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Functional and morphological changes in shoulder girdle muscles after repeated climbing exercise

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25 ABSTRACT

This study aimed to investigate the acute effect of repeated climbing actions on functional and morphological measures of the shoulder girdle. Fifteen male indoor climbers participated in this study. All the climbers declared route level 6a+, as the best climbing grade (French climbing grade scale). Functional (range of motion - ROM and muscle strength), and morphological measurements (muscle/tendon stiffness and thickness) after a repeated climbing exercise protocol were analyzed. The ROM and muscle strength showed significant decreases from baseline to Immediate-Post (IA) as well as significant increases from IA to 1h-Post for all movements (p \leq .001 for all). Muscle stiffness showed significant increases from baseline to IA after as well as significant decreases from IA to 1h-Post for all muscles (p \leq .001 for all). However, thickness showed significant increases from baseline to IA for supraspinatus tendon and muscle thickness and occupation ratio (p \leq .001 for all), while a significant decrease was observed in acromiohumeral distance (p \leq .001). Significant decreases from IA to 1h-Post were found for muscles/tendons and occupation ratio (p \leq .001 for all), while a significant increase for AHD (p \leq .001). Our data demonstrated acute alterations in tendon thickness due to acute signs of implement symptom in climbers.

Key Words: muscle stiffness, thickness, overhead, fatigue, climbing

41 INTRODUCTION

Indoor climbing is gaining popularity as a form of physical activity and recreation. Currently, indoor and outdoor climbing have developed on a competitive level culminating by the debut of indoor climbing at the 2020 Olympic Games in Tokyo (Lutter et al., 2017). Climbers are characterized by high physical performance in endurance, flexibility and strength (Grant et al., 1996;Mermier et al., 2000;Grant et al., 2001). Previous studies have shown that climbers exhibit greater strength of the grip and fingers, flexibility of the upper extremity and endurance of the shoulder girdle compared with non-climbing controls (Grant et al., 1996). Range of motion (ROM) of the shoulder is a key element in climbing, especially during overhead and horizontal movements, e.g., single and double arm pull ups as well as hangings during climbing (Deyhle et al., 2015;MacLean and Dickerson, 2019).

Repetitive upper extremity activity have been shown to alter morphological properties of tendons and muscles, including changes in thickness (McCreesh et al., 2017;Klich et al., 2020;Mifune et al., 2020;Porter et al., 2020;Pozzi et al., 2021) as well as shoulder ROM and strength (Yu and Lee, 2013;Oliver et al., 2020). Previous experimental studies have shown that a repeated exercise bout of an rotator cuff muscles exercise led to an acute alteration of increased supraspinatus tendon thickness (McCreesh et al., 2017;Klich et al., 2020). Similarly, a short exercise bout in overhead athletes (Klich et al., 2020;Porter et al., 2020), and longer term repetitive work activity (Pozzi et al., 2021) have led to an increase in supraspinatus tendon thickness. An increase in muscle stiffness has also been demonstrated after repetitive upper extremity activity of the pectoralis major, deltoid, infraspinatus and upper trapezius (Dashottar et al., 2014;Klich et al., 2020). And repeated exercise bout specifically of the rotator cuff caused increases in the infraspinatus stiffness and supraspinatus tendon thickness (Klich et al., 2020), and reduction in shoulder ROM (Dashottar et al., 2014).

Schöffl et al. (2011) reported shoulder impingement syndrome as the most frequently disorders of the shoulder in climbers. The understanding of both muscle stiffness with tendon morphology changes might be a novel approach to provide the advancement in our understanding of mechanisms of the development of shoulder pain related to tendinopathy. Therefore, the aim of this study was to investigate the acute effect of repeated climbing actions on functional and morphological measures of the shoulder girdle. Specifically, we hypothesized that after repeated climbing exercise protocol: (1) the rotator cuff tendons would be thicker with a concurrent reduction of the subacromial space, and the supraspinatus tendon will occupy a greater proportion of the subacromial space; (2) there would be an increase in rotator cuff muscle stiffness.

MATERIAL AND METHODS

Study design

An observational, case series study assessing functional and morphological changes after repeated climbing exercise measurements was conducted. The functional measurements were rate of perceived exertion (RPE), shoulder ROM and maximal strength. The morphological measurements were

myotonometry and ultrasonography at baseline, immediately after (Immediate-Post) and 1-hour post exercise protocol (1h-Post). The time elapsed from the end of the repeated climbing exercise protocol to the beginning of the measurements was 30 seconds or less, and all measurements were made within 5 minutes. Measurements were made in the same order starting with (1) RPE, (2) myotonometry including muscle stiffness of the shoulder girdle, specifically of the pectoralis major, deltoid anterior, deltoid posterior, and infraspinatus, (3) ultrasonography of the tendons and muscles of the supraspinatus and infraspinatus, and subacromial space via acromiohumeral distance (AHD); (4) ROM, (5) isometric maximal strength of shoulder flexion, abduction, and internal rotation. All measurements were taken on the dominant shoulder.

Participants read and signed an informed consent form approved by the Senate Research Ethics Committee (project identification code: 26/2016 approval date: 13.10.2016). The study was conducted according to the Declaration of Helsinki.

Participants

A group of healthy male recreational indoor climbers (n=15, age 28.8±7.4 years, body height 178±9.3 cm, body weight 74.5±6.7 kg, BMI 23.7±1.5 kg·m⁻²) voluntarily participated in this study. All participants were right-handed with training experience in indoor climbing of 6±2 years and training duration of 10±1 hours per week. The difficulty level of the route was strictly adapted to the climber's skills and performance, based on the French climbing grade scale (Draper et al., 2011).

The recruitment process consisted of two parts: (1) An interview including questions about experience, training frequency (per week), rate of advancement (route grade) as well as history of pain and injuries in the upper extremity. (2) A screening evaluation using ultrasound assessment of the rotator cuff tendons/muscles performed by a experienced physical therapist, and further evaluated by an experienced orthopedic surgeon (specialized in shoulder) to avoid acute and chronic rotator cuff tears, or other tendon/muscle damage. The images were blind evaluated to increase the quality of the recruitment process. During the recruitment process, the participants reported their best climbing grade achieved in the past 2 years during a free climbing route. All the climbers declared route level 6a+, as the best climbing grade. The inclusion criteria were: 1) training experience in indoor climbing ≤5 years, training duration ≥8 hours per week, and climbing best level at 6a. This climbing route has an overhang with small, smooth and more oval handholds placed at greeter distance involving multi-planar movements. Exclusion criteria consisted of no intensive strength training 2 weeks prior to the study, previous upper extremity trauma, previous upper extremity surgery, and previous pain in the shoulder in the past 6 months.

The G*Power software (version 3.1.9.2; Kiel University, Kiel, Germany) (Faul et al., 2007) was used to estimate the required sample size. We calculated the power $(1-\beta)$ for repeated measure ANOVA within factors, by defining the sample size as 13 (for all ultrasound measurements), set a minimum expected effect size (Cohen's f) of 0.5, and level of 0.05, and a power of 0.95 and correlation for repeated

measures of 0.6. Also, power of shoulder stiffness, ROM and strength was calculated by defining the sample size as 13, α as 0.05, and effect size as 0.45; 0.35 and 0.38 respectively.

Procedure

The measurements were obtained three times; baseline, Immediate-Post, and 1h-Post. The participants were asked to refrain from climbing and to avoid strenuous physical activity 2 days before participating in the study.

After baseline measurements, the participants took part in a 15-minutes warm-up exercise program based on global exercises (including mobility and stability) of the shoulder girdle, trunk and forearm joints, and muscles. The warm-up ended with a single climb on the wall at the grade level of 6a+, to familiarize the participant with the specific climbing route. Following a 5-minutes rest, each subject performed a repeated climbing exercise protocol on a climbing wall. The wall was 15 meters high, including an overhang of about 7° from the right angle (83°). Grips had different sizes, shapes and were placed at different distances to occur the shoulder movement both vertically and horizontally. The exercise procedure consisted of five times climbs (grade level 6a+) for a total time of 5-minutes per climb, followed by a 5-minute rest. Immediately after the exercise protocol and 1-hour post, measures were taken (**Figure 1**).

Measurements

Rate of perceived exertion (RPE)

The participants were asked to determine the level of fatigue with the 6 to 20 RPE scale (Borg, 1998). During the evaluation, participants had to indicate the RPE scale according to their upper extremity and shoulder.

Functional measurements

Range of motion (ROM)

A wired twin-axis electrogoniometer (Noraxon USA Inc., Arizona, USA) was used to record shoulder movement during flexion, abduction and internal rotation. This device consists of a central strain gauged flexible shim that runs the length of the device with two end plates attached to the shim.

For all ROM measurements, the participants were seated with on a chair, with arms placed at the side of the body in neutral position and thumb pointing forward. For internal rotation measurements, the arms were positioned in flexion of the elbow of 90°. To assess ROM during flexion an investigator placed the sensor over the lateral board of the scapula and the anterior part of the arm, with the center of the sensor positioned over the acromion. For abduction, the sensor was placed with over the spine of the scapula and the arm, with the center of the sensor positioned over the acromion (Ribeiro et al., 2016). The ROM in the transverse plane was measured using two sensors, where one was positioned for flexion, while the second was placed over the lateral side of the arm radial part of the forearm. The ROM was measured and collected at 1500 Hz by TeleMyo 2400T G2 and PC interface (MyoResearch XP Master

Edition 1.08.09, Noraxon). All sensors were attached using double-sided tape and supported by an elastic band to avoid extra movements of sensors. Before repeated measurement the electrogoniometer was calibrated and set to 0° . Each measurement was made twice and maximum values were extracted. The relative reliability ranged from good to excellent for all analyzed movements (ICC_{2,1} from 0.87 to 0.92) (Landis and Kock, 1977). The absolute reliability showed that SEMs were 2.3° to 4.5° , while MDC90% ranged from 6.3° to 12.4° .

Maximal strength

A handheld dynamometer (HHD) (Hoggan Scientific, Lafayette, IN) was used to measure strength peak force during maximal voluntary isometric contraction (Harrington et al., 2011). Shoulder strength testing was performed in a seated position with their feet flat on the floor, with knees and hips at approximately 90°. For flexion and abduction the arm was placed in 90° of elevation, while for external rotation the arm was positioned by the side with a towel roll under the axilla, and the elbow flexed to 90°. The HHD was stabilized with an external device and aligned with the posterior forearm just proximal to the ulnar styloid process for flexion and abduction strength. For internal rotation strength, the HHD was placed on the anterior forearm just proximal to the wrist (Michener et al., 2021). The order of strength testing was randomized to minimize potential effect of fatigue. During the measurements, participants were informed to "push as hard as you can" for 5 seconds. Thirty seconds and 1 min rest were given between each trial and testing position, respectively (Harrington et al., 2011). Each measurement was taken twice and the maximum values were averaged prior to statistical analysis. The relative reliability was good to excellent for all analyzed strength (ICC_{2,1} from 0.82 to 0.90). The absolute reliability showed SEMs were 5.5 N to 13.0 N, while MDC90% were 15.0 N to 34.0 N.

Morphological measurements

Muscle stiffness

A hand-held myotonometer device (MyotonPro, Myoton Ltd, Estonia) was used to measure the stiffness of the pectoralis major, deltoideus anterior and posterior, and infraspinatus muscle. Muscle stiffness is defined as the property that characterizes resistance to the contraction or to a stretching external force that deforms the initial shape of the tissue. Stiffness (N/m) was computed as $S=a_{max}m_{probe}/\Delta l$, where a is the acceleration of the damped oscillation; m_{probe} is the mass of the measurement mechanism and Δl is the probe displacement (Kawczynski et al., 2018). The examiner located the probe perpendicular to the tested area and then the probe generated three impulses exerted on the tested muscles (Kelly et al., 2018). The probe was placed perpendicular to the tested area and generated three impulses exerted on the testing area. The subject was seated with their back on a chair, arms on the table with the forearms pronated. Measures of stiffness were performed over the shoulder on four locations: (1) infraspinatus – two fingers width below the center spine of scapula (Kelly et al., 2018), (2) anterior deltoid, (3) posterior deltoid, and (4) pectoralis major – muscle belly halfway between

clavicle and humeral bone (Klich et al., 2020). The relative reliability was good to excellent for stiffness of all analyzed muscles (ICC_{2,1} from 0.86 to 0.94). The absolute reliability showed that SEMs ranged from 10 N/m to 17 N/m, while MDC90% ranged from 28 N/m to 48 N/m.

Tendon and muscle thickness, acromiohumeral distance (AHD) and occupation ratio

Ultrasonography was performed using an ultrasound scanner (HS-2200, Honda, Toyohashi, Japan) with a 7.5 (6.0 to 11.0) MHz and 40 mm linear array transducer (HLS-584 M, Honda, Toyohashi, Japan) in greyscale B-mode. The settings of the ultrasound system were standardized for all participants and kept identical for all measures. A single examiner (a certificated physical therapist with a practice in musculoskeletal ultrasonography) obtained ultrasound images of (1) supraspinatus tendon thickness in short axis (SST-S_{Thick}), (2) supraspinatus tendon in the long axis (SST-L_{Thick}), (3) supraspinatus muscle thickness (SSM_{Thick}), (4) infraspinatus muscle thickness (INFM_{Thick}) and (5) acromiohumeral distance (AHD). The positioning of the participant and measurement procedures for SST-S_{Thick}, SST- L_{Thick} and AHD (Figure 2a-f) were performed according to Michener et al. (2015) and , while Schneebeli et al. (2014) for SSM_{Thick} (**Figure 2g,h**) and Koppenhaver et al. (2009) for INFM_{Thick} (**Figure** 2i, j). Each measurement was made twice and averaged for data analysis. The ultrasound images were coded to blind the evaluator and ranked in a random order to decrease the potential learning effect. Additionally, after data collection, the occupation ratio was calculated to specify alterations in the shoulder. The occupation ratio was defined as supraspinatus cross-sectional tendon thickness a % of AHD (Michener et al., 2015). The relative reliability was good to excellent for all analyzed tendon and muscle thickness and AHD (ICC_{2,1} from 0.88 to 0.95). The absolute reliability showed that SEMs ranged from 0.2 mm to 0.4 mm, while MDC90% ranged from 0.3 mm to 0.5 mm.

Statistical analysis

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The SPSS 18 statistical software (SPSS Inc., Chicago, Illinois, USA) was used for data analysis. Mean values \pm standard deviation (SD) as well as mean differences with confidence interval (CI 95%) have been reported. Normality of the data distribution was applied through the Shapiro–Wilk tests, while homogeneity of variance was analyzed by Levene's test. The analyzed data was normally distributed for all parameters, while the variances for all parameters were equal. A one-way analysis of variance with repeated measure (RM-ANOVA) with time (baseline, Immediate-Post, 1h-Post) was used as a within-subject factor for differences in RPE, ROM, maximal strength, ultrasonography and myotonometry measurements. If an interaction between variables was found, the Bonferroni adjustment for multiple comparisons was used for post hoc tests (p=0.001). The effect size was estimated using partial eta square (η^2), classified as small (.2< η^2 <.49), medium (.5< η^2 <.79) or large (η^2 <.8) (Richardson, 2011). For all statistical tests, p-value < 0.05 was considered significant.

216 RESULTS

Functional measurements

Table 1 reports the mean±SD of the RPE, ROM and muscle strength at baseline, Immediate-Post and 1h-Post repeated climbing exercise protocol. The one-way RM-ANOVA revealed a statistically significant effect of *Time* in RPE ($F_{2,28}$ =403.7, p≤.001, η ²=.97). The post-hoc analysis showed significant increase from baseline to IA after, while a significant decrease from IA to 1-h-Post (p ≤001 for all). See Table 1.

The one-way RM-ANOVA revealed a statistically significant effect of *Time* in ROM ($F_{2,88}$ =317.5, $p \le .001$, η^2 =.88). Post-hoc analysis showed significant decreases from baseline to Immediate-Post after for flexion, abduction and internal rotation as well as significant increases from Immediate-Post to 1h-Post for flexion, abduction and internal rotation ($p \le .001$ for all).

Similarly, the one-way RM-ANOVA showed a statistically significant effect of *Time* in maximal strength ($F_{2,88}$ =1070.5, p≤.001, η^2 =.96). The post-hoc analysis showed significant decreases from baseline to Immediate-Post after for flexion, abduction and internal rotation as well as significant increases from Immediate-Post to 1h-Post for flexion, abduction and internal rotation (p ≤.001 for all). See Table 1.

Morphological measurements

Table 2 shows the mean±SD of muscle stiffness of pectoralis major, deltoideus anterior and posterior, and infraspinatus as well as shoulder tendon and muscle thickness, AHD and occupation ratio at baseline, IA and 1-h-Post repeated climbing exercise protocol. The one-way RM-ANOVA revealed statistically significant effect of *Time* in muscle stiffness ($F_{2,118}$ =306.8, $p \le .001$, η^2 =.84). The post-hoc analysis showed significant increases from baseline to IA after for pectoralis major, deltoideus anterior and posterior, and infraspinatus muscle as well as significant decreases from Immediate-Post to 1h-Post for pectoralis major, deltoideus anterior and posterior, and infraspinatus muscle ($p \le .001$ for all).

Finally, the one-way RM-ANOVA revealed statistically significant effect of *Time* in ultrasonographic evaluation ($F_{2,148}$ =27.2, $p \le .001$, η^2 =.27). The post-hoc analysis showed significant increases from baseline to IA after for SST-S_{Thick}, SSM_{Thick}, INFM_{Thick} and occupation ratio ($p \le .001$) for all) and SST-L_{Thick} (p < .01), while a significant decrease was observed in AHD ($p \le .001$). Significant decreases from Immediate-Post to 1h-Post were found for SST-S_{Thick}, SST-L_{Thick}, SSM_{Thick}, INFM_{Thick} and occupation ratio ($p \le .001$) for all), while a significant increase for AHD ($p \le .001$), See Table 2.

246 DISCUSSION

The current study revealed the presence of alterations in shoulder function and morphological properties afterrepeated climbing exercise in recreational indoor climbers. Changes in shoulder function were characterized by decreases in ROM and maximal strength during flexion, abduction and internal rotation, with a simultaneous increase in RPE immediately after the exercise protocol. Moreover, after 1-hour rest we observed an increase in ROM and maximal strength as well as a decrease of RPE. The morphological properties, expressed as rotator cuff tendon and muscle thickness, showed greater

supraspinatus tendon (SST- S_{Thick} , SST- L_{Thick}) and muscle thickness (SSM $_{Thick}$), infraspinatus muscle thickness (INFM $_{Thick}$), and occupation ratio, with a simultaneous decrease in AHD immediately after exercise protocol. In parallel, we observed a decrease in tendon and muscle thickness, and occupation ratio as well as an increase in AHD after 1-hour rest. The results of our study were in agreement with the hypotheses suggesting acute alterations in tendon and muscle thickness in the shoulder girdle after a climbing exercise protocol in indoor climbers.

This study evaluated shoulder function, expressed by ROM and maximal strength after a repeated climbing exercise protocol in indoor climbers. Beside the expected increase in RPE, the analysis of our results showed a decrease in flexion and abduction by 15%, while internal rotation decreased by 29% from baseline to Immediate-Post climbing. For maximal strength, flexion and internal rotation decreased 34 and 42%, respectively. Previous studies have investigated ROM (Seminati et al., 2015;Schwesig et al., 2016;Matthews et al., 2017;Moreno-Pérez et al., 2019) and maximal strength (Mullaney and McHugh, 2006;Andrade et al., 2016;Schwesig et al., 2016;Matthews et al., 2017;Moreno-Pérez et al., 2019) after specific exercise protocols in overhead athletes. Our results for ROM and maximal strength are in line with previous studies showing a decrease in ROM for flexion (10 to 15%), abduction (11 to 15%) and internal rotation (18 to 26%) as well as decrease strength in internal rotation (24 to 34%) after repetitive overhead movement protocols (Mullaney and McHugh, 2006;Seminati et al., 2015;Schwesig et al., 2016;Klich et al., 2021). The current protocol enabled to explore the acute morphological changes due to fatigue even if task failure was not achieved in line with previous studies (Duchateau and Enoka, 2008).

In this study we investigated morphological alterations in rotator cuff tendons/ muscles thickness and stiffness of the shoulder girdle Immediate-Post and 1h-Post an indoor climbing protocol. Previous studies have assessed rotator cuff tendon thickness and stiffness of shoulder muscles (Klich et al., 2020; Mifune et al., 2020; Porter et al., 2020) in overhead athletes. However, only Klich et al. (2020) and Mifune et al. (2020) investigated both thickness and stiffness analyzed rotator cuff muscle thickness (supraspinatus and infraspinatus), trapezius and shoulder muscles stiffness (pectoralis major, deltoid anterior and posterior, rhomboids and serratus anterior). The analysis of our results showed an increase in stiffness for all shoulder muscles; the highest increase was observed in the deltoideus posterior (73%), and the lowest in the infraspinatus (43%). Our results showed an increase in the supraspinatus tendon and muscle thickness (19 to 25%) and the infraspinatus muscle (10%), with simultaneous decrease in AHD (22%) from baseline to Immediate-Post. The present differences in thickness can be explained by differences in exertion and fatigability of these muscles (Enoka and Duchateau 2008). Finally, the supraspinatus tendon occupied a greater proportion of the subacromial space, expressed by an increase in occupation ratio by 36%. In our previous study (Klich et al., 2020), we reported lower stiffness and thickness, with greater AHD compared with current results. Moreover, after 1 hour we observed decrease in stiffness and thickness, with decrease of AHD. Porter et al. (2021) reported a greeter thickness in the supraspinatus tendon immediately after exercise swimming protocol. They reported thickness after 6 and 24 hours post showing a decrease compared with immediately after.

An acute increase in tendon thickness and decrease in AHD might last up to 6 hours and reduce to baseline after 24 hours (McCreesh et al., 2017). Changes observed in this current and previous studies (McCreesh et al., 2017;Klich et al., 2020;Porter et al., 2020) of acute fatigue due to different intrinsic mechanisms. Furthermore, it should be noted that acute alterations in supraspinatus tendon thickness may be associated with mechanical compression of the tendon in the subacromial space (Michener et al., 2015;McCreesh et al., 2017) or posteriorly-superiorly between the glenoid and humeral head (Do and Lim, 2017).

Repetitive mechanical overload has been described as the main factor causing muscle-tendon morphology and altering motor functions (Bissas et al., 2020;Pozzi et al., 2021). An increased thickness of the supraspinatus tendon might be the first symptom of repetitive loading related to shoulder pain (Pozzi et al., 2021), and may lead to occupy a greater portion of the subacromial space (Michener et al., 2015). Repetitive overhead movements in indoor climbers, e.g. bilateral muscle sub-maximal contractions followed by a short resting time may alter the rotator cuff coursing acute impingement (Schöffl et al., 2011).

Previous studies have investigated different models of tendon and muscle degeneration including the assessment of damage, distribution of fiber recruitment, intra-muscular inflammation or edema (Proske and Morgan, 2001;Nakama et al., 2005;Fung et al., 2010;McCreesh et al., 2017). Acute changes in soft tissue's histological alterations might also lead to increased stiffness. Fung et al. (2010) showed that fatigue might cause increased stiffness and decreased hysteresis at low- and mid-level, mostly due to changes in fibers recruitment in damaged and undamaged fibers. Increase in stiffness of the pectoralis major might be related with repetitive elevation during climbing causing tightness of this muscle (Page, 2011). Furthermore, a higher stiffness of the deltoid posterior and infraspinatus could be a result of climbing movement pattern coursed by positioning of holds on the wall to horizontal movements (Reinold et al., 2004). The present study revealed concomitant changes in function and morphological properties of the shoulder girdle in response to repeated climbing.

The current study has strength and limitations. A strength relies in the combined field assessment of functional and morphological changes. We opted for an experimental protocol composed of repetitive climbing exercise resulting in fatigue-related changes analyzed in a before-after settings in line with previous study (Côté et al., 2008). Concerning limitations, we only recruited men in our study, however future experiments should include women to evaluate sex differences in functional and morphological changes. Second, a control group would facilitate the interpretation of the observed changes. Third, we reported an acute effect of repeated exercise mimicking bouldering in healthy and recreational climbers. Future studies could investigate alterations in shoulder function and morphological properties in relation to speed, lead and bouldering.

325 CONCLUSIONS

The present study showed for the first time changes in shoulder function and morphological properties after repeated exercise protocol in recreational indoor climbers. Significant increase in supraspinatus thickness, with simultaneous reduction in AHD resulted in greeter occupation ratio. Our study demonstrated acute alterations in tendon thickness due to acute signs of implement symptom in climbers. This work provides an important finding about evaluation morphological properties using both ultrasonography and myotonometry in conjunction with functional measurements.

Declaration of Interest Statement

- The authors declare no conflict of interest and no funding. Written informed consent was obtained from
- the participants.

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BASELINE (15-min before WARM-UP)	WARM-UP	5-MIN REST	FATIGUE PROTOCOL	IMMEDIATE- POST	1H-POST
RPE Myotonometry Ultrasonography ROM Maximal strengt			5 x	RPE Myotonometry Ultrasonography ROM Maximal strength	RPE Myotonometry Ultrasonography ROM Maximal strength

Figure 1. Experimental procedure including rate of perceived exertion (RPE), myotonometry, ultrasonography, range of motion (ROM) and maximal strength evaluation at baseline, Immediately-Post and 1 hour Post exercise (1H-Post) after repeated climbing exercise protocol.

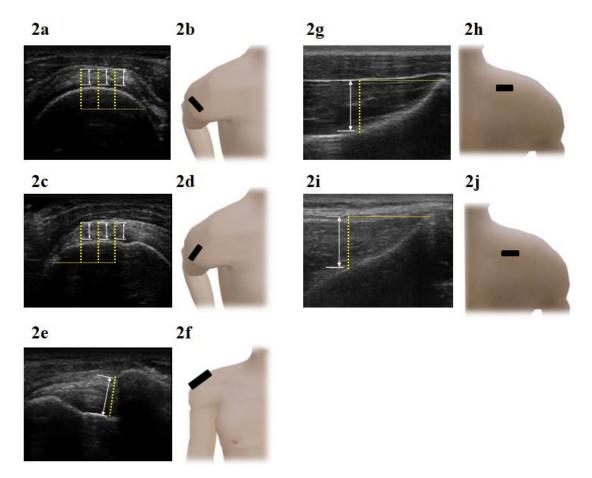


Figure 2. Ultrasound assessment and measurement of the rotator cuff: a) ultrasound measurement of the supraspinatus tendon thickness in short axis (SST- S_{Thick}); b) ultrasound transducer position (SST- S_{Thick}), c) ultrasound measurement of the supraspinatus tendon thickness in long axis (SST- L_{Thick}); d) ultrasound transducer position (SST- L_{Thick}), e) ultrasound measurement of the acromiohumeral distance (AHD); f) ultrasound transducer position (AHD); f) ultrasound measurement of the supraspinatus muscle thickness (SSM $_{Thick}$); g) ultrasound transducer position (SSM $_{Thick}$), h) ultrasound measurement of the infraspinatus muscle (INFM $_{Thick}$); i) ultrasound transducer position (INFM $_{Thick}$).

Table 1. Shoulder range of motion (ROM) [$^{\circ}$], shoulder maximum isometric strength [N], and rate of perceived exertion (RPE) at baseline-. Immediate-Post-and 1 hour-Post (1h-Post) repeated climbing exercise protocol. Mean \pm SD values

480

481

482

	Baseline	Immediate- Post	1h-Post	Baseline to Immediate-Post	P value*	Immediate- Post to 1h-Post	P value**
				Mean Difference (95% CI)		Mean Difference (95%CI)	
Shoulder Range	of Motion [°]						
Flexion	174.4±2.7	147.6±6.6	157.7±6.5	27 (22,32)	<i>p</i> ≤.001	-10 (-11, 9)	<i>p</i> ≤.001
Abduction	169.1± 1.5	142.8±5.5	156.9±4.3	26 (23, 30)	<i>p</i> ≤.001	-14 (-17, -11)	<i>p</i> ≤.001
Internal rotation	60.9± 1.1	43.3±1.9	53.5 ± 2.4	18 (15, 20)	<i>p</i> ≤.001	-11 (-14, -8)	<i>p</i> ≤.001
Shoulder Maxim Strength [N]	num Isometric						
Flexion	416.8±34.3	273.6±33.3	332.4±38.2	143 (125, 162)	<i>p</i> ≤.001	-58 (-70, -36)	<i>p</i> ≤.001
Abduction	383.4±20.6	240.3±24.5	299.1±22.6	143 (128, 150)	<i>p</i> ≤.001	-59 (-78, -32)	<i>p</i> ≤.001
Internal rotation	425.6±21.6	246.1±28.4	310.9±23.5	180 (158, 201)	<i>p</i> ≤.001	-65 (-90, -45)	<i>p</i> ≤.001
Rate of Perceive	d Exertion						
RPE	6±.0	14±1.7	9 ±.8	-8 (-9, -7)	<i>p</i> ≤.001	5 (4, 6)	<i>p</i> ≤.001

Significant differences *- within-group differences between baseline and IA, and **- within-group differences between 1h-Post and IA ($p \le 0.05$).

Table 2. Shoulder girdle stiffness (N/m) and rotator cuff tendon and muscle thickness [mm], acromiohumeral distance (AHD) [mm] and occupational ratio measured at baseline-. Immediate-Post- and 1 hour-Post (1h-Post) repeated climbing exercise protocol. Mean \pm SD values

	Baseline	Immediate- Post	1h-Post	Baseline to Immediate-Post	P value*	Immediate-Post to 1h-Post	P value**
				Mean Difference (95% CI)		Mean Difference (95%CI)	
Stiffness [N/m]							
Pectoralis major muscle	203.1±27.3	541.3±68.5	271.5±35.8	-338 (-375, -306)	<i>p</i> ≤.001	269 (238. 308)	<i>p</i> ≤.001
Deltoid anterior muscle	258.4±72.9	597.6±60.3	397.6±60.3	-339 (-362, -316)	<i>p</i> ≤.001	200 (156, 241)	<i>p</i> ≤.001
Deltoid posterior muscle	201.9±43.4	759.9±28.	298.2±28.2	-558 (-570, -543)	<i>p</i> ≤.001	462 (454, 483)	<i>p</i> ≤.001
Infraspinatus muscle	370.1±154.5	643.3±126.9	422.8±146.6	-273 (-301, -250)	<i>p</i> ≤.001	220 (135, 330)	<i>p</i> ≤.001
Thickness [mm]							
Supraspinatus Tendon							
Cross-sectional	4.7 ± 2.5	6.3±0.8	5.5±0.3	-1.6 (-2.1, -1.3)	<i>p</i> ≤.001	0.8 (0.6; 1.2)	<i>p</i> ≤.001
Longitudinal	4.4±0.5	5.7±0.7	4.8±0.2	-1.3 (-1.9, -0.8)	P=.007	0.9 (0.3, 1.3)	<i>p</i> ≤.001
Supraspinatus Muscle	11.2±0.3	13.9±0.8	12.3±0.2	-2.7 (-3.2, -2.2)	<i>p</i> ≤.001	1.6 (1.4, 2.1)	<i>p</i> ≤.001
Infraspinatus Muscle	18.1 ± 0.3	20.0±0.3	18.4 ± 0.4	-1.9 (-2.3, -1.4)	<i>p</i> ≤.001	1.6 (0.7, 1.8)	<i>p</i> ≤.001
AHD [mm]	9.6±0.5	7.5±0.3	8.5±0.2	2.1 (1.6, 2.4)	<i>p</i> ≤.001	-1.0 (-1.5, -0.6)	<i>p</i> ≤.001
Occupational ratio [%]	46.9±3.3	73.3 ± 2.9	57.5±3.2	-26 (-30, -23)	<i>p</i> ≤.001	15 (11, 18)	<i>p</i> ≤.001

Occupation ratio: Supraspinatus Cross-sectional tendon thickness as a % of AHD.

Significant differences *- within-group differences between baseline and IA, and **- within-group differences between 1h-Post and IA ($p \le 0.05$).