Eur Respir J 1999; 13: 1055–1061
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Cross-bridge kinetics in fatigued mouse diaphragm

C. Coirault*, P. Attal+, F-X. Blanc*, D. Chemla#, Y. Lecarpentier#

Cross-bridge kinetics in fatigued mouse diaphragm. C. Coirault, P. Attal, F-X. Blanc, D. Chemla, Y. Lecarpentier. ©ERS Journals Ltd 1999.

ABSTRACT: The aim of this study was to determine cross-bridge number and kinetics in the diaphragm during fatigue and early recovery.

Experiments were conducted in isolated mouse diaphragm (n=10). The force of a single cross-bridge (Π), the number of cross-bridges ($m \times 10^9 \cdot mm^{-2}$), the time cycle ($t_c$) and the rate constants for cross-bridge attachment (f_1) and detachment (g_2) were calculated from the equations of A.F. Huxley.

Following the fatigue protocol, peak isometric tension (P_0) and maximum unloaded shortening velocity fell by 40±1% and 17±2%, respectively. In fatigued diaphragm, m fell by approximately 40% and returned to baseline after 10 min. When compared to baseline, g_2 fell in fatigued diaphragm and remained significantly lower during the 15-min recovery period. In contrast, fatigue did not significantly modify Π, f_1, or t_c. There was a strong linear relationship between P_0 and m ($p<0.001$, $r=0.988$). No relationship was observed between t_c and g_2.

These results indicate that changes in tension during fatigue and recovery run parallel to changes in the number of active cross-bridges, with no change in the force generated per cross-bridge. It is conceivable that fatigue durably impairs adenosine diphosphate release from the actomyosin complex without modifying the total duration of the cross-bridge cycle.

Eur Respir J 1999; 13: 1055–1061.

*INSERM 451-Laboratoire d'Optique Appliquée-Ecole Polytechnique, Palaiseau, +Service d'ORL and #Service d'Explorations Fonctionnelles, Centre Hospitalier et Universitaire de Bicetre, Assistance Publique-Hôpitaux de Paris, France.

Correspondence: C. Coirault
INSERM 451-LOA-Ecole Polytechnique
Batterie de l'Yvette
91125 Palaiseau Cedex
France
Fax: 33 169319996

Keywords: Actomyosin interaction
fatigue
recovery
respiratory muscle
skeletal muscle

Received: July 23 1998
Accepted after revision January 28 1999

An impairment of the contractile machinery of the diaphragm muscle during fatigue might contribute to the onset of respiratory failure in humans. The fatigued diaphragm muscle shows impaired contractile performance, characterized by a fall in maximum shortening velocity and tension, and impaired relaxation rate [1–3]. Although the specific causes of diaphragm fatigue are not known, several known causes of skeletal muscle fatigue have been implicated (see [4] for a review). In particular, changes in the level of intracellular metabolites, such as increased H^+ and inorganic phosphate (Pi) and/or decreased myoplasmic calcium, might contribute to inhibiting force and velocity in the diaphragm [5, 6] and other skeletal muscles [4, 7–9]. The effects of H^+ and Pi on skinned skeletal fibres have been attributed to direct inhibition of the actomyosin interaction [10–12].

In striated muscle, force and velocity depend on both the total number of cross-bridges formed and the kinetics of the actomyosin interactions [13]. The aim of this study was to determine cross-bridge number and kinetics in mouse diaphragm during fatigue and early recovery. In the study, cross-bridge number and kinetics were calculated from mechanical data in isolated diaphragm muscle by using the classic equations of A.F. Huxley [13–16]. Despite potential muscle fibre heterogeneity and effects of series compliance, the equations of HUXLEY [13] have been demonstrated to accurately fit the mechanical properties of multicellular preparations. On the basis of the theory of HUXLEY [13], two hypotheses were tested: 1) that changes in diaphragm force generation during fatigue and recovery are associated with changes in the total number of cross-bridges and/or the amount of force per cross-bridge; and 2) that fatigue is associated with changes in the kinetics of the cross-bridge cycle. Three main steps in the cross-bridge cycle were investigated: the attachment step, the power stroke and the detachment step [14–16].

Materials and methods

Experimental protocol

Experiments were conducted on diaphragm muscle strips from adult male mice (n=10). Care of the animals conformed to the Helsinki convention. After the induction of anaesthesia with pentobarbital sodium (30 mg·kg body $weight^{-1}$, intraperitoneally), laparotomy was performed, followed by thoracotomy. A strip of the ventral part of the costal diaphragm was carefully dissected out from the muscle *in situ*. The insertions on the central tendon and ribs were kept intact. The diaphragm strip was rapidly mounted in a tissue chamber containing Krebs–Henseleit solution (in mM): 118 NaCl; 24 $NaHCO_3$; 4.7 KCl; 1.2 $MgSO_4$ $7H_2O$; 1.1 KH_2PO_4; 2.5 $CaCl_2$ $6H_2O$; and 4.5 glucose. The solution was bubbled with 95% O_2–5% CO_2 and maintained at 26°C and pH 7.4. The costal end of the muscle strip was held in a stationary clip at the bottom of the chamber, while the central tendon end was maintained with a second clip, attached to an electromagnetic force-transducer device. After a 15-min equilibration period the muscle was

supramaximally stimulated *via* two platinum electrodes arranged longitudinally on either side of the muscle. Peak tetanic tension was elicited with 1-ms rectangular pulses at 65 Hz. Experiments were carried out at the initial resting length (L_o) corresponding to the apex of the initial length–active tension curve. At the end of the experiment, the cross-sectional area (CSA; in mm^2) was calculated from the ratio of muscle weight to muscle length at L_o, assuming a muscle density of 1. The electromagnetic lever system has been described elsewhere [17]. The characteristics of the muscles were as follows (mean±SEM): CSA 0.39±0.05 mm^2; and L_o 6.9±0.2 mm.

Study design

The baseline parameters of the diaphragm muscles were first determined in tetanus mode at 65 Hz (30 trains·min^{-1} of 200 ms duration). Two baseline measurements were made at 10-min intervals to ensure the stability of the preparation. Fatigue was then induced by repeatedly stimulating each strip with 75 trains·min^{-1} of 200 ms duration at a stimulation frequency of 65 Hz. Stimulation continued until the muscle strip was fatigued to a point where it generated only 60% of the tetanic tension measured before the fatigue procedure. Mechanical parameters were then recorded from 8–10 contractions just after completion of the fatigue procedure and after 1, 5, 10 and 15 min of recovery.

Mechanical parameters

Maximum unloaded shortening velocity (V_{max}; in $L_o \cdot s^{-1}$) was measured from the contraction abruptly clamped to zero-load just after the stimulus. Peak isometric tension, *i.e.* peak force normalized per CSA (P_o; in mN·mm^{-2}), was measured from the fully isometric contraction.

The hyperbolic tension–velocity relationship was derived from the peak velocity (V) of 8–10 afterloaded contractions plotted against the isotonic load level normalized per CSA (P), by successive load increments, from zero-load up to isometric tension. The experimental P-V relationship was fitted according to the equation of HILL [18]:

$$(P + a)\ (V + b) = (cP_{max} + a)b,$$

where -a and -b are the asymptotes of the hyperbola as determined by multilinear regression and the least squares method, and cP_{max} is the calculated peak isometric tension at V=0. The curvature (G) of the P–V relationship is equal to (cV_{max}) / b = (cP_{max}) / a, where cV_{max} is the calculated peak velocity at zero-load.

Cross-bridge number and kinetics

Theoretical background. According to the theory of A.F. Huxley, which is the most widely accepted theory of muscle contraction, force and shortening are generated by cyclical interactions between myosin and actin, driven as one molecule of adenosine triphosphate (ATP) is hydrolysed [13]. Peak isometric force is the product of the number of cycling cross-bridges and the force of a single cross-bridge [13]. Recent mechanical and structural studies have provided insight into the molecular basis of the cross-bridge cycle [19, 20]. A condensed model of the cross-bridge cycle consistent with the theory of A.F. Huxley and recent studies is presented in figure 1. In this scheme, each myosin cross-bridge cycle occurs through multiple biochemical and structural changes, involving binding of ATP to the nucleotide site of the myosin head, ATP hydrolysis, and attachment and detachment of the cross-bridge to and from actin [19, 21]. The force-generating step of the cross-bridge cycle, *i.e.* the power stroke, is initiated by the release of Pi from the actomyosin–adenosine diphosphate (ADP) complex [22, 23]. The equations of A.F. Huxley make it possible to calculate the total number of cycling cross-bridges and the kinetics of the main steps of the cross-bridge cycle from mechanical data [13–16].

Characteristics of cross-bridge number and kinetics. The total time cycle (t_c; in s), the cross-bridge rate constants of attachment (f_1; in s^{-1}) and detachment (g_1 and g_2; in s^{-1}), the total number of cross-bridges per mm^2 ($m \times 10^9$), the peak mechanical efficiency (Meff; in %) and the elementary force per cross-bridge (Π, in pN) were calculated from the equations of HUXLEY [13], as previously reported [14–16]; g_1 is the maximum value of the rate constant for cross-bridge detachment at the onset of the stroke step, and g_2 is the maximum value of the rate constant for cross-bridge detachment at the end of the stroke step [13]. The duration of the power stroke (time stroke (t_s) in s) is given by:

$$t_s = L_o \times \frac{mh\Pi}{E_o}$$

where h is the step size (h=11 nm) [20] and E_o is the peak rate of total energy release in isometric conditions [13, 24].

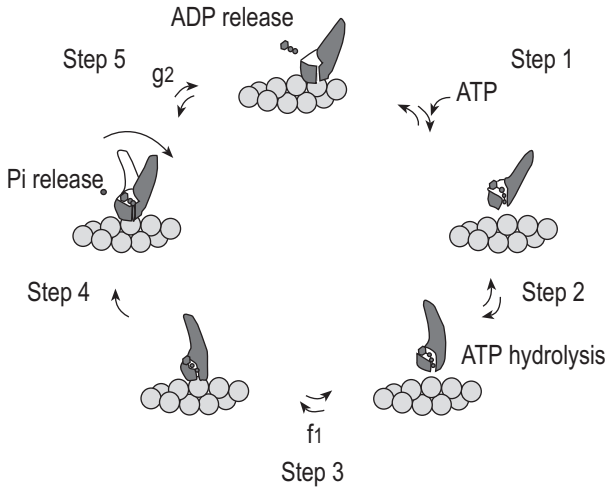

Fig. 1. – Mechanical and biochemical events in the cross-bridge cycle [19, 21]. Step 1: at the onset of the cycle, myosin is tightly bound to actin in the absence of nucleotide. Adenosine triphosphate (ATP) binding to the myosin head induces rapid dissociation of myosin from actin. Step 2: ATP is then rapidly hydrolysed to form the complex myosin–adenosine diphosphate (ADP)–inorganic phosphate (Pi). Step 3: attachment of myosin to actin, characterized by the rate constant for attachment (f_1). Step 4: power stroke initiated by the release of Pi. Step 5: ADP release step, which correlates with the rate constant of cross-bridge detachment (g_2). ATP can then rapidly bind, and myosin dissociates from actin.

Statistical analysis

Data are expressed as mean±SEM. Differences between mechanical parameters studied at different time points were identified by using ANOVA with repeated measures followed, if appropriate, by Student's paired tailed t-test with the Bonferroni correction. The threshold of statistical significance was set at $p<0.05$.

Results

Cross-bridge mechanics in fatigued diaphragm

The influence of fatigue on P_o and V_{max} is presented in figure 2. In fatigued diaphragm, P_o was reduced by 40±1% and V_{max} by 17±2% (each $p<0.001$). The total number of cross-bridges ($m \times 10^9$) changed similarly to P_o, with a fall of ~40% ($p<0.001$) (fig. 3). The force of a single cross-bridge (Π) did not change significantly in fatigued diaphragm (fig. 3). As peak isometric tension depends on both Π and m, this indicates that the number of cross-bridges is the main determinant of the fall in peak isometric tension during fatigue.

Neither Meff nor G changed significantly with fatigue (fig. 4). f_1, g_1 and g_2 are presented in figure 5. Compared to baseline, fatigue induced no significant changes in f_1 or g_1. In contrast, g_2 was significantly lower in fatigued diaphragm than at baseline ($p<0.001$). Fatigue did not significantly modify t_s (0.43±0.5 *versus* 0.42±0.04 ms, NS) or the total duration of the cross-bridge cycle (fig. 5).

Cross-bridge mechanics during recovery from fatigue

The time course of tension recovery from fatigue is shown in figure 2. P_o was 78±2% after 1 min of recovery, and gradually reached 96±2% of the prefatigue value after 10 min of recovery (fig. 2). At 10 and 15 min of recovery, P_o did not differ significantly from baseline (fig. 2). In contrast, V_{max} remained significantly lower than at baseline after the recovery period (fig. 2). The total number of cross-bridges per mm^2 recovered gradually (fig. 3) and did not differ significantly from baseline after 10 and 15 min. Throughout the recovery period there were no significant changes in Π, G or Meff (figs. 3 and 4). After 1 and 5 min of recovery f_1 did not differ significantly from baseline (fig. 5). In contrast, f_1 was significantly lower after 10 and 15 min of recovery than at baseline ($p<0.05$); g_l and t_c did not vary during recovery until 15 min (fig. 5), when there was a significant decrease in g_1 ($p<0.05$) and a significant increase in t_c ($p<0.05$) (fig. 5). g_2 remained significantly lower than at baseline throughout the recovery period ($p<0.001$). The t_s did not change significantly at any time during reco-very.

Relationships between parameters

As shown in figure 6 there was a close positive linear relationship between P_o and m ($Po=9.0\ m+ 10.0$, $r=0.988$, $p<0.001$), but no relationship between P_o and Π. The relationships between t_c and the reciprocal of f_1 and g_2 are

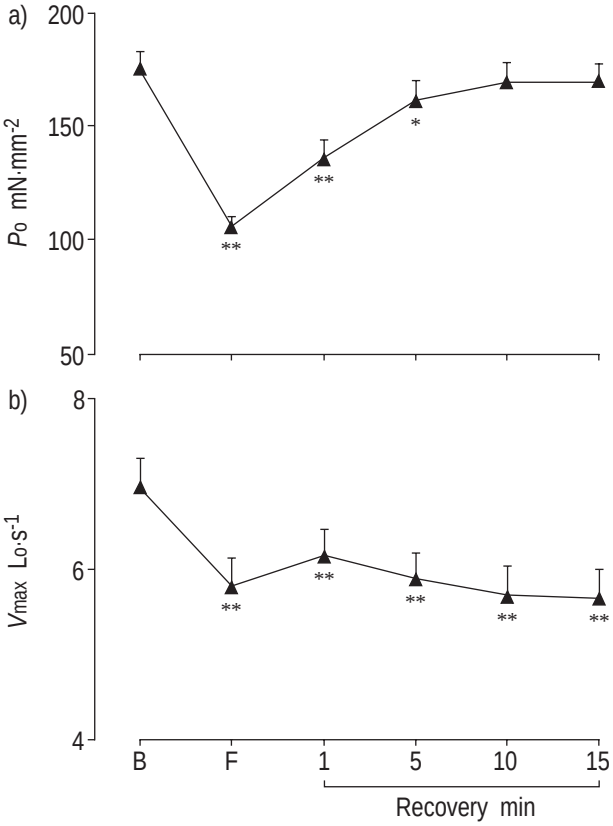

Fig. 2. – Effects of fatigue (F) and early recovery on peak isometric tension (P_o; a) and maximum unloaded shortening velocity (V_{max}; b) in tetanus mode. Values are mean±SEM. *: $p<0.05$; **: $p<0.01$, *versus* baseline (B).

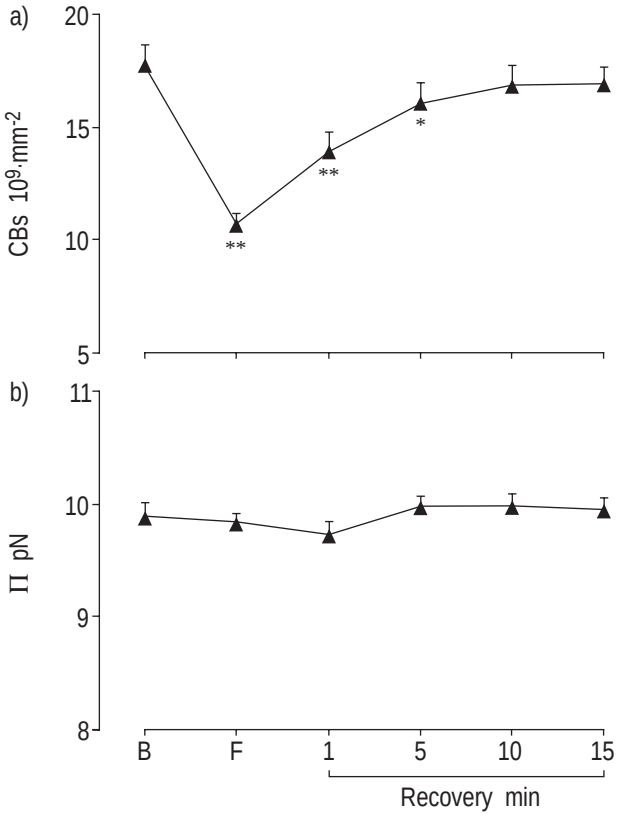

Fig. 3. – Effects of fatigue (F) and early recovery on total number of cross-bridges (CBs) per cross-sectional area ($m \times 10^9 \cdot mm^{-2}$; a) and the elementary force per single cross-bridge (Π; b). Values are mean± SEM. *: $p<0.05$; **: $p<0.01$, *versus* baseline (B).

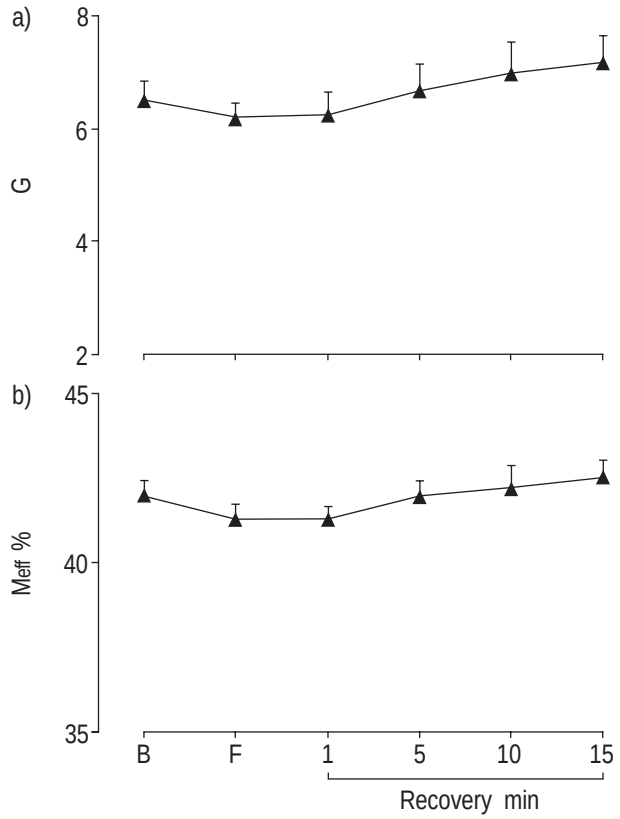

Fig. 4. – Effects of fatigue (F) and early recovery on the curvature (G) of the force–velocity relationship (a) and on maximum mechanical efficiency (Meff; b). Values are mean±SEM. As compared to baseline (B), fatigue modified neither the curvature G nor Meff.

shown in figure 7. t_C increased linearly with $1/f_1$; in other words, the lower the f_1, the longer the overall cycle. No relationship was observed between t_C and g_2 (fig. 7).

Discussion

The effects of fatigue and early recovery on cross-bridge kinetics in isolated mouse diaphragm muscle were studied. The results indicate that changes in tension during both fatigue and recovery run parallel to changes in the number of active cross-bridges, with no change in the force generated per cross-bridge. Moreover, fatigue was associated with a reduced g_2 that persisted throughout the 15-min recovery period.

Relevance of the experimental model

In this study, cross-bridge kinetics were calculated from mechanical data in isolated diaphragm muscle by using the equations of HUXLEY [13]. In muscle strips, series compliance and muscle fibre heterogeneity may affect mechanical properties and cross-bridge cycling kinetics. It is important to note that experiments designed to apply the principles of the theory of HUXLEY [13] were performed on isolated frog sartorius muscles, and not on isolated fibres. The equations can therefore be applied to multicellular preparations such as diaphragm muscle strips. One may also ask whether the model of HUXLEY [13] can be applied to muscles with heterogeneous myosin heavy chain (MyHC) isoform composition. HUXLEY [13] demonstrated that his equations accurately fitted the force–velocity characteristics shown by HILL [18] in frog sartorius muscle. Importantly, four MyHC isoforms have been identified in the hindlimb muscle of the frog [25]. Therefore, the model accurately fits the mechanical properties of a muscle whose fibre composition includes different MyHCs. In heterogeneous muscle, the force–velocity characteristics are thought to reflect the relative contribution of each fibre type [24]. Likewise, according to the equations of HUXLEY [13], cross-bridge characteristics are thought to reflect the average value of the myosin molecular motors.

The mouse diaphragm is mainly composed of fast MyHC isoforms, particularly types 2a and 2x [26]. Although V_{max} and isometric tension do not differ between type 2a and 2x fibres, the actomyosin adenosine triphosphatase (ATPase) activity of type 2a fibres has been found to be significantly lower than that of type 2x [27, 28]. It is likely that type 2a and 2x fibres have different force–velocity relationships and different cross-bridge cycling kinetics. Therefore, in the present study, the cross-bridge characteristics of the mouse diaphragm strip probably reflected the mean cross-bridge behaviour of the different MyHCs, mainly 2a and 2x MyHC isoforms.

Tension and cross-bridge number during fatigue

Depressed tension production during fatigue was associated with a fall in the number of cycling cross-bridges (fig. 2). Moreover, changes in tension during fatigue and early recovery ran closely parallel to changes in cross-bridge number (figs. 3 and 6). In contrast, the rate constant for cross-bridge attachment, t_S, the force per cross-bridge and the total duration of the cross-bridge cycle were not modified by the fatigue protocol (figs. 3 and 5). It is well established that fatigue increases intracellular Pi and decreases pH and Ca^{2+} concentration [7, 9, 11]. It is thus likely that such mechanisms also occur in fatigued mouse diaphragm. These changes in intracellular metabolite and electrolyte composition might contribute to reducing the number of cycling cross-bridges in fatigued muscle. Indeed, the rise in intracellular Ca^{2+} concentration is attenuated during fatigue so that contractile proteins might not be fully activated [9, 11]. In addition, acidosis and increased Pi concentration make myofibrils less sensitive to calcium [29, 30]. It is therefore possible that the reduced number of cycling cross-bridges documented in the present study was related to thin filament inactivation as a result of both reduced intracellular Ca^{2+} concentrations and reduced sensitivity of myofilaments to calcium in the fatigued diaphragm. Alternatively, the increased Pi concentration may inhibit the release of Pi from the actomyosin ADP–Pi complex, thereby decreasing the number of cross-bridges in the force-generating state or delaying cross-bridge entry into the stroke phase [31, 32]. Fatigue, by increasing the Pi concentration, would be expected to prolong the force-generating step, *i.e.* t_S and/or the immediate step before Pi release, which is the cross-bridge attachment step characterized by f_1 (fig. 1). However, in the experiments,

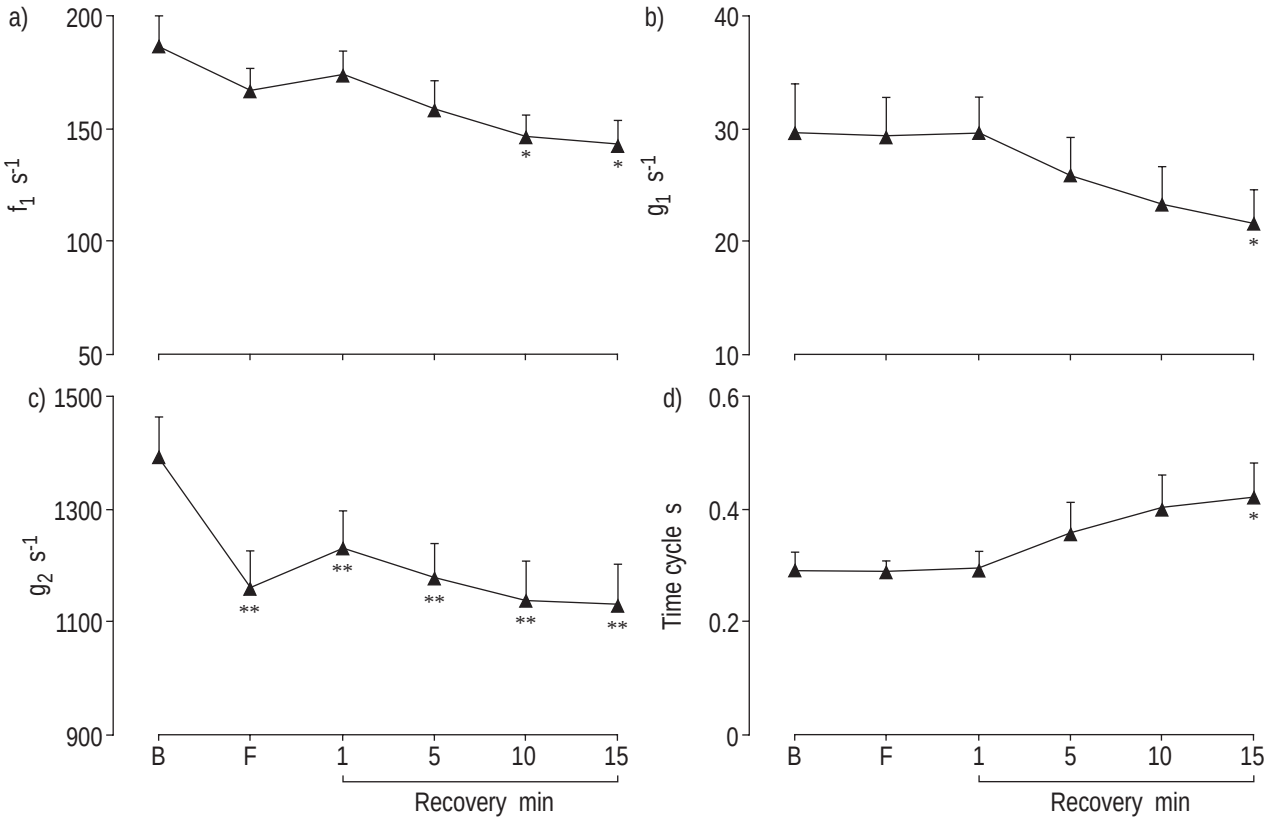

Fig. 5. – Effects of fatigue (F) and early recovery on kinetics of cross-bridge cycling; maximum values of the rate constant for cross-bridge attachment (f_1; a); peak values of rate constants for detachment g_1 (b) and g_2 (c); total duration of the time cycle (d). Values are mean±SEM. *: $p<0.05$; **: $p<0.01$, *versus* baseline (B).

fatigue modified neither *t*s nor f_1. This strongly suggests that fatigue does not significantly inhibit Pi release, at least in isolated mouse diaphragm muscle.

Muscle shortening and cross-bridge kinetics during fatigue

In fatigued muscle, the tension decline was associated with a decrease in V_{max}. It was found that the effect of fatigue on the muscle capacity to shorten was proportionally smaller than the effect on the capacity to generate tension, as previously reported in mammalian skeletal muscle [4] and diaphragm [2]. In contrast to tension and cross-bridge numbers, V_{max} did not recover to baseline levels after 15 min of recovery (fig. 2). These data are consistent with the delayed and/or incomplete recovery of shortening velocity observed in both *in vivo* and *in vitro* studies [1, 33] and suggest that certain functional, metabolic and/or structural alterations persist after muscle tension has returned to normal following fatigue.

Dissociation of complete recovery of tension and incomplete recovery of V_{max} may be explained by the fact that different intracellular mechanisms are involved in the decline in tension on the one hand, and the decline in V_{max} on the other [4]. It is also possible that ultrastructural changes in mitochondria, T-tubules and/or sarcoplasmic reticulum may contribute to the unrecovered V_{max}. Changes in cellular homeostasis such as increased free radicals during the fatigue run may result in damage to sarcolemma membrane and/or intracellular organelles [4]. Finally, the complete recovery of tension is consistent with previous studies showing that isometric contractions (but not eccentric contractions) do not induce myofibrillar injury [4].

According to the cross-bridge model of HUXLEY [13], V_{max} is proportional to the maximum value of g_2. Thus, biochemical or mechanical factors that influence g_2 are expected to modify the maximum velocity of muscle shortening. It has been proposed that the correlate of g_2 in the cross-bridge cycle is the rate of ADP dissociation from actomyosin [23, 24, 34]: the lower the g_2 value, the longer the time required for ADP release after the power stroke. In muscles with widely different shortening velocities, a strong correlation has been reported between V_{max} and the rate of ADP dissociation from actomyosin [34]. In rabbit psoas fibres, an increase in the [MgADP]/[MgATP] ratio causes a fall of ~12% in the V_{max} [11]. Moreover, *in vitro* motility assays have provided evidence that an increase in the Mg ADP concentration depresses the sliding velocities of fluorescence-labelled actin filaments of rat skeletal myosin [35]. This is thought to be due to competition between ADP and ATP for the nucleotide binding site of myosin, the increase in intracellular ADP concentration thereby slowing cross-bridge detachment by ATP [11, 35]. The increase in ADP concentration that occurs in fatigued muscles [4] is likely to reduce the rate of cross-bridge dissociation from actin at the end of the power stroke and to impede the V_{max} of the muscle, as observed in the present study. g_2 is not related to muscle tension and/or to the number of active cross-bridges [13,

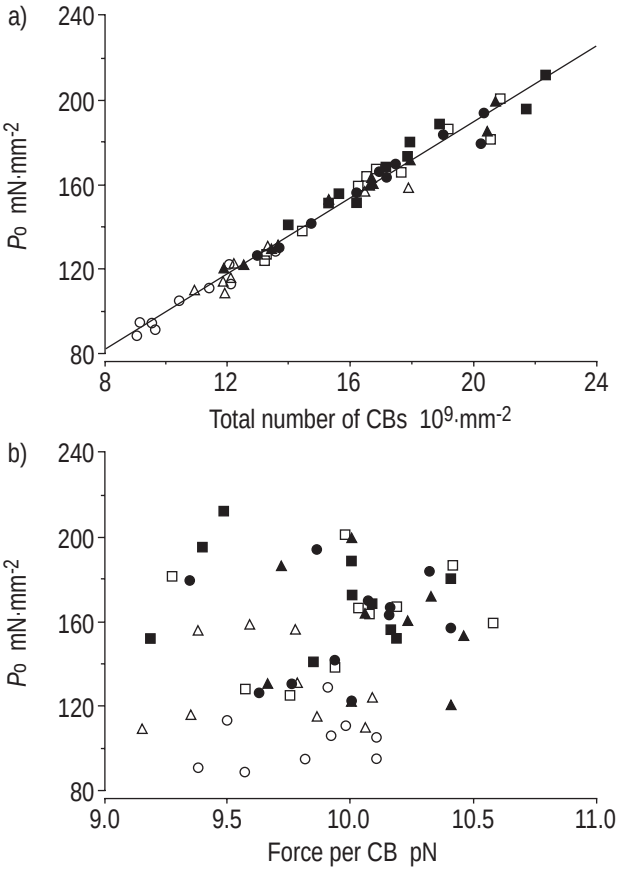

Fig. 6. – Relationship between peak isometric tension (P_o) and either the total number of crossbridges (CBs) per cross-sectional area (m × 10^9·mm^{-2}; a) or the single force per crossbridge (b). Over the study groups, P_o was equal to P_o=9.0 m + 10.0 (r=0.988, p<0.001). Conversely, there was no relationship between P_o and force of a single crossbridge (Π). ■: baseline; □: fatigue; △, ▲, ○, ●: 1, 5, 10 and 15 min recovery, respectively.

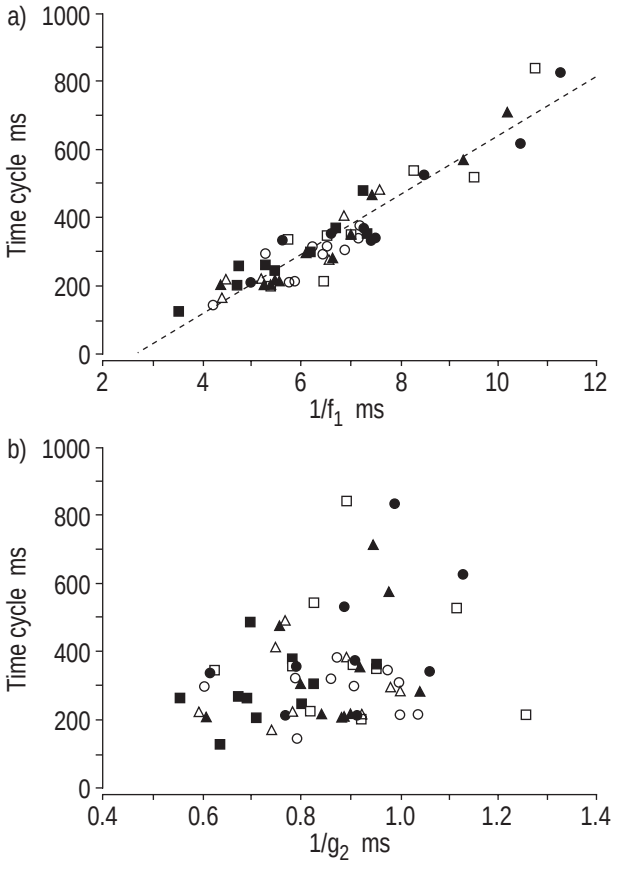

Fig. 7. – Relationship between the total duration of the crossbridge (t_c) and a) the duration of the attachment step ($1/f_1$), and b) the duration of the detachment step ($1/g_2$). Over the study groups, t_c=88.1 ($1/f_1$) - 235.7 (r=0.94, p<0.001). There was no relationship between tc and $1/g_2$. ■: baseline; □: fatigue; △, ▲, ○, ●: 1, 5, 10 and 15 min recovery, respectively.

24]. Therefore, reduced myoplasmic calcium with fatigue is unlikely to explain changes in g_2 although this has not previously been evaluated.

The slower rate of cross-bridge dissociation was not associated with an increase in the overall duration of the cross-bridge cycle, and no relationship was observed between $1/g_2$ and t_c (figs. 5 and 7). However, t_c increased linearly with the duration of the attachment step, *i.e.* $1/f_1$ (fig. 7). These findings are consistent with the proposal that the overall duration of the cross-bridge cycle does not depend on ADP release but is limited by the attachment step [21, 23].

Limitations of the study

The causal agents of fatigue are linked to the duration and intensity of work, the fibre type of the muscle studied, environmental conditions, and the degree of physical exercise. In the mouse diaphragm, it can be hypothesized that a predominant effect of fatigue on type 2x MyHC isoforms would be to shift the overall characteristics of the cross-bridge cycling kinetics towards those of the MyHC-2a fibres [28]. However, in fast skeletal muscle fibres, resistance to fatigue is more closely related to oxidative energy metabolism than to myosin ATPase activity [27]. In contrast to what has been observed in the rat diaphragm [28] and in other mammalian diaphragms [36], it has been demonstrated that in the mouse diaphragm the relative succinate dehydrogenase activity (a commonly used marker for oxidative energy metabolism) does not differ between fibre types [36]. Thus, both MyHC composition (85% 2a and 2x) and high oxidative homogeneity (>90%) indicate that mouse diaphragm is particularly homogeneous as compared to other mammalian diaphragms [36].

In conclusion, in the isolated mouse diaphragm, transient changes in tension during both fatigue and recovery run parallel to changes in the number of active cross-bridges, with no change in the force generated per cross-bridge. Moreover, fatigue reduces the rate of cross-bridge detachment, strongly suggesting that the rate of adenosine diphosphate release from the actomyosin complex is impaired by fatigue.

References

1. Road J, Vahi R, Del Rio P, Grassino A. *In vivo* contractile properties of fatigued diaphragm. *J Appl Physiol* 1987; 63: 471–478.

2. Mardini IA, McCarter RJM. Contractile properties of the shortening rat diaphragm *in vitro*. *J Appl Physiol* 1987; 62: 1111–1116.
3. Hervé P, Lecarpentier Y, Brenot F, Clergue M, Chemla D, Duroux P. Relaxation of the diaphragm muscle: influence of ryanodine and fatigue. *J Appl Physiol* 1988; 65: 1950–1956.
4. Fitts RH. Cellular mechanisms of fatigue. *Physiol Rev* 1994; 74: 49–94.
5. Mainwood GW, Cechetto D. The effect of bicarbonate concentration on fatigue and recovery in isolated rat diaphragm muscle. *Can J Physiol Pharmacol* 1980; 58: 624–632.
6. Yanos J, Wood LDH, Davis K, Keamy M III. The effect of respiratory and lactic acidosis on diaphragm function. *Am Rev Respir Dis* 1993; 147: 616–619.
7. Dawson MJ, Gadian DG, Wilkie DR. Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorus nuclear magnetic resonance. *J Physiol (London)* 1980; 299: 465–484.
8. Cooke R, Franks K, Luciani GB, Pate E. The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J Physiol (Lond)* 1988; 395: 77–97.
9. Westerblad H, Allen DG. Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *J Gen Physiol* 1991; 98: 615–635.
10. Nosek TM, Fender KY, Godt RE. It is diprotonated phosphate that depresses force in skinned skeletal muscle fibers. *Science* 1987; 236: 191–193.
11. Cooke R, Pate E. The effects of ADP and phosphate on the contraction of muscle fibers. *Biophys J* 1985; 48: 789–798.
12. Chase PB, Kushmerick MJ. Effects of pH on contraction of rabbit fast and slow skeletal muscle fibers. *Biophys J* 1988; 53: 935–946.
13. Huxley AF. Muscle structure and theories of contraction. *Prog Biophys Biophys Chem* 1957; 7: 255–318.
14. Lecarpentier Y, Coirault C, Lerebours G, *et al*. Effects of angiotensin-converting enzyme inhibition on cross-bridge properties of diaphragm in cardiomyopathic hamsters of the dilated Bio 53-58 strain. *Am J Respir Crit Care Med* 1997; 155: 630–636.
15. Coirault C, Lambert F, Joseph T, Blanc F-X, Chemla D, Lecarpentier Y. Developmental changes in cross-bridge properties and myosin isoforms in hamster diaphragm. *Am J Respir Crit Care Med* 1997; 156: 959–967.
16. Lecarpentier Y, Chemla D, Blanc F-X, *et al*. Mechanics, energetics and cross-bridge kinetics of rabbit diaphragm during congestive heart failure. *FASEB J* 1998; 12: 981–989.
17. Coirault C, Chemla D, Péry-Man N, Suard I, Lecarpentier Y. Effects of fatigue on force–velocity relation of diaphragm. Energetics implications. *Am J Respir Crit Care Med* 1995; 151: 123–128.
18. Hill AV. The heat of shortening and the dynamic constants of muscle. *Proc R Soc Lond Biol Sci* 1938; 126: 136–195.
19. Rayment I, Rypniewski WR, Schmidt-Bäse K, *et al*. Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* 1993; 261: 50–58.
20. Finer JT, Simmons RM, Spudich JA. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* 1994; 368: 113–119.
21. Spudich JA. How molecular motors work. *Nature* 1994; 372: 515–518.
22. Lymm RW, Taylor EW. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* 1971; 10: 4617–4624.
23. Eisenberg E, Hill TL, Chen YD. Cross-bridge model of muscle contraction. Quantitative analysis. *Biophys J* 1980; 29: 195–227.
24. Woledge RC, Curtin NA, Homsher E. Energetic aspects of muscle contraction. *Monogr Physiol Soc* 1985; 41: 27–117.
25. Lutz GJ, Cuizon DB, Ryan AF, Lieber RL. Four novel myosin heavy chain transcripts define a molecular basis for muscle fibre types in *Rana pipiens*. *J Physiol (Lond)* 1998; 508: 667–680.
26. Watchko JF, Daood MJ, Sieck GC, *et al*. Combined myofibrillar and mitochondrial creatine kinase deficiency impairs mouse diaphragm isotonic function. *J Appl Physiol* 1997; 82: 1416–1423.
27. Larsson L, Edström L, Lindegren B, Gorza L, Schiaffino S. MHC composition and enzyme-histochemical and physiological properties of a novel fast-twitch motor unit type. *Am J Physiol* 1991; 261: C93–C101.
28. Sieck GC, Zhan WZ, Prakash YS, Daood MJ, Watchko JF. SDH activity and ATPase activities of different fiber types in rat diaphragm muscle. *J Appl Physiol* 1995; 79: 1629–1639.
29. Fabiato A, Fablato F. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J Physiol (Lond)* 1978; 276: 233–255.
30. Godt RE, Nosek TM. Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. *J Physiol (Lond)* 1989; 412: 155–180.
31. Hibbert MG, Trentham DR, Goldman YE. Phosphate release and force generation in skeletal muscle fibers. *Science* 1985; 228: 1317–1319.
32. Kawai M, Güth K, Winnikes K, Haist C, Rüegg JC. The effect of inorganic phosphate on the ATP hydrolysis rate and the tension transients in chemically skinned rabbit psoas fibers. *Pflügers Arch* 1987; 408: 1–9.
33. Hatcher DD, Luff AR. Force–velocity properties of fatigue-resistant units in cat-fast twitch muscle after fatigue. *J Appl Physiol* 1987; 63: 1511–1518.
34. Siemankowski RF, Wiseman MO, White HD. ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc Natl Acad Sci USA* 1985; 82: 658–662.
35. Yamashita H, Sata M, Sugiura S, Momomura SI, Serizawa T, Iizuka M. ADP inhibits the sliding velocity of fluorescent actin filaments on cardiac and skeletal myosins. *Circ Res* 1994; 74: 1027–1033.
36. Green HJ, Reichmann H, Pette D. Inter- and intraspecies comparisons of fibre type distribution and of succinate dehydrogenase activity in type I, IIA and IIB fibres of mammalian diaphragms. *Histochemistry* 1984; 81: 67–73.