



**Figure 2**

**a)** Truncated force signal trace illustrating the fatigue protocol. (▲) The peak of three 5-second maximum voluntary contractions (MVC) was used to determine the fatiguing contraction intensity, while the interpolated twitch amplitude (IT) along with the associated control twitch (CT) elicited 5-seconds after the contraction were used to calculate the pre-fatigue level of motor unit activation. (♥) Ten 30-second fatiguing contractions with 30-seconds of rest were performed at 50% of MVC with an evoked twitch (ET) elicited 5-seconds after the contraction. The slope of the force plateau (SLP) was used as an indicator of fatigue accumulation. (♦) Recovery was monitored for 45-minutes where an MVC with IT was performed every 15-minutes, with ETs elicited at 5-minute intervals. **b)** Typical trace for one second of raw EMG data illustrating the 0.25 second overlapping epochs utilized to calculate mean root mean square and mean median frequency values.

and for evoking control doublets. Carbon rubber electrodes (4.45 cm × 10.16 cm) were placed on both limbs and delivered two 200 μs width square wave pulses from a continuously variable constant current stimulator (Digitimer DS7A, Hertfordshire, United Kingdom) separated by 10 ms. The electrodes were coated in a conducting gel and secured to the limb with tape. For the knee extension task, the cathode was placed across the lateral proximal quadriceps femoris muscle group approximately 3 cm from the anterior superior iliac spine, with the anode placed across the medial distal aspect approximately 2 cm from the superior border of the patella [25]. Due to the size of the electrodes relative to the surface area of the biceps brachii muscle group, the cathode was placed across the midline of the muscles below the ridge created by the deltoid and the anode was placed across the midline of the muscles above the anticubital space. The electrical stimulus was recorded as a 5 V pulse and collected simultaneously with the force signals.

It has been suggested that using an absolute supramaximal stimulation of the biceps brachii for twitch interpolation may stimulate the triceps brachii which may in turn affect the calculated MUA [26]. Therefore, in order to determine the greatest amount of twitch force that could

be electrically evoked in the elbow flexors, the current was increased from 200 mA in 25 mA increments until no further increase in force was observed. The intent was to reach maximal stimulation of the biceps brachii while employing a current that is adapted to each individual thereby controlling for differences in muscle thickness. A similar protocol was employed for the knee extensors where the current was increased from 250 mA in 50 mA increments. During each of the three MVCs performed prior to the fatiguing contractions and the four MVCs performed during recovery, the electrical stimulus was applied at the anticipated peak of the force and five seconds after the contraction was completed. During the 10 fatiguing contractions the electrical stimulus was applied at the 25 second mark and again five seconds after the contraction was completed. During the recovery period, doublets were evoked every five minutes, excluding those intervals when a MVC was performed.

#### EMG electrode placement and measurement

Two channels of an EMG cable telemetry system (Octopus, Bortec Biomedical Ltd., Alberta, Canada) connected to the National Instruments 32 channel A/D board were used to amplify (by a factor of two thousand) and collect all raw EMG signals at 1024 Hz, synchronized with the force and electrical stimulus data. The surface EMG was collected using a bipolar configuration of silver/silver chloride electrodes (Red Dot, 3 M Canada, London, ON) placed on the vastus lateralis during the knee extension task and on the short head of the biceps brachii during the elbow flexion task. For the knee extension task, the muscle belly of the vastus lateralis was palpated, and point stimulation was utilized to locate the innervation zone. A single 200 μs wide square wave pulse was applied at 40 mA using a constant current stimulator (Digitimer DS7A, Hertfordshire, United Kingdom) along the muscle belly and the site of the greatest muscular twitch was marked, denoting the innervation zone. The EMG electrodes were then placed on the middle of the muscle belly parallel with muscle fibres between the innervation zone and musculotendinous junction at an inter-electrode distance of 2 cm [27]. A single reference electrode was placed along the medial aspect of the right tibia. However, due to the limited surface area available on the biceps brachii muscle group, the EMG electrodes were placed along the surface of the short head between the two carbon rubber stimulating electrodes, with the reference electrode placed on the right acromion. All sites of EMG electrode placement were shaved, swabbed with alcohol and abraded to minimize potential electrical resistance [28].

The raw EMG data from the fatiguing contractions was processed using a zero lag 4<sup>th</sup> order band pass Butterworth filter with low and high cut-off frequencies set to those recommended by Basmajian and De Luca [28] for surface