

Implementation and ground validation of a facility for functional and structural analysis of proximal upper limb muscles in microgravity

Francesco Pacelli (1), Antonio Paoli (1), Valfredo Zolesi (2), Aleandro Norfini (2), Alessandro Donati (2), Carlo Reggiani (1)

*University of Padua, Department of Anatomy and Physiology, Padua (1)
Kayser Italia Srl, Italy (2)*

Abstract

The study of the activity of the proximal muscles of the upper limb and in particular of muscles acting on the gleno-humeral joint in microgravity condition has a special interest because: 1. The upper limbs play a major role in posture and locomotion for the subject living in the space station; 2. Muscle fatigue may have a significant effect on the hand and upper limb, for the ordinary work on board and in particular for the extra-vehicular activities (EVA); 3. Crewmembers may be at risk of shoulder injury during space walks because of decreasing muscle and tendon mass during long-duration space-flight. In this study a facility was developed to follow the changes in mass and force of proximal upper limb muscles. The facility is composed of a dynamometer, composed of a handle connected to a load cell, indicated as Pullgrip Dynamometer (PGD), and a specific software able to record force measurements and to drive the testing procedure (indicated as MAAT protocol). PGD was designed in a way to be easily mounted on the inner wall of the ISS. The results obtained in the testing session demonstrated that PGD and MAAT can reliably: 1. Discriminate between subjects in relation to the level of muscle strength develop in a pull effort applied on the PGD; 2. provide data for correlation between force and muscle size; 3. Evaluate the weight of the proprioceptive feedback in comparison with visual feedback to determine precision of force generation; 4. Evaluate muscle fatigue. The validation of the facility based on PGD and MAAT provides support to the plans to utilize them in experimental studies on alteration of proximal upper limb muscles on board of the International Space Station (ISS).

Key Words: muscle atrophy, microgravity, space flight, shoulder joint, upper limb, locomotion

Basic Applied Myology 19 (2&3): 77-86, 2009

The unloading of human muscles in microgravity during space flight is known to produce significant alterations in skeletal muscle structure and function [1]. Hind limb muscles with a defined postural role such as knee extensors and ankle plantar flexors are virtually completely unloaded in microgravity and it is not surprising that they undergo to significant loss in strength and size during space flight. The alterations of hind limb muscles after a period in microgravity or after ground based simulations of weightlessness (such as bed rest and unilateral lower limb suspension) have been the target of several studies [3,7,8]. There is however evidence that upper limb muscles also undergo to decrease in mass and strength after space flight [14,15]. The issue of microgravity adaptations of upper limb muscles is made complex by two specific reasons:

1. They are less involved in postural activity in normal gravity and therefore less severe effects of microgravity can be expected;

2. The upper limb muscles plays a major role in posture and locomotion during space flights as hands and arms are used to move and stabilize the body inside the space station as well as in extra-vehicular activities (EVA).

The contrast between disuse and atrophy on the one hand and intensive use for posture and locomotion on the other hand can determine significant muscle fatigue reducing the performance of the hand and upper limb during ordinary work on board and even more in extra-vehicular activity (EVA) and can increase the risk of shoulder injury during space flight for crewmembers [2].

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To our best knowledge only two studies have examined the upper limb functional impairment after space flight in humans [14,15] and in the latter only the distal portion has been carefully studied. Two distinct models have been employed to provide further data on the effect of disuse and unloading on upper limb muscles: the triceps and the biceps of a primate, *Macacus Rhesus*, exposed to two weeks of microgravity during a space flight [5,10,12] and the triceps of volunteers where simulated weightlessness has been obtained by wearing a sling or with a cast [6,9,11,13]. A reduction of contractile force both in handgrip and in elbow extension was consistently observed after few weeks of space flight [14,15] or arm disuse and immobilization [6]. By contrast, no decrease in performance has been observed after prolonged bed rest [3,8]. At muscle fiber level, studies on simulated weightlessness obtained with sling or plaster cast have demonstrated reduction in cross sectional area of slow and fast fibers [9,13]. A shift towards an increase in fast myosin isoform expression has been also observed [13], while the prolongation of the CrP recovery time and the increased fatigability have suggested a shift towards a more glycolytic phenotype [11].

The muscle impairment in the upper limb is not only relevant for the performance but it is also important because the stability of the shoulder or gleno-humeral joint relies essentially upon muscle activity. In particular, rotator cuff muscles function to stabilize dynamically the spherical humeral head in the shallow glenoid fossa. Impairment of the rotator cuff muscles lead to shoulder instability and pain. Decreases in muscle and tendon mass due to microgravity combined with heavy loading during EVA raises the likelihood of shoulder injury and shoulder pain is common complain for astronauts [2]. In this frame, specific training to obtain high quality ultrasonography of the shoulder has been given to the crewmembers of the ISS, where a facility to collect ultrasonic images and transmit in real-time to remote experienced sonologists in Telescience Center at Johnson Space Center is now available [2].

In this study, we aimed to implement a facility which allows the evaluation of the loss of strength and mass of the upper limb proximal muscles in microgravity. The facility is a development of the HPA system already present on the ISS and has been designed to be fully compatible with the ISS requirements. The facility is composed by a dynamometer, called pullgrip (PG), and a specifically designed software which drives the muscle testing. The following criteria have guided the development of the facility:

1. Non invasive testing of the proximal upper limb muscles;
2. user-friendly software;

3. close reference to the to the conditions in which shoulder muscles are utilized in microgravity;

4. short duration for assembling/tests performance/dismantling the system (total time 15 minutes);

5. ability to identify changes in strength, in nervous control of muscle force (proprioceptive control), in muscle fatigue.

Materials and Methods

Subjects

In ground simulation tests, the PGD hardware and software and the MAAT protocol were tested on 40 young and healthy volunteers (age, $25 \pm 2,02$ years; body mass $71,7 \pm 16,3$ kg; height $174,8 \pm 9,71$ cm). In an initial interview each respondent was provided all details about the test protocol. All subjects gave their informed consent and the study was approved by the Ethical Commission of the Department. After providing informed consent, the participant was interviewed regarding his or her health status. A modified version of the Health Status Questionnaire [4] was used as a screening tool. Respondents provided informed consent to participate in the study and were screened for the presence of diseases or conditions that would place them at high risk for adverse responses to exercise [4]. Exclusion criteria for the study included recent upper-body injury and poorly controlled hypertension. The groups trained/untrained differed in number of training sessions/week attended: subjects who performed more than four hours of physical activity a week were considered as "trained". After this assigning criterion, participants were equally divided in 4 groups: 1. 10 trained men (TM); 2. 10 trained women (TW); 3. 10 untrained men (UM); 4. 10 untrained women (UW).

Pullgrip Dynamometer (PGD)

In collaboration with the team of Kayser srl a new tool for testing the function of the gleno-humeral joint on the ISS, whose name is Pull Grip Dynamometer (PGD), was designed and assembled (figure. 1). The tool is based on a dynamometer shaped as a handle, designed to be easily fixed to and removed from the wall of the IIS, connected to a computer where a specific software records force measurements and drives the testing procedure. The PGD handle is mechanically connected to a load cell. A hinged joint allows alignment of force direction with the load cell axis to perform a proper measurement of the pull force. Exploiting previous experience of HPA facility, a testing protocol called MAAT (Methods for Astronaut Arm Testing) was implemented.

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% MVC	ANG LE	HAN D	VF b	no Vfb	Vfb	RES T
100% MVC	90°	R	5"			55"
100% MVC	90°	L	5"			55"
75% MVC	90°	R	5"	5"	5"	30"
75% MVC	90°	R	5"	5"	5"	30"
50% MVC	90°	R	5"	5"	5"	30"
50% MVC	90°	R	5"	5"	5"	30"
25% MVC	90°	R	5"	5"	5"	30"
25% MVC	90°	R	5"	5"	5"	30"
100% MVC	135°	R	5"			55"
100% MVC	135°	L	5"			55"
75% MVC	135°	L	5"	5"	5"	30"
75% MVC	135°	L	5"	5"	5"	30"
50% MVC	135°	L	5"	5"	5"	30"
50% MVC	135°	L	5"	5"	5"	30"
25% MVC	135°	L	5"	5"	5"	30"
25% MVC	135°	L	5"	5"	5"	30"
Total time 15'						

Table 1. MAAT protocol: sequence of pull efforts and rest pauses. MVC, maximal voluntary contraction, Vfb, period with visual feedback, noVfb, period without visual feedback, REST, rest pause period

Methods for Astronaut Arm Testing (MAAT) testing protocol

The first steps in designing MAAT protocol were the choice of the gesture to be performed with muscles of the proximal upper limbs and of the body position at which the gesture had to be performed; The choice was based on two sets of data: 1) posture and locomotion inside and outside the ISS are generally based on upper limb push and pull, 2) video analysis shows that, during locomotion in the ISS, astronauts mostly perform pull-efforts with a trunk's inclination between 130-150°, while during EVA the angle between trunk and humerus is approximately 90°.

Therefore the chosen action to evaluate the "loss of strength in upper limb muscles" was a pull effort performed in two different positions: 1. astronaut's body in parallel with the ISS wall and the pull effort performed with an angle of 90° between trunk and humerus (figure 2A); 2. astronaut's body inclined

approximately 45° relative to the ISS wall and the pull effort performed with an angle of 130°-150° between trunk and humerus (figure 2B).

During tests, PGD was mounted on an handrail and subjects were either standing up or laying prone on an inclined bench to simulate the zero gravity condition of EVA (90°) or locomotion (135°).

Each tester performed "MAAT" protocol (Table 1), with a pre-defined sequence of isometric pulling efforts at 75%, 50% and 25% of maximal force in the two positions above described with periods with visual feedback and periods without and short rest pauses (figure 3).

Muscle mass determination

Before tests with PGD, in each subject the percentage of free fat mass in the dominant upper limb was assessed by skinfold measurement using Fitnext® (Montecchio Maggiore; Vicenza, Italy) software. The software utilizes 2 skinfolds (triceps, biceps), 2 bone circumferences (arm, forearm), 2 bone diameters

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Table 2. Mean values and SD in testers' age (years), height (cm), weight (kg).

Testers	N°	Age	Height (cm)	Weight (kg)
Trained women (TW)	10	23,2±4,5	170,9±6,2 [‡]	63,2±8,3 [‡]
Untrained women (UW)	10	23,9±3,3	163,6±7,8	54,4±5,7
Trained men (TM)	10	25,1±3,1	186,1±5,4 ^{*#} 	91,5±8,3 ^{*#}
Untrained men (UM)	10	27,8±6,8	178,6±5,4 ^{†§}	77,8±6,7 ^{†§}

Height: ‡TW > UW (p<0.05); *TM > UW (p<0.001); #TM > TW (p<0.001); ||TM > UM (p<0.05); †UM > UW (p<0.001); § UM > TW (p<0.05). Weight: ‡TW > UW (p<0.05); *TM > UW (p<0.001); #TM > TW (p<0.001); ||TM > UM (p<0.001); †UM > UW (p<0.001); § UM > TW (p<0.001).

(elbow, wrist) measurements. The determination of muscle mass in proximal part of upper limb, based on anthropometric measurements, was validated with NMR measurements in 15 subjects.

Statistical analysis

One-way multivariate analysis of variance (ANOVA) was used to compare scores across all groups. Whenever significant differences in values occurred, multiple comparisons tests (aimed to determining where significant differences occur between pairs of groups) were performed using a post-hoc Tukey-Kramer test, considered the most powerful method for all pairwise comparisons. Alpha significance level was set at 5% (and was adjusted for multiple comparisons).

Pearson's correlation coefficient was used to compare maximal force with upper limb free fat mass.

Results

All the 40 respondents passed the screening and elected to continue with the study. Therefore, the data presented below come from 40 subjects equally distributed among the four groups (UW N 10; TW N 10; UM N 10; TM N 10). Variance analysis revealed inter-group differences in height and weight before the PGD test. As can be seen in Table 2, TM subjects were significantly heavier compared to the other three groups (p<0.001); minor but significant diversity was detectable in height intra the same gender-group (p<0.05) and in weight between TW and UW (p<0.05).

Forces developed by the testers in pulling on PGD

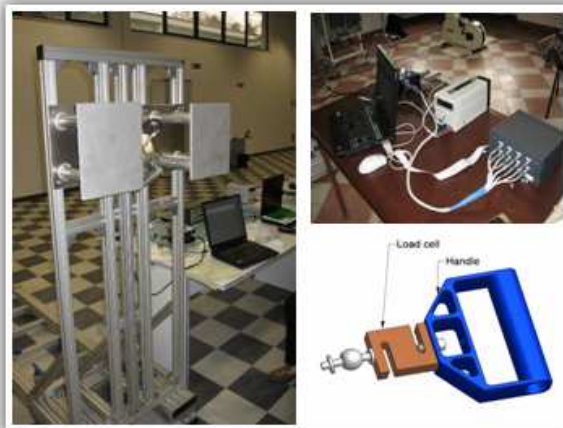


Fig. 1 Pullgrip Dynamometer, Software and Handle with load cell.

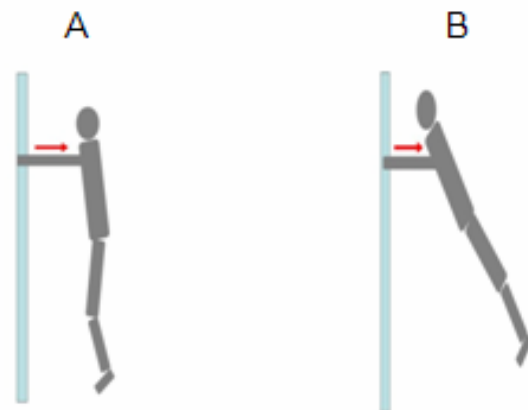


Fig. 2 Schematic drawings of pulling efforts at: A. 90°, B. 135° angle trunk-humerus.

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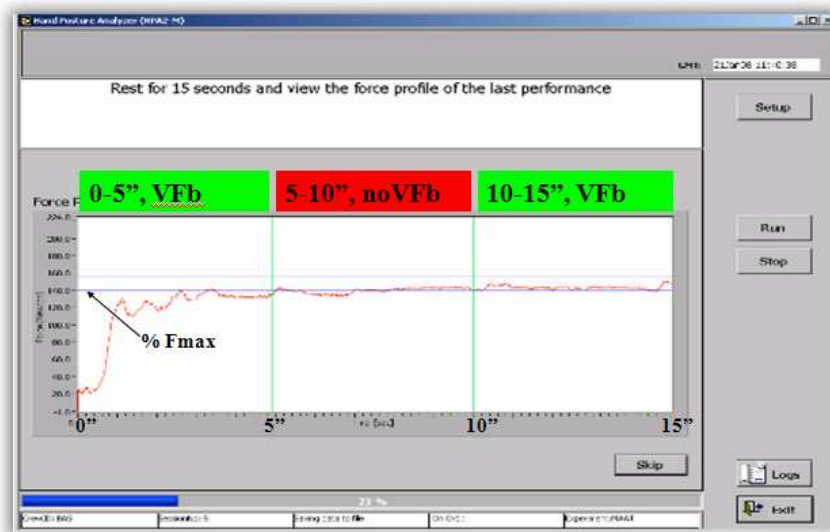


Fig. 3 PGD software display. The three phases of the pull effort can be clearly seen, together with their duration and the line which is offered as a reference to control pulling strength. Fmax is the force developed in MVC, maximal voluntary contraction, Vfb, period with visual feedback, noVfb, period without visual feedback, REST, rest pause period

with maximal voluntary contractions are shown in Figure 4 with the testers divided as described above in four groups with specific gender, training level and anthropometric parameters. Figure 4-A shows the average values of force developed in maximal voluntary contraction at an angle of 90° between trunk-humerus by the subjects divided in four groups: trained women (TW), untrained women (UW), trained men (TM), untrained men (UM). As can be seen tension developed by male subjects ($580,3 \pm 35,4$ N) was significantly greater ($p < 0.001$) than tension developed by female subjects ($306,9 \pm 50,2$ N), whereas a minor diversity was detectable also between trained and untrained subjects. The difference between the four groups reached statistical significance for TM compared UW and TW ($p < 0.001$) and for UM compared to UW and TW ($p < 0.001$). Figure 4-B shows the average values of force developed in maximal contractions at an angle of 135° between trunk-humerus by the four groups. As observed at 90°, tension developed by male subjects ($651,6 \pm 33,7$ N) was significantly greater ($p < 0.001$) than tension developed by female subjects ($373,2 \pm 72,9$ N) and the difference between the four groups reached statistical significance for TM compared UW and TW ($p < 0.001$) and for UM compared to UW and TW ($p < 0.001$); additionally can be seen a minor but significant difference for TW compared to UW ($p < 0.05$).

The direct comparison between the pulling efforts at the angle of 90° and 135° is shown in Figure 4C-D. Figure 4-C shows the average values of force developed in maximal contractions by male subjects at the two different angles between trunk and humerus.

Tension developed at 135° angle was higher than at 90°, however the only statistical significant difference can be observed between TM at 135° and UM at 90° ($p < 0.01$). Finally Figure 4-D shows the average values of force developed in maximal contractions by female subjects at the two different angles between trunk and humerus. Major diversities were detectable compared to Men groups: statistical significant difference can be observed between TW at 135° both UW at 90° and 135°.

Figure 5 shows the lack of differences of force developed in maximal contraction between dominant (d) and contralateral (cl) upper limb intra trained subjects (T) and untrained subjects (UT), gender and angles of pulling being pooled together. As expected, statistical significant differences can be seen in Td compared to Ud and Ucl ($p < 0.001$) and in Tcl compared Ud and Ucl ($p < 0.01$).

The results of free fat mass determination of the proximal part of upper limb are shown in Figure 6. As can be seen in Figure 6A, dominant upper limb muscle mass was significantly greater in TM compared to UW, TW ($p < 0.001$) and UM ($p < 0.05$). Also in UM was significantly higher than UW and TW ($p < 0.001$), whereas there was no significant difference between UW and TW.

When maximal force and free fat mass were compared in individual subjects, a high correlation ($r^2 = 0.62$, $n = 40$, $p < 0.0001$) was found. Figure 3B shows the correlation between maximal contraction developed in pulling efforts at PGD and dominant upper limb's muscle mass (obtained by Fitnext protocol).

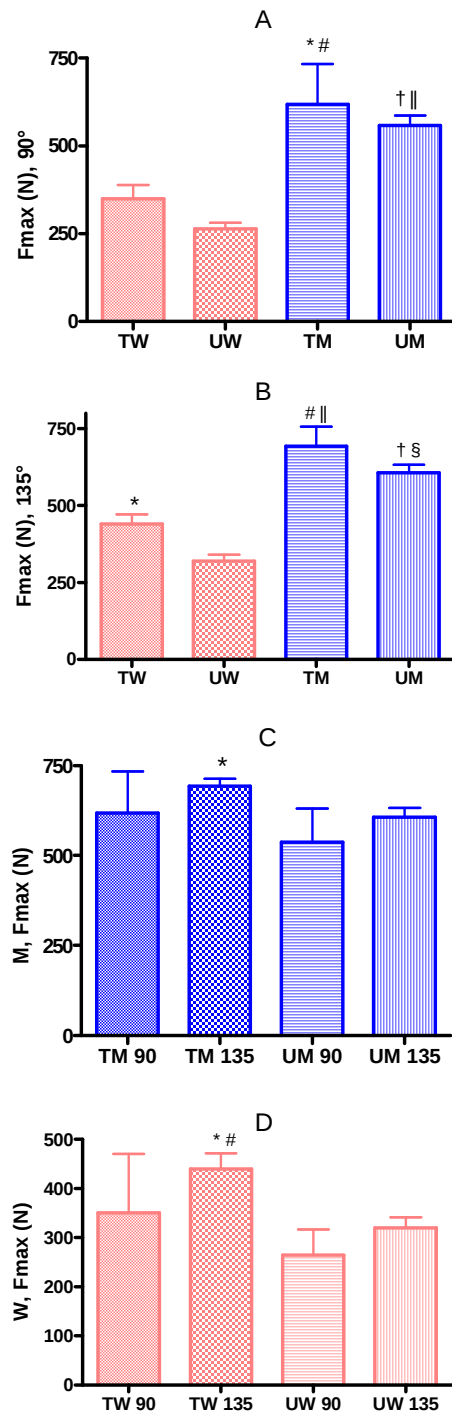


Figure 7 shows the relevance of feedback control in maintaining the required level of force during a submaximal efforts in the presence (periods between the third and the fifth second, between the tenth and the fifteenth second, see also figure 3) or in absence of Visual Feedback (period between the fifth and the tenth second). The parameter Δ Force (Expected Force – Recorded Force) was used to score the level of precision. In the example shown in figure 7, the pull

Fig. 4 Mean values and SD of force (Fmax) developed in Maximal Voluntary Contraction during isometric pulling effort at PGD in Trained Women (TW), Untrained Women (UW), Trained Men (TM) and Untrained Men (UM). Variance analysis and post-hoc tests revealed the following statistical differences:

A at an angle of 90° trunk-humerus: TW 350,6±119,4; UW 264,4±52,1; TM 618,5±114,96; UM 537,6±99,1.

*TM > TW ($p < 0.001$); #TM > UW ($p < 0.001$); †UM > TW ($p < 0.001$); ||UM > UW ($p < 0.001$).

B at an angle of 135° trunk-humerus: TW 439,8±101,9; UW 319,8±67; TM 693,4±63,8; UM 611,4±86,8.

*TW > UW ($p < 0.05$); #TM > UW ($p < 0.001$); ||TM > TW ($p < 0.001$); †UM > TW ($p < 0.001$); §UM > TW ($p < 0.001$).

C in Men (M) groups at different angles trunk-humerus of pulling effort: TM 90° 618,5±114,96; TM 135° 693,4±63,8; UM 90° 537,6±99,1; UM 135° 611,4±86,8.

*TM 135° > UM 90° ($p < 0.01$).

D in Women (W) groups at different angles trunk-humerus of pulling effort: TW 90° 350,6±119,4; TW 135° 439,8±101,9; UW 90° 264,4±52,1; UW 135° 319,8±67.

*TW 135° > UW 90° ($p < 0.001$); # TW 135° > UW 135° ($p < 0.05$).

effort was performed at 75% maximal contraction with an angle of 135° between trunk and humerus. Subjects' precision resulted inversely proportional to % of maximal force required (as expected by Weber's rule, not shown), was higher in presence of visual feedback, and, finally, decreased in the final part of the pulling effort (likelihood caused by fatigue effect). In particular in women groups, UW5-10 was significantly less precise than UW3-5 ($p < 0.01$) and TW5-10 was significantly less precise than TW3-5 ($p < 0.01$) and TW10-15 ($p < 0.05$). In men groups, statistical significant difference can be observed in UM5-10 compared to UM3-5 ($p < 0.01$) and UM10-15 ($p < 0.05$); finally TM5-10 was significantly less precise than TM3-5 ($p < 0.01$) and TM10-15 ($p < 0.05$). No significant differences were found in statistical analysis intergroup neither in 3-5" nor in 10-15" nor in 10-15" range.

Discussion

With the prolongation of the stays in zero gravity conditions and the perspective of long duration space flight (Earth-Mars, for example) the functional and structural evaluation of the proximal muscles of the upper limb and in particular of muscles acting on the gleno-humeral joint in microgravity conditions has achieved an increasing relevance. This is due to several reasons: upper limbs play a major role in posture and

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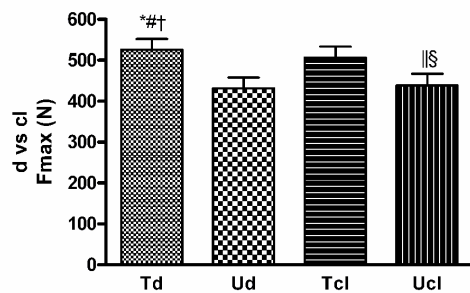


Fig. 5 Mean values and SD in maximal voluntary contraction (F_{max}) (N) developed during isometric pulling effort at PGD with dominant (d) and contralateral (cl) upper limb in trained (T) and untrained (U) groups. Td 525,6±169,8; Ud 432±162,5; Tcl 506,7±169,5; Ucl 438,7±179,1.

*Td > Ud ($p < 0.001$); #Td > Ucl ($p < 0.001$); ||Tcl > Ud ($p < 0.01$); §Tcl > Ucl ($p < 0.01$).

Therefore video and film recorded on board of ISS were analyzed to conclude that, during locomotion in the ISS, the shoulder muscles are employed mostly to perform pull-efforts with trunk's inclination between 30° and 45°, while during EVA the angle between trunk and humerus is mostly 90°. Therefore the action chosen to evaluate the "loss of strength in upper limb muscles" was a pull effort performed in two different positions to simulate the zero gravity condition of EVA (angle of 90° between trunk and humerus 90°) or locomotion (angle of 120°-150° between trunk and humerus 135°). A suitable sequence of pulling efforts and rest pauses was designed and indicated with the acronym MAAT.

The PGD hardware, its software and the MAAT protocol were validated in a transversal study on 40 young and healthy volunteers equally divided in 4 groups: trained men, trained women, untrained men, untrained women.

The results showed that PGD and MAAT could reliably discriminate between subjects in relation to their levels of muscle strength. This was confirmed by the fact that significant differences expected on the basis of gender and training were detected and by the fact that in individual subjects the strength values were linearly and significantly correlated with the values of upper limb muscle mass. It is, thus, reasonable to predict that PGD and MAAT will be able to detect all changes in strength occurring in astronauts during their stay on ISS.

Two points deserve some comments: 1) A greater maximal force was generated by all the subjects at 135° angle conditions (514,9±168 N) than at 90° (443±171,8 N). The greater strength showed by testers in the laying position than in the standing position could be

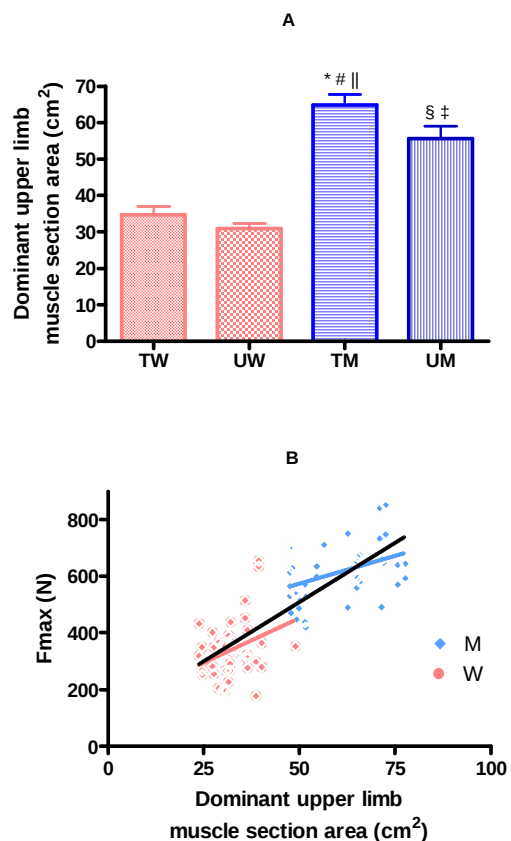


Fig. 6 Determination of muscle section area (cm²) in proximal dominant upper limb and its relation with maximal force in pulling efforts. A Mean values and SD in dominant upper limb muscle section area (cm²) in Trained Women (TW), Untrained Women (UW), Trained Men (TM) and Untrained Men (UM) group: TW 34,7±7,3; UW 30,9±4,6; TM 64,8±9,3; UM 55,6±10,7. *TM > UW ($p < 0.001$); # TM > TW ($p < 0.001$); || TM > UM ($p < 0.05$). § UM > UW ($p < 0.001$); ±UM > TW ($p < 0.001$). B Correlation ($r^2 = 0.62$, $n = 40$, $p < 0.0001$) between maximal contraction (F_{max}) expressed in pulling efforts at PGD and dominant upper limb's muscle mass (obtained by Fitnext protocol) in women (W) and men (M) groups (trained and untrained were pooled together).

explained by a pre-lengthening of latissimus dorsi, deltoid (posterior head) and triceps brachii (long head). The differential activation of individual muscles in the two positions was also checked with surface electromyography in a preliminary study. 2) No significant differences were found between dominant and contralateral upper limbs neither in trained nor in untrained groups. These surprising data could be explained by the specific feature of the action required: isometric pulling effort is not frequent in daily and is

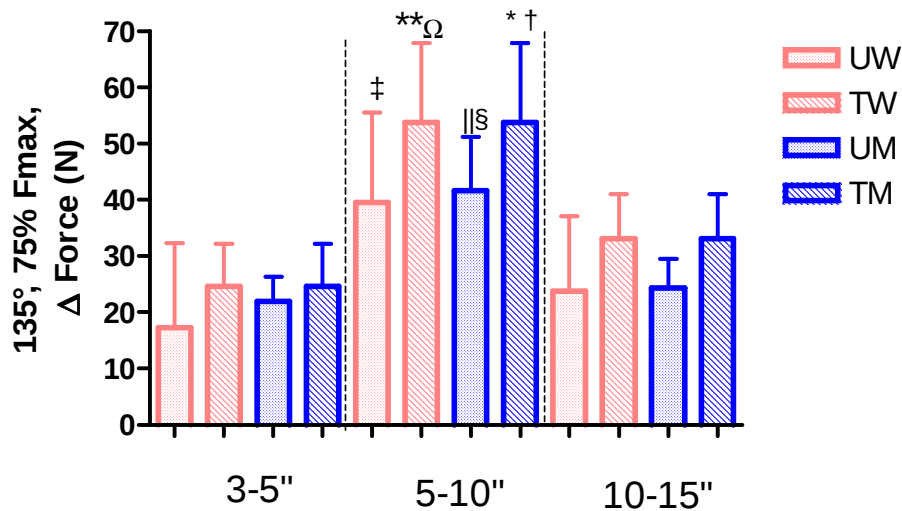


Fig. 7 Precision of the feedback force control during pulling efforts at 75% of maximal contraction (F_{max}). Means and SD of Δ Force (= Expected Force – Recorded Force) (N) recorded in isometric pulling effort executed at 75% maximal contraction (with an angle of 135° trunk-humerus) during the three phases of the test: range between 3-5'' (with visual feedback), 5-10'' (without visual feedback), 10-15'' (with visual feedback). Analysis of variance and post hoc tests showed that: UW3-5: 17,3±4,7,4; UW5-10: 39,5±5,0,7; UW10-15: 23,7±4,1,1. TW3-5: 24,6±2,3,8; TW5-10: 53,8±4,4,7; TW1 0-15: 33,1±2,5,2. UM3-5: 21,9±1,4,2; UM5-10: 41,7±2,9,9; UM10-15: 24,4±1,6,5. TM3-5: 24,6±2,3,8; TM 5-10: 53,8±4,4,7; TM10-15: 33,1±2,5,2. ‡UW5-10 > UW3-5 ($p<0.01$). ** TW5-10 > TW3-5 ($p<0.01$); ΩTW5-10 > TW10-15 ($p<0.05$). †§UM5-10 > UM3-5 ($p<0.01$); §UM5-10 > UM10-15 ($p<0.05$). *TM5-10 > TM3-5 ($p<0.01$); †TM5-10 > TM10-15 ($p<0.05$).

not a common sport action. For these reasons, we can hypothesize that neuromuscular recruitment was not different between the two upper limbs.

Beside the maximal strength in pull effort, PGD and MAAT showed a good discriminative ability to evaluate strength control mechanism and muscle fatigue. The precision in generating given required levels of force (25%, 50%, 75% of MCV, strength in maximal voluntary contraction) was found to be inversely proportional to % of maximal force, as expected by Weber's rule, was higher in presence of visual feedback, and, finally, it decreased in the final part of the pulling effort. At each given force level, the relevance of the proprioceptive feedback could be estimated as the increase of the Δ force in the second phase of the pulling effort (5-10 s) compared to the first phase (3-5 s) where also the visual feedback was present. The decrease in precision observed in the final part of the pulling effort could be due to muscular fatigue effect. However, due to the short duration of the single test (15 seconds) this fatigue effect was detectable at 75% of MVC better than at 50% and 25% of MCV.

In conclusion, the results of this study, taken together, support the view that the facility based on

PGD and MAAT is reliable and able to evaluate muscle strength and analyze muscle control in the proximal part of the upper limbs. Work can be done to further validate the facility on the ground such as, for example, a longitudinal study where the measurements are repeated in the same subjects at subsequent times during a training or disuse period or a transversal study including subjects with pronounced structural and functional impairment of shoulder muscles. The final goal was and remains, after this validation study on the ground, the use of the facility on the ISS, to identify and quantify the alterations of proximal upper limb muscles and, based on this, to propose the required countermeasures.

Acknowledgements

This study has been supported by ASI (Agenzia Spaziale Italiana) with the grant WP 1B235.

Address Correspondence to:

Prof. Carlo Reggiani, Department of Anatomy and Physiology, University of Padova, Via Marzolo 3, I-35131 Padova, Italy.

E-mail: carlo.reggiani@unipd.it

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