

## There is no experimental evidence for non-linear myofilament elasticity in skeletal muscle

Information on the stiffness of the actin and myosin filaments is a key parameter in the study of muscle contraction. For example, the number of motors attached to actin in each half-sarcomere of a muscle fibre can be obtained from measurements of the half-sarcomere stiffness, provided that the compliances of the actin and myosin filaments are known (Piazzesi et al., 2007). The stress-strain relationship in myofilaments has generally been considered to be linear (Kojima et al., 1994; Wakabayashi et al., 1994; Reconditi et al., 2004; Brunello et al., 2006), and data suggesting that the myofilament stiffness can vary with force could also find alternative explanations (Huxley et al., 1994; Higuchi et al., 1995).

The argument has been recently reinvestigated by Edman (Edman, 2009), who concluded that myofilament stiffness exhibits a strong non-Hookean behaviour. In Edman's paper (Edman, 2009), stiffness is measured by the change in force in response to 2–4 kHz length oscillations imposed on intact fibres isolated from the tibialis anterior muscle of *Rana temporaria* (2–2.5°C). Measurements are performed at two different sarcomere lengths ( $s_l$ ) in isometric contraction and during isotonic shortening at different pre-set loads. The changes in stiffness with sarcomere length for different loads are estimated from the ratio of the fibre stiffness at  $s_l=2.6\ \mu\text{m}$  ( $S_{2.6}$ ) to that at  $s_l=2.2\ \mu\text{m}$  ( $S_{2.2}$ ; the condition for full overlap between actin and myosin filaments). The pre-set loads are in the range of 0.4–0.7 the isometric tetanic force ( $T_0$ ) at  $s_l=2.2\ \mu\text{m}$ . The elements considered to contribute to the half-sarcomere stiffness are the array of actin-attached cross-bridges and the portions of both myosin and actin filaments beyond the overlap region. The stiffness of the cross-bridge array is proportional to the number of attached cross-bridges, which depends solely and linearly on the overall level of force, independent of how force is modulated, either with a different degree of overlap (Gordon et al., 1966) or with isotonic shortening at different loads. This latter assumption has been recently found valid in the force range 0.5–1 $T_0$  (Piazzesi et al., 2007).

The contribution of myofilaments to the half-sarcomere compliance is assumed proportional to the length of their non-overlapping regions. The compliance per unit length is considered the same for both actin and myosin. This is an arbitrary assumption but is acceptable as a first approximation for the purpose of determining whether or not the myofilament stiffness is Hookean. Other acceptable approximations are that the changes in the length of the fibre are used for estimating the changes in the half-sarcomere length and that a frequency of length oscillations in the range of 2–4 kHz is assumed sufficiently high to estimate the instantaneous stiffness, ignoring the effect of quick force recovery.

The conclusion that the myofilaments are non-Hookean is based on the results of the linear fit on the  $S_{2.6}/S_{2.2}-T$  data. In my reproduction of data from fig. 3 of Edman (Edman, 2009), the linear regression gives a best fit with  $m$  (the slope)=0.066±0.109 (best estimate ± s.e.m.) and  $q$  (the ordinate intercept)=0.861±0.067;  $R^2=0.03$ . The mean of the squared residuals is  $1.4\times 10^{-3}$ . This result is taken by Edman as evidence of a strong nonlinearity of the stress-strain relation of the myofilaments (Edman, 2009).

Equation 1 in Edman (Edman, 2009) can be rewritten as  $S_{2.6}/S_{2.2}=(T+S/F_0)/(T\times 1.61/0.81+S/F_0)$ , where  $S$  is the stiffness of the myofilaments at  $s_l=2.2\ \mu\text{m}$  and  $F_0$  is the stiffness of the cross-bridges for  $T=T_0$ .

Under the hypothesis that  $S/F_0$  is independent of force, the above equation represents an hyperbola in the variables  $S_{2.6}/S_{2.2}$  and  $T$ . The best hyperbolic fit to the  $S_{2.6}/S_{2.2}-T$  data gives a  $S/F_0$  estimate of  $5.24\pm 0.74$ . The mean of the squared residuals is  $1.7\times 10^{-3}$ , which is practically the same as that obtained with the linear fit. In the range of

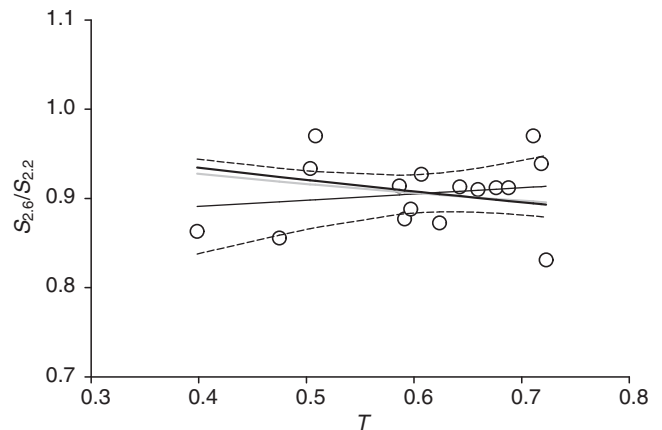


Fig. 1. Ratio of fibre stiffness at  $s_l=2.6\ \mu\text{m}$  ( $S_{2.6}$ ) to that at  $s_l=2.2\ \mu\text{m}$  ( $S_{2.2}$ ), plotted against force  $T$  [circles; replotted from fig. 3 of Edman (Edman 2009)]. Thin black line represents linear fit to data according to Edman's assumption of non-Hookean stiffness of myofilaments; broken lines represent 95% confidence limits for the linear fit; thick black line represents hyperbolic fit under the hypothesis of Hookean filament stiffness; thick grey line represents hyperbolic fit under the hypothesis of Hookean filament stiffness and using the correct formula for the compliance of the half-sarcomere.

forces used, the hyperbolic fit is very close to a straight line, as demonstrated by the linear fit through the values predicted by the hyperbolic fit that gives  $S_{2.6}/S_{2.2}=0.984-0.127T$  ( $R^2=0.999$ ), and lies well inside the 95% confidence limits of the linear fit to data, i.e. the bounds of the area that has a 95% chance of containing the true regression line (see Fig. 1). Thus, when uncertainties are taken into account, Edman's data are consistent with a linear stress-strain relation of myofilaments.

Edman reports a value of  $\sim 5$  for  $S/F_0$ , the ratio of myofilament stiffness over the cross-bridge array stiffness, much higher than previous estimates, which are close to 1 (Huxley et al. 1994; Wakabayashi et al. 1994; Reconditi et al. 2004). This discrepancy is the consequence of having neglected the contribution to the half-sarcomere stiffness of the myofilaments in the overlap region. When  $S$  is not much smaller than  $F_0$ , the half-sarcomere compliance ( $C_{hs}$ ) can be expressed with good approximation by the following equation:  $C_{hs}=c_A\times(l_A-2/3\zeta)+c_M\times(l_M-2/3\zeta)+1/fF_0$ , where  $c_A$  and  $c_M$  are the compliance per unit length of actin and myosin filament, respectively;  $l_A$  and  $l_M$  are the length of the actin and myosin filament in the half-sarcomere, respectively;  $\zeta$  is the length of the overlap region;  $f$  is the fraction of cross-bridges attached to actin, relative to the fraction attached at  $T_0$  [appendix A in Ford et al. (Ford et al., 1981)]. If, as in Edman (Edman, 2009), it is assumed that  $c_A=c_M=c$ , the contribution of myofilaments compliance  $C_f$  to  $C_{hs}$  can be expressed as:  $C_f=c\times(l_A+l_M-4/3\zeta)=c\times(2\times s_l-(l_A+l_M))/3$ . With  $l_A=0.970\ \mu\text{m}$  and  $l_M=0.775\ \mu\text{m}$  (Edman, 2009), the contribution of the myofilaments to  $C_{hs}$  at  $2.6\ \mu\text{m}$  is  $1.152/0.885$  that at  $2.2\ \mu\text{m}$ . Thus, Edman's eqn 1 (Edman, 2009) can be rewritten as:  $S_{2.6}/S_{2.2}=(T+S/F_0)/(T\times 1.152/0.885+S/F_0)$ .

The fit of the  $S_{2.6}/S_{2.2}-T$  plot with the above equation, in the hypothesis of  $S/F_0$  independent of  $T$ , gives  $S/F_0=1.15\pm 0.22$ , which is close to the values reported by the previous works quoted above.

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## Response to 'There is no experimental evidence for non-linear myofilament elasticity in skeletal muscle'

In his Correspondence article (p. 658), Massimo Reconditi discusses an alternative interpretation of previously presented results (Edman, 2009) concerning the nature of myofilament elasticity in striated muscle. In response to Reconditi (Reconditi, 2010), I should like to make the following remarks.

A pertinent finding in the study described by Edman (Edman, 2009) is the sarcomere length dependence of the measured fibre stiffness. The instantaneous stiffness was measured during tetanic stimulation while the active force was kept at a given level by load-clamp control within the range 0.4–0.7 of maximum tetanic force. The results showed that the measured stiffness was invariably lower as the sarcomere length was increased above optimal length, 2.20  $\mu\text{m}$ . This observation provides evidence that the myofilament compliance is indeed lower in the overlap region than in the region outside overlap where there is no interaction with the myosin bridges. The actual compliance of the filaments in the overlap region during tetanic activity cannot be assessed at the present time except that it must be effectively lower than that in the free portions of the filaments. In Edman (Edman, 2009), the assumption is made that the filaments in the overlap region, i.e. the portions involved in cross-bridge formation, are incompressible. On this basis, the results presented in fig. 3 of Edman (Edman, 2009) do suggest that the myofilaments in frog striated muscle have the character of a non-linear spring. To my knowledge, there is no clear-cut evidence that the myofilaments in the overlap zone are compliant in the particular way proposed by Reconditi (Reconditi, 2010), which would lead to a basically different conclusion from that reached in Edman (Edman, 2009). If a fraction of the filament compliance is actually assumed to reside in the overlap zone, this would provide lower values of the calculated myofilament stiffness than shown in fig. 4 of Edman (Edman, 2009), but the increase in myofilament stiffness with force would still hold true as illustrated.

As pointed out in Edman (Edman, 2009), the myofilaments of the intact muscle fibre are quite complex structures in that they are surrounded by, and interwoven with, a number of auxiliary filaments

that make up the cytoskeleton. These structures may be regarded as an integral part of the myofilament elasticity measured in intact muscle or intact muscle fibres and this will hold true irrespective of the measuring technique used [see Discussion and further references in Edman (Edman, 2009)]. The complexity of the myofilament structure may well be thought to be associated with non-linear elastic properties like those observed in, for example, muscle tendons (Cleworth and Edman, 1972; Edman and Josephson, 2007). Results of previous investigations based on mechanical measurements (Higuchi et al., 1995) and X-ray diffraction studies (Griffiths et al., 2006) provide evidence in favour of this view, thus supporting the conclusion reached in Edman (Edman, 2009). It is worth pointing out in this connection that no concrete evidence has been presented to show that the myofilaments in intact muscle fibres behave as Hookean springs.

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