximum value was taken as MIP.

4.6 Data reduction

US MEASUREMENT

US images were evaluated with the analyzing program 'ImageJ'. Thickness of the diaphragm was expressed in mm, which is a quantitative variable.

When the image was theoretical correct (as on Figure 2), the diaphragm thickness was measured perpendicular from the lower to the upper border of the diaphragm, five mm distally from the lung shade. However, sometimes the lung shade disappeared behind the left rib on the image, with only the diaphragm visible between the two ribs. In that case, the diaphragm thickness was measured five mm distally from the left rib. This data were collected in Microsoft Office Excel 2007.

Furthermore, the absolute difference between active and rest diaphragm thickness was calculated for as well ipsilateral as bilateral ASLR. Rest was subtracted from active diaphragm thickness. When the difference was positive, there was a thickening, and when the difference was negative, there was a narrowing. In addition, diaphragm thickening fraction (DTF %) was calculated for ipsilateral as well as for bilateral ASLR with the following formula: ((Active – Rest) / Rest)*100 (Kocjan J. et al.). Images of the first four participants were excluded, since these measurements were seen as trials.

MIP MEASUREMENT

The highest measured MIP and calculated predicted MIP values according to reference values (Rochester & Arora, 1983) were inserted in Microsoft Office Excel 2007 for each participant.

4.7 Statistical analysis

Inter- and intra-rater reliability were assessed through calculating a two-way random ICC (2,1), absolute agreement. Intra-rater reliability was calculated for the measurements of rater 1, for three different conditions, i.e. the 6 rest measurements, the 3 ipsilateral measurements and the 3 bilateral measurements. The inter-rater reliability between rater 1 and 2 was calculated for three different conditions, i.e. second rest measurement (See Table 1 trial 3), second ipsilateral ASLR (See Table 1 trial 4) and second bilateral ASLR (See Table 1 trial 10). ICC findings are interpreted by the classification of Fleiss. Using this classification, following categories are handled: ICC < 0.40 is poor reliability, ICC 0.40- 0.75 is fair to good reliability, ICC > 0.75 is excellent reliability (Fleiss, J.L., 1986). A confidence interval (CI) of 95% was handled. Repeated measures were used to examine differences between the three rest measurements (before ipsilateral ASLR) and the three ipsilateral measurements, as well as between the three rest measurements (before bilateral ASLR) and the three bilateral measurements. Likewise, repeated measures were used to examine differences between the different DTF's(%) and also to examine differences between the absolute change in thickness of the different trials. Correlations between diaphragm thickness, MIP and MIP predicted were calculated by the Pearson test, because these data were proven to be normal according to the Shapiro-Wilk test. Correlations between diaphragm thickness and gender, BMI and age were calculated by the Spearman test, because gender, BMI and age did not have a normal distribution according to the Shapiro-Wilk test. The r-value is used to interpret the data, where a r< 0 represents a negative correlation and a r> 0 represents a positive correlation. The statistical analysis was performed in JMP Pro 13 and SPSS 25. The level of significance was set at p< 0.05.

5. Results

5.1 Participants

Eventually 27 healthy participants took part in the study (14 males, 13 females). Baseline characteristics of the subjects are shown in table 2.

Table 2
Baseline characteristics

	Subject group (n=27)
Age (yrs)	23 ± 3
Height (cm)	175 ± 12
Weight (kg)	71 ± 15
BMI* (kg/m ²)	23 ± 3

* BMI= Body Mass Index

5.2 Inter-rater and intra-rater reliability

The inter-rater ICC [95% CI] during rest was 0.458 [0.122-0.700] (p< 0.002), the ICC during ipsilateral ASLR was 0.474 [-0.031-0.756] (p< 0.001) and the ICC during bilateral ASLR was 0.518 [-0.061-0.798] (p< 0.001). The reliability of these three measurements is considered fair to good (0.40-0.75). The intra-rater ICC [95% CI] during rest was 0.875 [0.806-0.929] (p<0.001), the ICC during ipsilateral ASLR was 0.849 [0.745-0.919] (p<0.001) and the ICC during bilateral ASLR was 0.889 [0.809-0.941] (p< 0.001), which all are considered as excellent reliability (> 0.75). In addition, there was no significant difference between the three repeated measures for the ipsilateral (p= 0.725) neither for bilateral ASLR (p= 0.145). Also, no significant difference was found between rest and change in thickness when comparing the three different measurements for ipsilateral (p=0.996) and bilateral ASLR (p= 0.330). There is no significant difference of the DTF(%) over the three different repetitions for ipsilateral neither for bilateral ASLR (p= 0.409).

5.3 Change in diaphragm thickness following ASLR

There was no significant difference in diaphragm thickness during rest and ipsilateral ASLR (p= 0.804). Likewise, there was no significant difference in diaphragm thickness between rest and bilateral ASLR (p= 0.762).

In terms of the direction of change in thickness, 22 to 30% of the subjects showed thickening during either ipsilateral or bilateral ASLR, whereas 18 to 48% showed narrowing and 29 to 48% showed no change. An overview of the percentages is shown in table 3.

Trial	Thickening	Narrowing	No Change
1 st ipsilateral ASLR*	29.6%	22.2%	48.2%
2 nd ipsilateral ASLR*	22.2%	48.1%	29.7%
3 rd ipsilateral ASLR*	22.2%	40.7%	37.1%
1 st bilateral ASLR*	25.9%	25.9%	48.2%
2 nd bilateral ASLR*	29.6%	18.5%	51.9%
3 rd bilateral ASLR*	29.6%	40.7%	29.7%

Percentages of participants, showing change in thickness of the diaphragm per trial

*ASLR= active straight leg raise

Table 3

DTF(%) is not significantly different when comparing ipsilateral to bilateral ASLR (p= 0.199). Also, the absolute change in thickness was not significantly different between the ipsilateral and the bilateral trials (p= 0.807). Furthermore, there was no significant difference in absolute change in thickness between the 3 repetitions, when comparing the ipsilateral and the bilateral trials (p= 0.144).



Figure 4. Average diaphragm thickness for the different experimental conditions.

5.4 Correlations with MIP

There was a significant positive correlation between MIP and diaphragm thickness at rest (r> 0.41, p< 0.030), during ipsilateral ASLR (r> 0.43, p< 0.023) and during bilateral ASLR (r> 0.49, p< 0.008), which indicates that the higher the MIP value, the greater diaphragm thickness (Figure 5). For the predicted MIP(%) there was no correlation with diaphragm thickness during the aforementioned conditions (r< 0.30, p> 0.050).



Figure 5. Correlation between MIP and diaphragm thickness.

5.5 Correlations with patient characteristics

There was a significant positive correlation between gender and diaphragm thickness (r> 0.46, p< 0.015), which indicates that males have greater diaphragm thickness. In addition, there was no significant correlation between diaphragm thickness and age (r= -0.09 - 0.08, p> 0.05). When exploring the correlation between BMI and diaphragm thickness, a significant positive correlation was found between BMI and diaphragm thickness during the first ipsilateral ASLR (r= 0.42, p= 0.025) and the third ipsilateral ASLR (r= 0.41, p= 0.031). Furthermore, there was a significant positive correlation between BMI and diaphragm thicknesm thicknesm thicknesm between BMI and diaphragm thicknesm between BMI and diaphragm thicknesm the diaphragm the first ipsilateral ASLR (r= 0.42, p= 0.025) and the third ipsilateral ASLR (r= 0.41, p= 0.031).

(r> 0.4, p< 0.05). This indicates that persons with higher BMI have greater diaphragm thickness during bilateral ASLR (Figure 6 and 7).



Figure 6. Correlation between BMI and diaphragm thickness during the six rest conditions.



Figure 7. Correlation between BMI and diaphragm thickness during ipsilateral and bilateral ASLR.

6. Discussion

The difference in diaphragm thickness when comparing rest to ASLR

The outcomes of this study suggest that there is no difference in diaphragm thickness when comparing rest to ASLR. In addition, there is no difference in thickening and DTF(%) between ipsilateral and bilateral ASLR, and between the different repeated measurements. Hellyer N.J. et al. demonstrated a greater thickness of the diaphragm in erect position compared to supine position, investigated with US (Hellyer et al., 2017b). This finding suggests that the diaphragm truly does contribute to postural control in erect postures, which are functional tasks. It could be concluded that the diaphragm does not contribute to every postural task; specifically in this research during ASLR, which is a less functional task, there was no change in activation of the diaphragm observable through US. One explanation could be that there is effectively no change in activation during ASLR, another explanation could be that US is not precise enough to measure this change in activation. However, this latter would be unlikely because the validity of US is already proven (Goligher et al., 2015).

Inter-rater and intra-rater reliability

The inter-rater reliability of the US measurement of diaphragm thickness during rest, ipsilateral and bilateral ASLR was fair to good. It could be further improved by standardizing the measurement procedure even more, for example by marking the mid-axillary line and the intercostal rib space (where de diaphragm's attachment is visible) on the skin of the participant. Hereby, the raters would place the probe of the US more consistent. Additionally, a custom made probe fixator could be used which makes it besides possible to hold the probe in place during more dynamic postural tasks such as standing, balance disruption or walking. A US probe fixator like this has already been used for measurement of abdominal muscle thickness during a standing postural task, which was proved to be beneficial compared to handheld US measurement (Bunce, Hough, & Moore, 2004). The intra-rater reliability was excellent, which means that it is reliable to measure diaphragm thickening with US within the same rater. In comparison, Grams et al. demonstrated moderate intra- and excellent inter-rater reliability when investigating diaphragm excursion measurement with US during quiet breathing (Grams et al., 2014).

Furthermore, for the measurement of the intra-rater reliability, no distinction can be made between the influence of the rater himself or the influence which is independent from the rater, i.e. fatigue as a possible confounder, which could make the repeated measures slightly different from each other.

The Correlation between MIP and diaphragm thickness

A positive correlation between MIP and diaphragm thickness was found. This suggests that a greater inspiratory muscle force is associated with greater muscle mass of the diaphragm. This is in line with the overall knowledge that muscle strength depends on muscle mass, and thus is related to the cross-sectional (Ikai & Fukunaga, 1968). Furthermore, the assumption was made that thickening of the diaphragm during a postural task, compared to rest thickness, could also be related to MIP. Thus, the greater the MIP value, the greater the thickening of the diaphragm. However, this correlation has not been investigated in this study, because there was no explicit thickening of the diaphragm during ASLR when compared to rest thickness. In addition, the correlation with MIP might not be as clear as thought, because no correlation is shown with the MIP pred.(%), which actually is a parameter that is more adjusted to the individual participants.

The Correlation between diaphragm thickness and personal parameters

There was a positive correlation for diaphragm thickness with gender and BMI. More specifically, in males the diaphragm was thicker. This was expected, because in general men have greater muscle mass than women, i.e. muscle fibers of the vastus lateralis muscle in men have a larger cross sectional area compared with women (Haizlip, Harrison, & Leinwand, 2015). Furthermore, participants with a greater BMI showed greater diaphragm thickness. It could be that a higher BMI includes greater abdominal adipose tissue, causing more effort needed from the diaphragm to inhale which eventually result in greater diaphragm thickness. In contrast, it was stated that in individuals with higher BMI caused by muscularity, the increase of diaphragm muscle mass rarely is the result of increased ventilation, but more so through types of work which involve thorax stabilisation (Arora & Rochester, 1982). However in this study, participants with BMI equal to or greater than 30 were excluded. Therefore, it is hard to make a statement about the relation between BMI and thickness of the diaphragm. Yet, Boon et al. also found a positive correlation between

diaphragm thickness and BMI, whereas participants with an even wider BMI range (16.1 – 51.2 kg/m²) were included. Furthermore, there was no correlation found with age. Possibly, this is because diaphragm thickness is linked to inspiratory effort (breathing), which is a vital function that has to be maintained throughout the lifespan. In contrast, it has been proved that by the age of 75, 30 - 40% of the muscle fibres are lost in the whole body, type I as well as type II (Piasecki, Ireland, Jones, & McPhee, 2016). Again for age, in this study there were specific inclusion criteria (18 - 29 years), which makes it hard to state something about this correlation. Yet, these are all expected outcomes according to previous studies, which found a positive correlation between diaphragm thickness at rest and gender, no effect of age (Harper et al., 2013), and a positive correlation with body composition (Boon et al., 2013).

Limitations

The fact that the postural task was done in supine lying is a relative limitation; this is not as functional as dynamic postural tasks from daily life, for example lifting weights, running, balance disruptions etc. However, it is unclear whether mobile US devices are reliable to measure diaphragm thickness during a dynamic task. Nevertheless, for measurement of abdominal muscle thickness, it has been investigated that US is a reliable tool during postural standing tasks (Ehsani, Arab, Jaberzadeh, & Salavati, 2016). In addition, the US measurement method could be more standardized for an eventual increment in inter-rater reliability. However, it seems unlikely that this reduced reliability has affected the main outcome of this study. Furthermore, when in- and exclusion criteria were less exclusive, it would be more likely to find proper correlations with for example age or BMI. Yet, in this research the emphasis was on the measurement of diaphragm thickening in healthy young participants.

Clinical implications

Since US is a reliable measurement tool for the investigation of diaphragm thickness, and MIP is a reliable measurement tool for investigation of inspiratory pressure, it is suitable for health care providers to use them in clinical practise or research for diagnosis or rehabilitation.

The results of our study suggest not using ASLR as a postural task, when investigating the postural function of the diaphragm. It might be recommended to select a more functional postural task, as mentioned previously. In addition, the US measurement might be more reliable when the procedure is further standardized. For example, it would be beneficial to use a probe fixator, a clear instruction list to improve the performance of the patient etc.

7. Conclusion

The main outcome of this study suggests that there is no difference in diaphragm thickness when comparing rest to ASLR. Also, there is no difference in thickening and DTF(%) between ipsilateral and bilateral, and between the different repeated measurements. This suggests that there is no change in thickness of the diaphragm during this specific postural task (ASLR) compared to rest thickness.

In addition, the inter-rater reliability of the US measurement of diaphragm thickness was fair to good, and could be further improved with a more standardized procedure. The intra-rater reliability was excellent, whereas US measurement is best used within the same rater.

Furthermore, a positive correlation between MIP and diaphragm thickness was found, which means that the higher the MIP, the greater the diaphragm thickness is.

Also, a positive correlation was found between BMI, gender and diaphragm thickness, which means that males and participants with a higher BMI have greater diaphragm thickness. No correlation was found between age and diaphragm thickness.

Taking these results into account, further research is needed to investigate the postural function of the diaphragm in other, more functional postural tasks.

8. Reference list

- Arora, N. S., & Rochester, D. F. (1982). Effect of body weight and muscularity on human diaphragm muscle mass, thickness, and area. J Appl Physiol Respir Environ Exerc Physiol, 52(1), 64-70. doi:10.1152/jappl.1982.52.1.64
- Ayoub, J., Cohendy, R., Dauzat, M., Targhetta, R., DeLaCoussaye, J. E., Bourgeois, J. M., . . . Pourcelot, L. (1997). Non-invasive quantification of diaphragm kinetics using m-mode sonography. *Canadian Journal of Anaesthesia-Journal Canadien D Anesthesie*, 44(7), 739-744.
- Black, L. F., & Hyatt, R. E. (1969). Maximal respiratory pressures: normal values and relationship to age and sex. Am Rev Respir Dis, 99(5), 696-702. doi:10.1164/arrd.1969.99.5.696
- Boon, A. J., Harper, C. J., Ghahfarokhi, L. S., Strommen, J. A., Watson, J. C., & Sorenson, E. J. (2013). Two-dimensional ultrasound imaging of the diaphragm: quantitative values in normal subjects. *Muscle Nerve*, 47(6), 884-889. doi:10.1002/mus.23702
- Bunce, S. M., Hough, A. D., & Moore, A. P. (2004). Measurement of abdominal muscle thickness using M-mode ultrasound imaging during functional activities. *Man Ther*, *9*(1), 41-44.
- Cohn, D., Benditt, J. O., Eveloff, S., & McCool, F. D. (1997). Diaphragm thickening during inspiration. *J Appl Physiol (1985), 83*(1), 291-296.
- de Groot, M., Pool-Goudzwaard, A. L., Spoor, C. W., & Snijders, C. J. (2008). The active straight leg raising test (ASLR) in pregnant women: differences in muscle activity and force between patients and healthy subjects. *Man Ther*, *13*(1), 68-74. doi:10.1016/j.math.2006.08.006
- Ehsani, F., Arab, A. M., Jaberzadeh, S., & Salavati, M. (2016). Ultrasound measurement of deep and superficial abdominal muscles thickness during standing postural tasks in participants with and without chronic low back pain. *Man Ther, 23*, 98-105. doi:10.1016/j.math.2016.01.003
- Goligher, E. C., Laghi, F., Detsky, M. E., Farias, P., Murray, A., Brace, D., . . . Ferguson, N. D. (2015). Measuring diaphragm thickness with ultrasound in mechanically ventilated patients: feasibility, reproducibility and validity. *Intensive Care Med*, *41*(4), 642-649. doi:10.1007/s00134-015-3687-3
- Grams, S. T., von Saltiel, R., Mayer, A. F., Schivinski, C. I., de, S. N. L. F., Nobrega, I. S., . . . Paulin, E. (2014). Assessment of the reproducibility of the indirect ultrasound method of measuring diaphragm mobility. *Clin Physiol Funct Imaging*, *34*(1), 18-25. doi:10.1111/cpf.12058
- Haizlip, K. M., Harrison, B. C., & Leinwand, L. A. (2015). Sex-based differences in skeletal muscle kinetics and fiber-type composition. *Physiology (Bethesda), 30*(1), 30-39. doi:10.1152/physiol.00024.2014
- Harper, C. J., Shahgholi, L., Cieslak, K., Hellyer, N. J., Strommen, J. A., & Boon, A. J. (2013).
 Variability in diaphragm motion during normal breathing, assessed with B-mode ultrasound. *J Orthop Sports Phys Ther*, 43(12), 927-931. doi:10.2519/jospt.2013.4931
- Hellyer, N. J., Andreas, N. M., Bernstetter, A. S., Cieslak, K. R., Donahue, G. F., Steiner, E. A., .
 . Boon, A. J. (2017a). Comparison of Diaphragm Thickness Measurements Among Postures Via Ultrasound Imaging. *Pm&R*, 9(1), 21-25. doi:10.1016/j.pmrj.2016.06.001

- Hellyer, N. J., Andreas, N. M., Bernstetter, A. S., Cieslak, K. R., Donahue, G. F., Steiner, E. A., .
 . Boon, A. J. (2017b). Comparison of Diaphragm Thickness Measurements Among Postures Via Ultrasound Imaging. *Pm r, 9*(1), 21-25. doi:10.1016/j.pmrj.2016.06.001
- Hodges, P. W., Eriksson, A. E. M., Shirley, B., & Gandevia, S. C. (2005). Intra-abdominal pressure increases stiffness of the lumbar spine. *Journal of Biomechanics, 38*(9), 1873-1880. doi:10.1016/j.jbiomech.2004.08.016
- Hu, H., Meijer, O. G., Hodges, P. W., Bruijn, S. M., Strijers, R. L., Nanayakkara, P. W., . . . van Dieen, J. H. (2012). Understanding the Active Straight Leg Raise (ASLR): an electromyographic study in healthy subjects. *Man Ther*, *17*(6), 531-537. doi:10.1016/j.math.2012.05.010
- Ikai, M., & Fukunaga, T. (1968). Calculation of muscle strength per unit cross-sectional area of human muscle by means of ultrasonic measurement. *Int Z Angew Physiol, 26*(1), 26-32.
- Jalan, N. S., Daftari, S. S., Retharekar, S. S., Rairikar, S. A., Shyam, A. M., & Sancheti, P. K. (2015). Intra- and inter-rater reliability of maximum inspiratory pressure measured using a portable capsule-sensing pressure gauge device in healthy adults. *Can J Respir Ther*, 51(2), 39-42.
- Janssens, L., McConnell, A. K., Pijnenburg, M., Claeys, K., Goossens, N., Lysens, R., . . . Brumagne, S. (2015). Inspiratory muscle training affects proprioceptive use and low back pain. *Med Sci Sports Exerc*, *47*(1), 12-19. doi:10.1249/mss.000000000000385
- Kolar, P., Sulc, J., Kyncl, M., Sanda, J., Cakrt, O., Andel, R., . . . Kobesova, A. (2012). Postural function of the diaphragm in persons with and without chronic low back pain. J
 Orthop Sports Phys Ther, 42(4), 352-362. doi:10.2519/jospt.2012.3830
- Lomax, M., & McConnell, A. K. (2009). Influence of Prior Activity (Warm-Up) and Inspiratory Muscle Training upon Between- and Within-Day Reliability of Maximal Inspiratory Pressure Measurement. *Respiration, 78*(2), 197-202. doi:10.1159/000211229
- O'Sullivan, P. B., Beales, D. J., Beetham, J. A., Cripps, J., Graf, F., Lin, I. B., . . . Avery, A. (2002). Altered motor control strategies in subjects with sacroiliac joint pain during the active straight-leg-raise test. *Spine (Phila Pa 1976), 27*(1), E1-8.
- Piasecki, M., Ireland, A., Jones, D. A., & McPhee, J. S. (2016). Age-dependent motor unit remodelling in human limb muscles. *Biogerontology*, *17*(3), 485-496. doi:10.1007/s10522-015-9627-3
- Priori, R., Aliverti, A., Albuquerque, A. L., Quaranta, M., Albert, P., & Calverley, P. M. (2013). The effect of posture on asynchronous chest wall movement in COPD. *J Appl Physiol* (1985), 114(8), 1066-1075. doi:10.1152/japplphysiol.00414.2012
- Rochester, D. F., & Arora, N. S. (1983). Respiratory muscle failure. *Med Clin North Am, 67*(3), 573-597.
- Romer, L. M., & McConnell, A. K. (2004). Inter-test reliability for non-invasive measures of respiratory muscle function in healthy humans. *Eur J Appl Physiol*, 91(2-3), 167-176. doi:10.1007/s00421-003-0984-2
- Smeltzer, S. C., & Lavietes, M. H. (1999). Reliability of maximal respiratory pressures in multiple sclerosis. *Chest*, *115*(6), 1546-1552.
- Terada, M., Kosik, K. B., McCann, R. S., & Gribble, P. A. (2016). Diaphragm Contractility in Individuals with Chronic Ankle Instability. *Medicine and Science in Sports and Exercise, 48*(10), 2040-2045. doi:10.1249/mss.00000000000994
- Volianitis, S., McConnell, A. K., & Jones, D. A. (2001). Assessment of maximum inspiratory pressure Prior submaximal respiratory muscle activity ('warm-up') enhances

maximum inspiratory activity and attenuates the learning effect of repeated measurement. *Respiration, 68*(1), 22-27. doi:10.1159/000050458

9. Attachments

Attachment 1. Informed consent.

INFORMATION LETTER

Dear participant,

Thank you for consideration to participate in our research. With this letter, we would like to provide you with information about the aim of the research, criteria for participation, the testing procedure, expectations and other important information for you as a potential participant. We would kindly ask you to carefully read through the information below. In a case of uncertainty during or after reading this information letter please do not hesitate to contact the researcher via email or phone number provided in the email you received.

What is the aim of the research?

Abdominal wall muscles, such as Transversus Abdominis, Internal and External Oblique muscles, diaphragm and intercostal muscles play a major role in spinal stability during functional activities. For this reason, the aim of this research is to measure above-mentioned muscle thickness at rest, during an active straight leg raise test and bilateral straight leg raise using musculoskeletal ultrasound imaging. Another aim is to observe a correlation between muscle thickness and a performance of a dynamic balance task and inspiratory muscle strength.

Who can participate?

We are looking for healthy participants in the age range of 18-29 years. Other requirements to participate are:

- 1. No pregnancy or delivered baby in past 6 months
- 2. No musculoskeletal pain in lower back and lower extremities in past 6 months
- 3. No orthopedic surgery in lumbar spine, pelvis, abdominal or thoracic area in the past year
- 4. No neurologic disorder affecting lumbopelvic and groin regions
- 5. No diagnosed neuromuscular diseases (e.g. weakness or paralysis of diaphragm)
- 6. No history of sensory nervous or vestibular system problems
- 7. No lower limb injuries in past year
- 8. BMI between 18.5 29 kg/m²
- 9. No previous diagnosed lung disorders/ illness (e.g. asthma)

What is the procedure of the testing?

If you match the participation criteria and decide to participate in the study, at the time of meeting you will be asked to sign consent form, which serves as a proof of your agreement to participate in the research. The data collection will begin with measuring your weight and height in order to calculate your body mass index. After which the measurements with ultrasound imaging, performance of a dynamic balance task and measurement of inspiratory muscle force will take place.

Your abdominal muscles (Transversus abdominis, internal and external oblique, intercostal muscles and diaphragm) will be measured at rest, during active straight leg raise and bilateral leg raise in laying position. For ultrasound imaging picture collection, a linear transducer with gel will be used and applied on your skin on both sides of your body. You will also be asked to perform a postural balance task called Dynamic One-leg Stance Test (DOLS). The test consists of 5 consecutive levels with three trials on each level. After which the measurement of inspiratory muscle strength will take place.

What is expected from you?

We expect you to independently come to the explore lab at D. Th. Fliednerstraat 2, 5631 BN Eindhoven. We expect you to wear comfortable clothes and agree on undressing your upper body until underwear.

What happens to data collected during testing procedure?

All the recorded data will be kept confidential and anonymous and will be accessible only to researchers, research supervisor who is a representative of Fontys University of Applied Sciences and the clients of the research. The collected data of ultrasound imaging will be stored for 15 years at Fontys University of Applied Sciences and deleted from researchers' personal computers once the research is over.

What happens if you decide to withdraw from testing procedure?

The participation in this research is voluntary; therefore you are allowed to withdraw your participation at any time without providing an explanation.

What happens after testing procedure?

The entire testing procedure is expected to last about 1 hour, after which you will be offered tea and snacks as an appreciation for participating in our research.

Are there extra costs/compensations for the participation?

There are no extra costs or monetary compensations for the participation in this research.

Are there any questions left after reading the information above?

If you have decided to participate or have any questions, please do not hesitate and contact one of the researchers, who will send you the schedule of available testing dates and times:

LAURA JANITE

l.jante@student.fontys.nl

+31648054664

Thank you for your time and interest, Sincerely, Laura Janite, Marija Gusiatina, Niki Budai, Eva Shalley and Josefine Smeets

Informed Consent form

Participant no: _____

I volunteer to participate in a research project "Ultrasound measurement of changes in thickness of internal and external oblique muscles during active straight leg raise" conducted by Laura Janite, 4th year student of Fontys University of Applied Sciences, Eindhoven, The Netherlands.

1. I have read and understood the information provided in information letter.

2. I was given enough time to decide whether to participate in the research or not.

3. I understand that I can withdraw and discontinue participation in the project at any time without explanation and penalty.

4. I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.

5. I have been given the opportunity to ask questions and have had them all answered to my satisfaction.

6. My signature on this form signifies that I freely give my consent to participate in this study.

My name:	Signature	Date: / /
	jignature.	Dale

I hereby declare that I have provided the participant with all the necessary information about the research. The participant was provided an opportunity to ask questions, and I answered them with my best ability. I declare that the all the gathered information about the participant will be kept confidential as stated in information letter.

Signature of the researcher: _____

	>	
www.uhasselt.be	UHA	SSELT
Campus Dissen () Material and a 1 () 2000 russen Campus Dispenbaek (Agoralaan gebouw D) 85:3390 Diepenbeek T + 32(0)11 26 81 11 E-mail: info@uhasselt.be	RNON	VLEDGE IN ACTION

VOORTGANGSFORMULIER WETENSCHAPPELIJKE STAGE DEEL 2

DATUM	INHOUD OVERLEG	HANDTEKENINGEN
05-10-'17	* Bespreking MP deel 1	Promotor:
	* Overleg verloop MP deel 2 en concretiseren aanpak schrijven	Copromotor:
		Student(e):
		Student(e):
23-10-'17	Contact via E-mail: Feedback onderzoeksvraag en hypotheses	Promotor:
		Copromotor:
		Student(e):
		Student(e): <****
30-10-'17	Contact via E-mail: Feedback inleiding	Promotor:
		Copromotor: V
		Student(e):
v		Student(e):
03-11-'17	* Bespreking introductie en hypotheses	Promotor:
	* Bespreking data reductie met 'ImageJ'	Copromotor:
		Student(e):
		Student(e):
10-04-'18	Contact via E-mail: Verbetering voorstal statistische analyse	Promotor:
		Copromotor: V
		Student(e):
		Student(e):
17-04-'18	* Bespreking en verdere uitwerking statistische analyse	Promotor:
	* Overleg resultaten sectie	Copromotor:
	* Afspraken omtrent verdere aanpak schrijven	Student(e):
		Student(e):
18-04-'18	Contact via E-mail: Feedback inleiding en methode	Promotor:
		Copromotor: V
		Student(e): Student
		Student(e):
09-05-'18	Contact via E-mail: Feedback methode en resultaten	Promotor:
		Copromotor:
		Student(e):
		Student(e): Contraction
14-05-'18	* Bespreking resultaten sectie, discussie	Promotor:
	* Ondertekening inschrijvingsformulier en verdediging aangehaald	Copromotor: Helley
		Student(e):
	-	Student(e):
23/5/12	Contact via E-mail: Feedback resultaten en discussie	Promotor:
1211/18	·	Copromotol: 1 lou
		Student(e):
		Student(e):

Auteursrechtelijke overeenkomst

Ik/wij verlenen het wereldwijde auteursrecht voor de ingediende eindverhandeling: Thickness of the diaphragm during active straight leg raise: an investigation with ultrasound

Richting: master in de revalidatiewetenschappen en de kinesitherapie-revalidatiewetenschappen en kinesitherapie bij musculoskeletale aandoeningen Jaar: 2018

in alle mogelijke mediaformaten, - bestaande en in de toekomst te ontwikkelen - , aan de Universiteit Hasselt.

Niet tegenstaand deze toekenning van het auteursrecht aan de Universiteit Hasselt behoud ik als auteur het recht om de eindverhandeling, - in zijn geheel of gedeeltelijk -, vrij te reproduceren, (her)publiceren of distribueren zonder de toelating te moeten verkrijgen van de Universiteit Hasselt.

Ik bevestig dat de eindverhandeling mijn origineel werk is, en dat ik het recht heb om de rechten te verlenen die in deze overeenkomst worden beschreven. Ik verklaar tevens dat de eindverhandeling, naar mijn weten, het auteursrecht van anderen niet overtreedt.

Ik verklaar tevens dat ik voor het materiaal in de eindverhandeling dat beschermd wordt door het auteursrecht, de nodige toelatingen heb verkregen zodat ik deze ook aan de Universiteit Hasselt kan overdragen en dat dit duidelijk in de tekst en inhoud van de eindverhandeling werd genotificeerd.

Universiteit Hasselt zal mij als auteur(s) van de eindverhandeling identificeren en zal geen wijzigingen aanbrengen aan de eindverhandeling, uitgezonderd deze toegelaten door deze overeenkomst.

Voor akkoord,

Schalley, Eva

Smeets, Josefine