Influence of muscle fatigue on electromyogram–kinematic correlation during robot-assisted upper limb training

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Abstract
Introduction: Studies on adaptive robot-assisted upper limb training interactions do not often consider the implications of muscle fatigue sufficiently.

Methods: To explore this, we initially assessed muscle fatigue in 10 healthy subjects using two electromyogram features, namely average power and median power frequency, during an assist-as-needed interaction with HapticMaster robot. Since robotic assistance resulted in a variable fatigue profile across participants, a completely tiring experiment, without a robot in the loop, was also designed to confirm the results.

Results: A significant increase in average power and a decrease in median frequency were observed in the most active muscles. Average power in the frequency band of 0.8–2.5 Hz and median frequency in the band of 20–450 Hz are potential fatigue indicators. Also, comparing the Spearman’s correlation coefficients (between the electromyogram average power and the kinematic force) across trials indicated that correlation was reduced as individual muscles were fatigued.

Conclusions: Confirming fatigue indicators, this study concludes that robotic assistance based on user’s performance resulted in lesser muscle fatigue, which caused an increase in electromyogram–force correlation. We now intend to utilise the electromyogram and kinematic features for auto-adaptation of therapeutic human–robot interactions.

Keywords
Human–robot interaction, upper limb training, muscle fatigue, electromyogram, kinematic correlation

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Introduction
A Human–robot interaction (HRI) study is used to understand, design and evaluate different robotic systems for use by or with humans, which involves communication between robots and humans. Designing a HRI system requires evaluating the capabilities of humans and robots, using suitable training and technologies allowing to produce the desirable interactions between them. Robotic systems have been used in the context of exercise/rehabilitation training for many years. Robots can also help to improve the quality of life by assisting people with disabilities. Assistive robots can sense, process the sensory information and perform actions that benefit seniors and people with disabilities. Hence, they can provide support when access to health care professionals is limited, e.g. out of hours or due to limited availability. To provide a purposeful interaction, knowledge of the person interacting with the robot is an important contributor to the success of the task. The current study explores the usability of electromyogram (EMG)-based features in HRI and the influence of muscle fatigue on EMG–kinematic correlation.

Muscle fatigue is defined as the decline in the ability of muscles to generate force/power during a physical task. Fatigue is also defined as any exercise or non-exercise-induced loss in total performance due to various physiological factors, athlete-reported...
Muscle fatigue usually results in a feeling of tiredness or forces a person to take rest because of lack of strength, and it develops gradually during a physical activity. Muscle fatigue is the consequence of a variety of physiological changes within the working muscle. Studies have observed that muscle fatigue is associated with lower median frequencies and higher amplitudes in EMG signals. However, this observation has not been tested sufficiently in the context of a 'shared control' strategy of a robot-assisted system. A shared control strategy, for example, 'assist as needed' is used in HRI systems, that are based on impedance/admittance control. With the help of this, it is possible to create virtual effects such as weight, virtual drag or haptic sensation in a rehabilitation training, and the resistive environment can be adjusted by a robotic algorithm. Shared control with robots has been reported to be helpful for regaining motor skills.

Past studies on EMG–kinematic correlation during HRIs have mainly concentrated on isometric contractions (where the length of muscles does not change during the contractions). However, the current context of research is related to non-isometric/isotonic muscle contractions (contractions, which generate force by changing the length of the involved muscles). Similar to the findings by Jenkins et al., the non-linearity in the relationship between the muscle force and EMG amplitude during fatigue might probably affect the correlation coefficient in an isotonic context as well. As stated by Dideriksen et al., the EMG signal amplitudes showed an increase when force was maintained at the target level. But during fatigue (i.e. beyond task failure), the EMG amplitude started reducing and the target force could not be maintained any longer. This indicates that during fatigue, the linear relation between EMG and force is not completely clear as it depends on multiple neuro-muscular conditions.

This paper presents two related studies. At first, a feasibility study is presented as the first experiment, which was conducted on healthy individuals to explore how upper limb muscle fatigue can be estimated using EMG features (average power and median frequency) in a robot-assisted environment. The experiment used the HapticMaster (HM) robot to provide assistance during upper limb tasks, which also allowed users to be monitored by sensing the user’s hand movements through its end-effector. In the active-assisted mode of the HM, the subject has to initiate the activity, after which the HM robot would assist/guide the subject for the rest of the movement. A self-reported fatigue questionnaire was used, which showed that the majority of the participants only reported slight pain and muscle weakness.

Muscular activation during the robotic interaction can be obtained through EMG measurements. EMG is a very useful resource and is being increasingly used by the research community. It has the potential to provide a measure of muscle tiredness/fatigue during training interactions. Few studies have explored the fatigue

Background

People with neurological impairments often need to use extensive rehabilitation training for regaining their lost motor functions. It has been suggested that exercising improves a patient’s capacity to undertake physical activities. Studies have shown that repetition and practice could cause plastic changes in the human brain and, hence, an improved task performance. Robots have the potential to improve the recovery process in stroke patients as evidenced by regaining functions. Robots have the capability to deliver many repetitions in training exercises and also to record movements during the interaction. Few studies have explored kinematic features measured by the robot to adapt the rehabilitation training environment using HM robot. However, most rehabilitation robotic studies do not consider fatigue as a driving parameter for an adaptive HRI. Since muscle fatigue may place patients at risk of further injury, training under high levels of fatigue may be avoided by monitoring fatigue and by adapting the training accordingly. Monitoring fatigue can also provide important feedback needed to adjust training loads accordingly. Such a system will also have potential applications in other HRI contexts such as robot-assisted muscle training. A system that can sense the muscular state (for example level of pain or stiffness) of patients has the potential to improve the adaptability of the training environment and hence, increasing the amount of training.

Muscular activation during the robotic interaction can be obtained through EMG measurements. EMG is a very useful resource and is being increasingly used by the research community. It has the potential to provide a measure of muscle tiredness/fatigue during training interactions. Few studies have explored the fatigue...
state of participants based on EMG signals of the upper limb during robotic interactions. However, the EMG fatigue parameter was only used to cross-validate the subjective measurement of fatigue. These studies have mainly suggested that the muscle fatigue parameters can be detected from upper limb muscles.

During HRIs, the relationship between EMG and kinematic force measurements has been reported to be unclear. During dynamic muscle actions, both linear and non-linear relationships between EMG amplitudes and resultant force have been identified. EMG amplitude in isometric muscle contractions is reported to be directly proportional to the square root of the resultant force when the motor units (MUs) are activated independently. A linear relation between EMG and force can occur when full MU is recruited before the MU firing starts increasing. A non-linear relation will start when MU recruitment and MU firing frequency contribute together. Hence, different muscles will have different EMG–force relationships since they have different strategies for MU recruitment. During low levels of muscular force, both the MU recruitment and the firing rate changes are used to change muscle force. But during higher levels of force (approximately more than 30% of maximum voluntary contraction value), most of the muscles MUs remain already recruited. In such a case, the changes in muscle force are caused by a change in firing rates of MUs.

The power spectrum for force and EMG was reported to contain the most of the power below 0.5 Hz and they were found to be correlated. The low-frequency components (low pass filter at \(\leq 0.5 \text{ Hz}\)) of a rectified EMG during constant force tasks were found to be correlated with the interference (actual) EMG signals in the frequency band of 35–60 Hz. On the other hand, a study by Lin et al. on fatigue effects during isometric contractions stated that the power spectrum of a rectified EMG signal displayed a reduction in the gamma EMG oscillations (40–60 Hz of EMG signals) when fatigue occurred. A correlation of EMG signals with exerted force parameters was also indicated by studies of Yoshitake and Shinohara, but the study concentrated on the steady sub-maximal force values rather than dynamic and varying forces. The study stated that the smoothed MU discharge rates were more correlated with the rate of change of force than with the force parameters directly. A correlation was also noticed with the low-frequency component of rectified and smoothed EMG.

**Experiment 1**

The first experiment was conducted to validate EMG features and to explore if they could represent the state of upper limb muscle fatigue in healthy participants during a robot-assisted experiment.

**Methods**

The experiment was designed as in Figure 2. Ethics approval was obtained from the University of Hertfordshire (COM/PGT/UH/02002) board of ethics. Written consent was obtained from all individual participants included in the study.

**Setup.** The study used the HM robot as an interaction tool while performing upper limb reaching tasks. When the user exerts a force on the HM arm, the device will react with the proper displacement, from which the position, velocity and acceleration can be calculated using a virtual model. The admittance control paradigm makes the HM robot capable of rendering high stiffness, near-to-zero friction and zero end-effector weight, giving a very low-impedance motion. HM measures the applied force using the force sensor in the end-effector. In this experiment, the robot was configured in an active-assisted mode, where the subject only had to initiate the activity, after which the HM robot assisted/guided the subject for continuing the rest of the movement. This mode was initially selected to create the same environment when a stroke patient performs upper limb training tasks with robotic assistance. The participants were asked to hold the ball attached to the end-effector of HM with their right hand and to move between various points. A C++ code running on a Windows 7 (64 bit) machine using Visual Studio 2009 was used to configure the virtual reality (VR) environment and the HM. The VR environment was developed with the help of OpenGL libraries. A simulated 2D environment was created to offer visual guidance for the planned movement and its correct execution. A small yellow ball in the VR environment represented the robot end-effector and this was directly mapped to the movement of the end-effector in the real space. Some level of challenge was introduced for having active participation and motivating the participants to win over the robot. A grey coloured cylinder represented the path to be followed by the robot according to minimum jerk trajectory. A red-coloured cylinder represented the actual path achieved by the robot when the participant interacted with the environment. When the participant moved slower than the robot, the red cylinder would lag behind the grey cylinder. When the participant is ahead of the robot, the red cylinder would lead the grey cylinder. The user interface also provided provision for configuring different robotic parameters such as stiffness, inertia and so on. EMG signals from upper
limb muscles were collected using a Biometrics Ltd DataLINK signal acquisition device as in Figure 1.

**Protocol.** Before starting the experiment, the participants were given a practice session to become familiar with the HM operation in the active-assisted mode. They were asked to hold the gimbal of the HM (its end-effector) and move according to the trajectory displayed on the monitor. The participants were asked to fill in a questionnaire in the beginning and at the end of the experiment indicating their fatigue status and difficulty of the task.

Ten right-handed healthy participants of at least 20 years old took part in the experiment. The study concentrated on the gross movements of upper limbs, which involve larger muscles like Biceps Brachii (BB), Triceps Brachii (TB), Anterior Deltoid (DLT) and Trapezius (TRP). A ‘rectangle’ shaped movement pattern was defined in the XY plane of the VR environment on a 24" wide LCD monitor as in Figure 2. By maintaining a 90° abduction angle for the shoulder, the arm movements were constrained to a plane that is in line with the shoulder centre of rotation. This position was thought to help create fatigue for the muscles around the shoulder, since the hand and the elbow were positioned at shoulder height. Participants were asked to sit straight on a non-rotating chair. Audio feedback was given regarding the start and end of each trial. Each trial consisted of 10 iterations, and one iteration was a sequence of four segmented movements named S1, S2, S3 and S4 traversing rectangle sides as in Figure 3. The path between a source point and a destination point is termed a segment. Each trial lasted around 6 min including the 5 sec break in between iterations. After each trial, there was a short break period of 1–2 min. The experiment was conducted until the subject reported fatigue or until a maximum of six trials were reached.

**Methodology.** EMG average power and median frequency were calculated for each segment. These features were analysed using IBM SPSS version 22 and MATLAB. Summary tables were generated by comparing the feature values during the first and last trials for each subject. Correlation between the EMG and the kinematic data (represented by the 3D Cartesian force as in \( F_x \), \( F_y \) and \( F_z \)) was studied after conducting a mapping between the EMG and kinematic measurements based on the time and segment information from the HM data. In order to do this mapping, the time-stamp from the HM and EMG log files were used. Based on this mapping, the EMG and kinematic data were divided into four segments in a mapped file. The study was conducted after splitting the data into small windows. The window size for the EMG analysis was decided based on the number of samples per segment of the kinematic data. The sampling rates for EMG (1000 Hz) and kinematic data were different (151 samples per segment). So, before
the correlation study, in order to make the number of features on both sides equal, each iteration per segment of EMG data was divided into 151 windows/blocks of equal length. A few numbers of replications had to be made at the end of each segment to make the total size divisible by 151 and thus to make them of equal length. However, these replications to adjust the window width would not propagate to the next segment since the total windows in each iteration of a segment were bounded by the start and end of the segment. Thus, for each of the 151 windows, the average power was calculated. The corresponding raw values of force components \( (F_x, F_y \text{ and } F_z) \) were low-pass filtered at 0.5 Hz for removing any high-frequency variations.

The objective was to compare how EMG features and force components varied over time using correlation analysis. The features derived from EMG were used to see if there was a relation with the kinematic force components, and it was investigated how the correlation was affected as the trials progressed. The average power of EMG and the force components \( (F_x, F_y \text{ and } F_z) \) were compared segment-wise for each of the four muscles (TRP, DLT, BB and TB) separately. Since the kinematic features were not normally distributed, Spearman’s correlation was used for the correlation study. Then, the correlation was studied between these two features for each segment separately. The correlation was studied by considering all the subjects together, and also by considering the subjects separately. Finally, each trial was considered separately for each of the subjects, and the results were then used to assess how the muscle fatigue would affect the correlation coefficients as the trials progressed.

### Results

The mean values of the potential fatigue indicators (average power and median frequency of the EMG) in the first and last trials were compared. The summary of the results of the EMG analysis is shown in Table 1.

The mean value of average power in the last trial was higher than that in the first trial, and the mean value of median frequency in the last trial was lower than the first trial in the majority of the participants. The answers to the fatigue questionnaire indicated that there was a slight level of fatigue experienced by the subjects after the experiment. A majority of the participants stated that they were ‘somewhat fatigued’, while one of them was ‘very fatigued’ and another participant was ‘not fatigued’. The difficulty level of the experiment was reported as easy/moderate by most of the participants. All the participants responded that they could still continue the experiment.

![Table 1](image.png)

**Table 1.** Experiment 1: Summary table for EMG average power. The average power for 10 subjects displayed an increase in its median value as the trials progressed. This was more visible in the TRP and DLT muscles. A ’1’ indicated that there was an increase in the average power and a ’0’ otherwise.
The majority of them stated that the duration and the difficulty level of the experiment were moderate.

**Correlation study.** A typical amplitude of a force component \((F_x)\) for Trial 1 during the different segment movements is shown in Figure 4. A change in their sign indicates a change in the direction on the movements.

Correlation table based on raw force components and EMG average power considering all the subjects together, separated by different segments and different muscles, is described in Table 2. Spearman’s correlation coefficients generated using SPSS showed a weak or moderate correlation between the EMG and kinematic features. The results were similar when individual subjects were considered for the correlation study.

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**Table 2.** Correlation table based on raw force components and EMG average power for all subjects together. 151 analysis windows were considered for each segment and each muscle. Spearman’s method was used for the correlation study.

| Segment 1 | AvgPower TRP | AvgPower DLT | AvgPower BB | AvgPower TB |
|-----------|--------------|--------------|-------------|-------------|
| Force_X   | Correlation coefficient | -.235\(^a\) | .283\(^a\) | .129\(^a\) | .370\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Y   | Correlation coefficient | .229\(^a\) | -.293\(^a\) | -.168\(^a\) | -.419\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Z   | Correlation coefficient | .290\(^a\) | -.087\(^a\) | .176\(^a\) | .101\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |

| Segment 2 | Spearman’s rho | Force_X | Correlation coefficient | .299\(^a\) | .164\(^a\) | -.187\(^a\) | -.492\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Y   | Correlation coefficient | .292\(^a\) | .116\(^a\) | -.099\(^a\) | -.471\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Z   | Correlation coefficient | .165\(^a\) | .199\(^a\) | .246\(^a\) | .045\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |

| Segment 3 | Spearman’s rho | Force_X | Correlation coefficient | .118\(^a\) | .259\(^a\) | .038\(^a\) | -.485\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Y   | Correlation coefficient | -.034\(^a\) | -.362\(^a\) | -.047\(^a\) | .175\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Z   | Correlation coefficient | .136\(^a\) | -.071\(^a\) | .065\(^a\) | .001 |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |

| Segment 4 | Spearman’s rho | Force_X | Correlation coefficient | .141\(^a\) | -.102\(^a\) | .414\(^a\) | .252\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |
| Force_Y   | Correlation coefficient | .003 | -.105\(^a\) | .378\(^a\) | .262\(^a\) |
| Sig. (two-tailed) | .504 | .000 | .000 | .000 |
| Force_Z   | Correlation coefficient | .043\(^a\) | .222\(^a\) | .130\(^a\) | .099\(^a\) |
| Sig. (two-tailed) | .000 | .000 | .000 | .000 |

\(^a\)Correlation is significant at the 0.01 level (two-tailed).
To see if fatigue caused any changes in the correlation coefficient, the initial and final trials were compared. A summary table was also formed based on the trend in correlation coefficients as the trials progressed as shown in Table 3. The hypothesis was that there would be a decrease in the value of the correlation coefficient as the trials progressed. A value of ‘1’ indicated that there was a decrease in the correlation in the last trial compared to the first trial.

### Experiment 2

The results of Experiment 1 gave an indication of the potential of EMG parameters to be used as fatigue indicators during HRI. However, the robot was configured in ‘active-assisted’ mode and, hence, the additional support resulted in less fatigue. The questionnaires also stated that there was a low level of fatigue experienced by the participants after the experiment. The majority of the participants stated that they were ‘somewhat fatigued’. The difficulty level of the experiment was reported as easy/moderate by most of the participants. All the participants responded that they could still continue the experiment. This did not allow the experimenters to fully evaluate the suitability of the EMG features for fatigue estimation, in the chosen context of HRI. Hence, it was decided to conduct a second experiment, designed to be inherently fatiguing, to verify if the low level of fatigue measured in Experiment 1 could be mainly due to the robotic assistance. The second experiment, therefore, considered a similar observation without the aid of a robot and using a dumbbell instead. The second experiment was formulated with assistance from colleagues in sports science studies, involving a biceps curl exercise.

### Methods

#### Setup

The experiment setup included an EMG acquisition device (g.USBamp) from g.tec medical engineering GmbH, which is a multimodal biosignal amplifier for multiple electrophysiological signals. An electrode cable with a clip lead was attached to disposable electrodes to measure EMG signals from three major upper limb muscles of the participants as shown in Figure 5. The data acquisition parameters (sampling rate, channel selection and so on) for the g.USBamp amplifier were configured using Simulink. Three EMG electrode channels were configured in bipolar mode with a sampling frequency of 1200 Hz. The measurements were taken during each trial.

#### Protocol

Twenty (14 males, 6 females) healthy participants of at least 18 years old with no history of injury to the upper limb and back were involved in this experiment. Participants were students or staff members of the University of Hertfordshire or volunteers from outside the university. Ethics approval was obtained from the University of Hertfordshire board of ethics (Protocol number: COM/PGR/UH/02741). Written consent was obtained from all individual participants included in the study. They were asked to sit straight on a non-rotating chair. Three gross upper limb muscles, BB, TB and Deltoid were studied and three EMG

| Table 3. Summary table based on the trend in correlation coefficients as the trials progressed in segment 1. |
|---------------------------------------------------------------|
| Hypothesis: There will be a decrease in the value of correlation coefficient as the trials progress (1 = TRUE, 0 = FALSE, NA = Unknown) |
| | Force\_X\_LPF | Force\_Y\_LPF | Force\_Z\_LPF |
| | TRP | DLT | BB | TB | TRP | DLT | BB | TB | TRP | DLT | BB | TB |
| Subject 1 | 1 | NA | 0 | NA | 1 | NA | 1 | NA | 0 | 1 | 0 | NA |
| Subject 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | NA | 1 | 1 | 0 | 1 |
| Subject 3 | 0 | 1 | NA | 1 | 0 | 1 | NA | 1 | 0 | 1 | 0 | 0 |
| Subject 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | NA | 1 | NA |
| Subject 5 | 1 | 0 | NA | 1 | 0 | 1 | NA | 1 | 1 | 0 | 1 |
| Subject 6 | 1 | NA | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Subject 7 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | NA |
| Subject 8 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 |
| Subject 9 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| Subject 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TOTAL | 7 | 6 | 6 | 8 | 8 | 6 | 7 | 7 | 8 | 5 | 6 |
| Percentage | 70% | 60% | 60% | 80% | 80% | 60% | 70% | 70% | 70% | 80% | 50% | 60% |
| Fatigue score | 27 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 | 28 |
| Overall percentage (%) | 68% | 70% | 70% | 65% |

TRP: Trapezius; DLT: Anterior Deltoid; BB: Biceps Brachii; TB: Triceps Brachii.
electrodes were attached to the participants' upper limb. The task involved biceps curl requiring elbow flexion and extension movements as directed by visual instructions on the screen. The instruction also enforced uniform timing of flexion and extension for all participants. Participants were asked to hold the weights/dumbbell using their dominant arm. The experiment progressed from no weight (Trial 1) to low-weight (Trial 2), and then high-weight (Trial 3). The initial trials also helped to warm up the muscles reducing the risk of injury. A short break period of 1 min was given between each experiment trial. The initial two trials were conducted until a defined number of iterations was reached. Trial 1 recorded the relaxed state of muscles, which involved elbow flexion and extension tasks with no weight. This task was repeated 10 times continuously, where the starting time was guided by a 'Beep' sound. The beep would repeat every 10 sec since each iteration was defined to take 10 sec to complete. In Trial 2, the participants were asked to hold a small load of 500 g weight, while performing the elbow flexion and extension tasks and repeating this 10 times continuously or until the muscles become fatigued. The start of each iteration was signalled by a beep. In Trial 3, the participants were asked to carry a heavy load (10 kg for a male participant and 7.5 kg for a female participant) and this involved elbow flexion and extension tasks continuously until the muscles were fatigued. The start of each iteration was signalled by a beep. The participants were allowed to stop the repetition when they were highly fatigued or unable to continue. In addition to the EMG measurements from muscles, a subjective measurement of fatigue at different stages was also taken at the end of the experiment using a questionnaire.

**Methodology.** Experiment 2 was conducted to verify if the low level of fatigue in many subjects measured through EMG fatigue indicators could be mainly due to the robotic assistance during Experiment 1. EMG average power was calculated from the measured EMG data. Two types of analysis were conducted. Initially, the variations in EMG average power were compared across trials 1, 2 and 3 in each participant. Second, the trend in EMG average power within Trial 3 was studied. Trial 3 was designed to be the most difficult task that would cause muscle fatigue in the participants. In both methods, linear regression coefficients were calculated. Regression line slopes with significant p-values were used to state if there was a trend in the EMG feature as the windows/trials progressed.

Since each iteration of flexion/extension tasks lasted for 10 sec, non-overlapping windows with a length of 10 sec were used to analyse the EMG data. Many participants did the first iteration very fast without looking at the screen or without keeping in sync with the visual directions on the computer monitor. Hence, during the analysis, the initial window (10 sec) was skipped.

The collected EMG signals were filtered using an Infinite Impulse Response (IIR) notch filter to remove the power line interference at 50 Hz. The signals were then band-pass filtered using two different frequency bands to explore which of the two EMG frequency bands was more useful as fatigue indicator. Initially, for the analysis of average power, the signals were band-pass filtered in the frequency band 0.8–2.5 Hz as used by Octavia et al. However, in contrast to this, in the current study, the signals were not full-wave rectified, since it was noticed that the rectification process altered the frequency content of the EMG and the median frequency analysis would be affected. The median frequency analysis was done within the whole frequency band of 20–450 Hz. A non-overlapping moving window of 10 sec width corresponding to each iteration was used for generating each EMG feature value. The existence of a trend in the EMG features was studied by performing a linear regression of the feature values as the analysis windows progressed. Summary tables were formed based on significant regression slopes of EMG features. The muscles BB and TB were studied separately. In the summary table, a trend in average power or median frequency as the windows progressed in Trial 3 was marked as a positive...
slope, negative slope or non-significant (NS) slope, where a positive slope represented an increase in the EMG feature as the windows progressed, whereas a negative slope represented a decrease in the EMG feature.

**Results**

Before the second experiment, most of the participants reported through a questionnaire that they were not fatigued. During the experiment, trials 1 and 2 were completed easily by most of the participants and Trial 3 was completed with difficulty. During the analysis, average EMG features across trials 1, 2 and 3 were compared. The average EMG power of the BB and TB muscles for Trial 3 was significantly higher compared to trials 1 and 2 in both male and female participants (shown in Figure 6). The median EMG frequency of the BB and TB muscles also displayed a significant difference between trials (shown in Figure 7). These significantly different EMG feature values could be due to the obvious need for increased muscle force to lift the heavy dumbbell during Trial 3 or due to muscle fatigue. Hence, Trial 3 data alone were also analysed to see how the EMG features varied as the windows progressed within the trial. Regression lines were plotted within the trial for different muscles in all the subjects. We observed a positive trend in average power and a negative trend in median frequency as the windows progressed in Trial 3.

The maximum number of iterations during Trial 3 for each subject was also analysed (see Figure 8), although we understand and note that this also depends on the muscle strength of the participants.

**Male participants.** As shown in the summary tables for male participants, Tables 5 and 6, the majority (92% and 85.7% for EMG average power and median frequency, respectively) showed indications of fatigue by the end of Trial 3. This was also supported by the post-experiment questionnaires. A non-parametric (Wilcoxon signed-rank) test as in Table 4 indicated that the fatigue level after Trial 3 (mean rank = 7.50) was rated higher than the fatigue level before starting the experiment (mean rank = 0), $Z = -3.309$, $p = 0.001$. It showed an increase in the level of fatigue after the experiment compared to the case before starting the experiment.

The regression slopes for average power in the frequency band 0.8–2.5 Hz were statistically significant (shown in Figure 6 for a typical participant ‘Subject 5’). However, Subject 4 displayed NS regression slopes even though the linear regression analysis appeared to provide a positive slope. Similarly, the regression slopes for median frequency in the frequency band of 20–450 Hz (shown in Figure 7) indicated significant negative regression slopes for the majority of the male participants. This was visible more in the BB and TB muscles than in the DLT muscle.

The answers to the questionnaires stated that 10 out of 14 male participants were fatigued or very fatigued. The remaining four male participants reported being

![Figure 6. Experiment 2: Regression slope across trials for average power – Subject 5. The regression slopes across Trial 3 showed a positive trend with significant $p$-values. The values for trials 1 and 2 were similar and very close to zero, but Trial 3 showed high and increasing values of average power for the BB and TB muscles as the windows progressed.](image)
‘Somewhat Fatigued’ as explained in the Fatigue Chart (Figure 9). The female participants reported different levels of fatigue (two of them reported ‘Very Fatigued’, one reported ‘Fatigued’, one reported ‘Somewhat Fatigued’ and another two reported ‘Not Fatigued’). All the female participants displayed statistically NS regression slopes within Trial 3 (using a 7.5 kg dumbbell) for average EMG power and median EMG frequency. The average power in the frequency band of 0.8–2.5 Hz in the majority of the female participants except for Subject 1 resulted in regression slopes tending towards positive, but lacking statistical significance. The case was similar for the regression slopes of the EMG median frequency in the band of 20–450 Hz. In their responses to the questionnaire, three female participants stated being ‘Fatigued’ or ‘Very Fatigued’, whereas one stated being ‘Somewhat Fatigued’ and two others stated ‘Not Fatigued’. The variation in average power during Trial 3 of the experiment was significantly different between the female and male participants as shown in Figures 10 and 11 for BB and TB muscles, respectively.

**Figure 7.** Experiment 2: Regression slope across trials for median frequency – Subject 5. Regression slopes across trials 1–3 showed a negative trend with significant p-values. The median frequencies for Trial 1 were significantly different compared to Trial 3 for BB and TB muscles. BB: Biceps Brachii; TB: Triceps Brachii.

**Figure 8.** Experiment 2: Iterations during Trial 3 for male and female participants. The number of iterations of flexion and extension tasks in Trial 3 were significantly different between male and female participants. Female participants were asked to lift a dumbbell of weight 7.5 kg, while the weight lifted by male participants was 10 kg.

**Female participants.** All the female participants displayed statistically NS regression slopes within Trial 3 (using a 7.5 kg dumbbell) for average EMG power and median EMG frequency. The average power in the frequency band of 0.8–2.5 Hz in the majority of the female participants except for Subject 1 resulted in regression slopes tending towards positive, but lacking statistical significance. The case was similar for the regression slopes of the EMG median frequency in the band of 20–450 Hz. In their responses to the questionnaire, three female participants stated being ‘Fatigued’ or ‘Very Fatigued’, whereas one stated being ‘Somewhat Fatigued’ and two others stated ‘Not Fatigued’. The variation in average power during Trial 3 of the experiment was significantly different between the female and male participants as shown in Figures 10 and 11 for BB and TB muscles, respectively.

**Discussion**

In Experiment 2, it was observed that the average EMG power and median EMG frequency after the tiring exercise represented the upper arm muscle fatigue. As suggested by muscle physiology, when there is a development of muscle fatigue, more recruitment of MUs occurs, which results in an increased EMG amplitude. However, the aim of Experiment 2 was not to literally check if the task resulted in higher fatigue; instead the goal was to verify if the parameters used in Experiment 1 were
Table 4. Wilcoxon signed-ranks test based on the questionnaire response from Experiment 2.

| Wilcoxon signed ranks test – Ranks | Test statistics<sup>a</sup> |
|-----------------------------------|-----------------------------|
|                                    | N | Mean rank | Sum of ranks | Fatigue_Level_Trial_3 – Fatigue_Level_Initial |
| Fatigue_Level_Trial_3 – Fatigue_Level_Initial | Negative ranks | 0<sup>b</sup> | 0.00 | 0.00 | Z | –3.309<sup>b</sup> |
|                                    | Positive ranks | 14<sup>a</sup> | 7.50 | 105.00 | Asymp. Sig. (two-tailed) | 0.001 |
|                                    | Ties | 0<sup>c</sup> |    |      |                    |    |
|                                    | Total | 14 |    |      | b. Based on negative ranks. |    |

<sup>a</sup>Fatigue_Level_Trial_3 < Fatigue_Level_Initial.
<sup>b</sup>Fatigue_Level_Trial_3 > Fatigue_Level_Initial.
<sup>c</sup>Fatigue_Level_Trial_3 ¼ Fatigue_Level_Initial.

**Figure 9.** Experiment 2: Fatigue chart for male and female participants. The responses about the state of fatigue from the post-experiment questionnaire were analysed separately for male and female participants. All the male participants reported some level of fatigue. The female participants reported different levels of fatigue (2 'Very Fatigued', 1 'Fatigued', 1 'Somewhat Fatigued' and 2 'Not Fatigued').

However, it was noted that the EMG average power displayed a further increase from its initial value within Trial 3 as the iterations progressed, as shown in Figure 6. Trial 3 iterations were carried out until the participants were completely exhausted or unable to continue. This ensured that the majority of the participants tried their best to maximise the number of iterations in Trial 3, which resulted in their gross upper limb muscles becoming fatigued. The EMG signals were compared using both frequency bands 0.8–2.5 Hz and 20–450 Hz. The average power analysis of the 20–450 Hz band resulted in less significant results than 0.8–2.5 Hz. This indicates that the amplitude-based study is better for low-frequency ranges of EMG as also used in the studies of Octavia et al.<sup>36</sup>

In female participants, it was noted that the EMG fatigue indicators (median frequency and average EMG power) displayed NS regression slopes. Five out of six female participants reported in the questionnaire that the weight of 7.5 kg during Trial 3 was too heavy to lift and, hence, could not continue the iterations properly. As noted from the questionnaire and the recorded videos, it was understood that the heavy-weight resulted in performing only a small number of iterations by the majority of the female participants, which was not sufficient for regression analysis. Even though the results were NS, the regression slopes seemed to be moving in a positive direction for average power and in a negative direction for median frequency.

In male participants, fatigue charts as shown in Figure 9 indicated that all the participants have some level of fatigue after lifting a weight of 10 kg during Trial 3. The average number of iterations among all the male subjects was approximately 10 as shown in Figure 8. Expectedly, there was a statistically significant difference between the number of iterations in male and female participants. Considering the fatigue indicator based on EMG average power in male participants, there was a significant difference between the
Figure 10. Experiment 2: Box plots for average power during Trial 3 for male and female participants in Biceps Brachii muscles. The variation in the feature for the female participants (F) was less than that of the male participants (M). BB: Biceps Brachii.

Figure 11. Experiment 2: Box plots for average power during Trial 3 for male and female participants in Triceps Brachii muscles. The variation in the feature for the female participants (F) was less than that of the male participants (M). TB: Triceps Brachii.
initial and final trials as indicated in Figure 6. This implied that there was a significantly increased muscle activity due to the larger force required to lift the higher weight. This increase was observed in all the participants. However, the progress of the EMG feature within Trial 3 can offer further information on how fatigue develops over time and with extraneous activity.

For the average power in male participants within Trial 3, the use of frequency band 0.8–2.5 Hz resulted in a summary table as shown in Table 5. The majority of the male subjects displayed statistically significant positive slopes with $p$-value $<0.05$ as shown in the summary table. It was noticed that the BB muscle displayed the highest indication of fatigue in 13 out of 14 male participants (92.85% of the d cases) compared to the TB muscle (57%). Both the BB and TB muscles were expected to play a major role in the flexion/extension tasks. However, the results for the TB muscles were not statistically significant in a few subjects, even though the slopes tended towards the positive direction. The lesser significance for the TB compared to BB muscles could be due to the participants resting their elbow on their laps and thus getting support during the extension movements. This support could potentially have resulted in a lesser EMG amplitude in the TB muscles. One of the male subjects (Subject 4) displayed unexpected results in terms of both EMG features, where the BB muscles displayed NS regression slopes. However, the participant stated being ‘Fatigued’ in the questionnaire. The smaller significance of EMG features from the BB muscles could potentially be explained by the EMG electrode positions.

Similarly, the summary table based on the EMG median frequency also displayed significant negative slopes in 12 out of 14 male subjects as the windows progressed within Trial 3. While using median EMG frequency as the fatigue indicator with a frequency band of 20–450 Hz, the summary table (Table 6) implies that BB and TB muscles had the maximum percentage of cases with significant regression slopes 85.71% and 85.71%, respectively. On the other hand, using the frequency band of 0.8–2.5 Hz for calculating median frequency resulted in a less clear indication of fatigue, where the BB and TB muscles only had significant slopes in 71.42% and 71.42% of the cases, respectively. Even though the EMG amplitude values for TB muscles were affected due to the elbow support during extension, this did not seem to affect the median frequency values possibly due to the physiologically different reason behind the reduction in median frequency.30–52

In comparison to the results for average power, we did

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**Table 5.** Experiment 2: Summary table for average power in male participants. The summary table shows significant regression slopes for the majority of the male participants as the iterations progressed in Trial 3. This was more significant in BB muscles than TB muscles. The ‘tick’ sign indicates statistically significant slope with $p$-values $<0.05$ and ‘NS’ indicates a non-significant slope. The reported fatigue and the fatigue score after Trial 3 are also shown. Subject 15 and 17 showed a mismatch in the reported state of fatigue, hence they are identified with different colours.

| Feature | Average power – Male participants |
|---------|----------------------------------|
| Hypothesis | There is a positive trend in average power as the windows progressed in Trial 3. (✓= Positive, – = Negative, NS = Non-significant) |
| Methodology | Linear regression test on the values of average power considering moving window of 10 sec duration corresponding to each iteration carrying 10 Kg weight. The EMG was band pass filtered at 0.8–2.5 Hz. |

| Subject | BB muscle | TB muscle | Fatigue reported in questionnaire | Fatigue score (0–10 scale) |
|---------|-----------|-----------|---------------------------------|---------------------------|
| Subject 2 | ✓ | NS | Very fatigued | 10 |
| Subject 3 | ✓ | ✓ | Very fatigued | 9 |
| Subject 4 | NS | ✓ | Fatigued | 5 |
| Subject 5 | ✓ | ✓ | Fatigued | 5 |
| Subject 6 | ✓ | ✓ | Very fatigued | 8 |
| Subject 7 | ✓ | NS | Very fatigued | 8 |
| Subject 9 | ✓ | NS | Very fatigued | 10 |
| Subject 11 | ✓ | ✓ | Fatigued | 8 |
| Subject 12 | ✓ | - | Somewhat fatigued | 9 |
| Subject 15 | ✓ | NS | Somewhat fatigued | 10 |
| Subject 16 | ✓ | NS | Somewhat fatigued | 6 |
| Subject 17 | ✓ | ✓ | Somewhat fatigued | 10 |
| Subject 18 | ✓ | ✓ | Very fatigued | 10 |
| Subject 19 | ✓ | ✓ | Fatigued | 8 |

EMG: electromyogram; BB: Biceps Brachii; TB: Triceps Brachii.
not notice less clear fatigue indication in TB muscles than for BB muscles while using the median frequency parameter.

In contrast to the second experiment, the first experiment with robotic assistance had shown that EMG features in the presence of robotic assistance displayed only slight indications of muscle fatigue. The horizontal position of the upper limb in parallel to the shoulder was expected to produce a good level of fatigue. However, since the HM robot was configured in the active assisted mode, we had only a limited reported-fatigue to validate our observations. Participants were not producing a maximum effort to actively involve in the interaction; instead many were seeking some assistance from the robot to complete the movements. This resulted in a less clear indication of fatigue through the EMG features in the majority of the participants. The questionnaires also supported this observation since 80% of the participants in Experiment 1 reported being ‘Somewhat Fatigued’. However, in the majority of the subjects, the EMG features indicated the presence of muscle fatigue in the most active upper limb muscles. The trend in EMG features was more visible in TRP and DLT muscles in comparison to BB and TB muscles (Table 1). A possible explanation for these differences between muscles is that TRP and DLT muscles played a more active role in lifting the arm to shoulder height for performing the tasks, while robot provided little support for their involvement.

Based on the findings from both experiments, we believe that EMG features (e.g. median frequency) can be indicative of fatigue, and the results from the first experiment could indeed be an indication for successful assistance offered by the robot. The results showed that with robotic assistance, the participants reported ‘Somewhat Fatigued’, whereas without robotic assistance, the participants reported high fatigue. The extent of fatigue with and without assistance varied significantly. Due to the complex nature of muscle fatigue, no literature has as yet clearly identified a standard method to quantify the level of muscle fatigue based on EMG features. In an effort towards this, based on our results, we have noted that a baseline range calculated from statistical significance test (two times the standard deviation) of the EMG features may be used to set the threshold to detect muscle fatigue by checking if a new value of EMG feature lies within the range.

In order to study how ‘High Fatigue’ could be compared to ‘Fatigued’ and ‘Somewhat Fatigued’, the fatigue scores reported through the questionnaire in all the subjects after Trial 3 of Experiment 2 were analysed. On a scale of 0–10, the participants who reported a state of ‘High Fatigue’ mostly gave a fatigue score

### Table 6. Experiment 2: Summary table for median frequency in male participants. The summary table for median frequency shows significant negative regression slopes for the majority of the male participants as the iterations progressed in Trial 3 for BB and TB muscles.

| Feature -> | Median frequency – Male participants |
|------------|-------------------------------------|
| Hypothesis -> | There is a negative trend in median frequency as the windows progressed in Trial 3. (Negative, NS => non significant) |
| Methodology -> | Linear regression analysis on the values of median frequency considering moving window of 10 sec duration corresponding to each iteration carrying 10 Kg weight. The EMG was band pass filtered at 20–450 Hz. |
| Muscles -> | BB | TB |
| Subject 2 | ✓ | NS |
| Subject 3 | NS | ✓ |
| Subject 4 | NS | ✓ |
| Subject 5 | ✓ | ✓ |
| Subject 6 | ✓ | NS |
| Subject 7 | ✓ | ✓ |
| Subject 8 | ✓ | ✓ |
| Subject 9 | ✓ | ✓ |
| Subject 10 | ✓ | ✓ |
| Subject 11 | ✓ | ✓ |
| Subject 12 | ✓ | ✓ |
| Subject 13 | ✓ | ✓ |
| Subject 14 | ✓ | ✓ |
| Subject 15 | ✓ | ✓ |
| Subject 16 | ✓ | ✓ |
| Subject 17 | ✓ | ✓ |
| Subject 18 | ✓ | ✓ |
| Subject 19 | ✓ | ✓ |

EMG: electromyogram; BB: Biceps Brachii; TB: Triceps Brachii.
between 7 and 10. Two subjects (Subjects 15 and 17) marked a score of 10 for ‘Somewhat Fatigued’. A score of 10 must correspond to the highest level of fatigue, hence this seems incorrect. However, further exploration is required to see for which values of the EMG features do the muscle state transit from a ‘Fatigued’ state to a ‘High Fatigued’ state and from a ‘Somewhat Fatigued’ state to a ‘Fatigued’ state. It is also interesting to compare the accuracy of fatigue state identification methods when the fatigue thresholds are based on 2*STD range, 3*STD range and so on.

It was commented by some participants that there was a big weight difference between 0.5 kg and 10 kg. The difference could have been smaller so as to notice more gradual progress of fatigue development in the EMG features. The maximum number of iterations in Trial 3 was different across the subjects probably because of the different muscle strengths of the participants. Hence, the user physiology needs to be considered while designing training interactions. In a robot-assisted training interaction, the task difficulty may be set based on the muscular strength and force generation capabilities of the participants. This is addressed in our ongoing study. We also realised that the dumbbell weight used in Trial 3 for female participants could have been selected better so that they could do more iterations to make the muscles tired through repetitions rather than making them unable to lift it or to use compensatory strategies for lifting. This, however, does not affect the findings of our study, since the intention here was to verify if the EMG fatigue indicators can indicate muscle fatigue and to let us proceed to next stage, regarding how they can be used to improve robotic adaptation as planned in our future experiments. The results obtained from the male participants’ EMG data have indeed verified that episodic fatigue measurement is possible.

In the correlation study between EMG and force, the effective kinematic force at the robotic end-effector was used instead of the force in the near proximity of muscles, where the EMG was measured. The force was the result of combined action by multiple upper limb muscles. The study used individual force components (\(F_x\), \(F_y\) and \(F_z\)), instead of using the resultant force values. The robotic interaction involved dynamic muscle contraction tasks, where the length of muscles changed during different segment movements. It was also noticed that the reaching activities had a need for different muscles, and hence, the correlation between the EMG and kinematic features varied based on the type of movement. Hence, the correlation analysis was conducted for different segments separately.

The correlation results using all the subjects and trials together gave a similar result as when individual subjects were studied. Only a weak or moderate correlation was observed in these cases as shown in Table 2. For example, in Segment 1, the correlation coefficients for TB muscle against the force components \(F_x\) and \(F_y\) were 0.370 and −0.419, respectively. For Segment 2, this was −0.492 and −0.471, respectively. As per the rule of thumb for interpreting the size of a correlation coefficient, we got \(\rho\) values ranging from 0.1 to 0.5, which indicated a weak or moderate correlation. The different reaching activities during Experiment 1 have a need for different muscles, and hence, the correlation varied for each segment. The upper limb movements defined in the experiment involved the end-effector movements in different directions including away-from-body and towards-the-body movements. So the force components had both positive and negative values due to the changes in their directions. The weak/moderate correlation could be a result of the robotic assistance received from the Active-Assisted mode of HM or due to muscle fatigue. However, it needs to be noted that most of the subjects reported ‘somewhat’ fatigue after the robotic experiment.

In order to confirm this, the correlation results were analysed for each trial for each subject separately as shown in Table 3. A gradual change (mostly decrease) in correlation coefficient was noticed as the trials progressed (noticed mainly in segments S1, S2 and S3). For example, as the trials progressed in Segment 1, the correlation coefficients showed a reduction in its value in 68% of the cases for \(F_x\), 70% of the cases for \(F_y\) and 65% of the cases for \(F_z\). This decrease in the correlation as the trials progressed could be an indication of fatigue. During fatigue, the non-linearity in the correlation between the muscle force and EMG amplitude might have caused the particular behaviour of correlation coefficients as the trials progressed. This is supported by the past studies. Previously, the EMG analysis indicated that DLT and TRP muscles were more fatigued than the BB and TB muscles. So, it seems that muscle fatigue affected the correlation between the EMG and kinematic force since the fatigued muscles displayed the least correlation in the majority of the subjects.

The muscles BB and TB did not seem to play a significant role in the shoulder position; instead, they played more role in determining the direction of movement along the four segments. Probably due to this reason, these muscles were found to have the strongest EMG–force correlation compared to TRP and DLT muscles. The TB muscle was found to be the most correlated in all the segments, whereas BB muscle was found to be more correlated in the towards-the-body and close-to-the-body segment S4.

It was noted that the kinematic force used in the study was not only representing the human-generated
force but was representing the interaction force between the robot and the human. The force required to be applied by the user to move the robotic end-effector also depended on the level of assistance provided by the robot in the active-assisted mode for different point-to-point movements. This created a mixed input space that was a combination of the force generated by the robot (in response to the performance of the user) and the force generated by the user. The robotic assistance worked here when the participant lagged behind the robot or deviated from the prescribed path. The participants lagged behind the robot probably because they were tired or fatigued. So the primary input here was the user performance or muscle fatigue. Probably due to the mixed input space, the EMG–force correlation was not very high as shown in the results. Interestingly, analysing the trend in EMG–force correlation as the different trials progressed gave a better understanding of this topic. The results showed that as the trials progressed, there was a decrease in the correlation coefficient for the involved muscles during the upper limb movements. This showed that even with the mixed input space, the analysis of EMG–force correlation was a useful method to understand the progress of muscle fatigue during a robot-assisted upper limb interaction. However, the influence of the mixed input space needs to be explored further in future work.

Conclusions

The main results of this study indicated an inverse relationship between the level of muscle fatigue and the EMG–force correlation. A high fatigue corresponded to a weaker EMG–force correlation and a low fatigue corresponded to a stronger correlation. The overall correlation between the EMG average power and kinematic force components was either weak or moderate, and this could be due to the presence of the HM assistance in the active-assisted mode. The correlation study also showed that there was a reduction in the correlation coefficient due to the effect of muscle fatigue as the trials progressed. Hence, the robotic assistance based on user's performance, which resulted in a lesser fatigue trials progressed. Hence, the robotic assistance based on user's performance, which resulted in a lesser fatigue, which was supported by the subjective reporting of fatigue through the questionnaire. Hence, a two times standard deviation (2*STD) check of the EMG features was found to be useful for fatigue detection during training interactions with a constant load. However, this method needs to be tested further in training environments with varying loads, for example during progressive muscle strengthening exercises and adaptive environments. It was also noted that the lower band of frequencies (0.8–2.5 Hz as used by Octavia et al.36) was more suitable for the amplitude/average power-based features than considering the whole band of 20–450 Hz. Interestingly, for the median EMG frequency as the fatigue indicator, the EMG frequency band of 20–450 Hz was found to provide the best fatigue indication as compared to the band of 0.8–2.5 Hz.

Both the experiments were conducted on healthy participants. However, in a real scenario of rehabilitation training, the patients (for example stroke survivors) will exhibit reduced muscular or cognitive capabilities. It is likely that their muscles can easily come to a state of fatigue even in a robot-assisted environment. The state of fatigue in the patients can deplete their limited resources if there is no mechanism to detect this and avoid them becoming highly fatigued. A fatigue indicator has the potential to be used to alter the training intensity by changing the robotic assistance parameters like stiffness, training duration and so on based on the level of muscle tiredness before damaging their muscles. As indicated by the results, the EMG features (average power and median frequency) are potential parameters, which can be used to improve the adaptation of robot-assisted rehabilitation. Hence, future studies will explore utilising the fatigue indicators together with user intention for the auto-adaptation of therapeutic HRIs.

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Contributors

The design of the study was agreed by ATP, FA and VS. ATP recruited the participants, conducted the experiment and acquired the data. ATP, FA and VS were involved in analysing the data and interpreting the results. ATP drafted the manuscript with critical revision of the manuscript from time to time by FA and VS. All the authors read and approved the final manuscript.

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