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Conventionally assessed voluntary activation does not represent relative voluntary torque production

R. D. Kooistra · C. J. de Ruiter · A. de Haan

Abstract The ability to voluntarily activate a muscle is commonly assessed by some variant of the twitch interpolation technique (ITT), which assumes that the stimulated force increment decreases linearly as voluntary force increases. In the present study, subjects \( (n=7) \) with exceptional ability for maximal voluntary activation (VA) of the knee extensors were used to study the relationship between superimposed and voluntary torque. This includes very high contraction intensities (90–100%VA), which are difficult to consistently obtain in regular healthy subjects (VA of \(~90\%\)). Subjects were tested at 30, 60, and 90° knee angles on two experimental days. At each angle, isometric knee extensions were performed with supramaximal superimposed nerve stimulation (triplet: three pulses at 300 Hz). Surface EMG signals were obtained from rectus femoris, vastus lateralis, and medialis muscles. Maximal VA was similar and very high across knee angles: 97 ± 2.3% (mean ± SD). At high contraction intensities, the increase in voluntary torque was far greater than would be expected based on the decrement of superimposed torque. When voluntary torque increased from 79.6 ± 6.1 to 100%MVC, superimposed torque decreased from 8.5 ± 2.6 to 2.8 ± 2.3% of resting triplet. Therefore, an increase in VA of 5.7% (from 91.5 ± 2.6 to 97 ± 2.3%) coincided with a much larger increase in voluntary torque (20.4 ± 6.1%MVC) and EMG (33.9 ± 6.6%max). Moreover, a conventionally assessed VA of 91.5 ± 2.6% represented a voluntary torque of only 79.6 ± 6.1%MVC. In conclusion, when maximal VA is calculated to be ~90% (as in regular healthy subjects), this probably represents a considerable overestimation of the subjects’ ability to maximally drive their quadriceps muscles.

Keywords Voluntary activation · Neural drive · EMG · Muscle length · Twitch interpolation

Introduction

When a supramaximal electrical stimulus is applied to the nerve of a muscle during a voluntary isometric contraction, the contractile response of any motor units not yet (fully) recruited will lead to a force increment. Merton (1954) first reported a linear decrease of this superimposed force increment as voluntary contraction intensity increased in the adductor pollicis muscle. Hence, as voluntary activation (VA) increases, the force increment as a result of electrical stimulation decreases. For the most accurate assessment of maximal VA, Behm et al. (1996) recommended using the maximal voluntary contraction (MVC) with a superimposed electrical stimulus, which is now common practice (Allen et al. 1995; Babault et al. 2003; Becker and Awiszus 2001; Behm et al. 2001; de Ruiter et al. 2004; Maffioletti and Lepers 2003; Newman et al. 2003; Oskouei et al. 2003; Suter and Herzog 1997). Maximal VA is generally quantified by expressing the ‘twitch’ force increment on an MVC as a percentage of resting twitch force. Subtraction from 100% results in a value for maximal VA.

Generally, high levels of VA (~90%) for the knee extensors are achieved by regular healthy subjects (e.g.
Babault et al. 2003; Behm et al. 2001; Bulow et al. 1993; de Ruiter et al. 2004; Newman et al. 2003; Oskouei et al. 2003; Suter and Herzog 1997). An important implicit meaning of a VA level of 90% is that 90% of the maximal torque capacity (MTC) is reached, and that at most, a 10% further increase in torque is possible. In our experience, however, a greater increase in torque can be observed than the obtained VA level suggested when subjects succeed in improving their MVC in a later attempt during the same session. This suggests that VA can be overestimated using the conventional method of superimposed stimulation. This would have important consequences for conclusions drawn regarding calculated VA. The inability of patient groups to access their muscles’ potential could be strongly underestimated. This has implications for the estimations made of the effects of paralytic poliomyelitis on strength and endurance for instance (Grabljevec et al. 2005). Furthermore, many studies have been performed using target force levels expressed as %MVC (de Ruiter et al. 2006; Grabljevec et al. 2005; Hisaeda et al. 2001; Kalmar and Cafarelli 1999; Ng et al. 1994; Place et al. 2005). For example, the %MVC at which full occlusion of blood flow of the knee extensors occurs during isometric contraction (de Ruiter et al. 2006). Clearly, knowledge of the potential of a far greater torque increase than, for example, the 10% implied by a VA of 90% is very important.

In our experience, in subjects capable of consistently high levels of maximal VA (~97%), the superimposed torque increment is already very small at high contraction intensities (~70%MVC). Further increases in voluntary torque show only minor reductions of the superimposed torque increment. This is in accordance with the modelling study of Herbert and Gandevia (1999); for the adductor pollicis muscle they show that large increases of motor-neuronal excitation near MVC will be accompanied by only minor reductions in the size of the superimposed force. At high contraction intensities, therefore, the %VA does not necessarily represent the same percentage of the muscles’ maximal torque capacity. The first aim of the present study was therefore to assess the relationship between calculated VA and voluntary torque of the knee extensors at high contraction intensities. To achieve the best possible assessment of the relationship between conventionally assessed VA and voluntary torque (as %MVC), in the present study, only those individuals capable of consistently high levels of maximal VA were included.

An additional concern of using the ITT is the dependence of the calculated VA on the size of the resting twitch. Following a single electrical stimulus, tendon slack must first be taken up before resting peak twitch torque is reached. Therefore, during an isometric contraction, where most of the tendon slack has already been taken up, the twitch torque increment will be relatively large compared to the twitch torque at rest (Belanger and McComas 1981). By scaling the superimposed twitch to resting twitch torque, calculated VA will thus be lower than it otherwise would have been. The effect of tendon slack on the resting twitch has been reported to be of minor influence on the relationship between the superimposed response and voluntary torque, at flexed knee angles (Behm et al. 2001; Bulow et al. 1993; Rutherford et al. 1986). However, tendon slack is greater at short compared to long muscle length (Muraoka et al. 2004). Therefore, particularly at short muscle lengths, scaling the superimposed torque response to the response obtained at rest may lead to a relatively lower calculated VA at short (extended knee angles) compared to long muscle lengths (flexed knee angles).

In subjects with a high ability for maximal VA, a neural drive may be reached that is usually unattainable for regular healthy subjects. Moreover, during voluntary fast isometric torque development, rectified surface EMG levels of the knee extensors may be substantially higher than at the torque plateau of an MVC (de Ruiter et al. 2004). These findings indicate a potential to increase neural drive at the torque plateau of a MVC (see also Herbert and Gandevia 1999). However, based on the shape of the stimulation frequency–force (de Ruiter et al. 1999; Deutekom et al. 2000) and pCa–force curve (Stephenson and Williams 1982) at high force levels large increases in neural drive and calcium are required for a comparatively small increase in force. As EMG is often measured to quantify the neural drive at different contraction intensities, this has important consequences for the EMG–torque relationship. EMG is generally normalized to the EMG obtained during MVC, a disproportionate increase in EMG at very high force levels will consequently lead to a more curvilinear EMG–torque relationship. However, this can only be demonstrated in subjects who are able to consistently reach high levels of neural drive. The shape of a subjects’ EMG–torque relationship would therefore be determined by the subjects’ ability for VA. This would explain why both linear (Woods and Bigland-Ritchie 1983) and non-linear (Alknerr et al. 2000) EMG–torque relationships have been reported. Therefore, this study further aims to investigate the EMG–torque relationship of the knee extensors in subjects with high ability for VA.

Methods

Subjects

Seven healthy male subjects (23–32 year, 69–83 kg, and 1.73–1.93 m) volunteered to be subjects for this investigation. Before participation, each subject was thoroughly
informed about the procedures and provided written informed consent prior to testing. Only subjects capable of >95%VA across knee angles (see below) were included in the present study. The study was performed according to the Declaration of Helsinki and approved by the local ethics committee. Subjects did not perform any fatiguing exercise 48 h prior to measurements.

Experimental procedures

The subjects visited our laboratory on two occasions with at least 1 day in between. All subjects were familiar with the set-up, electrical stimulation, and isometric knee extension from previous studies (de Ruiter et al. 2004; Kooistra et al. 2005). During the first experimental day, several contractions with superimposed stimulation (see below) were performed at 30° (extended) and 60 and 90° (flexed) knee angles. At the same time, surface EMG of the rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM) was recorded. To gain information concerning test-retest variability, the experimental procedure performed on experimental day 1 was repeated during experimental day 2.

Torque measurements

Isometric knee extension torque of the right leg was measured using a custom-built dynamometer (Kooistra et al. 2005). Subjects were seated with their hips at 70° (0° = full extension). Shoulders, hips, and lower thigh were strapped to the dynamometer. By very tight strapping of the hips and lower thigh, a contribution of the hip extensors to knee extension was absolutely impossible. Moreover, during contractions, subjects had to lean forward about 10 cm with their shoulders (while grasping the strap around their shoulders) pushing their lower back into the back rest. This further guaranteed that no hip extension occurred. The distal part of the shank was strapped to a force transducer (KAP, E/200 Hz, Bienfait B.V. Haarlem, The Netherlands) that was attached to the lever arm of the dynamometer. A shin guard ensured subjects could exert maximal forces without discomfort at the shin. The compliance of the dynamometer at the position of the force transducer was 1.4 × 10⁻⁴ deg/Nm. The backrest, force transducer height, and its medio-lateral position were adjusted for precise alignment of the knee axis with the axis of rotation of the dynamometer arm. A cranck enabled changing of the knee angle of the subject. Knee joint angles were determined with a handheld goniometer (model G300, Whitehall Manufacturing) using the greater trochanter and lateral epicondyle of the femur, and the lateral malleolus of the fibula as references. The dynamometer arm was positioned so that the indicated knee angles were angles in an active state, with subjects delivering ~50% of MVC. During pilot experiments using an electrogoniometer attached to the lateral side of the knee, the changes (active–passive) in knee angle were found to be 3–7°, independent of knee angle, with minimal (<1°) changes above ~50% of MVC. Real-time force applied to the force transducer was displayed on line on a computer monitor and digitally stored (1 kHz) on computer disc. The force signals were automatically corrected for gravity at each angle: the average force applied by the weight of the limb to the transducer during the first 50 ms after the start of a recording, with the subject seated in a relaxed manner, was set to zero force by the computer program. Extension torque was calculated by multiplication of force with the individual lever arm and data will be presented as torque.

Electrical stimulation

A cathode (self-adhesive stimulation electrode, 5 × 5 cm, Schwa-Medico, The Netherlands) was placed over the femoral nerve. The anode (13 × 8 cm) was placed over the gluteal fold. The quadriceps femoris muscle was stimulated transcutaneously with rectangular pulses of 100 μs using a computer-controlled constant current stimulator (Digitimer DS7H, Digitimer Ltd., Welwyn Garden City, UK). Stimulation current was increased until torque measured in response to a triplet (three 100 μs pulses applied at 300 Hz) levelled off at each knee angle tested. The current (in mA) was then increased by a further 50 mA to ensure supramaximal stimulation (range: 250–600 mA). At this point, we considered all muscle fibres of the knee extensors to be activated. Pilot studies had shown that triplet torque did not increase after an MVC at any knee angle, indicating that any potential potential post-activation potentiation did not result in a triplet torque increase (see also Discussion). Usually, torque generated by the triplet is about ~35% of the MTC (Kooistra et al. 2005). Furthermore, in pilot studies, the stimulation current was increased far beyond supramaximal levels (up to 200%) without a subsequent decrease in triplet torque. Hence, the hamstring muscles were assumed not to be activated during supramaximal stimulation of the knee extensors (see also Discussion).

Voluntary activation

During experimental day 1, the level of maximal VA was determined in random order at 30°, 60°, and 90° knee angles. At each knee angle, subjects were asked to maximally generate isometric torques for about 3–4 s to determine MVC extension torque. Real-time torque was visible on a computer monitor and subjects were vigorously encouraged to exceed their maximal value, which was also displayed. MVC torque was defined as highest torque within a
stable 1,000 ms plateau of the torque signal. MVC torque was taken as the highest value, which did not exceed preceding attempts by >5%, allowing a maximum of four attempts to prevent fatigue. In the incidental case a trial had no stable plateau, the trial was repeated. Subjects then performed a series of superimposed measurements consisting of a triplet applied to a fully relaxed muscle and another triplet superimposed on the stable part of the torque signal (Fig. 1). If the torque level was unstable (>1% torque fluctuation) prior (100 ms) to stimulation, the contraction was repeated. As all our subjects were very experienced at maintaining stable torque levels at all contraction intensities, it was rarely necessary to repeat a measurement. As an indication, for all subjects, less than 1 in 10 contractions (across all knee angles and intensities) were repeated. Several different contraction intensities with an emphasis toward higher intensities were chosen (25, 50, 70, 80, 90, and 100%MVC). At each knee angle, the order of the contraction intensities with superimposed triplet was randomized, and the order of the knee angles was randomized per subject. A 3-min rest period separated contractions. For each superimposed contraction, the accepted formula for the interpolated twitch torque technique (Allen et al. 1995; Babault et al. 2001, 2006; Becker and Awiszus 2001; de Ruiter et al. 2005; Kooistra et al. 2005; Maffioletti and Lepers 2003; McKenzie et al. 1992; Newman et al. 2003; Oskouei et al. 2003) was used to determine the level of voluntary activation (VA):

\[
VA(\%) = 100 - \left( \frac{\text{triplet torque increment}}{\text{resting triplet torque}} \times 100 \right)
\]

With this method, the triplet torque increment is expressed as a percentage of the resting triplet torque, and subtraction from 100% results in a value for VA (Fig. 1). Maximal VA was obtained from the 100%MVC superimposed contraction. To illustrate the relationship between superimposed and voluntary torque from 90 to 100%VA, only subjects capable of an average maximal VA of >95% across knee angles were included in the present study. MVC torques as close to the muscles’ MTC as possible were thus obtained. Due to our strict inclusion criteria (average VA of >95%) at each knee angle, we were able to select a contraction at a lower than maximal torque level where the superimposed triplet was ~10% of resting triplet. At this torque level, VA is conventionally calculated to be ~90%, and we expressed this torque level as a percentage of MVC. We could thus compare the theoretical 10% torque increase implied by a conventionally calculated VA level of 90% with the torque increase actually achieved by our subjects during MVC. This provides us with a good indication of the potential torque increase that may be possible in regular healthy subjects with a maximal VA level of ~90%. The ~90% is a VA level that is frequently reported in the literature for regular healthy subjects (Babault et al. 2001, 2003; Bulow et al. 1993; de Ruiter et al. 2004; Newman et al. 2003; Oskouei et al. 2003; Suter and Herzog 1997).

Furthermore, for each subject and for each superimposed contraction, the increment in torque as a result of the superimposed triplet was plotted as a function of the torque reached just prior to the superimposed stimulation (e.g. Figs. 2, 3a). Curvilinear relationships between voluntary and superimposed torque are often reported in the literature for group data (e.g. Behm et al. 2001; Dowling et al. 1994; Oskouei et al. 2003; Suter and Herzog 1997). In the present study, however, neither a linear nor a curvilinear fit closely followed the data points for any of the individual subjects at any knee angle. However, linear regression was performed on the highest four contraction intensities (70, 80, 90, and 100%), and \( R^2 \) values were calculated at each knee angle for each subject. This was done to show that although superimposed torque increments were small at high contraction intensities, the torque increment continued to decrease with contraction intensity (e.g. Fig. 3a, b).

Surface electromyography

Electromyographic activity of the RF, VL, and VM muscles was recorded using surface EMG electrodes (Blue Sensor, Ambu, Ølstykke, Denmark, lead-off area: 1.0 cm²). After shaving, roughening, and cleansing the skin with 70% ethanol, electrodes were placed on the muscle belly in a bi-polar configuration in line with the muscle fibre direction with a centre to centre inter-electrode
distance of 25 mm. Reference electrodes were placed on bone structures, on each patella, and on the lateral epicondyle of the femur of the right leg. Surface EMG signals were amplified (×100), digitized (1 kHz), and stored with the force signal on computer disc. All EMG signals were band-pass filtered (10–400 Hz). Rectified surface EMG amplitude (rsEMG) was calculated for the RF, VL, and VM for 1,000 ms segments just before the superimposed stimulation. RsEMG values obtained during the highest torque level on each experimental day were set to 100%.

Statistics

All results are presented as mean ( SD. Knee angle effects were tested for significance with repeated measures ANOVA. If significant main effects were observed, Bonferroni tests were performed for post hoc analysis. Test-retest reliability was tested for using the intraclass correlation coefficient. The level of significance of all statistical analyses was set at $P < 0.05$.

Results

Torque levels during the superimposed contractions at the 25, 50, 70, 80, 90, and 100%MVC levels were not different from the intended values or different between experimental days. Linear extrapolation of the torque increment as a result of the triplet on the high intensity contractions (70,
80, 90, and 100\%MVC) provided high $R^2$ values at each knee angle (30\°: 0.89 ± 0.09; 60\°: 0.90 ± 0.10; and 90\°: 0.87 ± 0.10 averaged over days), which were not different between knee angles or the two experimental days (e.g. Fig. 3b). The continuing consistent decrement of the triplet torque increment with increase in contraction intensity was denoted by the overall negative slope (~0.13 ± 0.05 across days and angles), which was not different between days and angles. The relationship between the triplet torque increment and voluntary torque is shown in Fig. 4 for the 30, 60, and 90\° knee angles.

There was no difference in resting triplet torque or MVC between experimental days and average values across days are presented. At the 60\° knee angle, MVC was significantly ($P < 0.05$) greater compared to both 30 and 90\° knee angles (Table 1). Resting triplet torque, however, was significantly ($P < 0.05$) lower at 30\° compared to 60 and 90\° (Table 1). Reproducibility for both MVC and resting triplet torque was very high, which was illustrated by high ICC values (Table 1).

Voluntary activation

Overall, there was no difference in maximal VA, determined by applying the ITT to the highest MVC, between experimental days and average values across days are presented. The significantly lower triplet torque at 30\° compared to the 90\° knee angle did not result in a lower maximal VA at 30\° compared to 90\°. At each knee angle, maximal VA was very high and similar between knee angles (Fig. 5). Furthermore, at each knee angle, at torques where the triplet increment was ~10\% (8.5 ± 2.6\%), VA was calculated to be ~90\% (91.5 ± 2.6\%, across angles). This VA level turned out to be significantly higher compared with the relative voluntary torque level at which the triplet was superimposed. The torques at which VA was calculated to be 91.5 ± 2.6\% represented only 79.6 (± 6.1\%) of MVC (across knee angles), a finding that was similar at each knee angle ($P > 0.05$). Note that at this torque level, the triplet torque increments were already small (3.2 ± 1.1\% MVC) compared to the torque generated when the triplet was applied on the resting muscle (~37\% MVC). The key point we want to address with our study is illustrated in Fig. 6. For both subject nos. 1 and 7, a VA level of ~90\% was calculated at the 60\° knee angle at a torque level of ~250 Nm. This suggests a potential further increase of maximal torque by 10\%. However, subject no. 1 was able to significantly increase his torque production by more than 30\%.

EMG

The rsEMG values obtained during MVC were similar between days at each knee angle ($P = 0.96$). For all submaximal contraction intensities, normalized rsEMG values for each muscle (RF, VL, and VM) at each contraction

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**Table 1** Torque

| Knee angle | 30° | 60° | 90° | ICC  |
|------------|-----|-----|-----|------|
| MVC (Nm)   | 186.1 ± 36.6 | 278.4 ± 44.5* | 189.6 ± 19.7 | 0.99*** |
| Triplet torque (Nm) | 65.9 ± 10.0** | 91.6 ± 15.8 | 80.1 ± 10.8 | 0.96*** |

All values are means ± SD, averages across days shown, $n = 7$. Maximal voluntary isometric knee extension (MVC) and resting triplet torque for the 30\°, 60\°, and 90\° knee angle
* Significantly different from 30\° to 90\° knee angle ($P < 0.05$)
** Significantly different from 60\° to 90\° knee angle ($P < 0.05$)
*** Significant ICC, the intraclass correlation coefficient (ICC) was calculated for the measurements made on experimental days 1 and 2 and is shown in the last column
The torque increment as result of the triplet is shown as a function of voluntary torque for subject no. 1 (grey squares) and 7 (open circles) at the 60° knee angle on day 1. At the 60° knee angle, the maximal VA level of subject no. 7 calculated at 250 Nm was 90.7%, which resembled that of a regular subject, and is exceptionally low in our study (see lowest point in Fig. 5). A VA of 90.0% is also calculated at 240 Nm for subject no. 1. In both subjects, this implies a further potential increase in torque of 10%, yet a >30% torque increase (to 330 Nm) could be demonstrated in subject 1. This is most likely due to the exceptional neural drive of subject 1 (~98%VA) during his best attempts at this knee angle.

Discussion

In the present study, the relationship between calculated VA and voluntary torque was assessed in subjects with high ability for maximal VA at different knee angles. At the same time, the EMG–torque relationship in these selected subjects was examined. The first and main finding of the present study was that when VA is calculated to be ~90% (as in regular healthy subjects), this probably represents a considerable overestimation of the subjects’ ability to maximally drive their quadriceps muscles. An additional finding was that, although resting triplet torque was lower at 30° versus 90°, this was of minor influence on the calculated maximal VA in our subjects. Furthermore, a relatively large increase in normalized rsEMG was observed as MVC was approached, making the shape of the normalized EMG–torque relationship of the knee extensors curvilinear.

intensity was similar between days; hence, averaged values across days are presented. Furthermore, no difference between knee angles or muscles was found, and with each increase in contraction intensity, there was a significant increase in normalized rsEMG (Fig. 7a, VL shown). An increase of normalized rsEMG of ~21% (across angles and muscles) was seen for a 25% increase in torque from 25 to 50% MVC (Fig. 7a). When contraction intensities approached MVC, there was a much larger increase in normalized rsEMG (~34%) relative to the torque increase of only ~18% at all knee angles (Fig. 7a). In regular healthy subjects, the highest MVC would have occurred at torque levels where VA would have been calculated to be ~90%, and consequently the EMG–torque relationship would most likely have been considerably less curvilinear. This is illustrated by the two EMG–torque relationships shown in Fig. 7b for the VL at the 90° knee angle. The first EMG–torque relationship includes all contraction intensities measured, whereas for the second EMG–torque relationship, the 90 and 100%MVC contraction intensities have been removed. In the latter case, all rsEMG values are normalized to a torque level that corresponds to the maximal VA level of ~90% (generally obtained by regular healthy subjects), the result being a near linear EMG–torque relationship (Fig. 7b).

As a consequence of the disproportionate increase in EMG as MVC is approached, normalized rsEMG values obtained at intensities below MVC are normalized to a relatively large value. Hence, these normalized rsEMG values are relatively smaller compared to the relative torques at which they have been obtained. For example, at 25 and 50%MVC, normalized rsEMG for the RF, VL, and VM muscle was significantly less than would be expected based on the %MVC at which they were obtained at all knee angles (Fig. 7a, VL shown). By normalizing the rsEMG levels to the rsEMG values obtained at a torque level that corresponds to the VA level of 90% as found in regular healthy subjects, normalized rsEMG values are closer to the line of identity, and a more linear EMG–torque relationship is seen (Fig. 7b).
Maximal voluntary activation and knee angle

Subjects in the present study exhibited a consistent high level of maximal VA of the knee extensors (Fig. 5), which is higher than the maximal VA levels of 90% that are generally reported for the knee extensors in regular healthy subjects when applying the ITT (Babault et al. 2001, 2003; Bulow et al. 1993; de Ruiter et al. 2004; Newman et al. 2003; Oskouei et al. 2003; Suter and Herzog 1997). Although full activation of the knee extensors has been reported in the past (Bigland-Ritchie et al. 1986; Rutherford et al. 1986), in those studies a single superimposed twitch was used. Due to the declining signal-to-noise ratio with increase in contraction intensity, the detection of a single superimposed twitch is difficult, and 100% VA may mistakenly have been assumed (Dowling et al. 1994). To improve the signal-to-noise ratio, multiple stimuli have been suggested (Gandevia and McKenzie 1988; Suter and Herzog 2001). In the present study, triplet stimulation provided considerable resting triplet torque levels at each knee angle (Table 1), which ensured a good signal-to-noise ratio. Furthermore, pilot studies had shown no increase in triplet torque before and after an MVC at any knee angle. By using supramaximal and high frequency (300 Hz) triplet stimulation, we avoided the potential influence of post-activation potentiation on our data.

A limitation of the ITT may be the scaling of the twitch increment to the resting twitch. When muscle is stimulated by a twitch at rest, all tendon slack has to be taken up; conversely, for a twitch superimposed on an ongoing isometric contraction, no further slack has to be taken up (Suter and Herzog 1997). Since calculated VA is determined by the scaling of the triplet torque increment to the resting triplet, calculated VA will be reduced by the influence of tendon slack. As the effect of tendon slack is greater at short versus long muscle length (Muraoka et al. 2004), VA will be influenced to a greater degree at short versus long muscle length. Besides tendon slack, length-dependent $\text{Ca}^{2+}$ sensitivity may also influence the size of the resting triplet by
shifting the resting triplet torque–length relationship to shorter muscle length compared to the MVC torque–length relationship. The length-dependent effect of tendon slack and \( \text{Ca}^{2+} \) sensitivity were reduced by the use of a triplet (Kawakami et al. 2001), but not abolished as triplet torque was significantly smaller at the 30° versus 90° knee angle (Table 1), whereas MVC levels were similar. This implies that despite using triplet stimulation, compared to the MVC, resting triplet torque remains relatively lower at the 30° versus 90° knee angle. The latter is supported by Figs. 2 and 3A, where the triplet response is similar at 0 and 25%MVC at the 30° knee angle. It is evident that calculated VA will be influenced by tendon slack, as the resting triplet is influenced by tendon slack, especially at short muscle length, whereas the triplet torque increment is not (Suter and Herzog 1997).

Despite a lower resting triplet torque at the 30° compared to the 90° knee angle, maximal VA was not different between the 30° and 90° knee angle in our subjects. From the literature, it is unclear whether maximal VA is knee angle-dependent. Kubo et al. (2004) report a lower level of maximal VA at extended compared to flexed knee angles, whereas Suter and Herzog (1997) find maximal VA to be highest at the most extended knee angle (15°). Newman et al. (2003) and Babault et al. (2003), however, find no effect of knee angle on maximal VA, which is in line with our current findings. Note that due to our selection criteria (average maximal VA of 95%, and at least 90% at each knee angle), maximal VA was very high (~97%) and close to 100%. Potential knee angle-dependent differences in maximal VA could consequently only occur within a very small range. Furthermore, the small size of the superimposed triplet torque makes the calculation of maximal VA insensitive to large differences in the size of the resting triplet torque. By hypothetically increasing the resting triplet torque at 30° by 20%, making it similar to the 90° knee angle, an increase in maximal VA of less than 0.5% is calculated at the 30° knee angle. In subject populations possessing lower maximal VA levels (e.g. patient groups), a relatively greater increment will be obtained on an MVC. This greater increment is more susceptible to the muscle-length dependent influence of, for example, tendon slack on the size of the resting triplet. The influence of tendon slack would have been greater still had a twitch instead of a triplet been used (Kawakami et al. 2001), leading to an even greater difference in resting twitch size at 30° versus 90°. Combined with the use of a resting twitch, therefore, a muscle length-dependent effect of tendon slack is expected to significantly influence the calculation of VA in this subject population.

Calculated voluntary activation in relation to relative voluntary torque

For the most accurate assessment of maximal VA, Behm et al. (1996) recommended using the MVC with a superimposed electrical stimulus. As mentioned previously, in regular healthy subjects a maximal VA level of ~90% is often reported for the knee extensors using this method (Babault et al. 2001, 2003; Bulow et al. 1993; de Ruiter et al. 2004; Newman et al. 2003; Oskouei et al. 2003; Suter and Herzog 1997) implying that the MTC has almost been reached. In the present study, with our selected subject group, we were able to demonstrate that 90%VA is already calculated at torque levels that represent only ~79% of MVC (across knee angles). In some subjects, this phenomenon is very pronounced. For the subject shown in Fig. 3a and b, already at a torque of ~148 Nm, VA using the ITT was calculated to be 95.3 and 97.1% for experimental day 1 and 2, respectively. These are very high VA levels that have been calculated at 148 Nm, especially when compared with the MVC of >200 Nm that was obtained on both experimental days. Note that the higher part of the curve (in this example over 140 Nm), as shown in Fig. 3b can usually not be obtained in regular healthy subjects. In our experience, we can only demonstrate the existence of this part of the curve in subjects with a very high ability for maximal VA (>99% in this extreme example, subject no. 1 in Fig. 5). This finding strongly suggests that the 90%VA reported for regular healthy subjects is a large underestimation of the MTC of the muscle. A far greater torque increase therefore seems possible than the 10% implied by a calculated VA of 90% (Fig. 6). The main new finding, therefore, is that the present study for the first time quantifies the difference between calculated VA and relative voluntary torque.

Experimental and methodological factors

When relating the triplet torque increment to the triplet torque obtained at rest, it is important to maintain supra-maximal stimulation during both conditions. In a previous study examining superimposed stimulation of the knee extensors (Behm et al. 1996), the authors reported significantly lower M-wave amplitudes during a superimposed stimulation on an MVC compared to during rest. They presumed that the contraction of the knee extensors resulted in a displacement of the stimulating electrode from its optimal position over the femoral nerve. In pilot studies of the present investigation, M-wave amplitude at rest and superimposed on an MVC was similar. Moreover, a substantial (50%) increase in stimulation current did not lead to an increase in the size of the triplet increment on high intensity contractions (70 and 80%MVC). This strongly
suggests that even during high intensity contractions, there was supramaximal stimulation of the femoral nerve during superimposed triplet stimulation. By using multiple stimuli during superimposed stimulation, spinal reflexes may have more time to diminish the superimposed response (Herbert and Gandevia 1999; Herbert et al. 1997). Modeling suggests that the use of twin stimuli at 100 Hz has a minimal effect on the estimates of VA (Herbert and Gandevia 1999). By applying a stimulation frequency of 300 Hz in the present study, triplet stimulation occurred within 10 ms, which is even less when compared to the use of twin stimuli at 100 Hz. The influence of spinal reflexes on the superimposed stimulation is therefore considered negligible. Furthermore, although the current passes through the sciatic nerve that innervates the hamstring muscles, its stimulation was regarded negligible. This is illustrated by the very small EMG recording of the m. biceps femoris compared to the VL muscle during stimulation of the knee extensors [Fig. 8, data from a previous study of ours (Kooistra et al. 2005)]. We accordingly assumed a marginal activation, if at all, of the hamstring muscles during superimposed stimulation and therefore no influence on our calculation of VA. In a previous study by our group (de Ruiter et al. 2004), we measured the contribution of co-activation during isometric knee extensions during brief MVCs and found it to be very small (5–10%). As this finding is in accordance with previous findings (Newman et al. 2003; Psek and Cafarelli 1993), we did not measure coactivation in the present study and considered it minimal.

The EMG–torque relationship

As expected, when MVC was approached we observed a relatively large increase in normalized rsEMG (Fig. 7a). This large increase makes the shape of the EMG–torque relationship of the knee extensors curvilinear. This is in contrast to the findings of Woods and Bigland-Ritchie (Woods and Bigland-Ritchie 1983) who report a linear EMG–torque relationship, yet in accordance with Alkner et al. (2000) and Pincivero and Coelho (2000) who show a non-linear relationship for the knee extensors.

Based on a modelling study of motor-unit pools, Fuglevand et al. (1993) indicate that the difference between muscles that exhibit the linear as opposed to the nonlinear form of the EMG–torque relationship may be related to differences in firing rate behaviour rather than due to differences in recruitment organization. It is reasonable to expect that only subjects with a high ability for maximal VA (high neural drive) will achieve very high motor unit firing rates. For this large increase in firing rate, however, little gain in torque may be predicted (Herbert and Gandevia 1999). The large increase in excitation, as represented by the disproportionate increase in normalized rsEMG when MVC was approached, confirms the model study by Herbert and Gandevia (1999). However, in contrast to their model where essentially 100%MVC was achieved at 60% of maximal voluntary excitation, in the present study, we experimentally determined that the disproportionate increase in excitation lead to a more substantial increase in torque (~18%). However, many factors may affect the EMG–torque relationship that have not been taken into account. Those include, signal cancellation from overlapping positive and negative phases of action potentials (Day and Hulliger 2001); the sigmoid relationship between motor unit force and firing rate (Herbert and Gandevia 1999); and the nonlinear distribution of recruitment thresholds (Fuglevand et al. 1993). Interestingly, in a review, de Luca (1997) states that the amplitude of the

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Fig. 8 Representative EMG recordings are shown for the vastus lateralis (VL, upper panel) and biceps femoris (BF, lower panel) muscle. On the left, the M-wave for the VL as a result of supramaximal twitch stimulation applied to the n. femoralis. The simultaneous EMG recording for the BF muscle is shown in the lower left panel. On the right, the EMG recording during maximal voluntary extension (top, right) and flexion (lower, right) torque is shown to illustrate that the lower M-wave of the BF is not due a lower sensitivity of the BF recordings compared to the VL recordings.
EMG signal should be normalized to values less than 80%MVC. Above this level, the EMG signal is said to be exceptionally unstable and, hence, is unable to provide a suitable reference point. As shown in the present study, very large increases in EMG coincide with very small increases in calculated VA (Fig. 7a). This is consistent with unstable EMG measurements for similarly high torque levels.

EMG, superimposed torque and voluntary activation

When combining the non-linearity of the EMG–torque relationship and non-linearity of the superimposed triplet-torque relationship, the EMG–torque relationship indicates that a large amount of extra EMG produces relatively little extra torque at high intensities. The triplet given to evoke the superimposed triplet could be considered as extra EMG in that three extra action potentials are added. As voluntary EMG is reduced in its effectiveness at producing torque in strong voluntary contractions, the extra activation from the triplet might be reduced similarly. This is in accordance with a model study of the adductor pollicis motoneuron pool (Herbert and Gandevia 1999). The authors predict large increases in motoneuronal excitation for minor increases in voluntary force and VA (as shown by small decreases in the superimposed twitch, their Fig. 9c). As mentioned above, this is consistent with the EMG–torque data in the present study (Fig. 7a). The small decreases in modelled interpolated twitch amplitude are also consistent with the current findings, where the triplet torque increment was only 3.2 ± 1.1%MVC at a torque level that corresponded to 79.6 ± 6.1%MVC and showed only a minor further reduction in size (to 1.0 ± 0.9%MVC).

Note that the minor reduction in triplet increment with increasing voluntary torque is, and can only be, observed in those subjects consistently able to achieve high levels of VA, as would be expected from the model (Herbert and Gandevia 1999). This would indicate that these subjects are capable of excitation levels that (almost) evoke their MTC. Having noted this, it is important to point out that a 100% excitation may not be necessary to evoke the MTC as ‘‘only’’ 60% excitation was modelled to elicit 100% MTC (Herbert and Gandevia 1999). This is in agreement for example with the much greater excitation (EMG) levels attained during voluntary fast isometric knee extensions compared to during the plateau of an MVC (de Ruiter et al. 2004).

How can we be certain that our selected subjects did indeed approach their true MTC? The only way to truly determine the MTC of a muscle is by the use of tetanic nerve stimulation. In two subjects with exceptional ability for maximal VA (>99%), supramaximal tetanic nerve stimulation (2 s, at 150 Hz) of the knee extensors was performed and torque levels showed no further increase above MVC (data not presented). Applying supramaximal tetanic nerve stimulation is not recommended, however, as it is highly unpleasant and could be harmful to certain vulnerable subject groups (e.g. subjects with an ACL deficiency).

Currently, the ITT is the only feasible available technique used to determine VA of the knee extensors and, as mentioned previously, it is used extensively (Allen et al. 1995; Babault et al. 2003; Becker and Awiszus 2001; Behm et al. 1996, 2001; de Ruiter et al. 2004; McKenzie et al. 1992; Newman et al. 2003; Oskouei et al. 2003; Suter and Herzog 1997). The findings of the present study indicate that for the knee extensors, despite a commonly reported maximal VA of ~90% for regular healthy subjects, a far greater relative torque increase seems possible than the 10% implied by 90% VA. This has important implications for conclusions that are drawn regarding calculated VA. Especially because many studies have used target force levels expressed as %MVC to investigate: effects of caffeine neuromuscular function (Kalmar and Cafarelli 1999), the %MVC at which full occlusion of the blood supply occurs (de Ruiter et al. 2006), and fatigue of the quadriceps in patients with multiple sclerosis for instance (de Haan et al. 2000). Clearly, knowledge of the potential of a far greater torque increase than, for example, 10% implied by a VA of 90% is very important.

In conclusion, when maximal VA is calculated to be ~90% (as in regular healthy subjects), this probably represents a considerable overestimation of the subjects’ ability to maximally drive their quadriceps muscles. Moreover, the effect that the length dependent size of the resting triplet has on the calculation of VA with the conventional method is only minimal when VA is greater than 95%. Furthermore, a curvilinear shape of the EMG–force relationship may be caused by a disproportionately large increase in normalized rsEMG when MVC is approached in subjects with very high capacity to drive their muscles maximally.

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