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Research paper

Neuromuscular ultrasound of the scapular stabilisers in healthy subjects

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ABSTRACT

Objectives: To obtain normative high-resolution ultrasound (HRUS) data for thickness of the serratus anterior, the trapezius and the rhomboid major muscles and diameter of their corresponding nerves, the long thoracic, the spinal accessory and the dorsal scapular nerve. Moreover, we aimed to examine intra- and inter-examiner agreement of the HRUS measurements.
Methods: We included 41 healthy subjects. Muscle thickness and nerve diameter were measured bilaterally, resulting in 82 ultrasound measurements for each structure. Normative data were calculated using regression equations for the lower limit of muscle thickness and upper limit of nerve diameter, taking into account various variables. For intra- and inter-examiner agreement, ten subjects underwent two extra ultrasound examinations and Bland-Altman plots were calculated.
Results: This normative data set showed significant correlations between decreasing muscle thickness with increasing age and height and increasing muscle thickness with increasing weight and with male sex. Muscle thickness was larger on the dominant side compared to the non-dominant side for the trapezius and rhomboid muscles, whereas the opposite was found for the serratus anterior muscle. For all nerves, significant correlations were found between decreasing nerve diameter with increasing age and height. Intra-examiner agreement was acceptable in all sites. Inter-examiner agreement was acceptable for all sites but one site for the serratus anterior muscle and long thoracic nerve, and not acceptable for five out of six sites for the trapezius muscle.
Conclusion: This study provides HRUS normative data and intra- and inter-examiner agreement data for muscle thickness and nerve diameter for the muscles stabilizing the scapulae and their corresponding nerves.
Significance: The normative HRUS data reported may be useful in future studies investigating neuromuscular disorders.

1. Introduction

Stability of the scapula is of importance for movement of the shoulder joint and therefore important for the function of the upper extremity. The most important muscles stabilising the scapula are the serratus anterior muscle innervated by the long thoracic nerve, the trapezius muscle innervated by the spinal accessory nerve, and the rhomboid muscle innervated by the dorsal scapular nerve. Injury to these muscles can result in scapular winging and is often evaluated by electrodiagnostic examination (Seror et al., 2018) and in some cases radiographs, computer tomography or magnetic resonance imaging (Mohsen et al., 2006; Nguyen et al., 2016; Orth et al., 2012).

High resolution ultrasound (HRUS) is a simple technique to visualize muscle and nerve tissue and has an excellent compliance as it is non-invasive and well tolerated by patients. It has been used to evaluate muscles by describing and measuring the structure, thickness and echogenicity (Arts et al., 2010; Pillen and van Alfen, 2011).

Despite the increasing use of muscle ultrasound, normative data for some of the above mentioned muscles and nerves are either derived from small groups of healthy subjects (Adigozali et al., 2016; Bentman et al., 2010; Day and Uhl, 2013; O'Sullivan et al., 2007; Talbott and Witt, 2013, 2014)), or has a narrow age-range (Adigozali et al., 2016; Bentman et al., 2010; Day and Uhl, 2013; Jeong et al., 2016; O'Sullivan et al., 2007; Talbott and Witt, 2013,

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2014). Intra- and inter examiner reliability has been reported in few studies and was reported as moderate to good when measuring the thickness of the serratus anterior muscle (Talbott and Witt, 2013, 2014), of the lower, middle and upper part of the trapezius muscle (Adigozali et al., 2016; Bentman et al., 2010; Day and Uhl, 2013; O'Sullivan et al., 2007) and of the rhomboid major muscles (Jeong et al., 2016; Yang et al., 2011).

Most studies on HRUS normative data for the nerves have been scarce and most publications have reported values for single nerves such as for the spinal accessory nerve (Mirjalili et al., 2012) and the long thoracic nerve (Lieba-Samal et al., 2015). To our knowledge, there are no studies reporting nerve measurements of the dorsal scapular nerve. Good intra-examiner reliability of the diameter of the spinal accessory nerve was reported in one study and inter-examiner reliability was not assessed (Mirjalili et al., 2012).

Thus, the aim of this study was to obtain normative data for muscle thickness of the serratus anterior muscle, the trapezius muscle and the rhomboid major muscle and nerve diameter of the long thoracic nerve, the spinal accessory nerve and the dorsal scapular nerve using HRUS in a representative group of healthy subjects with a wide age distribution. Furthermore, we aimed to examine intra- and inter examiner agreement for the HRUS scan of all these structures. The overall aim is that ultrasound of these structures, could be used in the diagnostic work-up of neuromuscular diseases causing a winged scapula.

2. Methods

2.1. Ethics approval

The study received ethical permission from the regional Committee on Biomedical research Ethics and the Danish Data Protection Agency. All subjects signed an informed consent statement after receiving written and oral information. The study complies with the 2013 update of the Declaration of Helsinki.

2.2. Study design

This study is a prospective, single blinded study. The HRUS data collection was not blinded. However, the final measurements were done after anonymization and randomization with US data from a group of patients with scapular winging. In this way, the examiner who did the measurements did not know if the images were from patients or healthy subjects. The patient group is not a part of this study. Data from the patient group are under preparation and will be submitted elsewhere.

2.3. Subjects

Between April 2018 and January 2019, we invited 42 healthy subjects. The healthy subjects were recruited by http://www.forsoegsperson.dk/, by advertisement at Aarhus University and by advertisement at different departments of Aarhus University Hospital. The inclusion criteria were age 18 or above and the ability to speak Danish. The exclusion criteria were a history of shoulder trauma or shoulder disease, cervical radiculopathy, upper extremity peripheral neuropathy/plexopathy or peripheral vascular disease. Forty-one healthy subjects were included in the study as one was excluded due to earlier diagnosis of rotator cuff syndrome.

2.4. HRUS examination

HRUS was performed with a Siemens ACUSON 1000 ultrasound machine with a high-frequency linear array transducer (18L6HD, 5 cm). B-mode was used constantly, and the frequency, depth and focus were adjusted depending on the individual variations. In order to avoid deformation of the structures, the probe was held gently over the skin and the transducer was adjusted perpendicularly to the nerve or muscle, thus the clearest image was obtained. Colour Doppler mode was used to differentiate between arteries/veins and nerve fascicles.

The HRUS examination of muscles included bilateral images of the upper part of the trapezius muscle (Fig. 1a and b), the mid part of the trapezius muscle and the rhomboid major muscle in the same image (Fig. 1c and d), the inferior part of the trapezius muscle (Fig. 1e–g) and the serratus anterior muscle at three different levels (Fig. 2). The HRUS examination of the nerves was done in a standardized way (see session 2.5 below) and included bilateral images of the long thoracic nerve and the dorsal scapular nerve (Fig. 3), and the spinal accessory nerve (Fig. 4). The images were taken in the mentioned order. One image was taken at each site. In each subject 14 muscle sites and 10 nerve sites were examined, adding up to 574 muscle sites and 410 nerve sites overall.

HRUS measurements of muscle thickness and nerve diameter were done after anonymisation.

For ten healthy subjects two extra scans were performed for intra- and inter examiner agreement.

The HRUS was performed by SSB, a medical student, who prior to the start of this study completed the Basic Ultrasound course and the Musculoskeletal Ultrasound course from the Danish Society of Diagnostic Ultrasound and received training in neuromuscular ultrasound in the department of Neurophysiology, Aarhus University Hospital.

Another examiner (EQ), who supervised the examinations, has extensive practical and research experience in nerve and muscle ultrasound and clinical neurophysiology and participated as examiner 2 for the inter-examiner agreement.

2.4.1. HRUS of muscles

HRUS of muscles was performed in a standardized fashion and following the suggestions of a previous study (Krzesniak-Swinarska et al., 2017) making sure to include an osseous landmark and another muscle for comparison of echogenicity. Moreover, we chose the following probe positions for the different muscles to standardize the examination and to reduce the variation as much as possible.

For the upper trapezius muscle, the probe was placed in an oblique position between C7 and acromion where acromion is our osseous landmark at the most lateral end of the picture (Fig. 1). For the middle trapezius muscle and the rhomboid major muscle, the probe was placed in a horizontal manner medial to the medial border of scapulae right below the spine of the scapulae (Fig. 1). The medial border of scapulae was used as our osseous landmark. For the lower trapezius muscle, the probe was right on the spinal column in level with the inferior angle of scapula. From here, the probe was moved laterally to see the muscle belly and at the same time keeping the lateral edge of the transverse processes in the picture, which was used as our osseous landmark (Fig. 1).

For the serratus anterior muscle (Fig. 2), **t**he probe was placed behind the post axillary line in line with the armpit. Here, visualizing the second (level 1), third (level 2) and fourth (level 3) rib respectively from the armpit in the middle of the picture and turning the probe in a manner where the rib is shown in cross section. When doing this the cranial part of the probe was turned anteriorly.

Measurements of muscle thickness was done inside the muscle fasciae, between the superficial and the profound muscle fasciae. For the trapezius muscle, the measurement was made at the thickest point of the muscle belly. For the rhomboid major muscle, the measurement was made at 2,5 cm from our osseous landmark,

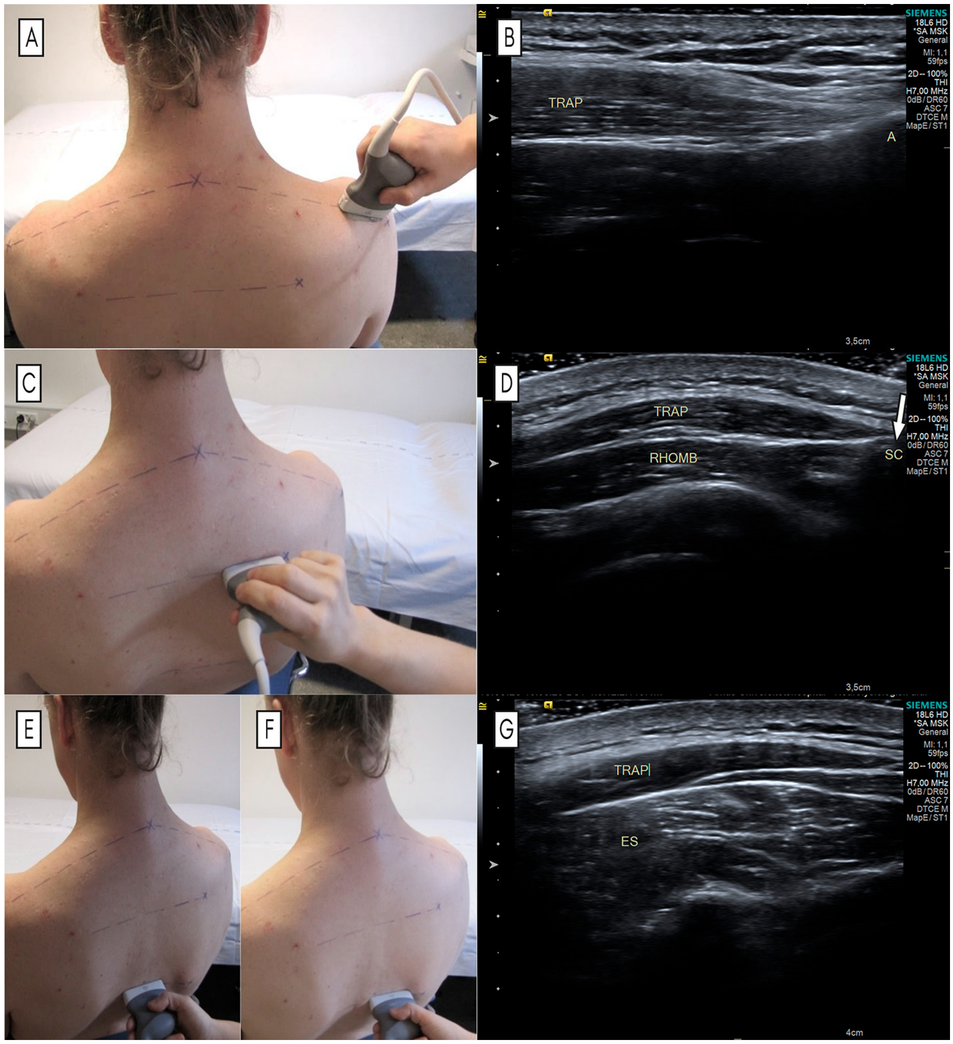

Fig. 1. The trapezius and rhomboid major muscle. The upper trapezius muscle: A) The probe was placed in an oblique position between C7 and acromion where acromion is our osseous landmark at the most lateral end of the picture. B) The ultrasound (US) image of the upper trapezius muscle. Here, the supraspinatus muscle could be seen in the bottom of the image if depth was increased. To the right in the image we have the acromion (A). The middle trapezius muscle and the rhomboid major muscle: C) The probe was placed in a horizontal manner medial to the medial border of scapulae right *below* the spine of scapulae. D) The US image. Here, the trapezius muscle could be seen above the rhomboid major muscle. The medial border of scapulae was used as our osseous landmark. The lower trapezius muscle: E) Firstly, the placement of the probe was right on the spinal column on a level with the inferior angle of scapula. F) From here, the probe was moved laterally to see the muscle belly and at the same time keeping the lateral edge of the transverse processes in the picture. G) The US image of the lower trapezius muscle with the erector spinae muscle (ES) lying below.

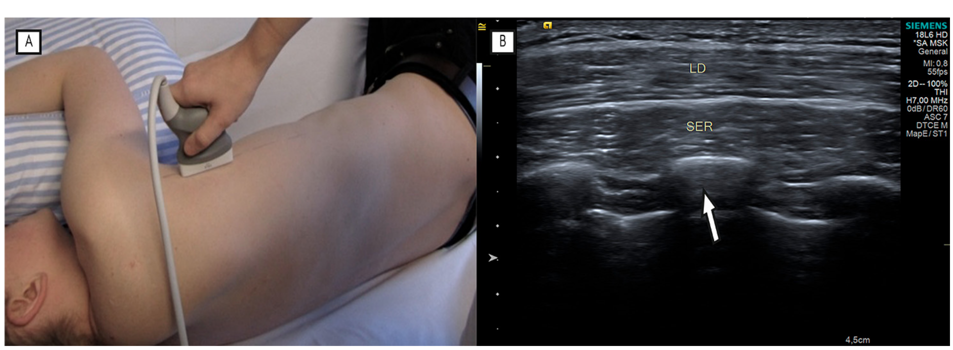

Fig. 2. The serratus anterior muscle. A) The probe was placed behind the post axillary line in line with the armpit. Here, visualizing the second (level 1), third (level 2) and fourth (level 3) rib respectively from the armpit in the middle of the picture and turning the probe in a manner where the rib is showed in cross section. When doing this the cranial part of the probe was turned anteriorly. B) US image of the serratus anterior muscle with the latissimus dorsi muscle (LD) lying above and ribs (white arrow) lying below.

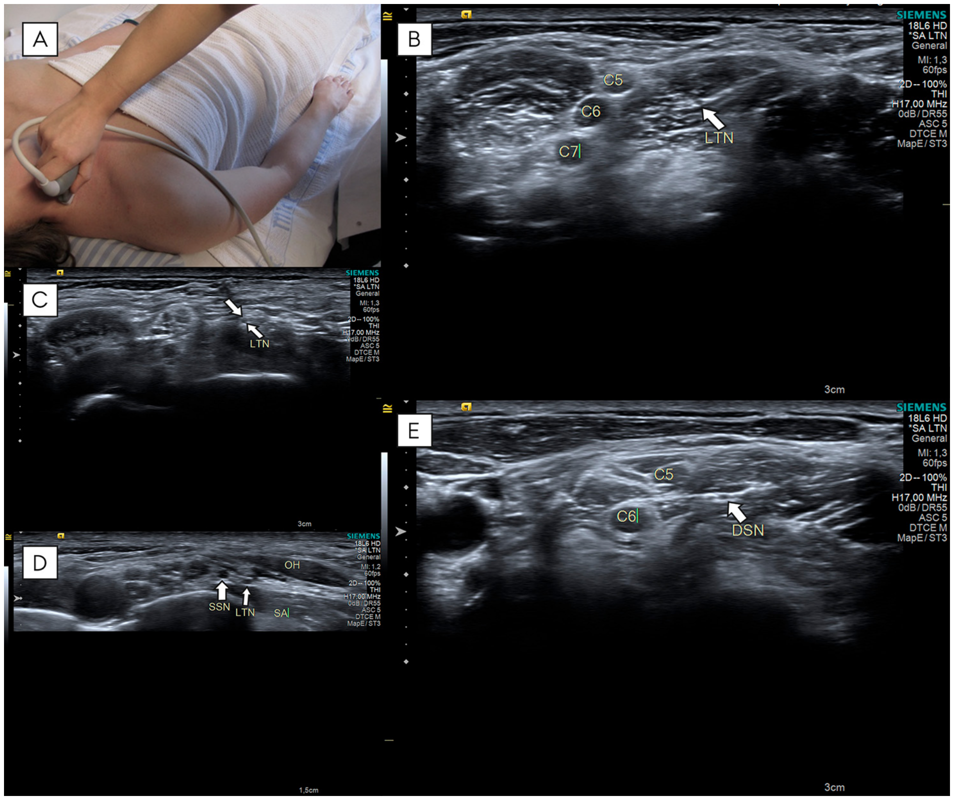

Fig. 3. The long thoracic (LTN) and dorsal scapular (DSN). All ultrasound images are of the left side on a healthy subject. A) The probe was placed on the neck on/in front of the sternocleidomastoid muscle. B) LTN1: The LTN (white arrow) in or under the middle scalene muscle at the largest point; lying in a hyperechoic fascial line. C) LTN2: The LTN (white arrows) above the scalene musculature at the largest point. D) LTN3: The LTN above the serratus anterior muscle (SA), under the omohyoid muscle (OH) and in front of the suprascapular nerve (SSN) at the largest point. E) The DSN (white arrow) in the middle scalene muscle at the largest point; lying in a hyperechoic fascial line.

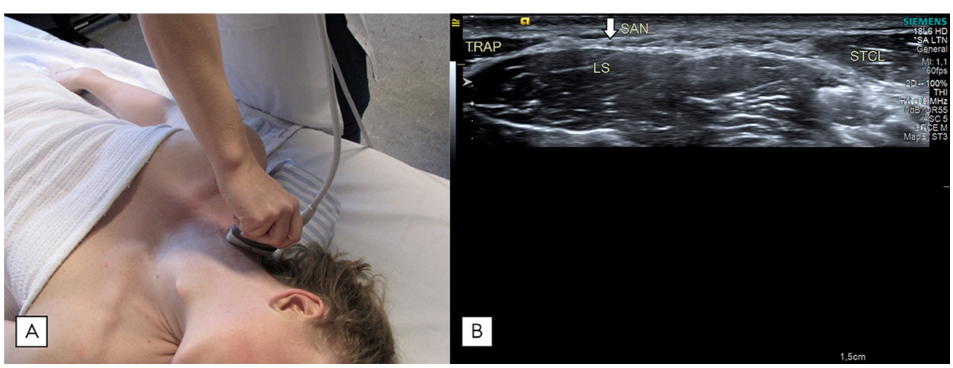

Fig. 4. The spinal accessory nerve (SAN). A) The probe was placed on the neck behind the sternocleidomastoid muscle (STCL) in the posterior triangle of the neck. B) The SAN (white arrow) lying on the levator scapulae muscle (LS). The trapezius muscle (TRAP) to the left and the STCL to the right. The image was taken where the nerve was found to be largest either before the nerve branched in two and if after the largest of the two branches was used.

the scapula. For the serratus anterior muscle, the measurement was made at the most convex point of the underlying rib; if the rib was flat on the entire surface the line was drawn from the midpoint.

2.4.2. HRUS of nerves

The subject lay on the back with a small roll-pad under the lower neck/upper back. Herby, extending the neck backwards and giving more space for the transducer. When scanning the right side of the neck, the subject was looking to the left and vice versa (Figs. 3 and 4).

In order to ensure a correct and consistent identification of the nerves, we developed a standardized procedure of searching for the long thoracic nerve and the dorsal scapular nerve as follows: 1) we identified the cervical roots C5, C6 and C7 by first recognizing C7 which transverse process only has a posterior tubercle and then moved cranially to identify the C6 and C5 roots; 2) we identified the scalene-muscles and looked for nerve tissue in or behind the middle scalene muscle; 3) we traced the nerves back to the cervical roots in order to establish the root of origin. Then specific criteria for the individual nerves were as follows:

For the long thoracic nerve (LTN) (Fig. 3), the criteria for nerve identification were (Hanson and Auyong, 2013; Lieba-Samal et al., 2015; Tubbs et al., 2006; Wang et al., 2008; Yazar and Comert, 2009):

1. Obvious emergence from C6
2. Lying in a hyperechoic fascial line inside or under the middle scalene muscle

3. Lying deeper than the dorsal scapular nerve in a view where both nerves are visible or when comparing a view with the long thoracic nerve and the dorsal scapular nerve, respectively, the long thoracic nerve is the one lying deeper
4. Ability to follow the nerve from above the scalene muscles and down to serratus anterior muscle: here, lying profound of the omohyoid muscle and above the serratus anterior muscle and lying in front of the suprascapular nerve (tracking the suprascapular nerve from C5).

For the dorsal scapular nerve (Fig. 3), the criteria for nerve identification were (Hanson and Auyong, 2013; Kim et al., 2016):

1. Obvious emergence from C5
2. Lying in a hyperechoic fascial line inside the middle scalene muscle
3. Lying more superficial than the long thoracic nerve in a view where both nerves are visible or when comparing a view with the long thoracic nerve and the dorsal scapular nerve, respectively, the long thoracic nerve is the one lying deeper

For the spinal accessory nerve (Fig. 4), the criteria for nerve identification were (Aramrattana et al., 2005; Durazzo et al., 2009; Lu et al., 2002; Mirjalili et al., 2012; Symes and Ellis, 2005; Tubbs et al., 2005):

1. Lying in the posterior triangle of the neck.
2. Passing under or through the two heads of the sternocleidomastoid muscle
3. Running in or under the trapezius muscle

Measurements of the nerves were done within the nerves outer rim. The largest diameter of the nerve was used. Nerve diameter and not CSA was used, since the latter gave us a less precise number due to technical limitations with the Acuson 1000 machine. Since the nerves in this study are very small, we could not always get a CSA larger than 0.00 cm^3, whereas we could get the diameter in mm and therefore were able to get meaningful numbers on much smaller nerves.

2.5. Data analysis

Statistical analysis was performed in STATA 15.1. For continuous data mean $\pm$ 1.96 standard deviations were calculated. Difference between sides within the same subject was tested using a paired *t*-test. For assumptions of normal distribution, Bland-Altman plots (paired data) were used. For the muscle data overall, a correlation was seen between the difference and the average in the Bland-Altman plots. Therefore, data was analyzed in log-transformed form in order to reduce or remove this correlation. The results were back-transformed and presented in their geometric form. When back-transforming a difference, the data can only be presented in ratios. For this reason, nerve diameter data are presented as differences between sides and muscle thickness data are presented as ratios between sides. A two-tailed p-value of <0.05 was considered significant. Data are presented as mean and standard deviation, unless otherwise stated.

A linear multiple regression for muscle thickness and nerve diameter taking into account age, sex, weight, height and hand dominance was performed. This took into account the random subject effect due to the fact that each subject was in the analysis twice (both sides were used for each subject). For intra- and inter examiner agreement, Bland-Altman plots with 95% limits of agreement were calculated.

Since the normative data calculated in this study are created as reference material for patients with neuromuscular disorders, in whom we would expect an atrophic, thin muscle and probably an enlarged nerve, we present the lower limit of muscle thickness and the upper limit of nerve diameter.

3. Results

3.1. Demographics

A description of the study population is shown in Table 1. The gender distribution was equal, and the age distribution was wide.

3.2. Intra- and inter-examiner agreement

Values for the Bland-Altman plots for intra- and inter–examiner agreement can be seen in Supplementary Table 1 and Supplementary Table 2. The Bland-Altman plots did not show any fixed bias for any of the muscle or nerve measurements.

For intra-rater agreement, no differences were found between the two measurements for any of the muscles or nerves.

For inter-examiner agreement, no differences were found between the two examiners for all the nerves except for the right LTN3, for the rhomboid muscle and for the serratus anterior muscle, except left mid-level measurement of the latter. Differences were found for all parts of the trapezius muscle on both sides.

3.3. Normative data

Regression equations for the prediction of the lower limit of muscle thickness and the upper limit of nerve diameter taking into account age, height, weight, sex and hand dominance can be seen in Supplementary Table 3.

We found significant correlations between decreasing muscle thickness with increasing age and height, increasing muscle thick-

Table 1
Demographic characteristics of the study population (n = 41).

Demographics	Healthy subjects (n = 41)	Median [min, max]
Sex, n		
Female	21 (51%)	
Male	20 (49%)	
Age, years	43.59 (SD: 15.42)	44 [25, 73]
BMI, kg/m^2	24.99 (SD: 4.35)	24.84 [18.00, 36.13]
Height, cm	174.76 (SD: 9.69)	175 [158, 190]
Weight, kg	76.66 (SD: 16.15)	75 [50, 122]
Training status[‡], n		
Inactive	7 (17%)	
Cardio	19 (46%)	
Strength +/− cardio	15 (37%)	
Occupation 1, n		
Office worker	24 (59%)	
Manual worker	3 (7%)	
Retired	6 (15%)	
Student	8 (20%)	
Occupation 2 [†], n		
Non-physical job	38 (93%)	
Physical job	3 (7%)	

SD: standard deviation.
[†] Occupation 2: "physical job" contains the manual labour workers and "non-physical job" contains the office workers, students and retired.
[‡] Training status: Inactive: No cardio or strength training, Cardio: Cardio training one hour per week or more, Strength +/- cardio: Training of stregth one hour per week or more with or without cardio training.

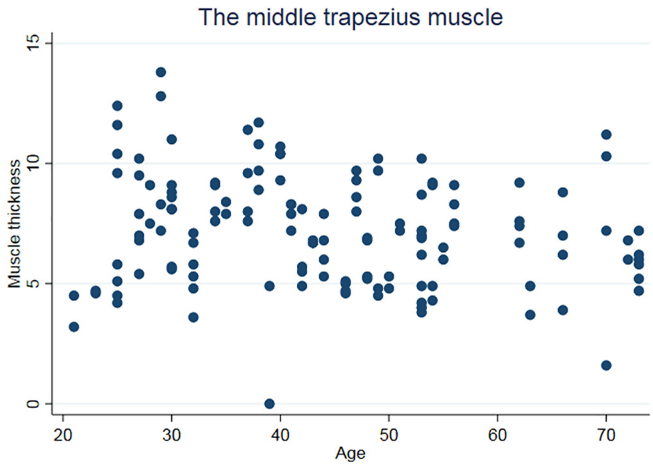

Fig. 5. The thickness of the middle trapezius muscle and age. A significant decrease in muscle thickness of the middle trapezius muscle was found with increasing age. This association was found while adjusting for height, weight, sex and hand dominance.

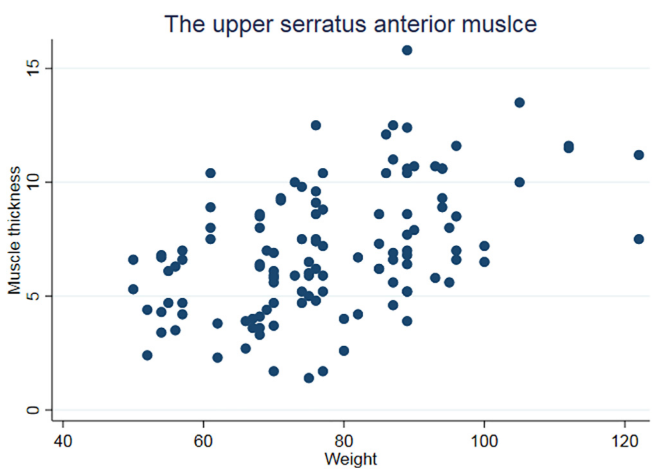

Fig. 6. The thickness of the upper serratus anterior muscle and weight. A significant increase in muscle thickness of the upper serratus anterior muscle was found with increasing weight. This association was found while adjusting for age, height, sex and hand dominance.

ness with increasing weight and with male sex in most muscle sites (Supplementary Table 3). In Figs. 5 and 6 some of the significant correlations are depicted.

We found significant correlations between decreasing nerve diameter with increasing age and height for all nerves and increasing nerve diameter with increasing weight for all nerves except for the spinal accessory nerve and with male sex for all nerves except for the LTN1 site. Furthermore, a trend towards a larger nerve diameter on the dominant side compared to the non-dominant side for all nerves except for the LTN2 was found (Supplementary Table 3).

For the superior and inferior part of the trapezius muscle and for the rhomboid major muscle, we found a significantly larger muscle thickness on the dominant side compared to the non-dominant side. For the serratus anterior muscle, we found a trend towards the opposite, namely a larger muscle thickness on the non-dominant side compared to the dominant side.

When choosing which variables we needed to use to adjust muscle thickness and nerve diameter, we looked into significant confounders, but also if the variable had a trend towards a positive or a negative impact on muscle thickness or nerve diameter. No constant tendency was found with regard to training status and therefore, we chose not to use it in our regression equations.

Unfortunately, we had only 3 healthy subjects who were workers engaged in manual labour, thus, we were not able to see whether this factor had any influence on muscle thickness or nerve diameter.

3.4. Nerve identification

For the spinal accessory nerve, we identified all nerves except for one nerve on one side on one healthy subject.

For the identification of the long thoracic nerve, 4 out of 82 (5%) nerves could not be found. For 32 nerves not all criteria were met. In 10 cases, the reason was that the dorsal scapular nerve was not found, since the dorsal scapular nerve is part of criteria 3. For the identification of the dorsal scapular nerve, 20 out of 82 (24%) nerves could not be found (See Fig. 7).

4. Discussion

One of the strengths of this study is the presentation of the regression equations for the prediction of the lower limit of muscle

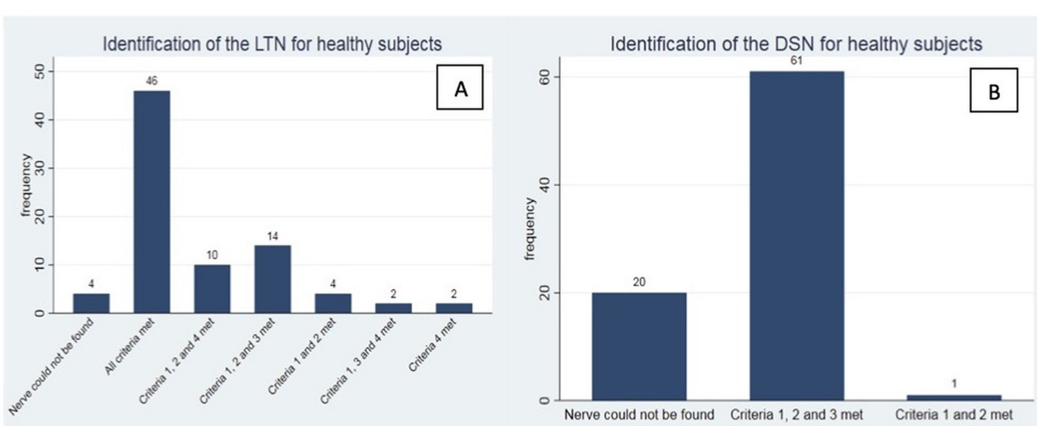

Fig. 7. Identification of the long thoracic nerve (LTN) and dorsal scapular nerve (DSN). A) Criteria for LTN identification. 1: Obvious emergence from C6. 2: Lying in a hyperechoic fascial line inside or under the middle scalene muscle. 3: Lying deeper than DSN in a view where both nerves are visible or when comparing a view with LTN and DSN, respectively, the LTN is the one lying deeper. 4: Ability to follow the nerve from above the scalene muscles and down to the serratus anterior muscle: here, lying profound of the omohyoid muscle and above the serratus anterior muscle and lying in front of the suprascapular nerve (following the suprascapular nerve from C5). B) Criteria for LTN identification. 1: Obvious emergence from C5 2: Lying in a hyperechoic fascial line inside the middle scalene muscle. 3: Lying more superficial than LTN in a view where both nerves are visible or when comparing a view with LTN and DSN, respectively, the LTN is the one lying deeper.

Table 2
Muscle thickness and nerve diameter.

Muscle/nerve	Side	N †	Mean (mm) 95% CI	SD	95% PI	Median [min, max]
SER Upper level	Dominant	41	7.1 [6.4, 7.9]	2.4	[2.5, 11.8]	6.9 [2.3, 12.1]
	Non-dominant	41	7.4 [6.4, 8.3]	2.9	[1.7, 13.0]	6.8 [1.7, 12.5]
	Ratio (d/nd)	41	1.0 [0.9, 1.1]	1.4	[0.5, 2.0]	1.0 [0.5, 2.8]
	Ratio (nd/d)	41	1.0 [0.9, 1.1]	1.4	[0.5, 2.0]	1.0 [0.4, 1.9]
SER Mid-level	Dominant	41	8.1 [7.1, 9.0]	3.0	[2.3, 13.9]	7.3 [2.9, 17.6]
	Non-dominant	41	8.5 [7.6, 9.3]	2.6	[3.3, 13.6]	7.9 [4.9, 15.3]
	Ratio (d/nd)	41	0.9 [0.9, 1.0]	1.3	[0.5, 1.6]	0.9 [0.5, 1.7]
	Ratio (nd/d)	41	1.1 [1.0, 1.2]	1.3	[0.6, 1.9]	1.1 [0.6, 1.9]
SER Lower level	Dominant	41	6.9 [6.1, 7.6]	2.4	[2.2, 11.6]	6.6 [2.4, 12.4]
	Non-dominant	41	7.0 [6.3, 7.8]	2.5	[2.2, 11.9]	6.7 [2.4, 12.2]
	Ratio (d/nd)	41	1.0 [0.9, 1.1]	1.4	[0.5, 1.9]	1.0 [0.4, 2]
	Ratio (nd/d)	41	1.0 [0.9, 1.1]	1.4	[0.5, 2.0]	1.0 [0.5, 2.4]
TRAP pars superior	Dominant	41	12.5 [11.7, 13.3]	2.6	[7.5, 17.6]	11.6 [7.1, 19.5]
	Non-dominant	41	11.9 [11.1, 12.6]	2.4	[7.1, 16.6]	11.3 [8.1, 17. 6]
	Ratio (d/nd)	41	1.1 [1.0, 1.1] *	1.1	[0.8, 1.3]	1.0 [0.7, 1.4]
	Ratio (nd/d)	41	0.9 [0.9, 1.0]	1.1	[0.8, 1.2]	1.0 [0.7, 1.3]
TRAP pars medius	Dominant	41	7.1 [6.3, 7.8]	2.2	[2.7, 11.4]	6.8 [3.8, 12.8]
	Non-dominant	41	6.9 [6.1, 7.6]	2.4	[2.3, 11.5]	6.8 [3.6, 13.8]
	Ratio (d/nd)	41	1.0 [1.0, 1.1]	1.2	[0.8, 1.4]	1.0 [0.7, 1.5]
	Ratio (nd/d)	41	1.0 [0.9, 1.0]	1.2	[0.7, 1.3]	1.0 [0.7, 1.4]
TRAP pars inferior	Dominant	41	5.7 [5.2, 6.3]	1.7	[2.4, 9.0]	5.5 [2.7, 9.6]
	Non-dominant	41	5.4 [4.8, 5.9]	1.7	[2.1, 8.7]	5 [2.4, 10]
	Ratio (d/nd)	41	1.1 [1.0, 1.1] *	1.2	[0.7, 1.6]	1.1 [0.6, 1.7]
	Ratio (nd/d)	41	0.9 [0.9, 1.0]	1.2	[0.6, 1.4]	0.9 [0.6, 1.7]
RHOMB	Dominant	41	7.9 [7.3, 8.5]	2.0	[4.0, 11.9]	7.7 [4.3, 13.5]
	Non-dominant	41	7.4 [6.9, 7.9]	1.5	[4.4, 10.4]	7.2 [4.2, 11.1]
	Ratio (d/nd)	41	1.1 [1.0, 1.2]	1.2	[0.7, 1.5]	1.1 [0.7, 1.8]
	Ratio (nd/d)	41	0.9 [0.9, 1.0]	1.2	[0.6, 1.4]	0.9 [0.6, 1.4]
LTN1	Dominant	37	1.5 [1.4, 1.6]	0.4	[0.8, 2.2]	1.5 [0.8, 2.5]
	Non-dominant	39	1.5 [1.4, 1.6]	0.3	[0.9, 2.1]	1.4 [1.0, 2.4]
	Difference (d-nd)	37	0.0 [-0.1, 0.1]	0.3	[-0.6, 0.6]	0.0 [-0.5, 0.9]
LTN2	Dominant	34	1.3 [1.2, 1.3]	0.2	[0.9, 1.6]	1.2 [1.0, 1.7]
	Non-dominant	35	1.3 [1.2, 1.3]	0.2	[0.8, 1.7]	1.3 [0.8, 1.8]
	Difference (d-nd)	33	0.0 [-0.1, 0.1]	0.3	[-0.5, 0.5]	0.0 [-0.6, 0.5]
LTN3	Dominant	32	1.2 [1.2, 1.3]	0.2	[0.9, 1.6]	1.3 [0.9, 1.5]
	Non-dominant	29	1.2 [1.2, 1.3]	0.3	[0.9, 1.5]	1.2 [0.9, 1.5]
	Difference (d-nd)	29	0.0 [-0.1, 0.1]	0.2	[-0.4, 0.4]	0.1 [-0.6, 0.5]
SAN	Dominant	41	1.0 [1.0, 1.1]	0.2	[0.7, 1.3]	1.0 [0.7, 1.3]
	Non-dominant	41	1.0 [1.0,1.0]	0.1	[0.7, 1.3]	1.0 [0.8, 1.2]
	Difference (d-nd)	41	0.0 [0.0, 0.1]	0.2	[-0.3, 0.3]	0.0 [-0.3, 0.4]
DSN	Dominant	26	1.1 [1.0, 1.2]	0.3	[0.6, 1.7]	1.2 [0.7, 1.6]
	Non-dominant	28	1.0 [0.9, 1.0]	0.2	[0.6, 1.4]	1.0 [0.6, 1.5]
	Difference (d-nd)	23	0.1 [0.0, 0.2] *	0.3	[-0.4, 0.6]	0.1 [-0.2, 0.8]

N: number of subjects included, d: dominant side, nd: non-dominant side, SER: Serratus anterior muscle, TRAP: Trapezius muscle, RHOMB: Rhomboid major muscle, LTN: Long thoracic nerve, SAN: Spinal accessory nerve, DSN: Dorsal scapular nerve, PI: Prediction interval.
* Significant difference.
† Not all nerves were found, therefore, for the nerves, it is specified how many subjects that are included in the calculation.

thickness and upper limit of the nerve diameter by taking into account age, height, weight, sex and hand dominance, thus enabling evaluation of abnormality on the individual level.

The muscle thickness of the serratus anterior muscle in our study was found to be a bit larger, though with some overlap (6.9–8.5 mm in our study compared to 3.8 mm-7.6 mm in other studies (Day and Uhl, 2013; Talbott and Witt, 2013, 2014)). We found similar results for all the parts the trapezius muscle compared to one study (O'Sullivan et al., 2009), for the middle part of the trapezius muscle compared to one study (Bentman et al., 2010) and for the lower part compared to two studies (Day and Uhl, 2013; O'Sullivan et al., 2007)

Methodological differences in regard to reference point, choice of place of measurement (Day and Uhl, 2013; O'Sullivan et al., 2007), probe placement (Bentman et al., 2010), subject positioning and the state of the muscle (in rest or contracted) may account for these differences. Furthermore, the number of subjects in these studies was low (14–20 healthy subjects), and the age group was younger than in this study. As for the rhomboid muscle, we found only one study (Jeong et al., 2016) that reported thickness of this muscle as a part of a reliability study in 24 young adults. We found a larger muscle thickness than this study (7,8 mm in our study vs. 4,6 during rest and 6,8 with abducted arm). The reason for this could be a younger population, and different methodology and place of measurements.

There are few studies of HRUS identification of the small nerves of the neck, and with the exception of the spinal accessory nerve (Mirjalili et al., 2012), these nerves cannot always be identified using ultrasound.

We were able to identify the long thoracic nerve in 95% of times (78 out of 82 nerves found) but we were not able to follow the nerve all the way down to the serratus anterior muscle for all detected nerves (Fig. 7). One study(Lieba-Samal et al., 2015) reported the nerve was identified on both sides all the way down to the serratus anterior muscle in all healthy subjects (n = 20) participating in the study. We could identify the dorsal scapular nerve in 76% of times (62 out of 82 nerves found) (Fig. 7) similar to one study of 50 patients getting an interscalene-block (Hanson and Auyong, 2013) where they reported identification of the nerve in 77% of the cases. In this study a stimulating needle was used to identify the nerve.

Fewer studies have reported measurements of the nerves, most commonly the diameter of these nerves. In one study using HRUS, the largest nerve diameter of the long thoracic nerve was 1.6 mm ± 0.3 on average (Lieba-Samal et al., 2015), compared to our study where we found a slightly smaller long thoracic nerve diameter of 1.2–1.5 mm (Table 2) depending on the site where the nerve was measured. Regarding the spinal accessory nerve, the mean nerve diameter in our study (1.0 mm) is similar to an earlier study (Mirjalili et al., 2012) where the mean diameter was found to be 0.76 ± 0.12 mm. To our knowledge, no other study has measured the dorsal scapular nerve size using HRUS.

The normative data in our study are derived from 82 observations for all the muscles since we used both sides for each subject (41 subjects) and performed statistics that took into account the random subject effect due to the fact that each subject was in the analysis twice. For the nerves this number was not always true as not every nerve on each side was identified. This can be mentioned as one of the limitations of this study as according to the recommendations from American Association of Neuromuscular and Electrodiagnostic Medicine (Dillingham et al., 2016) a number of 100 or more healthy subjects are suggested in order to ensure wide representation of the distribution.

The current technique for imaging of the serratus anterior muscle at the posterior axillary line can pose a disadvantage when the nearby muscles such as the latissimus dorsi muscle are affected as well, as this might make it difficult to obtain a clear image of the serratus anterior lying beneath.

Lastly, we did not use a stimulating needle to confirm our findings regarding the nerves, as it has been done in some earlier studies(Hanson and Auyong, 2013; Kim et al., 2016), thus, we depended on knowledge of the anatomy. Nevertheless, well-defined and accessible criteria, taking into account earlier cadaver and US studies, were created prior to the inclusion of subjects, thus we believe that the structures were identified correctly.

5. Conclusion

We found that HRUS is a feasible method to visualise the muscles stabilizing the scapula and their corresponding nerves with acceptable intra- and inter-examiner agreements. Furthermore, we present HRUS normative data of muscle thickness and nerve diameter using regression-based prediction formulas based on age, weight, height, sex and hand dominance. Moreover, to our knowledge, this study presents for the first-time normative data of nerve diameter of the dorsal scapular nerve. Further research is needed to investigate the use of HRUS of these structures in neuromuscular diseases and improve the diagnostic practice in those patients.

6. Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cnp.2021.01.003.

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