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Changes in phosphorus compounds and water content in skeletal muscle due to eccentric exercise

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Abstract. The interrelationship of the time courses of soreness and oedema, and of force and phosphorus metabolites after eccentric exercise was studied. Eight male subjects performed 120 maximal eccentric contractions with their left forearm flexors. Soreness, maximal force, flexion and extension elbow angle, and creatine kinase and myoglobin efflux were followed for 96 h after exercise. For equal periods T1 and T2 relaxation times and muscle cross-sectional area were calculated from magnetic resonance images as indications of oedema, and inorganic phosphate (P_i) and phosphocreatine (PCr) were measured with magnetic resonance spectroscopy. Soreness on extension increased at 1 h ($P=0.043$), T1 and T2 (both $P=0.01$) and soreness when the arm was pressed ($P=0.028$) at 24 h, and muscle cross-sectional area increased at 48 h ($P=0.01$) after exercise. Soreness on extension reached a maximum at 48 h, the other four parameters at 72 h. All parameters related to oedema, and soreness, showed an increasing pattern for the period after exercise as a whole, but the largest increase between two points of measurement occurred earlier for soreness than for oedema. Creatine kinase increased significantly from baseline from 24 h onwards ($P=0.017$) and myoglobin from 1 h onwards ($P=0.012$). The P_i:PCr ratio differed from baseline for the first time 24 h after exercise ($P=0.018$), increased to 225%, and then remained on a plateau until 72 h. Maximal isotonic force decreased to 53% at 1 h ($P=0.012$), and recovered from then on. It was concluded firstly that the largest increase in soreness precedes that of oedema, and secondly that the decrease in force after eccentric exercise is not related to an altered metabolic state. The combined imaging and spectroscopy results gave the impression that changes in phosphorus metabolites were homogeneously distributed over the flexor muscles whereas oedema was not.

Key words: Magnetic resonance imaging – Magnetic resonance spectroscopy – Oedema – Muscle injury

Introduction

Vigorous eccentric exercise has been shown to lead to delayed onset muscle soreness (DOMS) and changes in functional parameters like force and range of motion (Clarkson et al. 1992; Rodenburg et al. 1993). The exact causes of these changes are still unclear. It has been thought that DOMS may be associated with the development of oedema (Friden et al. 1988), and decreases in force may be associated with muscle fibre disruption (Clarkson et al. 1992) or changes in phosphorus metabolites (Dawson 1986). However, it is not possible to study cause-effect relationships for the above changes, since one cannot induce or block oedema, morphological disruption or metabolism alone in vivo in humans, without changing other factors. However, the comparison of the time courses of the different parameters may be a first useful step in determining whether relationships between the two factors are likely to exist at all.

To date, the time courses of several biochemical changes such as the efflux of creatine kinase (CK) from muscle (Newham et al. 1983a) and release of myoglobin (Mb) (Nosaka et al. 1992; Rodenburg et al. 1993), of morphological disruption of muscle fibres (Friden et al. 1983; Newham et al. 1983b), and of acute inflammation (Smith 1991; Stauber et al. 1990) have been studied in relation to DOMS. However, none of these factors followed exactly the same time course as DOMS. Also the time courses for oedema have been studied, and were not found to be in parallel with the time course of DOMS (Bobbert et al. 1986; Clarkson et al.

Parts of this study have already been presented during a conference as preliminary results (Rodenburg et al. 1992)

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1992). However, in these studies oedema was quantified by measuring the circumference of the affected limb, which it has been shown may change over periods of days due to factors other than oedema (Bobbert et al. 1986). Furthermore, this technique only provides an overall impression and does not focus on the affected muscle specifically. Oedema has also been quantified by measuring intramuscular pressure after exercise, which has been found to increase during periods of DOMS in some studies (Friden et al. 1986, 1988), but not in others (Newham and Jones 1985). Time courses of intramuscular pressure have not yet been studied. Magnetic resonance images (MRI) can be used to study the site at which oedema develops, and T1 and T2 relaxation times, which have been shown to be related to the water content of muscle tissue (Fried et al. 1988), can be obtained from the images to quantify the amount of oedema (Fleckenstein et al. 1989; Shellock et al. 1991). This way of measuring oedema and measuring its complete time course may lead to new information on how oedema develops.

It has been suggested that disruption of myofibrils may play a role in the decrease of muscle force after eccentric exercise (Newham et al. 1987; Rodenburg et al. 1993): Friden et al. (1983) have found disruption in about 32% of the fibres 1 h after exercise. However, they have measured that the relative area occupied by these disturbances was only 1.6%, and hence, it cannot account for the large decreases in force of about 40% (Rodenburg et al. 1993). Another possibility is that metabolic dysfunction causes the decrease in force: using phosphorus magnetic resonance spectroscopy (^{31}P-MRS) changes in the ratio between inorganic phosphate (P_i) levels and phosphocreatine (PCr) have been found, indicating some metabolic dysfunction (Aldridge et al. 1986; McCully et al. 1988). However, the P_i:PCr ratio was only measured up to 48 h after exercise in one of these studies (Aldridge et al. 1986), and in neither study was the time course of the P_i:PCr ratio measured together with the time course of the decrease and recovery of force.

So far, no studies have been presented in which MRI and ^{31}P-MRS are combined in the study of muscle damage. We feel that such an approach is important to establish whether the ^{31}P spectra describing the metabolic changes due to damage are collected from the appropriate area, i.e. the area which shows oedema on the images (Jeneson et al. 1992). The aim of the present study was:

1. To determine whether oedema precedes DOMS or is concurrent,
2. To determine whether changes in phosphorus metabolites precede a decrease in force or are concurrent after eccentric exercise, and
3. To combine MRI and ^{31}P-MRS data to interpret the latter properly.

Also, biochemical indicators of muscle damage CK (Bär and Amelink 1992) and Mb efflux (Bär et al. in press) and range of motion were measured, since MRI and MRS measurements and physiological parameters have not yet been measured simultaneously.

Methods

Subjects. Eight male subjects [mean 23 (SD 3) years, height 1.88 (SD 0.07) m, mass 76.0 (SD 5.1) kg] participated after giving informed consent. In the week before the tests they did not participate in more exercise than they were accustomed to. They had not participated in studies involving eccentric exercise before or in sports activities requiring specific eccentric arm exercise for the last 3 years.

Exercise. The subjects performed eccentric exercise with their left arm. The exercise protocol is described in full elsewhere (Rodenburg et al. 1993). To summarise, the subject sat on a chair with his chest against a support. The upper arm was placed on a horizontal support at shoulder level and pointed forwards in the sagittal plane. The hand was kept in a supinated position. A rope with a tray was attached to the wrist via a pulley system. Weights were placed on the tray to impose an extending momentum on the arm.

The exercise consisted of 120 maximal forced extensions of the forearm around the elbow joint from 45° flexion to 170° extension. Every 15 s, the subject had to counteract the extending momentum of the weights on the tray, thus performing only eccentric work with the biceps brachii and brachialis muscles. At the beginning of the exercise the load equalled the maximal isotonic force of the subject, which was determined beforehand. Every time the subject could no longer lower a given load gradually in 3 s, the weight on the tray was reduced by 0.5–1.0 kg, so that at the end of the exercise about 30% of the initial load remained. Total exercise time was 30 min.

Protocol and measurements. One hour before the exercise and at five set times afterwards (1, 24, 48, 72 and 96 h), MRI and ^{31}P-spectra were collected, and DOMS, maximal isotonic force, relaxed-extended and flexed elbow angle (RANG and FANG), CK activity and Mb concentration were assessed.

Magnetic resonance imaging. The subjects were examined in a 1.5 T whole-body scanner (Philips Gyroscan S15/HP), while laying prone with their left arm stretched out in front of their head; the right arm was placed beside the body. The belly of the biceps brachii muscle of the left arm was marked with semi-permanent ink to ensure that images and spectra were collected at the same site at the set times.

For two subjects, two transverse Spin-Echo-images (SE-images) were collected at each set time with a wrap-around receiver coil (slice-thickness: 10 mm). The first SE-image was collected with a long repetition time (TR) of 2000 ms and the second SE-image was collected with a short TR of 707 ms. For both images two echoes were collected: echo time (TE) = 30 and 60 ms. The T1 and T2 images were calculated from the four resulting images according to a method developed for this kind of minimal data set (Bakker and Moerland 1989). For the other six subjects, images were collected with a mixed sequence in which a SE-experiment and an inversion recovery experiment were combined, according to the following parameters: spin-echo TR = 680 ms, inversion recovery TR = 2160 ms, inversion delay = 360 ms, and TE = 40 and 80 ms. From this sequence T1 and T2 images were calculated by the Philips Gyroscan software (In Den Kleef and Cuppen 1987). Both methods led to similar results for the T1 and T2 images and required total imaging times of around 17 min.

On the T1 and the T2 images, three areas were defined based on either the anatomy or the difference in contrast between affected and non-affected areas after eccentric exercise: the triceps brachii muscle was clearly separated from the flexor muscles by fat and connective tissue; the biceps and the brachialis muscles, however, could not be separated from each other on the basis of such demarcations on the MRI before exercise. Therefore, based on clearly visible differences in signal intensity on the images 72 and 96 h after exercise, two flexor sites were discriminated: an

'affected' and a 'less-affected' site. The signal intensity was sampled five times on each of the three sites on the T1 image and five times on each of the three sites on the T2 image. These samples were averaged, resulting in one T1 and one T2 value for each site. From the images the cross-sectional area of the total muscle mass was determined. The contour of the muscles was traced with a cursor, whereafter the cross-sectional area of the indicated muscle mass was calculated using the Gyroscan software. For T1, seven images could be quantified at 1 h and 48 h, and six at 72 h after exercise. For T2, seven images could be quantified at 48 h, and six at 72 h after exercise. At the other set times the images of all eight subjects could be quantified.

^{31}Phosphorus-magnetic resonance spectroscopy. The ^{31}P-spectra were collected in the same MR-scanner with a 5-cm, single-turn, double-tuned surface coil (Philips) after shimming on the proton signal (full width at half height: 20–30 Hz). The coil was placed against the skin over the belly of the biceps brachii muscle. The acquisition parameters were: adiabatic rapid half passage pulse, adiabatic 90° pulse length = 3.04 ms, 128 averages, TR = 3000 ms, sample frequency 3000 Hz, number of data points = 1024. Phase correction was performed manually (zero and first order). The signal-to-noise ratio of the spectra was calculated with LABONE NMR1 spectroscopy processing software (New Methods Research Inc., Michigan, USA). The baseline of the spectra was automatically corrected and the spectra were fitted in the frequency domain (with a Lorentzian function) with the same software. The areas of the peaks of P_i and PCr were calculated thereafter.

Saturation effects were calculated for 24 measurements in four subjects, of whom two participated in the eccentric exercise study. Spectra were obtained with TR = 10000 ms and with TR = 3000 ms. Then a single exponential curve for longitudinal relaxation was fitted on three intensities: zero signal intensity for TR = 0 ms, and the signal intensities measured for TR = 3000 and 10000 ms. The curve was fitted according to the formula: $SI = SI_{max} \times (1 - \exp^{t/T1})$, where SI is signal intensity. The T1 was determined from this fit and maximal signal intensity was calculated for complete relaxation. The mean T1 values thus obtained were 4.58 (SD 1.08) ms for PCr and 3.74 (SD 1.64) ms for P_i. Correction factors for PCr and P_i were then calculated by dividing SI_{max} by SI at TR = 3000 ms. Mean correction factors thus obtained were 2.10 (SD 0.36) for PCr and 1.83 (SD 0.51) for P_i. These factors were applied on all spectra.

Muscle intracellular pH (pH_i) was calculated from the chemical shift of P_i relative to PCr (Taylor et al. 1986). The ^{31}P-MRS data at 72 h after exercise were only obtained for six subjects due to failure of the scanner. For the other set times ^{31}P-MRS data for all subjects were obtained.

Delayed onset muscle soreness. The DOMS was scored on a qualitative scale ranging from 0–6 as used previously (Rodenburg et al. 1993). Each number corresponded to a verbal description of soreness (0 = no soreness, 1 = dull feeling of soreness, 2 = light continuous soreness, 3 = more than light soreness, 4 = annoying soreness, 5 = very sore, 6 = intolerable soreness). The subjects were allowed to score in half points. One score was obtained while the subject pressed with the contralateral hand simultaneously on the belly of the biceps brachii muscle and, where it reached the skin, of the brachialis muscle as hard as he could sustain. A second score was obtained while the subject extended the arm. When determining this score, the subjects indicated the presence of soreness both at the bellies of the flexor muscles, and at the musculo-tendinous junctions. They gave one single DOMS-on-extension score in which both sites were taken into account.

Maximal isotonic force. Maximal isotonic force was measured on the same apparatus as used for the exercise protocol. It was defined as the maximal mass subjects could hold for 5 s at an elbow angle of 90° (Rodenburg et al. 1993).

FANG and RANG. The FANG and RANG of the elbow joint were measured with a goniometer while the subject was erect. To obtain the FANG, the subjects were asked to reach with their thumb to the shoulder as far as possible. They were asked to keep the elbow in line with the body and to stretch their fingers. For measurement of RANG, the subjects stood with their arm relaxed at their side. To measure these angles each day in the same way, the acromion, the epicondylus lateralis of the humerus and the point halfway between the processi styloidei of the ulna and the radius were marked with semi-permanent ink.

CK activity and Mb concentration. Blood was collected from an antecubital vein in a heparinized tube (CK) and a tube without additive (Mb). Serum or plasma was separated and stored at −20° C until analysis. The CK activity in plasma was assessed by means of a spectrophotometric method (N-acetylcysteine activated kit, Boehringer Mannheim). The Mb concentration was assessed in serum using a radio-immuno-assay (Ria-mat, Byk-Sangtec Diagnostica).

Statistics. Changes over the six set times were tested with a non-parametric Friedman test for repeated measures for each parameter. If a significant change was found, a Wilcoxon test was used to detect the first significant difference from baseline in time. A Page test for ordered alternatives was performed on the postexercise values, to test whether a specific ordered pattern of change (increasing or decreasing) existed after exercise (Siegel and Castellan 1988). The results of this test were used to determine whether the time courses of oedema and DOMS, and of phosphorus metabolites and force were similar.

Results

Magnetic resonance imaging

Large increases in signal intensity on the images were found in the upper arm muscles due to the eccentric exercise, especially from 48 h after exercise onwards (Fig. 1). On the basis of these changes in signal intensity, the damaged site could be discerned. The largest increase in signal intensity was found for the site close to the humerus, the 'affected' site, which contained the brachialis muscle and possibly part of the biceps brachii muscle (Fig. 1).

The increases in signal intensity are reflected by the T1 and T2 values which were calculated from these images (Table 1, Fig. 2a, b). The T1 and T2 of the 'affected' site ($P = 0.003$, $P = 0.001$), and T1 of the 'less-affected' site ($P = 0.01$) showed a significant time effect over the whole period of measurement (Friedman). They differed from baseline for the first time at 24 h after exercise (Wilcoxon, $P = 0.01$). The highest value for the relaxation time constants was found at 72 h after exercise. Tested over the postexercise period as a whole, T1 and T2 followed an increasing pattern in both the 'affected' area, and the 'less-affected' area (Page, $P < 0.05$). Figure 2a and b shows that these increases did not occur again after 72 h, which indicated that the significance of the Page test was caused by the changes from 1 h to 72 h after exercise. Muscle cross-sectional area differed significantly with the passage of time (Fig. 2c, Friedman, $P = 0.014$), and its first change from baseline occurred at 48 h after exercise (Wilcoxon, $P = 0.042$). There was a significantly increasing

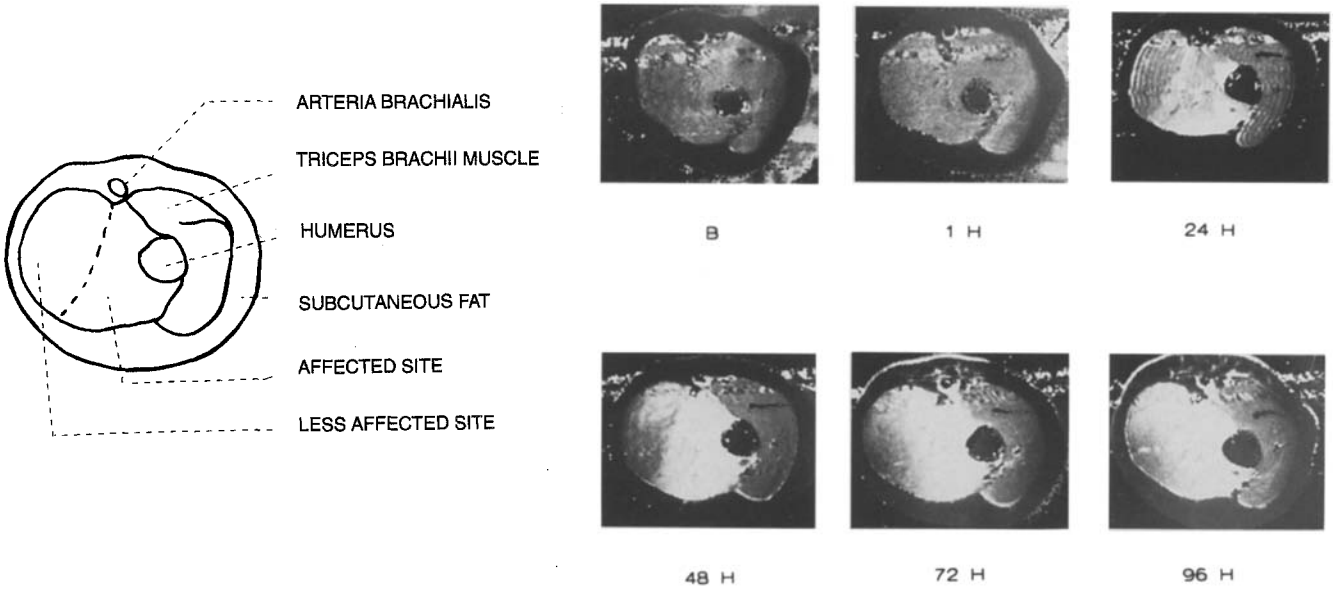

Fig. 1. Transversal T1 images of the upper arm for one subject before (*B*) eccentric exercise and at five set times afterwards (1 h, 24 h, 48 h, 72 h and 96 h). The images were collected at the height of the belly of the biceps brachii muscle. A diagram of the anatomical structures is given *to the left of the figure.* The shape of the arm differed somewhat with the passage of time, due to differences in the adjustment of supporting pillows on different days. These differences did not influence surface area measurements

trend over the five postexercise values (Page, $P<0.05$). This trend originated from the changes between 1 and 72 h; muscle surface area remained the same between 72 and 96 h (Fig. 2c).

^{31}Phosphorus-magnetic resonance spectroscopy

The ^{31}P-spectra were collected with signal-to-noise ratios ranging from 7 to 17 for the P_i-peak (Fig. 3a). The P_i:PCr ratio showed a significant time effect over the whole measurement period (Fig. 3b, Friedman, $P=0.001$). The first significant difference from baseline occurred 24 h after exercise (Wilcoxon, $P=0.018$). The subjects reached maximal values of 0.16–0.31 between 24 and 72 h. Tested over the postexercise period as a whole, the P_i:PCr ratio did not significantly recover (Page, $P<0.05$). This significance originated from the period between 1 and 72 h; after 72 h, recovery of the ratio started (Fig. 3b).

The pH_i showed a significant time effect (Fig. 3c, Friedman, $P=0.016$). Immediately after exercise, the pH_i had decreased (not significant), but after 24 h it had reached higher levels than before exercise. After exercise, the pH_i followed an increasing pattern (Page, $P<0.05$). The first time at which it was significantly different from baseline was 96 h after exercise (Wilcoxon, $P=0.017$).

Delayed onset muscle soreness

The DOMS showed a significant time effect both when the muscle was pressed and when it was extended (Fig. 4a,b, Friedman, $P=0.006$ and $P=0.008$). The DOMS when the muscle was pressed first appeared at 24 h (Wilcoxon, $P=0.028$) and did not differ significantly from baseline at 96 h. The DOMS, when the arm was extended, was already present 1 h after exercise (Wilcoxon, $P=0.043$) and remained present over the whole period after exercise. For both DOMS parameters, a significantly increasing trend was found over the postexercise period as a whole (Page test, $P<0.05$). However, when single times were observed, recovery was

Table 1. Resting values (before exercise) and maximal values (72 h afterwards) for T1 and T2 for three different sites of the upper arm muscles after eccentric exercise

	T1 (ms)				T2 (ms)			
Site	Before		72 h		Before		72 h	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Less affected site	744	33	904	63*	25	1	33	3*
Affected site	803	34	1257	71*	30	1	56	6*
Triceps muscle	797	67	915	158	31	2	29	1

*Significantly different from baseline (Wilcoxon, $P<0.05$)

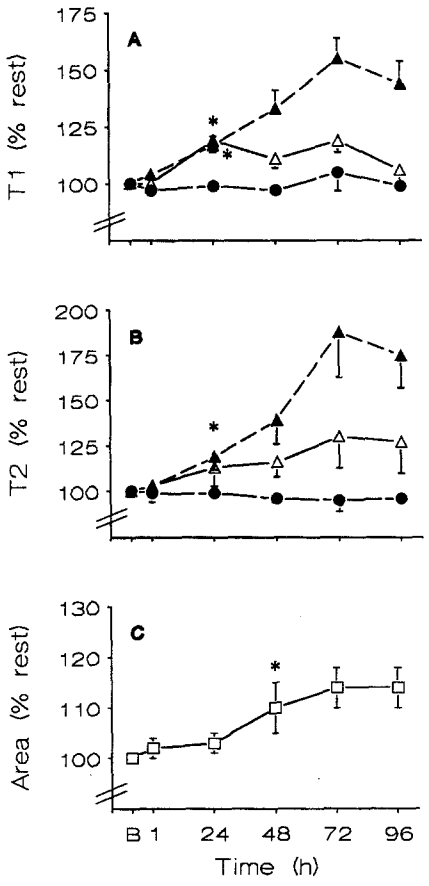

Fig. 2. Parameters indicating the amount of oedema in the upper arm before (*B*) exercise and 1, 24, 48, 72 and 96 h after exercise. All parameters are presented as a percentage of the initial value. Mean and SEM are given. **A**, T1 values; **B**, T2 values; **C**, total muscle cross-sectional area. *First time differing from baseline (Wilcoxon, $P<0.05$). —△— Less affected site; —▲— affected site; —●— triceps muscle; —□— total muscle area

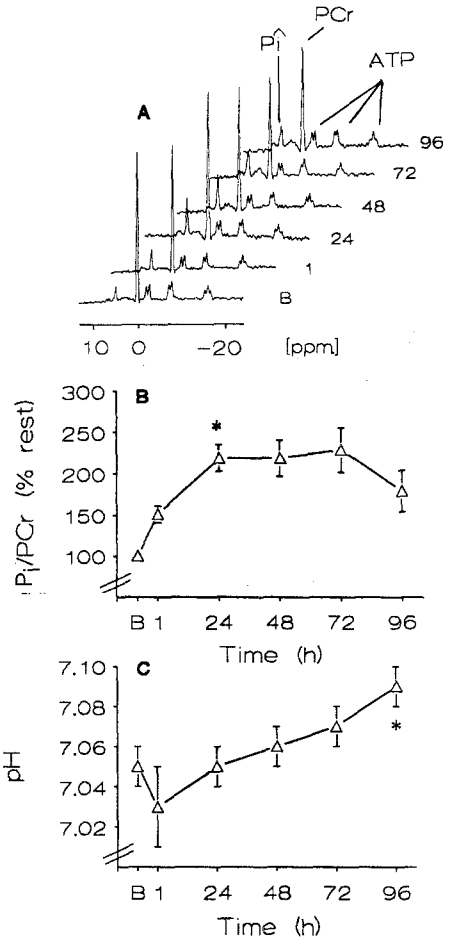

Fig. 3. Parameters collected by means of ^{31}Phosphorus-magnetic resonance spectroscopy (^{31}P-MRS) before (*B*) and 1, 24, 48, 72 and 96 h after exercise. **A**, a typical example of a time pattern of ^{31}P-spectra for one subject; **B**, the ratio between inorganic phosphate and phosphocreatine ratio (*P_i:PCr*); **C**, the intracellular *pH* (pH_i). Mean and SEM are given for **B** and **C**. *First time differing from baseline (Wilcoxon, $P<0.05$). *ATP*, Adenosine triphosphate

seen after 72 h for DOMS upon pressure (Fig. 4a) and after 48 h for DOMS upon extension (Fig. 4b).

Maximal isotonic force, FANG and RANG

The functional parameters, maximal isotonic force, FANG and RANG, showed significant time effects (Fig. 4c,d,e, Friedman, $P=0.001$, $P=0.002$ and $P=0.003$ respectively). They were all significantly different from baseline for the first time 1 h after exercise (Wilcoxon, $P=0.012$, $P=0.012$, $P=0.017$ respectively) and were maximally affected at that time (Fig. 4c,d,e). Maximal isotonic force and extension angle showed a significant recovery over the postexercise period as a whole (Page, $P<0.05$). The FANG did not show a significant time effect after exercise.

CK activity and Mb concentration

A significant time effect was found for CK efflux over the whole observation period (Fig. 5a, Friedman, $P=0.002$). The CK activity started to increase 24 h after exercise (Wilcoxon, $P=0.017$). The Mb concentration showed a significant time effect (Fig. 5b, Friedman, $P=0.001$), but, in contrast to CK, Mb had already increased 1 h after exercise (Wilcoxon, $P=0.012$), and increased further after 24 h. Both CK and Mb showed an increasing pattern over the period after exercise as a whole (Page, $P<0.05$). The Mb concentration started to recover after 72 h (Fig. 5b).

Discussion

Oedema and DOMS

To determine whether oedema and DOMS follow a similar time course, it is important to know whether changes in T1 and T2 indeed represent the development of oedema. Fried et al. (1988) have shown a linear relationship between intramuscular water content on the one hand, and T1 and T2 relaxation times on

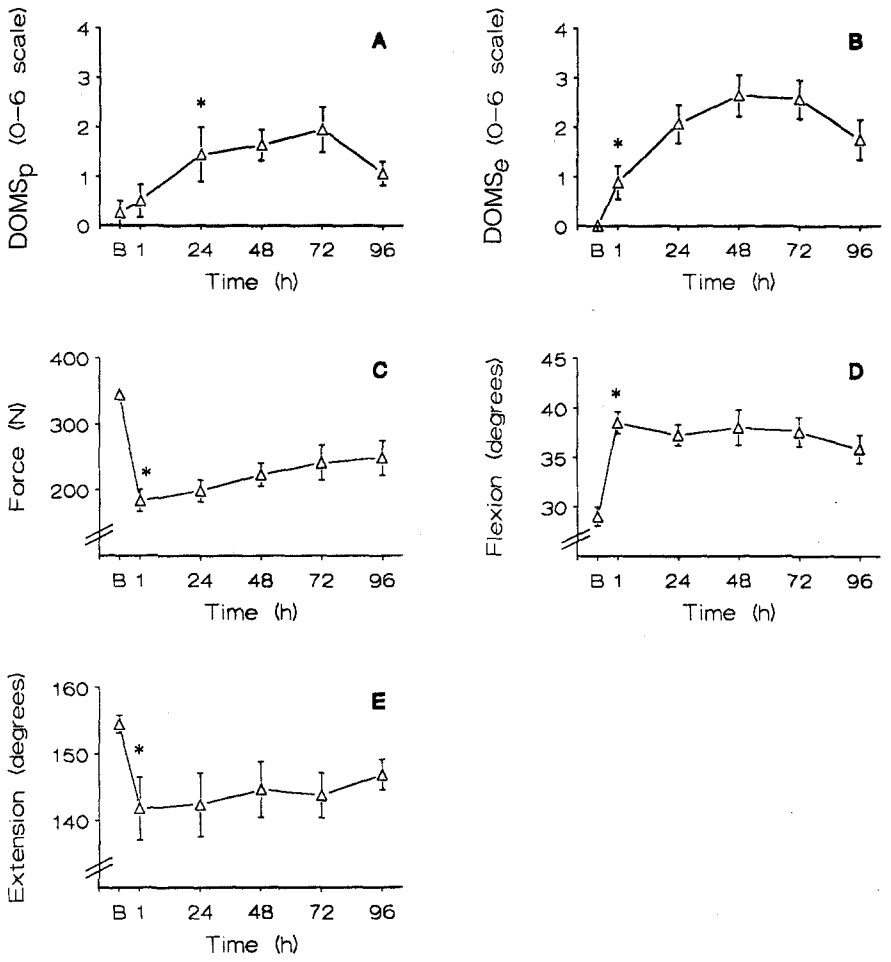

Fig. 4. Functional parameters before (*B*) and 1, 24, 48, 72 and 96 h after eccentric exercise. **A**, delayed onset muscle soreness while pressing the muscle ($DOMS_p$); **B**, delayed onset muscle soreness while extending the arm ($DOMS_e$); **C**, maximal isotonic force; **D**, flexion elbow angle; **E**, extension elbow angle. Mean and SEM are given. *First time differing from baseline (Wilcoxon, $P<0.05$)

the other for the myocardium and for quadriceps muscle during maturation in the rabbit. Polak et al. (1988) have also shown this relationship for the gastrocnemius muscle in the rat, which contains fast type muscle fibres, but could not find a relationship between T1 and T2 and water content for the soleus muscle, which contains slow type muscle fibres. They suggested that this difference arose because the relationship between water content and T1 and T2 only exists for intracellular water, and not for extracellular water, which forms the largest part of intramuscular water in the soleus muscle. The existence of a linear relationship between T1 and T2 and the intramuscular water content is also supported by the finding that the increases in T1 and T2 occurred simultaneously with increases in the cross-sectional area of the upper arm muscles in the present study. However, increases in T1 and T2 may also partly originate from fluid shifts between the intracellular, interstitial and vascular space in the muscle, since some authors have indicated that T2 and T1 are larger in one compartment than in the other (Hazlewood et al. 1974; LeRumeur et al. 1987).

The time course of DOMS upon pressure is quite similar to the time courses of the parameters for oedema: T1, T2, and muscle surface area. All of them show changes from baseline after the exercise, show an increasing pattern when tested over the postexercise period as a whole, and show a recovery after 72 h, when individual postexercise times are evaluated from the figures. Furthermore, T1, T2 and DOMS on pressure show their first change from baseline at the same time: 24 h after exercise, which is in agreement with the results of Bobbert et al. (1986), who have found an increase of limb volume 24 h after eccentric exercise. However, there is one important difference: whereas DOMS upon pressure showed a steep rise between 1 and 24 h, the parameters for oedema rose only slightly between these two times, and showed large increases between 24 and 72 h. This, together with the later start of the increase of muscle surface area (at 48 h), would indicate that DOMS upon pressure slightly precedes the development of oedema.

The DOMS upon extension precedes the development of oedema even more than DOMS upon pressure. Although DOMS on extension showed an increasing pattern when tested over the postexercise period as a whole, it showed recovery after 48 h when individual times were studied. Furthermore, DOMS upon extension already differed from baseline 1 h after exercise, at which time the parameters related to oedema were still at the normal level.

Both for DOMS upon pressure and for DOMS upon extension, there must have been a factor other than oedema which produced soreness in the first

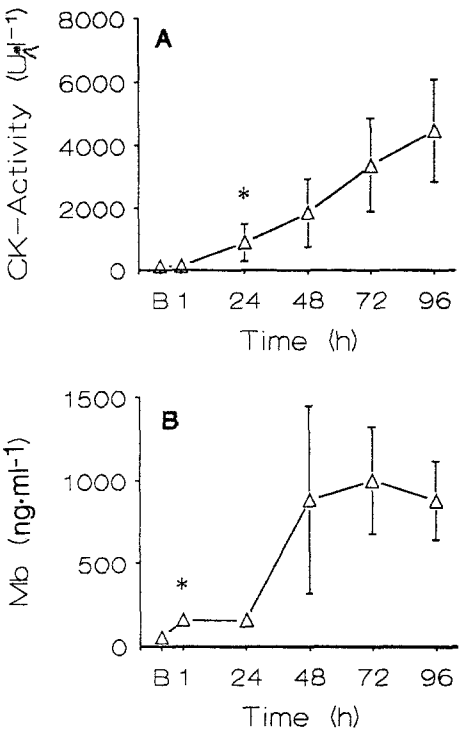

Fig. 5. Efflux of muscle proteins before (*B*) and 1, 24, 48, 72 and 96 h after eccentric exercise. **A**, Creatine kinase (*CK*) efflux; **B**, efflux of myoglobin (*Mb*). Mean and SEM are given. *First time differing from baseline (Wilcoxon, $P<0.05$)

hours. It remains unclear whether oedema plays a role later after exercise or not. It may account for soreness after some days, when other factors no longer act on the pain afferents, or it may aggravate already existing pain. However, it must be born in mind that there are some indications in other studies that oedema does not relate to DOMS: T1 and T2 also have been shown to increase for a short period after concentric, non-painful exercise (Fisher et al. 1990; Fleckenstein et al. 1988), as did intramuscular pressure during exercise when no pain was felt (Friden et al. 1986; 1988).

At first glance, the average increases in the P_i:PCr ratio followed the same time course as the occurrence of DOMS upon pressure and upon extension, but statistically the pattern of change of the P_i:PCr ratio was not the same as that of DOMS: in contrast to the DOMS scores, the P_i:PCr ratio did not follow an increasing pattern over the postexercise period as a whole. Furthermore, in some studies, elevations in resting P_i:PCr ratio have also been found without DOMS, e.g. in myopathies (Griffiths et al. 1985; Aldridge et al. 1986; McCully et al. 1988) or until 28 h after concentric exercise (Newham and Cady 1990), making the existence of a relationship between them unlikely.

Decreased force and P_i

Elevated concentrations of P_i are known to induce decreases in force in skinned muscle fibres (Hajjar and Gwathmey 1990) and in intact muscle fibres (Dawson 1986). Therefore, it can be deduced that the decrease in force, which has been seen in many studies after eccentric exercise (Newham et al. 1987; Rodenburg et al. 1993), is linked with the increases in P_i observed in other studies (Aldridge et al. 1986; McCully et al. 1988). In this study we measured force and the P_i:PCr ratio simultaneously, and found that force recovered gradually after an initial decrease 1 h after exercise, whereas the P_i:PCr ratio remained elevated at a plateau until 72 h and only recovered thereafter. We also calculated the relative amounts of $H_2PO_4^-$ and HPO_4^{2-} from P_i and pH_i (Dawson 1986), since Dawson has indicated that only $H_2PO_4^-$ shows a linear relationship with force decline (Dawson 1986), and because the pH_i changed with time in our data. However, the ratios from these compounds over PCr reached a plateau from 24 to 72 h, similar to the ratio of P_i over PCr. Therefore, at least one additional factor must have been present to limit force generation. This unknown factor must already have returned to normal the first day after exercise, explaining the recovery of force. This factor cannot be morphological degeneration, since morphological degeneration has been found to be present for more than one day (Friden et al. 1983).

Combination of MRI and ^{31}P-MRS

The combination of both MR techniques (MRI and ^{31}P-MRS) provides a means to verify whether spectra are collected from the appropriate area. In our study the surface coil was placed over the skin covering the biceps brachii muscle, since this muscle was expected to be one of those primarily affected beforehand. Placement of the surface coil for ^{31}P-MRS could not be guided by the site of oedema on the images for the first three times in the present study, since oedema only became apparent 48 h after eccentric exercise. By measuring the ^{31}P-MRS signal of two phantoms each 2 cm thick, we verified that 80% of that signal arose from the 2 cm closest to the surface coil. Since the 'less-affected' area on the images was about 3 cm thick, more than 80% of the ^{31}P signal probably originated from the 'less-affected' area in the present study and at the most 20% originated from the 'affected' area. Provided that the changes in the P_i:PCr ratio followed the same time course in the 'affected' and the 'less-affected' area, the nonoptimal placement of the surface coil had consequences only for the absolute values of the P_i:PCr ratios and not for the pattern of change. The absolute value of the P_i:PCr ratio may have been 0.60 in reality when a value of 0.28 was measured (which was the mean value at 72 h). This can be deduced as follows:

1. When the P_i:PCr ratio is heterogeneously distributed over the flexor muscles as is oedema.
2. When the P_i:PCr ratio of the 'less-affected' site is imagined to be only slightly elevated, e.g. 0.20.
3. When it is imagined that only 20% of the signal has originated from the 'affected' site, then:

$$P_i:PCr_{measured} = 0.80 \times P_i:PCr_{\text{'less-affected' site}} + 0.20 \times P_i:PCr_{\text{'affected' site}}$$

and hence,

$$P_i:PCr_{\text{'affected' site}} = (0.28 - 0.80 \times 0.20)/0.20 = 0.60$$

This fivefold increase compared to rest may have a profound effect on performance after eccentric exercise, since it has been shown that P_i induces decreases in force (Hajjar and Gwathmey 1990).

To verify this value of 0.60 experimentally, the surface coil was placed directly over the place where the 'affected' site reached the skin surface in one extra subject, 24 h after eccentric exercise of the flexor muscles, and a P_i:PCr ratio of 0,27 was found. In another subject a time consuming phase encoding experiment was performed in which the P_i:PCr ratios were measured in different slices of 1-cm width 24 h after eccentric exercise. The ratio for the slice including the 'less-affected' site was 0.21, compared with 0.20 for the slice including the 'affected' site. Since values of 0.60 were not reached for the 'affected' site in these extra experiments, the P_i:PCr ratios did not seem to be heterogeneously distributed over the 'less-affected' site as was supposed on the basis of the heterogeneous distribution of oedema. This would indicate that there was a dissimilarity between the localisation and development of oedema and the localisation and development of the changes in the P_i:PCr ratio.

Additional findings

An interesting similarity could be seen between the time courses of CK and Mb on the one hand, and T1 and T2 relaxation times on the other hand: they all remained at the resting level or increased slightly 1 h after exercise, and showed the largest increase between 24 h and 72 h. This similarity may be explained as follows: when the sarcolemma lost its integrity due to mechanical disruption, CK and Mb efflux to the interstitium resulted and the ionic compartmentization may have changed. This in turn, may have led to fluxes of fluid from the vascular space to the interstitium and the cell, so that oedema occurred, resulting in increases of T1, T2 and muscle cross-sectional area. The fact that Mb already showed small increases 1 h after eccentric exercise may have been caused by the smaller molecular mass compared to CK (Bär and Amelink 1992).

The loss of sarcolemmal integrity was also indicated by the occurrence of phosphodiesters in the spectrum 48 h after exercise, which were not present before. At 72 h and 96 h after exercise two of the eight subjects showed a very broad peak of low intensity around 2.8 ppm relative to PCr, which was too small to be quantified properly. In another four subjects there seemed to be a peak at this chemical shift position at 48 h or 72 h, but it was not easily detectable.

McCully et al. (1988) have suggested that the P_i:PCr ratio may be elevated after eccentric exercise due to an increase in cellular metabolism for repair processes, due to an increased ion transport pumping activity to compensate for leakage of the membranes, or due to an increased amount of extracellular P_i because of the elevated levels of extracellular fluid. If the latter two reasons were to be valid, the P_i:PCr ratio should follow the same time course as CK efflux and oedema, since sarcolemmal disintegration is the central factor in both. Therefore, we are inclined to think that an increased cell metabolism was a more likely reason for the elevation of the P_i:PCr ratio.

In conclusion, the present data support the view that muscle soreness after eccentric exercise precedes the development of oedema. The changes of force with time after eccentric exercise are not related to an altered metabolic state of the cell. Finally, the combination of MRI and MRS data led us to the impression that changes in the P_i:PCr ratio are homogeneously distributed over the flexor muscles, whereas oedema occurs to different extents at different sites after eccentric exercise.

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