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A. Ramírez, J. Grasa, A. Alonso, F. Soteras, R. Osta, et al.. Active response of skeletal muscle: n Vivo experimental results and model formulation. *Journal of Theoretical Biology*, 2010, 267 (4), pp.546. 10.1016/j.jtbi.2010.09.018 . hal-00637811

**HAL Id: hal-00637811**

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# Author's Accepted Manuscript

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PII: S0022-5193(10)00492-3  
DOI: doi:10.1016/j.jtbi.2010.09.018  
Reference: YJTBI6158



[www.elsevier.com/locate/jtbi](http://www.elsevier.com/locate/jtbi)

To appear in: *Journal of Theoretical Biology*

Received date: 12 April 2010  
Revised date: 9 September 2010  
Accepted date: 10 September 2010

Cite this article as: A. Ramírez, J. Grasa, A. Alonso, F. Soteras, R. Osta, M.J. Muñoz and B. Calvo, Active response of skeletal muscle: *I n Vivo* experimental results and model formulation, *Journal of Theoretical Biology*, doi:[10.1016/j.jtbi.2010.09.018](https://doi.org/10.1016/j.jtbi.2010.09.018)

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# Active response of skeletal muscle: *In Vivo* experimental results and model formulation

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## Abstract

The contractile properties of the Tibial Anterior (TA) of Wistar rats were measured by means of a multipurpose testing machine. The muscle was isolated from the connective tissues, preserving the proximal insertion. The distal tendon was transected and fixed to the machine actuator. The leg was immobilized using a pin drilled through the femoral condyle. In this way the force response was studied *in vivo* at different constant lengths for some voltages and frequencies. Mathematical functions are proposed for adjusting the force-length, force-frequency and force-time relations. The model includes a novel formulation for the depression response during muscle tetanization.

*Key words:* Active muscle test, force-length relationship, force-frequency relationship, sag phenomena, muscle mathematical model.

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## 1 Introduction

Skeletal muscle is responsible for force generation. Its performance is governed by contextual properties related to aspects such as muscle position and function in the body, and by intrinsic properties defined exclusively at the molecular, subsarcomeric or sarcomere level of the muscle fibre (Huijing, 1998).

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The effect of the intrinsic properties of muscle in generating force has frequently been studied experimentally (Edman and Reggiani, 1984; Zajac, 1989; Baratta and Solomonow, 1990). For instance, the influence of fibre stretch has been measured in the whole muscle (Abbott and Aubert, 1952; Davis et al., 2003), or bundles of muscle fibres (Gordon et al., 1966b; Bagni et al., 1999). Fibre size and its distribution inside the muscle has been studied both histologically and morphologically (Burke et al., 1973; Barker et al., 1992) and has been used to measure muscle power and muscle fatigue (Gorgey et al., 2009). Moreover, the excitation contraction process also depends on properties such as the action potential impulse (Herbert and Gandevia, 1995; Major and Jones, 2005) and propagation through the muscle cell (Arabadzhev et al., 2008), calcium diffusion in the sarcolemma (Cannell and Allen, 1984; Konishi and Watanabe, 1998), and the actin-myosin coupling for the force production (Huxley, 1957). Most of these studies were elaborated by isolating muscle fibers in controlled experimental conditions and they allowed a set of well known micromechanical properties of the tissue to be obtained.

Moreover, electrical stimulation of nerves can be used to study muscle force production from a macroscopic point of view. For instance, nerve stimulation has been applied to study muscle response during a time interval for different stimuli frequencies (Gilson, 1952; Walker, 1976; Stein and Parmiggiani, 1981; Davis et al., 2003). Thus, electrical stimulus is commonly used as a medical protocol in case of nerve muscle disfunction (Gordon et al., 2009; Roig and Reid, 2009). The force-time response can also be used as a criterion for classifying muscle physiological properties and to define its sensitivity to fatigue (Burke et al., 1973). This sensitivity could be studied using the decrease in the plateau force during time (sag phenomena (Parmiggiani and Stein, 1981)).

Biomechanical models which take into account the multiple factors involved in muscle behaviour have been derived from experimental measurements. These models have been implemented computationally for different coupling relationships to develop more realistic simulations and obtain robust predictions. The goodness of these models depends on both the mathematical formulation and the accuracy of the experimental parameters. Various unidimensional models have been implemented for specific stages of the excitation contraction coupling process. For instance, a mechanical model for the muscle-tendon unit was created by Hill (1938), improved by Hatze (1978) and applied by Zajac (1989). One of the most comprehensive phenomenological models of the electrical signal and the mechanical response was developed by Fuglevand et al. (1993). Although this model includes signal driving, firing times and fibre recruitment, the sag phenomena is not represented (Stein and Parmiggiani, 1981).

Despite all these studies, these models do not account for mechanical equilibrium in the muscle and the available data for completely characterizing the three-dimensional material properties of skeletal muscle tissue are still sparse (Odegard et al., 2008).

In a previous work by the authors, Calvo et al. (2010), the passive behaviour of the tibialis anterior (TA) muscle of Wistar rats was presented. That work was the first step in the process of developing a validated 3D FE model of the TA capable

of simulating muscle contractions in healthy conditions. The aim of this paper is to develop a phenomenological mathematical model of the active response of the TA, fitting its parameters to experimental results. The model tries to address the deficiencies in currently available data for simulating the tissue within the scope of continuum mechanics. The active response of the muscle is characterised under isometric conditions obtained at different frequencies and voltages and at different muscle lengths. In addition, a relationship to predict the force-time response including the sag phenomena is proposed.

## 2 *In vivo* experimental study

In this section, a series of experimental tests to characterise the rat TA muscle are described. These experiments were performed to establish the relationship between the active force exerted by the whole muscle and different input factors such as muscle length, voltage ( $V$ ), frequency ( $f_r$ ) and time ( $t$ ).

### 2.1 *Experimental set-up*

This study was conducted on fifteen ( $n = 15$ ) female adult Wistar rats with body mass  $226.6 \pm 19.7g$ . The animals were maintained in a temperature controlled room ( $22 \pm 1^\circ C$ ) with 12 hour light-dark cycles and free access to water and food. The rats were anaesthetised with an intraperitoneal injection of pentobarbital sodium solution ( $30mg/kg$ ). All experiments were approved by the University of Zaragoza Ethics Committee for the use of animals in experimentation.

For the *in vivo* characterisation, a midline incision was made from the ankle to the mid-section of the thigh. The TA was released and the fascia was completely removed. The distal tendon was transected and attached to sandpaper tabs using cyanoacrylate. Two points were marked to measure muscle length before and after cutting the tendon. The sciatic nerve was exposed and an electrode coupled to it. The electrode consisted of two stainless steel pins 2 mm apart partially surrounding the nerve to maximize the contact area. The animals were placed supine on a platform and the leg was immobilised using a pin previously drilled through the femoral condyle. The muscle was aligned in the actuator direction of an INSTRON 5548 test machine equipped with a 10 N full scale load cell. The tendon was secured to the upper clamp and fixed at the physiological length when the knee and ankle were completely flexed. This set-up allowed the preservation of muscle function and blood supply.

To avoid specimen drying, an ultrasonic humidifier provided a sub-cooling steam allowing a constant temperature of  $25^\circ C$ . Displacement control tests were performed, forcing the clamps to maintain a fixed distance to ensure isometric contractions. In this position each stimulus protocol was performed using a CIBERTEC CS-20 electrical signal generator.

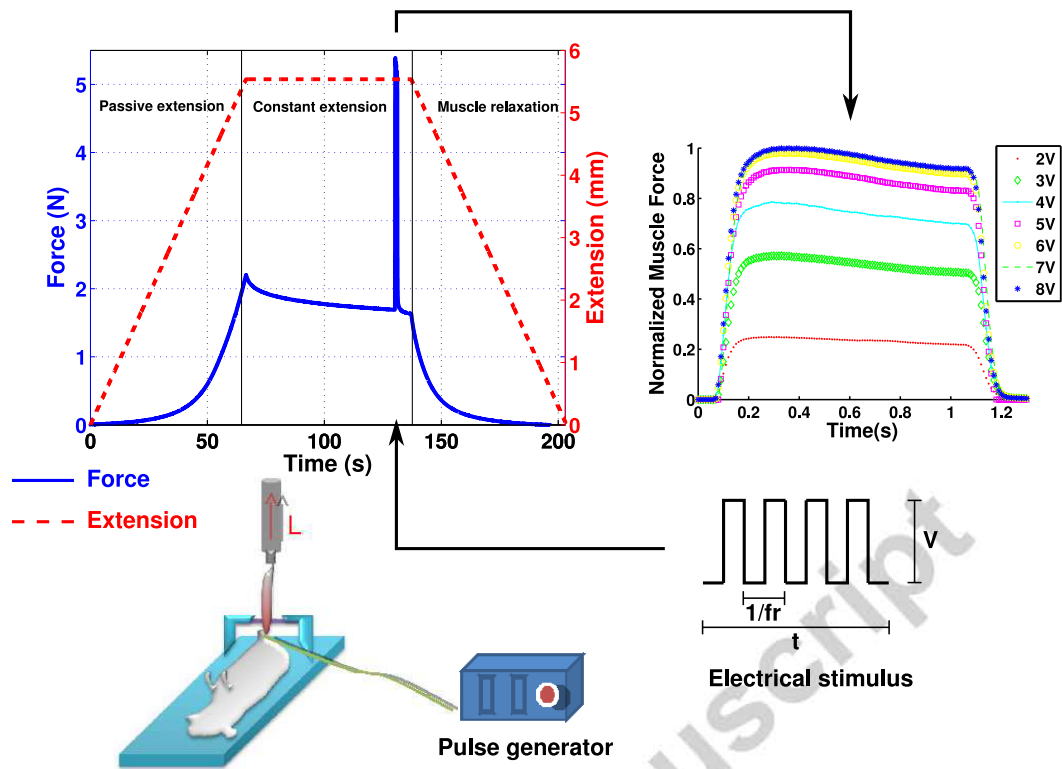


Fig. 1. Experimental Method. TA muscle was clamped to the test machine and stimulated by an electrode connected to the sciatic nerve. The signal generator was able to send pulses or train waves at different frequencies and voltage amplitudes. Results were registered as seen in the upper side of the figure, where displacement versus test time is plotted in dashed line and load in continuous line. When the muscle was stimulated, the force increased according to the electrical stimuli. The force peak is magnified to show in detail the active force for different signal amplitudes.

## 2.2 Force-stretch relationship and optimal muscle length

Firstly, an experiment was carried out to find the muscle force-length relationship. A total of 9 rats were distributed in 3 groups. The right TA muscle was tested in all the animals. For each group a different stimulus frequency (30, 60 and 90 Hz) was applied to avoid errors in the results due to fibre breakage or plastic deformation at long passive lengths. Hence, each rat was stimulated with a single frequency at different muscle lengths. These lengths were varied to subject the muscle to different stretch levels. The stretch ( $\lambda$ ) can be defined as the quotient between the final and the initial muscle length ( $L_f/L_i$ ), so the different levels vary from  $\lambda = 0.8$  to  $\lambda = 1.2$ . In order to stretch the muscle, an imposed displacement velocity of  $5 \text{ mms}^{-1}$  was applied. Once the muscle had reached its final position, it was maintained during 60 s before the stimulus was applied (see Fig. 1) in order to obtain a constant passive force without viscoelastic effects. Then the muscle was returned to its initial position at the same displacement velocity rate. The active component of total force in the muscle was obtained as the difference between force registered by the machine during stimulus and force needed to elongate the tissue before stimulus. The possible muscle fatigue during repeated tetanic contractions was avoided with 2 minutes resting time

between stimuli.

This first experiment allowed the optimum length of the muscle to be defined, described as the length where maximum twitch force is reached (Monti et al., 2003). This length coincides with the length when the knee is flexed at  $90^\circ$  (mean length  $22.32 \pm 1.29$  mm). Thus, the optimal muscle stretch ( $\lambda_{opt}$ ) was fixed at this length with  $\lambda_{opt} = 1$ .

The whole-muscle isometric tetanus was achieved with a 5 V square wave signal and 1 s duration. The maximum force generated at optimum length and 90 Hz stimulus frequency was  $7.2 \pm 0.23$  N. The force-time results for the three different frequencies and for three different stretches (optimal stretch, maximum stretch and minimum stretch) are shown in Fig. 2.

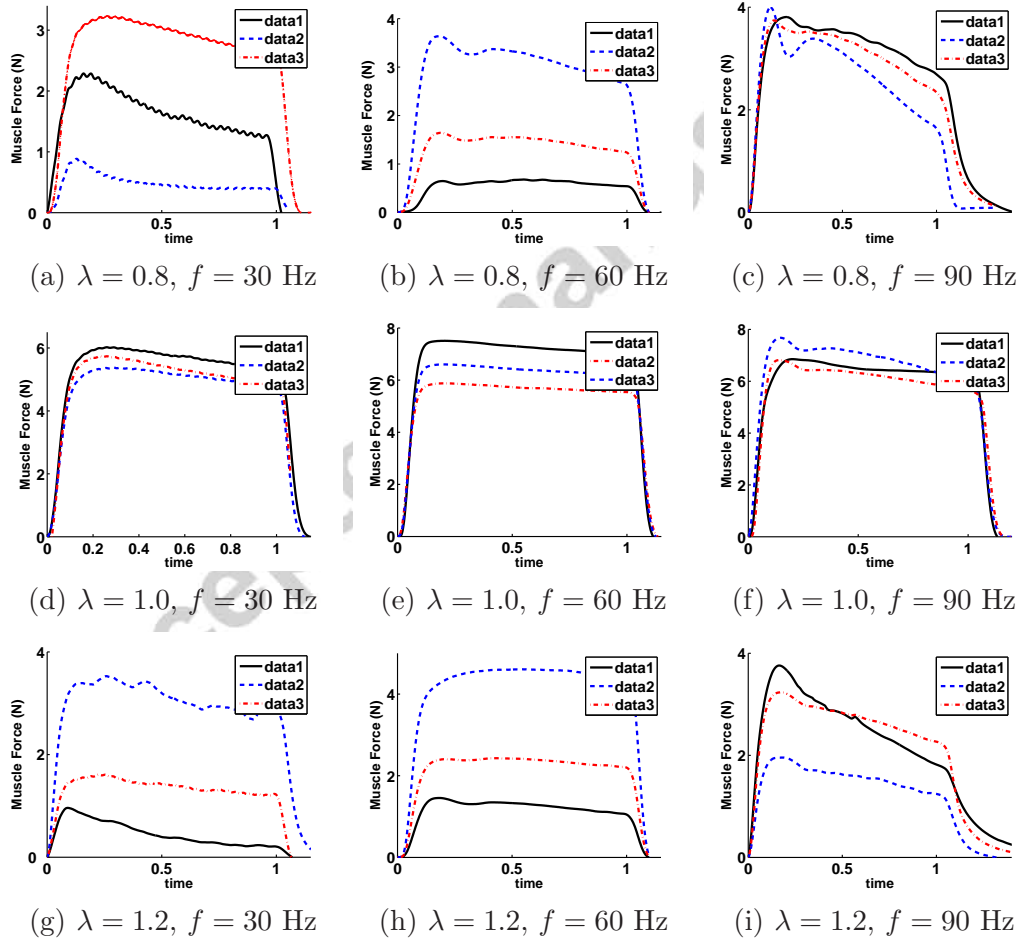


Fig. 2. Active muscle force vs time at different stretches ( $\lambda = 0.8$ ,  $\lambda = \lambda_{opt} = 1$  and  $\lambda = 1.2$ ) and frequencies (30, 60 and 90 Hz) for three different animals.

The maximum values of active muscle force registered at different stretches and frequencies were normalized for the maximum averaged value obtained at  $\lambda = 1$  and 90 Hz (7.2 N). These results are plotted for different stretches at different stimuli frequencies in Fig. 3.

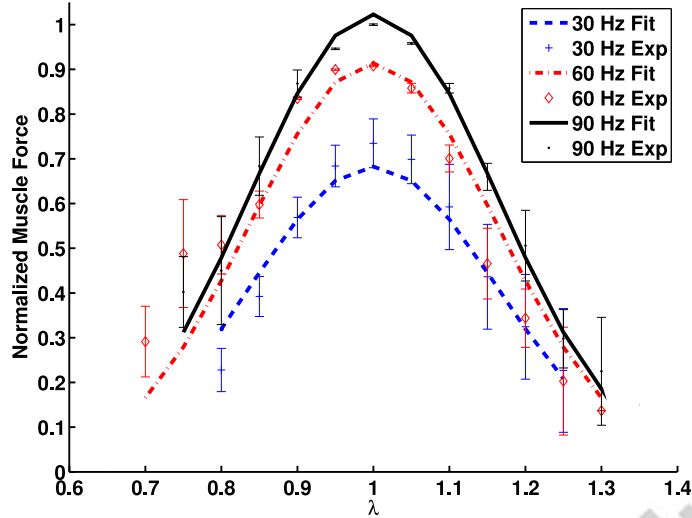


Fig. 3. Normalised muscle force-stretch curves at 30 Hz, 60 Hz and 90 Hz (mean and standard deviation of experimental force and fit data,  $\lambda = \lambda_{opt} = 1$ ).

### 2.3 Force-voltage relationship

A second experiment was performed to ensure that all muscle fibres were recruited and to determine the change in force for increasing voltages. A total of 3 right TA muscles were released and fixed to the testing machine as described above. A series of square waves of voltage amplitude varying at 1 V intervals from 2 to 8 V with a duration of 1 s were applied to the animals. Frequency and muscle length were fixed at 90 Hz and optimal length respectively. The force was normalised with the maximum force at 8V ( $8.0 \pm 0.2$  N). A Force-time curve is displayed in Fig. 4 showing an increase of the normalised force for increasing values of the input signal voltage. Although the maximum force was reached at 8 V, experiments were carried out at 5 V to avoid muscle fibrillations observed during rest periods between tests when applying higher voltages.

### 2.4 Force-time relationship

A third experiment was conducted to define the contraction time and the twitch force of the whole muscle. The nerve was stimulated with an electrical pulse of 10 ms and 5 V amplitude while the muscle was held at the optimal stretch. Different tests demonstrated the independence between twitches (mechanical response) and the duration of the pulse (electrical input signal), i.e. for the pulse duration of 1, 10, 100 or 500 ms, the amplitude and time of twitch contraction remained constant, around 1.8 N and 200 ms, respectively. The normalised mean twitch response for 3 specimens is plotted in Fig. 5.



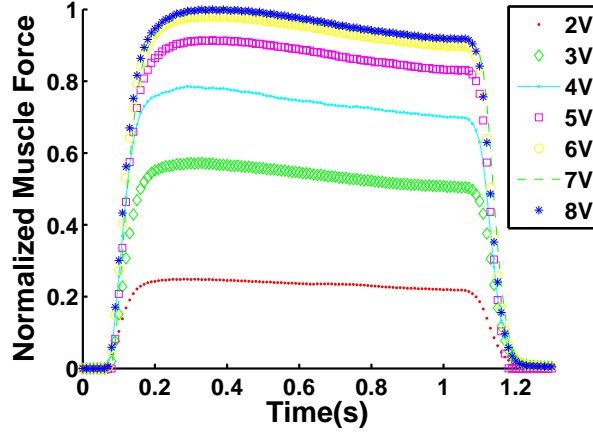


Fig. 4. Force-voltage relationship. Mean normalised muscle force at 2, 3, 4, 5, 6, 7, 8 V with a stimulus of 90 Hz during 1 s, at the optimal length. Force was normalised with a maximum force value of 8 N.

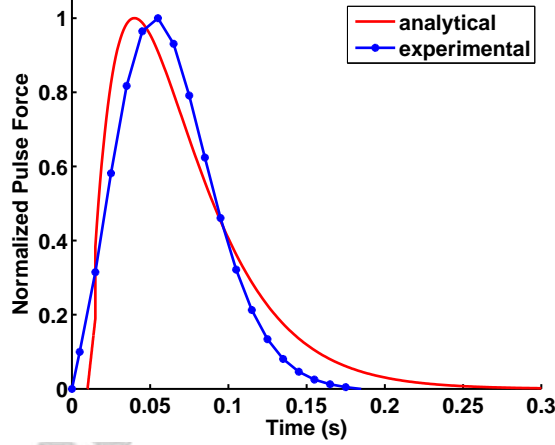


Fig. 5. Experimental versus analytical Force-time relationship. Muscle twitch normalised to maximum pulse force. The stimulus was of 5 V and 10 ms of duration.

### 3 Modelling of active muscle function

Traditionally, muscle force has been formulated in an uncoupled form as the sum of the passive and active contributions (Hill, 1938; Blemker et al., 2005; Böl and Reese, 2008). The total force could be represented in this way as:

$$F_{Muscle} = F_{passive} + F_{active} \quad (1)$$

The passive force  $F_{passive}$  is the force produced by the muscle while the tissue is passively compressed or stretched. A mathematical model for the TA passive behaviour was proposed in Calvo et al. (2010).

In this section, a one-dimensional phenomenological model for muscle force is proposed focusing on its active component and its dependence factors to fit experimental

data. The active force  $F_{active}$  produced in muscle contraction can be expressed as a series of relationships that scale the magnitude of a maximum isometric force  $F_0$  when the muscle is at the optimal length  $\lambda_{opt}$  (Zajac, 1989). The number of relationships can increase with the complexity of the model, e. g. taking into consideration other factors such as velocity or temperature. However, in this work, as all experiments were done at constant muscle length (isometric contractions) and constant room temperature, these factors have not been considered.

The model proposed here for the active isometric force is expressed as the product of the maximum isometric force  $F_0$  and adimensional factors obtained by the force-length relationship  $f_\lambda$  and the force-activation signal relationship  $f_\alpha$ . While this force is observed experimentally at the macroscopic level, with the model proposed, we are able to take into account information from both micro and macro-level. Active force can be expressed as:

$$F_{active} = F_0 f_\lambda f_\alpha \quad (2)$$

The force-activation signal relationship  $f_\alpha$ , describes the excitation-contraction coupling and depends on the electrical signal properties such as voltage  $f_V$ , and the fibres intrinsic characteristic response  $f_t$ . A multiplicative relation is considered  $f_\alpha = f_V f_t$  so:

$$F_{active} = F_0 f_\lambda f_V f_t \quad (3)$$

Results for the maximum isometric force were obtained from the experimental test described in section 2.3 ( $F_0 = 8 \pm 0.23$  N). The definition of the remaining factors and their constitutive parameters are described below.

### 3.1 Force-stretch relationship ( $f_\lambda$ )

It is known that as the muscle shortens or lengthens, moving away from its optimal stretch  $\lambda_{opt}$ , it is able to develop less force. Fig. 3 represents this effect, showing the normalized active forces for each stretch  $\lambda$  and frequency.

Several force-stretch relationships have been proposed by other authors, considering different characteristic parameters. For instance, Blemker et al. (2005) described force-stretch as a piecewise function depending on five different muscle stretches. Böl and Reese (2008) proposed a smooth function using a one dimensionless constant, namely  $\lambda_{opt}$ , which defines the optimal fibre stretch at which the sarcomere reaches its optimal length.

In this work a sigmoid function that presents a better fit to the obtained experimental data is proposed:

$$f_{\lambda} = e^{\left(\frac{-(\lambda - \lambda_{opt})^2}{2(1-\beta)^2}\right)} \quad (4)$$

where  $\lambda_{opt}$  is the optimum muscle stretch,  $\lambda$  is the actual stretch and  $\beta$  corresponds to the curvature of the sigmoid function. Fig. 3 also represents the fitting of the experimental data when using this sigmoid function. The parameter  $\lambda_{opt}$  was fixed to  $\lambda_{opt} = 1$  and  $\beta$  was set to  $\beta = 0.83616$ . Since this relation provides values for  $f_{\lambda}$  between 0 and 1, in this figure, the fitting was performed using the value of  $F_0$  proposed above and a voltage of 5V for the three different frequencies. The other terms and parameters involved in the multiplicative expression of equation (3) are described in the following sections.

### 3.2 Force-voltage relationship ( $f_V$ )

Fibre recruitment was determined by the input signal applied to the nerve as described in section 2. The voltage was increased until all the fibres were completely recruited (Fig. 4). The same stimulus current is assumed to flow from the electrode throughout all the axons in the sciatic nerve that innervate the TA.

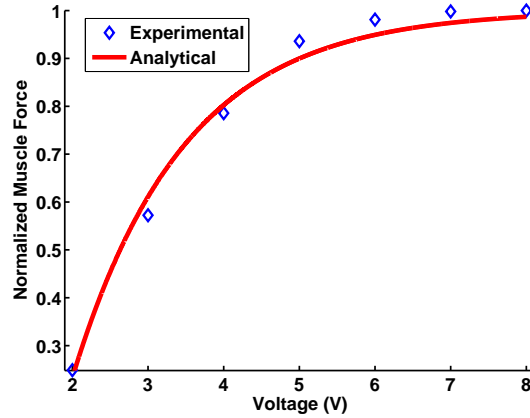


Fig. 6. Normalized force voltage relationship at optimal length and 90 Hz.

Increasing the voltage of the excitation signal produced an initial increase of the muscle force until a threshold was reached, indicating that all the motoneurons had been recruited. Thus, the force-voltage relationship proposed is:

$$f_V = 1 - e^{(a-V)/d} \quad V \geq 2 \quad (5)$$

where  $a$  is the cutting point with the  $Y$  axis and  $d$  controls the curvature. In order to best fit the experimental data (Fig. 6), these parameters were fixed at  $a = 1.609$  and  $d = 1.4737$ .

### 3.3 Force-time relationship ( $f_t$ )

If the stimulus is only a pulse, a single motor unit responds with a twitch characterised by only two parameters: the twitch amplitude or peak force  $P$ , and the contraction time  $T_c$  or time to reach the peak force (Fuglevand et al., 1993). Considering the whole muscle, an apparent contraction time  $T'_c$  must be defined.  $T'_c$  represents the time to reach the maximum force if the muscle were composed of a single fibre type. This assumption implies that the average contraction time of all the fibres is the same. The force apparent amplitude  $P'$  is defined as the peak force reached at  $T'_c$ , supposing an ideal isometric contraction. The function  $f_{Pulse}$  that represents this apparent twitch force could be expressed as:

$$f_{Pulse}(P', T'_c, t) = \frac{P't}{T'_c} e^{1 - (\frac{t}{T'_c})} \quad (6)$$

where  $P' = 0.110$  and  $T'_c = 0.04$  s were determined from the experimental data. In Fig. 5 this relationship, normalised by  $P'$ , is represented together with the experimental data obtained for the apparent muscle twitch force.

When trains of stimuli over a certain frequency are applied, motor unit twitches are added in a nonlinear way (Fuglevand et al., 1993). When pulse frequencies are high enough, a plateau zone is reached in the force time relationship. In the case that load oscillations are not discernible in the muscle force response, it is said that a fused tetanus has been reached.

Assuming the whole muscle as a unit, the total force is given by the addition of pulse responses depending on the time interval of the stimulus  $\Delta_{stim}$ . This parameter represents the time when each impulse  $n$  is triggered and is related with the inverse of the frequency (Böl and Reese, 2008). The function  $f_{train}$  that represents the sum of all motor unit twitches is:

$$f_{train} = \sum_{i=1}^n P' \frac{t - \Delta_{stim}(i)}{T'_c} e^{1 - (\frac{t - \Delta_{stim}(i)}{T'_c})} f_{fr} \quad (7)$$

where  $f_{fr}$  is:

$$f_{fr} = 1 - r e^{(-fr_{norm})/c} \quad (8)$$

$fr_{norm}$  is the product between the frequency  $fr$  and the contraction time  $T'_c$ ,  $r$  determines the ratio of twitch and tetanus forces, and  $c$  determines the rate of increase in force with increasing normalized frequency. Fig. 7 represents the normalised muscle

force versus the normalised  $f r_{norm}$ . Fitting equation (8) to the experimental data (Fig. 7) resulted in  $r = 1.0535$  and  $c = 1.1245$ .

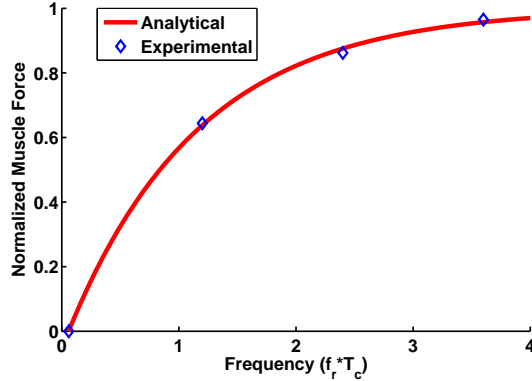


Fig. 7. Experimental versus analytical normalised force-frequency relationship at the optimal length

While most studies assume the plateau of the force-time curve to be constant (Fuglevand et al., 1993; Herbert and Gandevia, 1995), a sag phenomenon was observed here. This phenomenon can be caused by a gradual saturation of the troponin sites with the  $Ca^{2+}$  that is released by successive stimuli (Parmiggiani and Stein, 1981; Cannell and Allen, 1984).

Cannell and Allen (1984) demonstrated that the saturation in the muscle cell depends on the  $Ca - Calsequestrin$  association. The *Calsequestrin* is a protein in the sarcoplasmic reticulum with the function of storing calcium in the form of  $[Ca^{2+}] - Calsequestrin$ . The  $[Ca^{2+}]$  falls rapidly during a twitch and then recovers more slowly, principally due to release of calcium bound to the *Calsequestrin*. There is thus a slow fall in calcium bound calsequestrin during a tetanus.

This effect can be modelled with a Hill equation like relationship proposed by Konishi and Watanabe (1998) for fitting  $[Ca^{2+}]$ -force experimental data:

$$f_{Sat} = \frac{[A]^h}{[A_m]^h + [A]^h} \quad (9)$$

where  $[A]$  is the concentration of a generic substance  $A$  in the sarcoplasm. This concentration changes over time as it does in Fernandez et al. (2005). The Hill coefficient  $h$  is a measure of the slope and  $[A_m]$  is the concentration of  $A$  that gives half maximum force. Neither calcium nor any other substance concentration were measured in the experimental procedure, so defining the substance  $A$  as uniquely a calcium concentration was avoided. The time-dependent response of this substance could be expressed as (Fernandez et al., 2005):

$$[A] = [A_0] + ([A_{max}] - [A_0]) \frac{t - \Delta_{stim}(i)}{t_{[A_{max}]}} e^{(1 - (t - \Delta_{stim}(i)) / t_{[A_{max}]})} \quad (10)$$

where  $[A_0]$ , the resting concentration level, is considered for simplicity to be equal to zero,  $t_{[A_{max}]}$  is the time in which the maximum value  $[A_{max}]$  is achieved ( $t_{[A_{max}]} = 40ms$ ) and  $[A_{max}]$  is the maximum concentration of the substance is considered to vary linearly over stimulus time between the values presented in Table 1.

Fig. 8 displays the decrease of the force in the force-time relationship for the three frequencies tested.

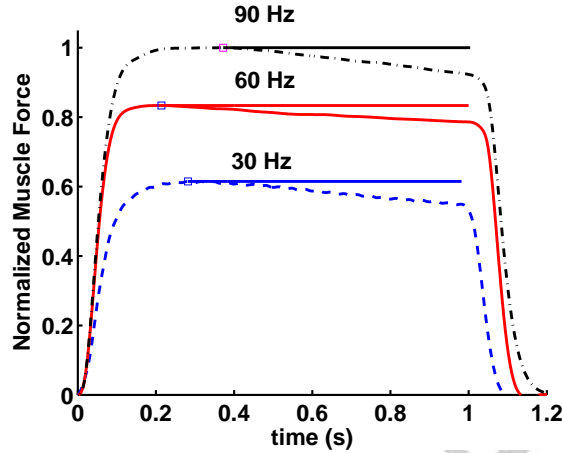


Fig. 8. Normalised load decreasing during tetanus effect for 30, 60 and 90 Hz.  
Table 1

Parameters for the  $f_{Sat}$  relation.

$Freq$	$h$	$[A_m] (\mu mol)$	$[A_{max}] (\mu mol)$
30	1.5	0.1	0.5 – 0.3
60	1.25	0.4	0.5 – 0.3
90	1.05	0.55	0.5 – 0.24

Finally, taking the relationship that include all the previous expressions that determine muscle contraction:

$$F_{active} = F_0 f_{\lambda} f_V f_{train} \quad (11)$$

where  $f_{train}$  includes frequency ( $f_{fr}$ ) and saturation ( $f_{Sat}$ ) effects. The normalised TA force along time at optimal length is represented in Fig. 9. This force is plotted for the three frequencies, comparing the experimental results and the fitting model.

#### 4 Discussion

Isolated TA muscles were prepared to measure the active force generated when varying different factors such as stretch and activation signal voltage and frequency.

The total muscle force developed by the muscle depends on several different factors. One of the most extensively studied is the influence of muscle length (Gordon et al.,

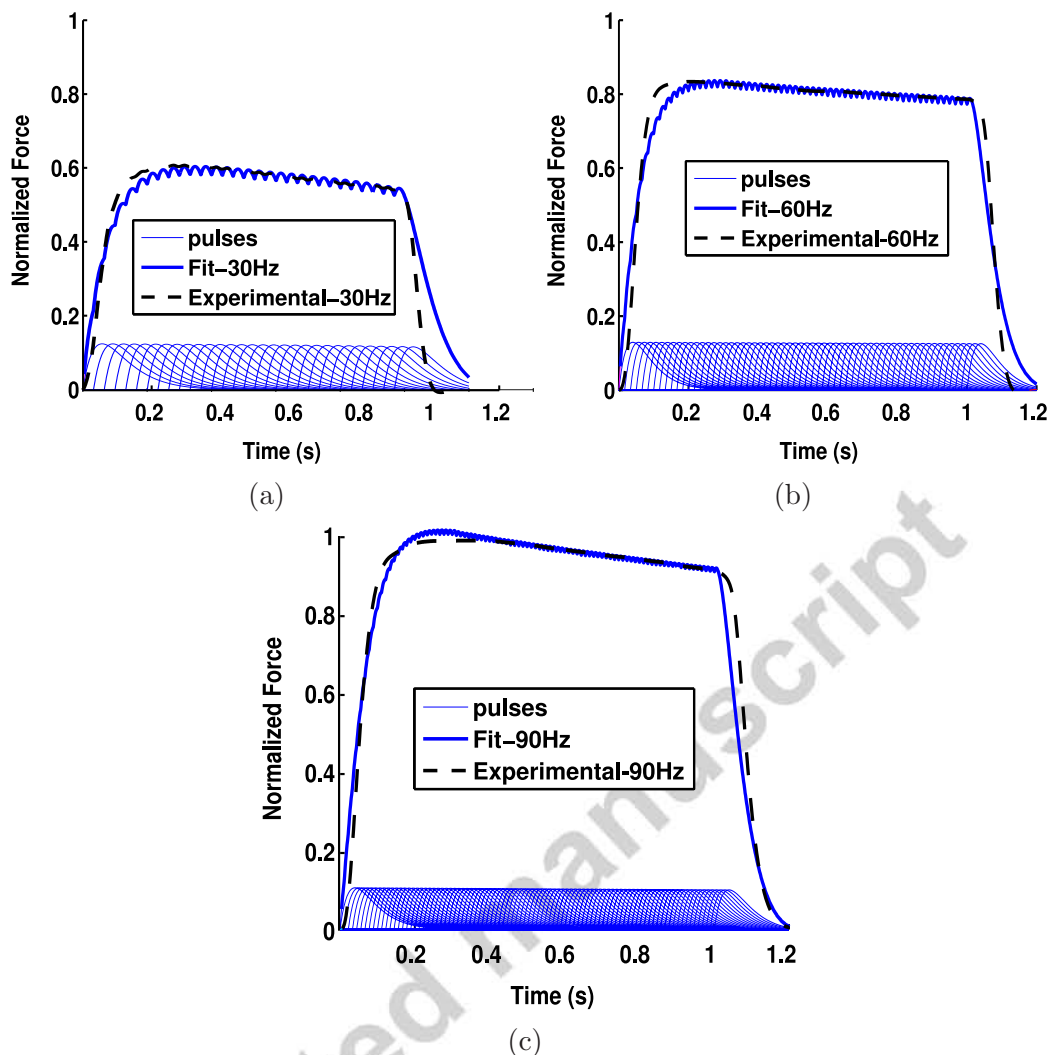


Fig. 9. Normalised force versus time at optimal length, at (a) 30 Hz, (b) 60 Hz and (c) 90 Hz. Pulses that form the total active force are also represented.

1966a; Hawkins and Bey, 1997). The present study considers this effect in the total range of movement of the rat ankle. The curves obtained for the force-length relation (see Fig. 3) are similar to those proposed by Edman and Reggiani (1987).

The maximum average force  $F_0$  determined in the experimental model for the TA rat muscle was  $8 \pm 0.23$  N. This value was reached at the optimal muscle length and at the maximum frequency tested (90 Hz). The results obtained for different muscle lengths are in good agreement with those obtained by Hawkins and Bey (1997) although maximum levels are higher in the present work. Differences in the animal strain and stimulation protocol could explain this.

Another important factor that affects the active force is the amplitude or voltage of the excitation signal. In this work, voltage and frequency replace the neural signal in a voluntary contraction. The size principle states that the motor axon size of fast fibres (FF) is larger than the motor axon of slow fibres (SF) (Henneman, 1957). In the experimental test proposed, low voltages started recruiting fibers with faster responses, producing less resultant force. Increasing voltages lead to recruiting a

greater number of motor units and the resultant force was higher. On the other hand, not only did the increasing voltage rate increase the total force, but also the ascending limb shape of the force-time curve was caused by the input signal frequency. For instance, at 90 Hz the ascending limb rose more steeply than for 30 Hz. However, this also depends on the fibre type. Due to slow twitch of SF, the curve rate rise more steeply at comparative lower stimulus and attains tetanic fusion at lower rates than for FF (Botterman et al., 1986). In the model proposed, the muscle was considered as a unique fibre type, obtaining its characteristic twitch parameters for the whole muscle.

In most studies, the force-time relationship is expressed at the optimal length and this force is considered constant during tetanus. However, experimental evidence has demonstrated that force decreases during stimulation (Burke et al., 1973; Parmiggiani and Stein, 1981; Cannell and Allen, 1984; Hawkins and Bey, 1997). This effect, is assumed to be related to  $Ca^{2+}$  (Cannell and Allen, 1984) and this was taken into account in the proposed mathematical model.

The principal contribution of this work is a complete *in vivo* animal model for determining total active muscle force during isometric contractions at different lengths, frequencies and voltages. In addition, a new approach to the decreasing force during constant length contraction in skeletal muscles has been developed. Although this model has been fitted to a specific animal, its results could be extended to any similar skeletal muscle. Also, the formulation could easily be implemented in a finite element 3D model evaluating active stress at each gauss point dividing the active force by the cross sectional area of the muscle. An estimated cross sectional area in the reference configuration was obtained by Calvo et al. (2010).

One of the limitations of the model is that an average contraction time  $T'_c$  was assumed for the whole muscle, neglecting different fibre types. However, if different fibre types had been considered, it would have been necessary to study each motoneuron and define its contraction time, which lies outside the scope of the experiments and the model considered here. In addition, Calcium concentration in the muscle was not measured, so the saturation relationship presented could not be directly associated with calcium as the cause of the depression in the force plateau. Another important fact to take into account is that fixing muscle ends does not guarantee that fibres inside muscle belly are under isometric conditions. This is a consequence of a series of elastic component elongations leading to shortening and re-lengthening of active muscle components. This effect influences the amplitude of the twitch force response that was approximated as an apparent twitch force ( $P'$ ).

Finally, the experimental set-up developed for fixing the animal to the test machine ensured the accuracy of the data collected and is deemed suitable for future studies that may include drug effects and dystrophies.



## 5 Acknowledgments

The authors gratefully acknowledge research support from the Spanish Ministry of Science and Technology through the research projects DPI2008-02335, DPI2009-14115-C03-01 and grant PI071133 from the “Fondo de Investigación Sanitaria of Spain”

The authors also want to thank the Tissue Characterisation Platform of CIBER-BBN for their technical support during the experimental tests. CIBER-BBN is an initiative funded by the VI National R&D&I Plan 2008-2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

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