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## **Title page**

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### **Title**

The role of agonist and antagonist muscles in explaining isometric knee extension torque variation with hip joint angle

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## **Abstract**

**Purpose:** The biarticular rectus femoris (RF), operating on the ascending limb of the force-length curve, produces more force at longer lengths. However, experimental studies consistently report higher knee extension torque when supine (longer RF length) compared to seated (shorter RF length). Incomplete activation in the supine position has been proposed as the reason for this discrepancy, but differences in antagonistic co-activation could also be responsible due to altered hamstrings length. We examined the role of agonist and antagonist muscles in explaining the isometric knee extension torque variation with changes in hip joint angle.

**Method:** Maximum voluntary isometric knee extension torque (joint MVC) was recorded in seated and supine positions from nine healthy males ( $30.2 \pm 7.7$  years). Antagonistic torque was estimated using EMG and added to the respective joint MVC (corrected MVC).

Submaximal tetanic stimulation quadriceps torque was also recorded.

**Result:** Joint MVC was not different between supine ( $245 \pm 71.8$  Nm) and seated ( $241 \pm 69.8$  Nm) positions and neither was corrected MVC ( $257 \pm 77.7$  Nm and  $267 \pm 87.0$  Nm, respectively). Antagonistic torque was higher when seated ( $26 \pm 20.4$  Nm) than when supine ( $12 \pm 7.4$  Nm). Tetanic torque was higher when supine ( $111 \pm 31.9$  Nm) than when seated ( $99 \pm 27.5$  Nm).

**Conclusion:** Antagonistic co-activation differences between hip position do not account for the reduced MVC in the supine position. Rather, a reduced voluntary knee extensor muscle activation in that position is the major reason for the lower MVC torque when RF is lengthened (hip extended). These findings can assist standardising muscle function assessment and improving musculoskeletal modelling applications.

**Keywords:** electrical muscle stimulation, muscle activation capacity, quadriceps function, seated torque, supine torque

**Abbreviations:** None

## **Introduction**

Isometric knee extensor muscle torque changes with hip joint angle (e.g. Maffiuletti and Lepers 2003; Rochette et al. 2003). This is because hip joint angle impacts on the length of the biarticular rectus femoris muscle, which has been estimated to contribute up to ~17% to the quadriceps torque output (McNair et al. 1991). However, a marked disparity exists between the expected knee extensor torque at different hip joint angles based on the force-length characteristics of the rectus femoris muscles and actual measurements. Specifically, as the rectus femoris operates on the ascending limb of the force-length curve (Herzog and ter Keurs 1988), a higher muscle force would be expected by the rectus femoris (and the quadriceps group as a whole, since the vastii muscles are crossing the knee joint only) when the muscle is lengthened, which corresponds to more extended hip joint angles. In contrast, experimental studies consistently show a lower knee extension torque values as the hip joint angle is extended (e.g. Maffiuletti and Lepers 2003; Rochette et al. 2003).

One possible reason for this unexpected effect might be an altered rectus femoris muscle activation at different muscle lengths (Herzog et al. 1991). This notion was supported by Maffiuletti and Lepers (2003), who used femoral nerve stimulation and found a significant 4.2% increase in quadriceps activation when the tests were performed in the seated position (shortened rectus femoris) compared to supine (lengthened rectus femoris).

A second, and currently unexplored, possibility is that the lower knee extension torque when the hip is extended compared to flexed could be linked with differences in antagonistic muscle co-activation between hip joint angles. Joint torque generated during knee extension is the 'net' sum of agonistic muscles positive moment and the antagonistic knee flexor muscles negative moment (Kellis and Baltzopoulos, 1999). A change in hip joint angle will result not only change the rectus femoris length, but also the hamstrings length. In isometric knee extension experiments, where the hip joint angle was kept constant and the hamstrings

length was manipulated by changing the knee joint angle, hamstrings co-activation increased at more knee flexed positions (i.e. shorter hamstring muscle-tendon unit length) (Kubo et al. 2004). Based on these results, shorter hamstrings muscle-tendon unit length, caused by extending the hip (rather than flexing the knee), could also increase co-activation of the hamstrings, subsequently increasing antagonistic torque and ultimately affecting the measured net isometric knee extension torque. Indeed, in experiments involving the ankle joint angle, antagonistic activity was shown to significantly affect the measured agonist torque (Billot et al., 2011; Maganaris et al., 1998), consequently impacting on the torque-angle relationship.

To examine whether antagonistic co-activation contributes to the knee torque variation with hip joint angle we designed a novel study with the aim of quantifying and comparing agonist activation and antagonistic co-activation in the seated and supine positions. We utilised electrical stimulation of the quadriceps muscle to allow examination of muscle function bypassing the subject's voluntary neural input, and thus exploring the muscle-tendon mechanical behaviour independently to voluntary neural activation. We hypothesised that during isometric knee extension an increased hamstring co-activation would be found in the supine position, thus partly contributing to the reduced measured knee extension torque compared to the seated position.

## **Materials and Methods**

### ***Subjects***

The study complied with the Declaration of Helsinki and the study and the procedures followed were approved by the Institutional Ethics Committee. Nine healthy, active males (age  $30.2 \pm 7.7$  years, stature  $1.78 \pm 0.09$  m, body mass  $81.7 \pm 11.2$  kg) free from any musculoskeletal injuries gave written, informed consent to participate in the study. In order to

reduce variability in performance, all subjects were familiar with the experimental procedures (Button and Behm 2008) and all testing took place on a single testing session.

### *Isometric knee extension torque measurements*

The subjects were tested in two positions, seated (hip joint angle =  $90^\circ$ ), and supine (hip joint angle  $160^\circ$ ) (full hip joint extension =  $180^\circ$ ), with the knee and ankle joint angles at  $90^\circ$  for both conditions. A custom-made dynamometer, sampling at 200Hz, was used for the study. The dynamometer was specifically developed for assessing isometric contractions and as such, the lever arm and the bed had very limited compliance while the restraints allowed for a better fixation of the pelvis and the body during the supine position. For the seated position, the subject sat in the chair of the dynamometer and straps were positioned over the pelvis and tested thigh, to prevent extraneous movement, while the lower leg was securely strapped, above the lateral malleolus, to a force-transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands). For the supine position, the subject lay in the chair of the dynamometer and the lower leg was securely strapped to the force-transducer above the lateral malleolus while straps were positioned over the pelvis and tested thigh. Pilot testing indicated extraneous movement occurred with the upper body moving upwards along the dynamometer's backrest. Hence, to prevent this movement, mechanical blocks were fixed securely in place in contact with the shoulders, which held the body in position without allowing any upwards movement (Figure 1).

FIGURE 1 ABOUT HERE

Subsequent testing confirmed that this set-up kept extraneous movement to an absolute minimum and was superior compared to our experience with other commercially available isokinetic dynamometers.

With the subjects sat in the dynamometer and their leg relaxed, the force trace was zeroed removing any passive force due to passive tension of the muscle-tendon unit of the knee extensors, and real-time, unfiltered force readings were displayed online (Matlab, The Mathworks, Natick, MA) and recorded for further analysis. For both positions, the mechanical output of the isometric knee extension was measured as the force applied externally in the sagittal plane at the level of the ankle (at right angles to the longitudinal axis of the lower leg), and converted to torque by multiplying that force by the external moment arm length, which was defined as the distance between the point of the external force application and the knee joint centre.

### ***Tetanic electrical stimulation***

Two 7 x 12.5-cm self-adhesive carbon rubber electrodes (Versa-Stim, ConMed, New York, USA) were placed on the proximal and distal regions of the quadriceps muscle group with the cathode being the proximal electrode. Stimuli of 200- $\mu$ s pulse width and 10-ms inter-stimulus gap were generated by an electrical stimulator (model DS7, Digitimer stimulator, Welwyn, Garden City, UK) and applied for a duration of 1s. Electrical stimuli application was displayed online along with the force signal. Percutaneous stimulation was selected over nerve stimulation, as the outcome between the two is comparable (Rutherford et al. 1986) and it reduces the discomfort induced to the subjects (Delitto et al. 1992).

### ***Procedures***

To obtain a baseline of each subject's strength, two isometric knee extension maximum voluntary contractions (with torque obtained as described above; joint MVC) (Figure 2A) were performed and averaged. If the coefficient of variation (calculated as standard deviation / average \* 100) between the two joint MVC's was >5%, a third trial was performed and the closest two were averaged. Subjects were given standardised verbal encouragement to motivate them to exert maximum effort.



Supramaximal electrical stimulation intensity was examined in both positions to confirm that the same level of stimulation intensity could activate the muscles to respectively similar (maximal) levels with the rectus femoris lengthened and shortened. Each subject's supramaximal stimulation intensity was determined by the application at rest of single twitches of 200- $\mu$ s duration, with the voltage set at 300 V and the current intensity increasing by 50 mA for each twitch application. The stimulation intensity that did not elicit any further increase in force output, despite an increase in current by 50 mA, was determined as supramaximal stimulation intensity and subsequently used. For eight subjects, the supramaximal intensity was identical between seated and supine positions, while for one subject the seated position required one additional increment of 50 mA and that was used as the supramaximal intensity for that subject in that position.

Following confirmation of the supramaximal stimulation intensity in both positions, a tetanus of 100Hz, duration of 1 s and of intensity sufficient to yield a force equivalent to one third of the respective MVC (Bampouras et al. 2012) was delivered to the muscle at rest (Figure 2B), to effectively 'standardise' the muscle's force output in the absence of any voluntary neural input and examine the true influence of rectus femoris muscle length changes on knee extensor torque output. Submaximal stimulation intensity was used, as it would not affect the outcome while it would reduce the subjects' discomfort (Bampouras et al. 2012), enabling a valid comparison of the knee extensor muscle group capability between the two conditions.

For electromyography (EMG) measurements, two surface Ag-AgCl electrodes of 10mm diameter each, were placed on the long head of the biceps femoris (BF) muscle. The electrodes were placed in a bipolar configuration and with a centre-to-centre distance of 20mm, preceded by shaving and cleansing of the placement area. EMG signal was collected at a sampling rate of 1000Hz, and filtered with a high- and low-pass filter of 10 and 500Hz,

respectively. The signal was subsequently smoothed using root mean square over 30ms and a mean value from a 500ms window was taken during the plateau phase of the contraction. Antagonistic BF muscle torque was estimated with the use of EMG (Beltman et al. 2003; Kubo et al. 2004; Maganaris et al. 1998; Reeves et al. 2004), based on the BF muscle activation when acting as an agonist. Subjects performed one maximal and three submaximal (at 20%, 40% and 60% of maximal effort) isometric knee flexions, with BF muscle torque and EMG synchronously recorded. Torque was plotted against the EMG signal and a line was fitted through these four data points to provide a regression equation; a minimum coefficient of determination value of 0.9 was set as the fit acceptance criterion (Figure 3). BF EMG was recorded during joint MVC and using the regression equation obtained, the torque contribution from the co-activation of the knee flexor muscles was estimated from the EMG activity. This antagonistic torque was then added to the measured knee extension MVC torque to obtain the 'corrected MVC'. This method has been reported to provide a reasonable estimation of antagonistic torque, even if it underestimates the true antagonistic torque (Simoneau et al. 2012). Finally, the mechanical ratio (torque achieved when the muscle acted as antagonist normalized to the maximal torque achieved when the muscle acted as agonist) was calculated to evaluate the level of coactivation (Billot et al. 2011).

FIGURE 2A ABOUT HERE

FIGURE 2B ABOUT HERE

FIGURE 3 ABOUT HERE

The above procedures were followed for both hip joint positions. Subjects were given at least two minutes rest between any contractions (joint MVC, tetanic, or knee flexion). All

measurements took place on the right leg. The initial MVCs were always performed in the seated position, but the order of the rest of the procedures was randomised.

### ***Statistical analysis***

Normality of distribution of the data was checked for and subsequently confirmed using Shapiro-Wilk test, while Grubb's test indicated no outliers were present in any data set. Differences between the two positions for all variables were examined using a dependent Student's t-test. Effect size (ES) was calculated for significantly different comparisons to provide an indication of the magnitude of the effect, with 0.8, 0.5 and 0.2 representing large, moderate and small effects (Fritz et al. 2012). For all statistical analysis IBM SPSS Statistics v19 was used. Data are presented as means  $\pm$  SD. Statistical significance level was set at  $p < 0.05$ .

### **Results**

The results showed that knee extension joint MVC was not significantly different between seated (shortened rectus femoris muscle) and supine (lengthened rectus femoris muscle) positions, while tetanic stimulation followed modelling predictions, with supine joint MVC torque being higher than seated ( $p = 0.001$ ). The antagonistic torque was significantly different ( $p = 0.025$ ) between positions, however, in a direction contrary to our hypothesis, as the seated position yielded higher antagonistic torque. The mechanical ratio between positions was not significantly different. Further, when the antagonistic torque was accounted for, the corrected MVC still did not follow the same pattern as the tetanic stimulation, as there was no difference between the two positions, suggesting that antagonistic muscle co-activation was not responsible for the observed experimental results.

Mean supramaximal stimulation intensity was identical between seated and supine positions ( $512 \pm 124.6$  mA for both positions), suggesting similar portion of the muscle was activated.

Descriptive statistics for joint MVC, tetanic torque, antagonistic and corrected MVC torque can be seen in Table 1.

TABLE 1 ABOUT HERE

## **Discussion**

The aim of the present study was to determine whether antagonistic co-contraction contributes to the unexpected, yet documented, decrease in isometric knee extension torque when the hip joint is extended compared to when the hip is flexed, in contrast to the rectus femoris force-length characteristics. We have used a unique approach by combining voluntary and electrical stimulation of the quadriceps muscle to investigate the mechanisms of the muscle-tendon when investigated for the mechanical behaviour alone (muscle electrically stimulated) and when the neural and mechanical behaviour was investigated together (joint MVC). The results suggest that differences in antagonistic knee flexor co-activation with hip joint angle do not contribute to the variation in MVC torque. Rather, a reduced voluntary knee extensor muscle activation in the supine position is the major reason for the lower MVC torque when the rectus femoris is in a lengthened position (hip extended), despite this muscle operating on the ascending arm of the force-length relationship (Herzog and ter Keurs 1988).

Contrary to our hypothesis that the antagonist muscles would co-contract more in the supine position to stabilise the pelvis, antagonistic co-activation torque was higher at longer biceps femoris muscle lengths (seated position). Further, despite the significant difference in antagonistic co-activation torque between the two positions, corrected MVC (i.e. with the antagonistic torque accounted for) was not significantly different between positions. The most likely explanation for the reduced antagonistic co-activation in the supine position lies in the common drive hypothesis (Basmajian and De Luca 1981). The agonist quadriceps and antagonist hamstrings have a common central motor drive, meaning that quadriceps activation and hamstrings co-activation will change concurrently. As the quadriceps muscle demonstrated lower activation in the supine position, antagonistic co-activation would also have to be lower, which concurs with our findings.

The tetanic stimulation results confirm biomechanical models predicting higher isometric knee extension torque in the supine (lengthened rectus femoris) position compared to the seated (shorter rectus femoris muscle) position (Herzog and ter Keurs 1988; Herzog et al. 1990; Hoy et al. 1990; Lewis et al. 2009). As the rectus femoris muscle operates on the ascending limb of the force-length curve, elongating it would force it to operate closer to or on the plateau region, generating higher forces and consequently, so do the quadriceps muscle.

Previous experimental studies reported higher MVC torque at the seated position (Maffiuletti and Lepers 2003; Rochette et al. 2003). These studies utilised maximum voluntary contractions and the MVC output reflected both the mechanical behaviour and voluntary activation capacity of the agonist muscles, but also the level of antagonist muscle co-contraction. Maffiuletti and Lepers (2003) reported higher isometric MVC quadriceps torque by approximately 10%, with a respective increase in activation of 4.2% in the seated position compared to supine, suggesting that increased agonist activation levels at that position resulted in the increased torque. The joint MVC results agree with the direction of change of previous experimental studies (e.g. Maffiuletti and Lepers 2003; Rochette et al. 2003), while the tetanic stimulation results agree with theoretical predictions (Herzog and ter Keurs 1988; Hoy et al. 1990; Lewis et al. 2009). When the subjects contracted their knee extensors voluntarily, the difference that existed between hip joint positions during tetanic stimulation disappeared, suggesting higher activation in the seated position.

Surprisingly, the extended hip joint position, which corresponded to the lower antagonistic co-activation, was also the position with the lower agonist activation. It seems, therefore, that the reduction in agonist activation in the supine position is independent of reciprocal inhibition mechanisms (Tyler and Hutton 1989), possibly due to inadequate pelvic fixation. Studies typically stabilise the subject by straps placed over shoulders and / or abdomen, to

prevent extraneous movement (Hart et al. 1984; Magnusson et al. 1993). These experimental set-ups, however, are unlikely to ensure adequate pelvis stabilisation in the supine position, where the direction of force applied pushes the body in a different direction to the one the straps are counteracting and can negatively impact on the subject's ability to exert maximal volitional effort (Hart et al. 1984; Magnusson et al. 1993). With reduced volitional effort, there would be a concurrent reduction in both agonist and antagonist muscle activation in line with the common drive hypothesis described above.

One other possible reason for the difference in agonist muscle activation between the two hip joint positions could be differences in vestibular feedback. Lewek et al. (2006) examined the effect of hip (afferent feedback) and head (vestibular feedback) position on quadriceps EMG during an isometric knee extension MVC, by altering the hip joint angle on its own as well as in combination with the head orientation. When the head position followed the hip position (head in alignment with upper body), there was significantly higher quadriceps EMG activity in seated position compared to supine. This pattern was not seen when the head orientation (vertical to the horizontal) was maintained the same for all hip positions, suggesting that vestibular, and not afferent input, was the prime reason for the change in EMG activity between positions (Lewek et al. 2006). In our study, the subjects maintained their head in alignment to the body (Figure 1) in both positions, which is likely to have resulted in reduced quadriceps activation in the supine position.

One methodological consideration with our study is the use of percutaneous stimulation. The electrode fixed placement over the muscle belly presents a possible limitation in that moving from the seated to the supine position, a different portion of the rectus femoris muscle may have been stimulated, which could have affected the results (Newman et al. 2003). However, the size of the electrodes we used was large, selected because of increased comfort and for enabling stronger contraction (Alon 1985), and, importantly, covered a wide quadriceps area.

Thus, it is unlikely that a significantly different portion of the rectus femoris would have been stimulated. Another issue with percutaneous stimulation is possible current overflow, which could induce antagonistic co-contraction (Alon et al. 1994). However, the overflow should be of sufficient level to increase activation of the muscle by at least 5% before any effect on torque takes place (De Serres and Enoka 1998). Given that during joint MVC antagonistic co-contraction was ~5-10% of the agonistic torque, any antagonistic co-contraction induced by current overflow would have been even less (as a percentage of the tetanic stimulation torque) and thus, of negligible impact on the outcome.

It has also been reported that subject variability in the operating range of the rectus femoris varies in healthy but untrained individuals (Winter and Challis 2010). From 28 subjects used in that study, 14 operated on the ascending limb of the force-length curve, 7 over the plateau region and 9 subjects on the descending limb (Winter and Challis 2010). In our study, tetanic stimulation being higher in the supine position, compared to seated, suggested that all our subjects were operating on the ascending limb of the force-length curve. However, such variability poses limitations on the generalisability of the findings and should be considered in future studies examining mechanical effects through hip joint manipulation. Further, muscle activation stimulation studies are typically difficult to recruit subjects, due to the discomfort levels caused by the delivery of the noxious stimuli (Bampouras et al. 2012; Billot et al. 2011), potentially affecting the statistical power of the study. A post-hoc power analysis (G\* Power; Faul et al. 2009) on the current study's results, suggested that the tetanic torque comparison had sufficient power (0.99) while the antagonistic torque power was lower (0.67) and a sample size of 12 would be required to achieve power of 0.8 (for a two-tailed t-test with alpha level at 0.05 and effect size of 0.9).

The present study shows that although antagonistic hamstrings co-activation torque is substantial and affects the estimation of knee extensor MVC, it is not a contributing factor to



the variation in isometric knee extension torque between different hip angles. This variation is explained, however, by the lower activation capacity in the supine position. Biomechanical models must consider variations with knee and hip joint positioning in both agonist activation and antagonist co-activation to more accurately reflect experimental observations of quadriceps muscle function.

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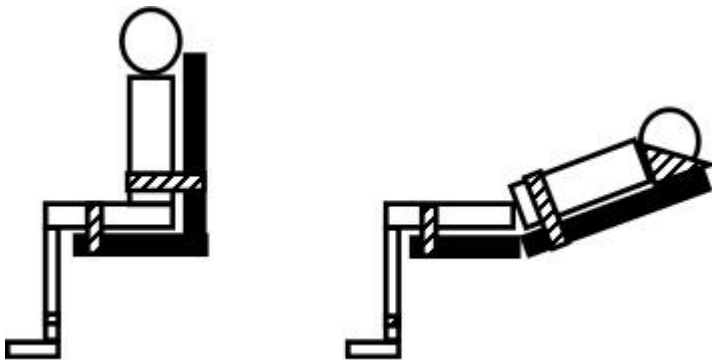
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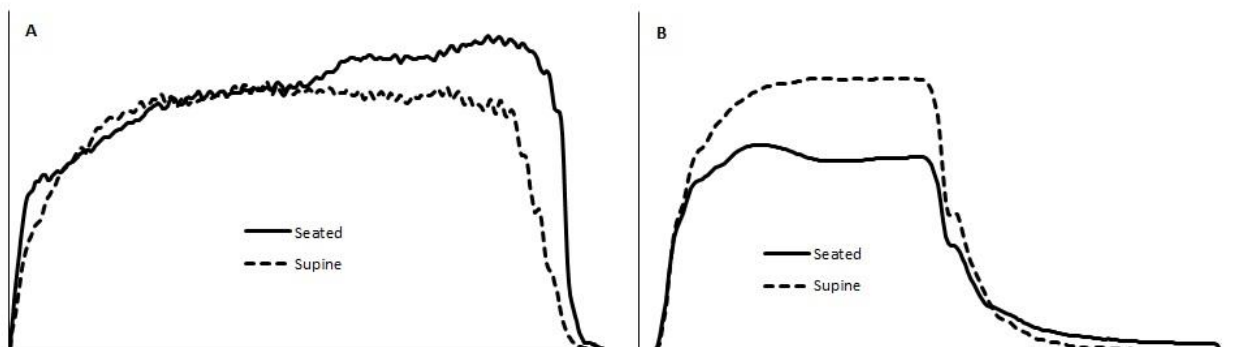
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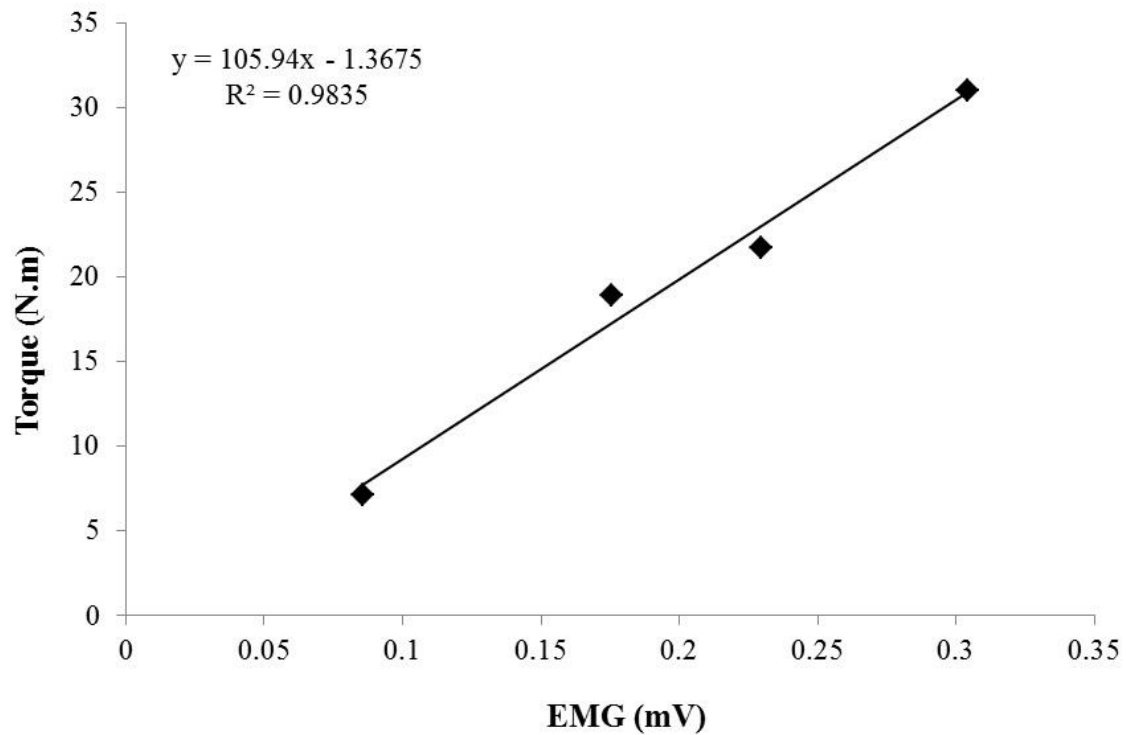
## FIGURES AND TABLE



**Fig. 1.** Schematic diagram of the experimental set up for seated (left) and supine (right) positions. Seated position hip joint angle was set at  $90^\circ$ , while supine  $160^\circ$  ( $180^\circ =$  full extension) with the knee and ankle joint angle at  $90^\circ$  in both positions. Solid rectangular shapes represent the dynamometer seat. Rectangular patterned shapes indicate straps placed on the lower leg (securely strapped to a force transducer), tested thigh and pelvis, for both conditions. Triangular patterned shapes indicate mechanical blocks used in the supine position to avoid extraneous movement upwards.



**Fig. 2.** Typical Joint MVC (panel A) and tetanic stimulation (panel B) torque traces from one subject for both seated and supine positions. Note the direction of change in the results with the joint MVC torque being higher for the seated position but the tetanic stimulation torque higher for the supine position.



**Fig. 3.** Typical results from one subject showing electromyography (EMG) and torque during four submaximal contractions of the BF when acting as an agonist. Torque was plotted against the EMG signal and a line was fitted to provide a regression equation, with a minimum coefficient of determination value of 0.9 set as the fit acceptance criterion. The regression equation was then used to estimate torque when the BF acted as antagonist.

**Table 1.** Descriptive statistics for maximum voluntary contraction (MVC) torque, tetanic stimulation torque, antagonistic torque and corrected MVC torque for both conditions (seated and supine). Data are presented as mean  $\pm$  SD. \* denotes significant difference at  $p < 0.05$  between seated and supine conditions. Effect size (ES) in brackets.

	Joint MVC torque(N•m)	Tetanic torque(N•m)	Antagonistic torque (N•m)	Mechanical ratio (%)	Corrected MVC torque (N•m)
Seated	241.2 $\pm$ 69.8	98.9 $\pm$ 27.5	26.0 $\pm$ 20.4	34.2 $\pm$ 24.5	267.3 $\pm$ 87.0
Supine	244.9 $\pm$ 71.8	110.6 $\pm$ 31.9*	12.0 $\pm$ 7.4* (0.9)	31.2 $\pm$ 13.1	256.9 $\pm$ 77.7

(1.1)