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### EMG normalization to study muscle activation in cycling

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#### Abstract

The value of electromyography (EMG) is sensitive to many physiological and non-physiological factors. The purpose of the present study was to determine if the torque-velocity test (T–V) can be used to normalize EMG signals into a framework of biological significance. Peak EMG amplitude of gluteus maximus (GMAX), vastus lateralis (VL), rectus femoris (RF), biceps femoris long head (BF), gastrocnemius medialis (GAS) and soleus (SOL) was calculated for nine subjects during isometric maximal voluntary contractions (IMVC) and torque-velocity bicycling tests (T–V). Then, the reference EMG signals obtained from IMVC and T–V bicycling tests were used to normalize the amplitude of the EMG signals collected for 15 different submaximal pedaling conditions. The results of this study showed that the repeatability of the measurements between IMVC (from 10% to 23%) and T–V (from 8% to 20%) was comparable. The amplitude of the peak EMG of VL was  $99 \pm 43\%$  higher (p < 0.001) when measured during T–V. Moreover, the inter-individual variability of the EMG patterns calculated for submaximal cycling exercises differed significantly when using T–V bicycling normalization method (GMAX:  $0.33 \pm 0.16 \ vs. 1.09 \pm 0.04$ , VL:  $0.07 \pm 0.02 \ vs. 0.64 \pm 0.14$ , SOL:  $0.07 \pm 0.03 \ vs. 1.00 \pm 0.07$ , RF:  $1.21 \pm 0.20 \ vs. 0.92 \pm 0.13$ , BF:  $1.47 \pm 0.47 \ vs. 0.84 \pm 0.11$ ). It was concluded that T–V bicycling test offers the advantage to be less time and energy-consuming and to be as repeatable as IMVC tests to measure peak EMG amplitude. Furthermore, this normalization method avoids the impact of non-physiological factors on the amplitude of the EMG signals so that it allows quantifying better the activation level of lower limb muscles and the variability of the EMG patterns during submaximal bicycling exercises.

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Keywords: Torque-velocity bicycling test; Isometric maximal voluntary contractions; Peak EMG amplitude; Bicycle-ergometer; Inter-individual variability

#### 1. Introduction

Electromyography (EMG) is a research tool that has been largely used over the last 50 years to gain insight into how neural control strategies adapt to environmental and task demands. The control strategy refers to the output from the nervous system used to accomplish a movement. It can be observed non-invasively by collecting EMG signal using surface electrodes. Different authors notably contributed to gain insight into the processes underlying the organization of the neuromuscular control of lower limb movements during bicycling by analyzing the impact of task conditions (Li, 2004; MacIntosh et al., 2000; Neptune et al., 1997; Raasch et al., 1997; van Ingen Schenau et al., 1992) on temporal variables (amplitude and timing of activation) of the EMG signals collected over the lower limb muscles.

The amplitude of EMG signal is generally defined after a first processing step consisting into filtering and calculating rectified, integrated or root mean square value (RMS) from raw data. However, large variations in the amplitude of the EMG signal can be observed between subjects (Kasprisin and Grabiner, 1998) because of the impact of physiological and non-physiological factors on the surface EMG signals (Farina et al., 2004b). The influence of ana-

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tomic, geometrical and physical factors and detection system differences on the EMG variables (Beck et al., 2005; Malek et al., 2006: Soderberg and Knutson, 2000) makes difficult the interpretation of the experimental data obtained for different subjects, days, muscles or studies. The influence of these non-physiological factors on the temporal characteristics of surface EMG signals has been largely described in the literature (Farina et al., 2004b). Different authors have documented the effect of factors such as inter-electrode distance (Beck et al., 2005; Malek et al., 2006; Melaku et al., 2001), electrode placements (Beck et al., 2006; Campanini et al., 2006; Jensen et al., 1993; Malek et al., 2006), subcutaneous fat (Kuiken et al., 2003; Nordander et al., 2003), perspiration and skin temperature (Petrofsky and Laymon, 2005), or EMG detection system (Farina et al., 2002a; Hunter et al., 2003) on the surface EMG signals collected.

The use of normalization procedures has been proposed to compare experimental EMG results obtained for different muscles, subjects or days within a subject. EMG normalization is a technique that permits to access the relative level of activation of a given muscle (Hsu et al., 2006) by expressing the absolute amplitude of the signal measured during the exercise as a percentage of a meaningful reference EMG value. The most powerful solution for physiologic interpretation is to measure the reference EMG value while the subject performs a muscle contraction for a calibrated test condition. The reference EMG value corresponds to the peak or mean values of the EMG signal that is measured during isometric maximal voluntary contractions (IMVC) (Ericson et al., 1985; Hunter et al., 2002; Marsh and Martin, 1995; Neptune et al., 1997), standard isometric contractions (SIC) (Marras and Davis, 2001; Marras et al., 2001), or dynamic contractions (Yang and Winter, 1984).

Although the impact of task conditions on the EMG activity of the lower limb muscles has been largely investigated in the literature (Li, 2004; MacIntosh et al., 2000; Neptune et al., 1997; Raasch et al., 1997; van Ingen Schenau et al., 1992), the validity of the normalization procedures used in these studies has not been discussed. This issue has to be considered because one can assume that the selection of a normalization procedure depends on the type of descriptions or comparisons to be made (i.e. between subjects, days, muscles, exercise conditions or studies). Theoretically, the choice of a normalization procedure is a prerequisite allowing the interpretation of the signal into a framework of physiological significance. It seems that the normalization procedure has to be selected so that it ensures that changes in the EMG signals reflect physiological modifications in the neural drive (Farina et al., 2004b). Referring to the recommendations proposed in the literature (Burden et al., 2003; Hunter et al., 2002; Yang and Winter, 1984), the validity of a normalization procedure depends on: (1) the repeatability and reliability of the EMG measurements; (2) the relevance and feasibility of the standardized efforts retained; (3) the use of reference values permitting to better estimate the level of activation of the muscles and the variability of the EMG patterns.

If normalization methods have been largely investigated in the literature (Burden and Bartlett, 1999; Burden et al., 2003; Hunter et al., 2002; Soderberg and Knutson, 2000), few studies have addressed the issue of the choice of normalization procedures to study the influence of task conditions on the activation of the lower limb muscles during bicycling at submaximal intensities. The joint angle(s) and/or muscle length(s) have to be controlled when using isometric contractions whereas the range of joint angle. muscle length, velocity of shortening/elongation and load applied have to be standardized for dynamic contractions. The normalization procedure the most frequently employed to analyze the neural drive during cycling exercise consists into a series of IMVC performed off (Ericson et al., 1985; Marsh and Martin, 1995; Neptune et al., 1997) or even on the cycle ergometer (Hunter et al., 2002). However, the use of IMVC method to study muscle activation during cycling is questionable. It is not obvious that the reference EMG signals collected during IMVC tests can be used to represent the maximal neural drive during cycling. Besides, testing separately the muscles in tasks and positions that differed from pedaling movement is time and energy-consuming (Hsu et al., 2006).

In this study, it was hypothesized that all-out torquevelocity (T–V) test performed on a bicycle-ergometer is an alternative normalization method that can be used to measure the amplitude of the reference EMG signal corresponding to the neural drive addressed to the different lower limb muscles during a maximal pedaling exercise. This effort was selected because it offers the opportunity to measure reference EMG values within a very short time period (less than 10-s) in a standardized condition that does not require any supplementary apparatus and that ensures identical type of contraction and muscle length changes. As a consequence, it was assumed that the use of T–V normalization procedure improves the quantification of the neural drive of the lower limb muscles during cycling at submaximal intensities.

The overall aim of the present study was to validate the use of torque–velocity test performed on a bicycle-ergometer to normalize the EMG signals collected over six of the main lower limb muscles recruited during bicycling exercise. To discuss the opportunity of using T–V test as a normalization method, the ability of this later to satisfy the selection criteria retained in the literature was evaluated in comparison with the classical IMVC method.

#### 2. Methods

### 2.1. Subjects

Nine male subjects [(mean  $\pm$  SD) age 24.3  $\pm$  1.4 years, height 1.77  $\pm$  0.08 m; mass 72.2  $\pm$  4.9 kg] volunteered to participate in this experiment after reading and signing an informed consent form. The experimental design of the study was approved by the

Ethical Committee of the Lyon University Hospital. All participants regularly trained in various sport activities.

#### 2.2. EMG data acquisition and processing

Electromyographic activity from six muscles of the right lower limb was continuously monitored [gluteus maximus (GMAX), vastus lateralis (VL), rectus femoris (RF), biceps femoris (long head), gastrocnemius medialis (GAS), and soleus (SOL)]. These muscles were selected because they are considered as the main muscles of the lower limb recruited to produce pedaling movement (Ericson et al., 1985; Hug et al., 2004; Li, 2004; MacIntosh et al., 2000; Marsh and Martin, 1995; Neptune et al., 1997; Raasch and Zajac, 1999; Raasch et al., 1997; Ryan and Gregor, 1992) (see Table 1). EMG signals were recorded using bipolar Ag-AgCl surface electrodes 10-mm in diameter, located 12-mm apart (Biochip, Emaltek S.A., Crolles, France).

The surface electrodes were aligned parallel to the muscle fibers over the belly of the muscle and positioned following the recommendations of the literature. For GMAX, electrodes were placed over the center of the muscle belly approximately 5-6 cm below its proximal origin (Lieberman et al., 2006). For BF (long head), electrodes were placed midway laterally on the posterior part of the thigh after having differentiated short and long heads by palpation of muscle contraction at the ischial attachment (Onishi et al., 2002). For RF, electrodes were placed midway along a line between the anterior superior iliac spine and superior border of the patella. For VL, electrodes were placed four fingerbreadths proximal to the superior lateral border of the patella (McHugh et al., 2002) and were oriented at 20° angle relative to a reference line drawn between the anterior superior iliac spine and superior lateral border of the patella to approximate the muscle fiber pennation angle (Fukunaga et al., 1997). For GAS and SOL, electrodes were fixed longitudinally on the distal half of the two muscles (Sanderson et al., 2006).

The skin was shaved, abraded and cleaned with alcohol swabs before placing the electrodes to improve the contact between the skin and the electrodes but also to reduce skin impedance. To reduce movement artifacts, the electrodes wires were taped to the skin and the subjects wore a skin suit. Raw EMG signals were sampled at 1000 Hz before being band-pass filtered (6–600 Hz) and differentially amplified (gain 600) at the level of the electrodes. Then EMG signals were electronically root mean squared (EMGrms) with a 25-ms moving rectangular window (536AJ, Analog Device, Norwood, MA) as proposed in a previous study (Hautier et al., 2000). In parallel to the recording of the EMG signals, the angular positions of the knee joint of the right lower limb was measured using a goniometer (Penny & Giles, M110, Blackwood, Gwent, UK) with a full range of motion of 180°.

Fig. 1. It shows the device developed to measure the right crank position during pedaling movement. A goniometer (model SEEB 502, SFERNICE, Nice, France) was positioned on a support fixed to the bicycle ergometer so that its axis of rotation (a) corresponded with the axis of rotation of the cranks (b). An aluminium stalk was welded in the pedal (c) and the displacement of the right crank was measured by the goniometer using a stiff foam device (d).

Simultaneously the angle of the right crank of the cycle ergometer (Figs. 1 and 2) was measured using a second goniometer (model SEEB 502, SFERNICE, Nice, France; accuracy =  $1^{\circ}$ ) with a full range of motion of 360°. The EMGrms signals collected over the six muscles and the signal of the two goniometers were sampled simultaneously at 200 Hz on a computer using an acquisition card (Keithley Metrabyte, type DAS8, Taunton, MA, USA). Subsequent analyses were performed with custom-written add-on software (Origin 6.1<sup>®</sup>, OriginLab, Northampton, UAS, EMG Toolbar add-on).

#### 2.3. Experimental protocol

After a standardized warm-up on the bicycle-ergometer (10 min at 100 W and 100 rpm), every subject performed two sets of tests that consisted of traditional IMVC situations and T–V test performed on a bicycle-ergometer. It was expected that the EMG values obtained during T–V test would be close or even higher than those measured during IMVC tests. The order of the

Table 1

Anatomical characteristics and functional roles of the lower limb muscles

Muscles	Muscle type	Action(s)	Testing and joint position	Attempted movement
BF	Bi-articular	Hip extension + knee flexion	Sitting (90° knee flexion + 90° hip flexion)	Knee flexion
GAS		Ankle plantar flexion + knee flexion	Prone + mid ankle position	Ankle plantar flexion
RF		Knee extension + hip flexion	Sitting (90° knee flexion + 90° hip flexion)	Knee extension
GMAX	Mono-articular	Hip extension	Prone + 45° hip flexion	Hip extension
SOL		Ankle plantar flexion	Prone + mid ankle position	Ankle plantar flexion
VL		Knee extension	Sitting (90° knee flexion + 90° hip flexion)	Knee extension

The testing conditions retained for the IMVC efforts are also described. For knee and hip angular positions, 0° is when the joints are fully extended. The mid ankle position was determined as the intermediate ankle position between maximal plantar flexion and maximal dorsiflexion positions.



Fig. 2. It illustrates the ensemble average of the changes in the knee joint angle pattern calculated over the pedaling cycle during T-V test and for three submaximal conditions of cycling. The knee joint is fully extended when angle equals  $0^{\circ}$ .

IMVC and T–V was randomized with at least a 5-min recovery period between the two methods.

### 2.3.1. IMVC tests

IMVC efforts consisted of two series of four brief maximum efforts so that each muscle group was evaluated separately in a standardized position for which the given group was maximally contracted. Subjects were positioned on an examination table to maintain constant joint angles during each isometric action and to stabilize the body during the maximal efforts. The examination table was equipped with padded cuffs attached to chains providing resistance. The length of the chains was adjusted so that the resistances were applied in standardized conditions for each muscle group. The testing positions and the movements attempted by the subjects for the IMVC tests are detailed in Table 1 and were defined according to the recommendations of the literature (Ericson et al., 1985; Hsu et al., 2006; Onishi et al., 2002). For each test, the subjects were asked to reach maximal force as soon as possible and maintaining it for a minimal 3-s period. When testing one muscle, the activation of the other muscles was not avoided. For each muscle group, two IMVC tests interspaced by a 3-min recovery period were performed. The order of the IMVC tests performed in the two testing positions was randomized. A 5-min recovery period was imposed between the IMVC of two different muscles to minimize the effects of fatigue.

#### 2.3.2. T-V bicycling test

T–V test consisted of two maximal cycling sprints performed on a bicycle ergometer (Ergomeca Sorem, Toulon, France). The subjects exerted an external force equaled to the sum of the forces required to accelerate the flywheel plus the frictional force (Arsac et al., 1996; Lakomy, 1986). The flywheel moment of inertia equaled 0.465 kg m<sup>2</sup>. Friction loads of 0.05 and 0.075 kg kg<sup>-1</sup> of body mass were imposed during the two maximal bicycling sprints by suspending calibrated masses on a counterbalanced lever arm. The use of this protocol has been demonstrated to be highly reliable to measure cycling peak power (Arsac et al., 1996; Dore et al., 2003). Posture was standardized to prevent from any influence of this parameter on muscle activation (Ericson et al., <u>1985</u>; Li, 2004) with the subjects being told to remain seated on the saddle throughout the entire sprint test. For each participant, the seat was adjusted so that the distance from the top of the saddle to the bottom dead centre position of the pedal matched 100% of the leg length, and handlebar positions were fixed to offer a comfortable position. The participant's feet were fixed on pedals with adjustable rubber straps. For each T–V test, the subjects were asked to push and pull the pedals as hard and as fast as they could to try to elicit maximal activation of both extensor and flexor muscles. The two cycling sprints were interspaced by a 5 min recovery period and the order of the sprints was randomized.

#### 2.3.3. Submaximal bicycling exercises

Each subject performed 15 times 30 s submaximal pedaling exercises on the same bicycle-ergometer, separated by at least 2min recovery period. The pedaling conditions were selected to record the EMG signals of the lower limb muscles for a large range of conditions that can be studied during submaximal pedaling exercise. The pedaling conditions investigated were composed of the combination of five pedaling rates (ranging from 50 to 150 rpm) and three friction loads (ranging from 1 to 3 kg). For each pedaling condition, the friction load was adjusted by the experimentalist prior to the start of the trial by suspending calibrated masses on a counterbalanced lever arm. Then subjects were provided with a visual feedback that permitted them to control their average pedaling rate for each pedaling cycle. Pedaling cadence was measured continuously using the goniometer positioned as shown in Fig. 1 and subjects were asked to maintain their average pedaling rate as close as possible from the target one for 30 s using the visual feedback provided by the display. After recording, goniometer's data were processed so that pedaling cycles for which the mean cadence varied for more than 3 rpm in comparison to the target one were not considered for the analysis.

### 2.4. Data analysis

#### 2.4.1. Amplitude of peak EMG during IMVC test

For each IMVC test, EMG data were collected over an 8 s period with the two first seconds corresponding to a baseline period, and the first and last seconds of the EMG burst being rejected to avoid EMG values to be affected by muscle shortening and lengthening phenomenon occurring at the onset and completion of the IMVC tests (Hsu et al., 2006). Therefore, 3–6 s time window of EMG was analyzed to calculate the peak EMGrms values (Fig. 3). The highest peak EMG amplitude of the two trials was selected as the reference value representing the maximal neural drive obtained during IMVC tests.

#### 2.4.2. Amplitude and time for peak EMG during T-V bicycling test

For each maximal cycling sprint, EMG data were collected over an 8 s period with the first second corresponding to a resting baseline. For 2–8 s time window, the EMG activity of each muscle was analyzed for 8–20 pedaling cycles (depending on the subjects and the friction load) by calculating the maximal amplitude of EMGrms obtained during the sprint (Fig. 3). For each muscle and subject, the EMG patterns were calculated over the pedaling cycle during which the maximal EMGrms value was calculated (i.e. 54 EMG patterns). The absolute values of EMGrms (in mV) were calculated for 36 crank position intervals with each interval



Fig. 3. EMGrms recorded during IMVC efforts and T-V tests from vastus lateralis (VL) and rectus femoris (RF) muscles.

consisting of  $10^{\circ}$  displacement. Then, time of maximal EMGrms was expressed as the crank angle during the pedaling cycle (in degrees with TDC corresponding to  $0^{\circ}$ ).

2.4.3. Time for peak EMG during submaximal bicycling exercises

For each submaximal bicycling condition, recordings lasted for more than 15 pedal revolutions. Average patterns were calculated from 10 consecutive pedaling cycles to describe the changes in knee joint angle and EMG activity of the six muscles. The values of absolute EMGrms (in mV) were averaged for 36 crank position intervals with each interval consisting of 10° displacement (Fig. 4). We calculated average EMG patterns for each of the nine subjects, the six muscles and the 15 submaximal exercise conditions (i.e. 810 EMG patterns). For three submaximal bicycling conditions (50 W at 50 rpm-200 W at 100 rpm and 450 W at 150 rpm), time of maximal EMGrms was expressed as the crank angle during the pedaling cycle. Finally, the EMGrms values (in mV) calculated for the different muscles, crank position intervals and exercise conditions were expressed as a percentage of the EMG reference values obtained from IMVC and T-V normalization procedures, respectively (Fig. 5).

## 2.4.4. Knee joint angles during T-V test and submaximal bicycling exercises

The signals of the goniometers measuring the position of knee joint were computed so that the average patterns of knee joint angle were calculated from 10 consecutive pedaling cycles for submaximal bicycling exercises and for the pedaling cycle for which maximal EMGrms value was obtained during T–V test. The mean values of the knee joint angle (in degrees) were calculated for 36 crank position intervals with each interval consisting of 10° displacement (Fig. 2). Maximum, minimum and ranges of knee joint angles were computed for three submaximal bicycling conditions (50 W at 50 rpm–200 W at 100 rpm and 450 W at 150 rpm) and T–V bicycling test for statistical analysis.

#### 2.4.5. Inter-individual variability of the EMG patterns

A variance ratio (VR in Eq. (1)) was calculated to determine the inter-individual variability of the EMG patterns obtained for each muscle and submaximal bicycling condition when using IMVC and T–V normalization procedures, respectively. The use of VR has been previously proposed in the literature (Burden et al., 2003; Hershler and Milner, 1978).

$$\mathbf{VR} = \frac{\sum_{i=1}^{k} \sum_{j=1}^{n} (X_{ij} - \overline{X}_i)^2 / k(n-1)}{\sum_{i=1}^{k} \sum_{j=1}^{n} (X_{ij} - \overline{X}_i)^2 / (kn-1)}$$
(1)

$$\overline{X} = \frac{1}{k} \sum_{i=1}^{k} \overline{X}_i \tag{2}$$

where k is the number of intervals (i.e. 36) considered over a crank revolution, n is the number of participants (i.e. 9),  $X_{ij}$  is the normalized EMGrms value at the *i*th interval for the *j*th participant, and  $\overline{X}_i$  is the mean of the normalized EMGrms values obtained at the *i*th interval calculated over the nine participants.  $\overline{X}$  is the mean of the normalized EMGrms values calculated as reported by Eq. (2).

#### 2.5. Statistics

Different statistical procedures were used to compare the ability of the IMVC and T-V methods to satisfy the selection criteria of a normalization procedure. The repeatability of the measurements of the amplitude of the peak EMG was evaluated by comparing the values of the coefficients of variation (calculated for each muscle and method from the data obtained from the two trials) using paired *t*-tests for each muscle separately. Analysis of the reliability of EMG measurements during T-V test was evaluated by applying the procedures proposed by Bland and Altman (1986). For this analysis, the mean difference and SD of the differences between the maximal values of RMS obtained during IMVC and T-V normalization procedures were calculated. The data were shown graphically comparing the difference between the two tests against their average value in mV. Relevance of the measurements of the amplitude of peak EMG was evaluated by determining if the time for peak EMG and the knee joint kinematics are influenced by pedaling conditions using a one-factor (pedaling condition) ANOVA with repeated measures for the different dependent variables considered. Beforehand, Kolmogorov-Smirnov test was employed to



Fig. 4. Examples of individual EMG patterns obtained for the six lower limb muscles during three submaximal bicycling exercises and for T-V bicycling test. The curves obtained for the submaximal intensities illustrate average EMG patterns calculated from 10 consecutive crank cycles. The T-V test curve has been drawn from the data obtained over the pedalling cycle for which peak EMG amplitude was measured.

check the normality of the data. Finally, the amplitude of the peak EMG and the inter-individual variability of the EMG patterns (VR values) obtained for each muscle and each method

(IMVC and T–V) were compared using paired *t*-tests. The significance level for all statistical tests was set at  $p \le 0.05$  and all data expressed as mean  $\pm$  SD.



Fig. 5. Inter-individual ensemble average ( $\pm$ SD) of EMG patterns from gluteus maximus (GMAX) and biceps femoris (BF) when pedaling at 100 rpm and 300 W using, respectively, IMVC and T–V normalization methods. The right crank arm is vertical when angles equal 0° (top dead centre) and 180° (bottom dead centre).

### 3. Results

3.1. Repeatability and reliability of the peak EMG measurements during T-V test

As shown in Table 2, the repeatability of the measurement of the absolute amplitude of peak EMG using T-V

and IMVC is quite low (i.e. CV values ranging from 8% to 23%). The coefficients of variation calculated from the data obtained at the two trials did not differ significantly between the two methods for any of the six muscles considered in the study. For T–V test, the amplitude of the peak EMG of RF, BF and SOL did not differ between the two friction loads used. The peak EMG of GMAX and GAS

Table 2

Presentation of the amplitude of the reference values (peak EMGrms), the repeatability of the measurements (CV) measured with IMVC and T-V tests

Criterion Variable	Amplitude Peak EMGrms (mV)			Repeatability CV (%)	
Normalization method	IMVC	T–V 0.05	T-V 0.075	IMVC	T–V
BF	$104\pm26$	$79\pm32$	$72\pm33$	$10\pm7^{ m d}$	$19\pm31$
GAS	$79\pm27$	$88\pm29^{ m b}$	$78\pm31$	$17 \pm 20$	$8\pm8$
RF	$89 \pm 43$	$71\pm28$	$70\pm37$	$18 \pm 16$	$18\pm18$
GMAX	$45\pm23$	$69 \pm 41^{\mathrm{b}}$	$52\pm21$	$23\pm25$	$20 \pm 12$
SOL	$51\pm28$	$64 \pm 13$	$67 \pm 16$	$16 \pm 13$	$12 \pm 14$
VL	$50\pm17^{\mathrm{a}}$	$85\pm24$	$94\pm25^{ m c}$	$19\pm18$	$9\pm9$

<sup>a</sup> When peak EMG is significantly lower when measured during IMVC test (p < 0.05).

<sup>b</sup> When peak EMG is significantly higher when measured during T–V at 0.05 kg kg<sup>-1</sup> of body mass (p < 0.05).

<sup>c</sup> When peak EMG is significantly higher when using T–V at 0.075 kg kg<sup>-1</sup> of body mass (p < 0.05).

<sup>d</sup> When CV is significantly higher when measured during T–V (p < 0.05).

was higher when T–V test was performed against the lowest friction load (0.05 kg kg<sup>-1</sup> of body mass; p < 0.05) whereas peak EMG of VL was more important when using the highest friction load (0.075 kg kg<sup>-1</sup> of body mass; p < 0.05). Bland and Altman procedure showed a good reliability for the absolute amplitude of the peak EMG obtained during T–V test compared with IMVC method for all the lower limb muscles investigated (Fig. 6).

# 3.2. Relevance of the peak EMG measurements during T-V test

As reported by Table 3, the time for peak EMG did not change within the pedaling conditions selected for the analysis for RF (p = 0.44); VL (p = 0.71); BF (p = 0.17); and SOL (p = 0.11). For GMAX (p < 0.05) and GAS (p < 0.05), the peak EMG amplitude shifted earlier in the pedaling cycle when pedaling cadence and external power output increased. The graphical (Fig. 2) and statistical analysis of the kinematics of the knee joint indicated that it was more extended near bottom dead center during T–V test compared to the other pedaling conditions ( $39 \pm 9^{\circ}$  for



Fig. 6. Bland and Altman procedures applied to peak EMG values recorded during T–V bicycling test and IMVC tests for the six lower limb muscles. Muscle plots: GMAX (yellow), RF (green), VL (red), BF (blue), GAS (purple), SOL (pink). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3

Crank position over the pedaling cycle, expressed in degrees, at which peak EMG was observed for each muscle at three submaximal intensities and during T-V test

Pedaling condition	50 W– 50 rpm	200 W– 100 rpm	450 W– 150 rpm	T-V test
BF	$140\pm31$	$130\pm33$	$109\pm15$	$120 \pm 21$
GAS <sup>a</sup>	$158\pm49$	$126\pm13$	$119\pm9$	$108\pm36$
RF	$331\pm29$	$321\pm26$	$302\pm30$	$326\pm37$
<b>GMAX</b> <sup>a</sup>	$112 \pm 31$	$93\pm39$	$41 \pm 46$	$26\pm18$
SOL	$124\pm36$	$108\pm29$	$101\pm 30$	$91\pm27$
VL	$37\pm 36$	$34\pm32$	$20\pm25$	$28\pm19$

<sup>a</sup> When crank position at peak EMG varies significantly with pedaling condition (p < 0.05).

#### Table 4

Presentation of the inter-individual variability (VR) of EMG patterns of the six muscles calculated using IMVC and T–V normalization methods from the EMG data collected for 15 submaximal pedaling conditions

Normalization method	Variance ratio			
	IMVC	T–V		
BF	$0.84\pm0.11^{\rm b}$	$1.47\pm0.47$		
GAS	$0.59\pm0.3$	$0.63\pm045$		
RF	$0.92\pm0.13^{\mathrm{b}}$	$1.21\pm0.20$		
GMAX	$1.09\pm0.04$	$0.33\pm0.16^{\rm a}$		
SOL	$1.00\pm0.07$	$0.07\pm0.03^{\rm a}$		
VL	$0.64\pm0.14$	$0.07\pm0.02^{\rm a}$		

<sup>a</sup> When VR value of EMG patterns is significantly lower when using T–V normalization (p < 0.05).

<sup>b</sup> When VR value of EMG patterns is significantly higher when using T–V normalization (p < 0.05).

the three submaximal conditions vs.  $28 \pm 13^{\circ}$  for T–V test; p < 0.05). The knee joint position  $(103 \pm 7^{\circ})$  was not changed at top dead center (p = 0.13). As a consequence, the range of motion of the knee joint was higher when cycling at 450 W and for T–V test ( $64 \pm 5^{\circ}$  for the other two submaximal conditions vs.  $77 \pm 6^{\circ}$  for T–V test; p < 0.05).

# 3.3. Amplitude of the peak EMG and inter-individual variability of the EMG patterns

Typical EMG signals collected during IMVC and T-V normalization procedures for VL are presented in Fig. 3. The statistical analysis showed that the peak EMG amplitudes measured did not differ significantly (p > 0.05 for the six muscles) between the two T-V test trials (performed, respectively, against friction loads of 0.05 and  $0.075 \text{ kg kg}^{-1}$  of body mass). Besides, the statistical analysis shows that the peak EMG amplitude obtained during T-V test for VL was significantly higher than that measured using IMVC (p < 0.05; see Table 2 and Fig. 3). However, no significant differences were observed between the peak EMG obtained during IMVC and T-V tests for BF (p = 0.08), GAS (p = 0.39), GMAX (p = 0.16), RF (p = 0.13) and SOL (p = 0.11). Examples of the ensemble average EMG patterns (in % of peak EMG amplitude) obtained for GMAX and BF when using the two normalization procedures are presented in Fig. 5. The VR values calculated from the EMG signals collected for the 15 bicycling conditions using IMVC and T-V methods revealed that inter-individual variability of the EMG patterns differed according to the normalization method used for all the muscles except for GAS. Table 4 reported that the inter-individual variability of the EMG patterns of GMAX, VL, and SOL was lower whereas VR values calculated for RF and BF were higher when using T-V test when using T–V normalization method (p < 0.05).

#### 4. Discussion

The possibility to normalize EMG using T-V tests in order to study the influence of the pedaling conditions on

muscle activation during submaximal bicycling exercise is discussed according to the different criteria retained in the literature (<u>Burden et al., 2003;</u> <u>Hunter et al., 2002;</u> Yang and Winter, 1984).

# 4.1. Repeatability and reliability of the peak EMG measurements during T-V test

The data obtained in this study indicated that the repeatability of the measurements of peak EMG amplitude of the six lower limb muscles is comparable when using IMVC and T-V normalization procedures. For the two methods, the differences in the absolute amplitude of the peak EMG observed between the two trials may be due to modifications in the motivation status (Soderberg and Knutson, 2000) that can be responsible for a change in the number of motor units that are recruited. For T-V test, the difference in the external resistance used for the two maximal sprints is not at the origin of supplementary variations in the amplitude of the peak EMG signals recorded. The EMG data obtained during T-V test are in line with those reported in the literature during pedaling exercises performed at submaximal intensities. First, the reproducibility of the measurement of peak EMG of BF is significantly lower during T–V test than for IMVC test (Table 2). Numerous authors (Hug et al., 2004; Laplaud et al., 2006; Ryan and Gregor, 1992) had previously reported that the EMG patterns of this bi-articular thigh muscle can vary considerably between the subjects during submaximal cycling exercises, especially at high pedaling rates. In line with Dorel et al. (2003), there is no influence of the external resistance on the peak EMG of BF during maximal cycling sprint. However, our data suggest that the activation level of some lower limb muscles change within efforts of maximal intensity depending on the combination between force, velocity and external power parameters. If such EMG activity changes are often described for pedaling exercises performed at submaximal intensities (MacIntosh et al., 2000; Marsh and Martin, 1995; Neptune et al., 1997), data are missing in the literature to describe the influence of the pedaling conditions on the neural drive during maximal cycling sprints. In this context, T-V test was proposed because it offers the opportunity to maximize the chance of measuring EMG signals of maximal amplitude when the neural drive is maximal. The data obtained in this study (Table 2) indicate that the peak EMG of GMAX and GAS muscles is higher when sprints are performed against a low friction load (0.05 kg kg<sup>-1</sup> body mass) whereas the peak EMG of VL is higher when a moderate friction load is used  $(0.075 \text{ kg kg}^{-1} \text{ body mass})$ . The data of the literature suggest that the differences in the absolute amplitude of the peak EMG may be due to physiological factors and/or anatomical constraints. To explain the differences in the peak EMG of GMAX and GAS, it is worth noting that the average muscle fiber conduction velocity increases

with pedaling rate (Farina et al., 2004a) and that this physiological factor influences the amplitude of the EMG signal (Farina et al., 2004b). Concerning VL muscle, one can assume that the neural drive addressed to this muscle may be limited at high pedaling rates (when low external resistance is employed during maximal sprint) in order to avoid any hyper-extension at the knee joint (van Ingen Schenau, 1989). Such a reduction in the number of motor units recruited may explain the differences in the peak EMG of VL observed between the two maximal cycling sprints (Farina et al., 2004b). Finally, the data of this study indicate that supplementary data have to be obtained to better describe the influence of the exercise conditions on the activation of the lower limb muscles during maximal cycling sprint.

# 4.2. Relevance and feasibility of the peak EMG measurements during T-V test

According to the relevance criterion, the choice of a maximal bicycling exercise is first justified by the fact that the contribution of the muscles of the lower limb are comparable for maximal sprint and submaximal bicycling conditions (Raasch et al., 1997). In line with the findings of previous studies (Marsh and Martin, 1995; Raasch et al., 1997), our data indicate that the time of peak EMG of GAS and GMAX shifted earlier when pedaling cadence is increased (Table 3). A comparable trend was observed for the other muscles even if it was not statistically significant. On a physiological point of view, it has been demonstrated that the phase advance in the activation of the different lower limb muscles is at least partly due to activation dynamics (Li, 2004). Some authors reported that the shift in the EMG bursts is identical for all muscles and concluded that there is a centrally generated primitive for pedaling movement (Raasch et al., 1997). In this study, we observed that the shift in the timing for peak EMG amplitude vary from one muscle to another (Table 3). Sensorimotor control mechanisms may induce some little changes in the neuromuscular activation strategy according to the modifications in the pedaling conditions (force, velocity and power). This was illustrated by some changes in the kinematics of the knee joint at bottom dead center, the knee joint being more extended at  $\sim 180^\circ$  crank position when pedaling at 450 W (and 150 rpm) and during T-V bicycling test (Fig. 2). One can assume that subjects did not remain seated and/or the range of motion of ankle joint is changed when high pedaling cadence and/or external power output are achieved. However, the analysis of the timing of activation (Table 3 and Fig. 4) supports the hypothesis that the biomechanical functions of the different lower limb muscles do not change when pedaling rate and external power output conditions vary (Raasch and Zajac, 1999). Because the ability of human to maximally activate a muscle or a muscular group is posture and task specific (Lehman and McGill, 1999),

one can consider that the use of T-V bicycling test is justified on a physiological point of view. Using T-V bicycling test, the activity of each muscle is calibrated in reference to the maximal activity obtained when its activation is regulated by identical afferent feedback and neural processing. At first, GMAX and VL are activated during the downstroke phase to deliver mechanical energy to the limb and the crank while they are shortening (van Ingen Schenau, 1989; van Ingen Schenau et al., 1992). Second, the activation of RF and BF is regulated so that these muscles transfer mechanical energy between the crank and the limb to ensure the smoothness of the pedaling movement over stroke transitions (Raasch and Zajac, 1999; Raasch et al., 1997). Third, GAS and SOL are activated during the downstroke phase to transfer the energy generated by GMAX and VL at the limb to the crank (Raasch and Zajac, 1999; Raasch et al., 1997). As a consequence, the biomechanical function and the regulation of the activation of the lower limb muscles (i.e. especially that of bi-articular) differ strongly between IMVC tests and pedaling movement whereas it is comparable between T-V tests and submaximal bicycling exercises.

During pedaling movement, the lower limb muscles move with respect to the recording electrodes due to changes in the joints angles so that the amplitude of EMG signals is affected by non-physiological factors (Farina et al., 2004b; Rainoldi et al., 2000). The use of T-V test permits to limit the impact of the displacement of the segments of the lower limb on the EMG signals because this later is close from that observed for submaximal bicycling exercises. Indeed, one can consider that the changes in the knee joint position at bottom dead center when pedaling at high intensity exercises (450 W and T-V test) had a limited impact on the muscle length and the displacement of the muscles with respect to the electrodes. Furthermore, because the muscles are activated at the same part of the pedaling cycle for the different pedaling conditions (Table 3 and Fig. 4), the EMG signals were obtained for comparable joint angle and/or muscle length so that the amount of muscle tissue in the pickup volume of the electrodes was similar. As a consequence, the use of T-V bicycling test permits to avoid the impact of geometrical factors on the EMG signals collected (Farina et al., 2004b; Onishi et al., 2002; Pincivero et al., 2004; Rainoldi et al., 2000). However, it is noteworthy that the reference EMG signals collected during T-V test are obtained for a given force- and power-pedaling rate combination so that it is not possible to avoid the effect of this parameter on the amplitude of the EMG activity (Farina et al., 2004a). This may explain the differences in the peak EMG of GMAX, GAS and VL muscles observed between the two maximal sprints. Nevertheless, the use of T-V test was considered as the best solution to allow all the mono- and bi-articular muscles to be recruited maximally. T-V bicycling tests against braking forces lower than 10% of body weight were chosen because the power output by the subjects is maximal (Dore et al., 2003) for these pedaling conditions. It was assumed that the power produced and transmitted by mono- and bi-articular muscles is maximal (van Ingen Schenau, 1989; van Ingen Schenau et al., 1992) during this maximal cycling effort even if it is measured at different force- and power-pedaling rate combinations. To conclude, T–V test is recommended because it maximizes the chances of measuring the EMG activity reflecting the maximal neural drive of the different lower limb muscles in bicycling within a short time period (less than 8 s).

# 4.3. Amplitude of the peak EMG and inter-individual variability of the EMG patterns

Different studies focused on the level of activation of the lower limb muscles (Li, 2004; MacIntosh et al., 2000; Neptune et al., 1997; Raasch et al., 1997; van Ingen Schenau et al., 1992) and the inter-individual variability of the EMG patterns within groups including cyclists and/or non-cyclists (Hug et al., 2004; Laplaud et al., 2006; Takaishi et al., 1998). The data collected by these authors demonstrated that the activation level of the muscles changes depending on the pedaling condition and that the nervous system has multiple ways of accomplishing pedaling movement. Because the variability of the EMG patterns depends on the differences in amplitude and timing of the EMG signals collected over the muscles, it is particularly important to avoid the impact of non-physiological factors on the amplitude of the EMG signals (Farina et al., 2002b). By limiting the impact of these factors, one can assume that it is possible to better quantify the variations in the neural drive addressed to the muscles during submaximal cycling exercises. In this study, it was assumed that the interindividual variability of the EMG patterns of the lower limb muscles could be modified by limiting the impact of the non-physiological factors on the amplitude of the EMG signals using T-V test as a normalization procedure. As shown in Tables 2 and 4, our data support this hypothesis.

In line with the data reported by Hautier et al. (2000), our results showed that the amplitude of the peak EMG of VL (p < 0.001; Table 2) was significantly higher during maximal cycling sprints compared to IMVC efforts. The difference in the peak EMG amplitudes measured during T–V and IMVC tests is too high (99 ± 43%) to be totally explained by the absence of specific training (i.e. responsible for a deficit ranging from 20% to 40%). Besides, one can observe that the inter-individual variability of the EMG patterns of this muscle is considerably decreased when eliminating the impact of non-physiological factors on the amplitude of the EMG signals (VR value divided by 8).

For RF muscle, the data of this study are in line with previous results showing that the EMG measured during maximal pedaling sprints performed against low and moderate friction loads is comparable with that collected for IMVC tests (Hautier et al., 2000). Referring to the data of Hunter et al. (2002), it is worth noting that maximal pedaling efforts performed against very high friction loads may not be selected to record the peak EMG of RF. A force inhibiting neural mechanism could reduce the voluntary neural drive to this muscle when the external resistance applied is very large (Aagaard et al., 2000). For RF, the non-physiological factors influence the relative amplitude of the EMG signals so that it partly hides the variability in the activation of this bi-articular muscle (VR value multiplied by 1.3). For this muscle, the variability in the neural drive may be underestimated when using IMVC normalization procedure.

For GMAX muscle, the peak EMG measured during T– V and IMVC tests are not significantly different (p = 0.16). However, the use of T–V test permits to reduce considerably the inter-individual variability of the EMG patterns calculated for this muscle from the data obtained during submaximal exercises (VR value divided by 3). In line with the data obtained for the other main power producer muscle in cycling (i.e. VL), the variability in the neural drive is likely to be overestimated when using IMVC tests because of the inability of this normalization procedure to eliminate the impact of non-physiological factors on the amplitude of EMG signals.

Concerning BF, the peak EMG levels measured during T-V tests tended to be lower than those obtained during IMVC efforts (p = 0.08). According to Takaishi et al. (1998), one can assume that the neural drive addressed to BF muscle during maximal sprint cycling is not maximal for non-cyclist subjects. Furthermore, the reproducibility of the peak EMG of this bi-articular muscle is low for T-V test (Table 2), suggesting that the neural drive addressed to BF strongly varies between two maximal cycling sprints. The data obtained using T-V test as a normalization procedure (VR value multiplied by 1.8) indicate that further studies are necessary to describe the inter-individual variability of the EMG patterns of BF muscle in cycling. For this bi-articular muscle, the variability of the EMG patterns may be underestimated when using the classical IMVC method.

For plantar flexor muscles, the peak EMG amplitude of GAS and SOL muscles did not differ between T–V and IMVC efforts (Table 2). This result was not really surprising because it has been demonstrated that the period of activation of plantar flexor muscles coincides with modest muscle shortening (Sanderson et al., 2006) and the amplitude of the EMG signal recorded over these muscles does not vary significantly with the position of the ankle joint (Miyamoto and Oda, 2003). So, the peak EMG amplitude of GAS and SOL can be measured during IMVC at an ankle position that does not correspond exactly with the one for which these muscles are activated during cycling. The impact of the normalization procedure on the variability of the EMG patterns differs between GAS and SOL

muscles. Whereas the inter-individual variability of the EMG patterns of GAS muscle does not differ significantly between the two normalization procedures, the use of T-V test results into a very large reduction in the VR for SOL (values divided by 13).

The data obtained in this study indicate that the variability of the EMG patterns may be underestimated for bi-articular muscles (except GAS) whereas it is likely to be overestimated for mono-articular muscles when using the classical IMVC normalization procedure. However, it is worth noting that a limit inherent to the use of normalization procedures such as T-V and IMVC tests is that one cannot ensure that the amplitude of the EMG signal reflect the maximal drive that can be theoretically addressed to the muscle. Finally, to our point of view, the use of T-V test as a normalization procedure offers the opportunity to estimate the variability in the ratio between the number of motor units recruited for submaximal bicycling exercises and during maximal cycling effort so to improve the analysis of the EMG signals into a framework of physiological significance.

### 5. Conclusion

The results of this study demonstrated the repeatability, the reliability, the relevance and the feasibility of the measurement of peak EMG amplitude of six lower limb muscles during T–V cycling test. Furthermore, the use of T– V test as a normalization procedure offers the opportunity to the researchers to quantify the impact of the only physiological factors on the inter-individual variability of the EMG patterns of the lower limb muscles during submaximal bicycling exercises. Referring to these elements, this alternative EMG normalization method seems to provide meaningful EMG reference values that have to be selected to study the activation of the lower limb muscles during submaximal cycling exercises.

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