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MUSCLE INJURY, CROSS-SECTIONAL AREA AND FIBRE TYPE DISTRIBUTION IN MOUSE SOLEUS AFTER INTERMITTENT WHEEL-RUNNING

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SUMMARY

1. It was previously noticed that mouse soleus, but not extensor digitorum longus (EDL) muscles, suffer fibre damage at the onset of voluntary wheel-running without further injuries thereafter.

2. In CBA/J mice trained continuously for 5 months and rested for periods of 1, 2, 3, 4 and 5 weeks acute muscle damage was found in soleus 7 days after the resumption of wheel-running. On single cross-sections damage was present on average in $8{\cdot}7 \pm 3{\cdot}5\%$ (mean $\pm$ S.D., $n = 15$) of the fibres, but only in $0{\cdot}47 \pm 0{\cdot}21\%$ ($n = 9$) and $1{\cdot}3 \pm 1{\cdot}1\%$ ($n = 4$) in control animals rested for 0–6 weeks after continuous running or in untrained controls.

3. Repeated muscle damage occurred when mice exercised for 4 days at intervals of 21–25 days, and after thirteen running episodes within 12 months marked changes in soleus, but not EDL muscles, were present. In cross-sections the total number of muscle fibre profiles was significantly larger in soleus of intermittent runners (768 ± 68, $n = 6$; $P < 0{\cdot}05$), compared to continuous runners (676 ± 54, $n = 3$) and sedentary animals (683 ± 33, $n = 4$). This is probably due to incomplete repair which results in 'split fibres'.

4. At the same time total muscle fibre cross-sectional area was significantly elevated in intermittent runners ($P < 0{\cdot}05$), mainly due to increase in fibre diameters. Net cross-sectional areas were $0{\cdot}59 \pm 0{\cdot}069$ mm^2 ($n = 6$) in intermittent, $0{\cdot}53 \pm 0{\cdot}076$ mm^2 ($n = 3$) in continuous runners and $0{\cdot}46 \pm 0{\cdot}031$ mm^2 ($n = 3$) in sedentary controls.

5. Tetanic and twitch force were also significantly elevated in soleus of intermittent runners while the ratio force/area remained the same.

6. There was an increase in the proportion of type I fibres in soleus from $75 \pm 0{\cdot}9\%$ ($n = 4$) in untrained controls to $90 \pm 4{\cdot}4\%$ ($n = 6$; $P < 0{\cdot}05$) in intermittent runners and $81 \pm 5{\cdot}6\%$ ($n = 3$; n.s.) in continuous runners.

7. Resistance to block of synaptic transmission in soleus was significantly higher in intermittent runners for two levels of curare, indicating enhanced safety margins.

8. EDL muscles in intermittent runners were not different from sedentary controls in any of the parameters studied. In particular, muscle fibres with signs of previous

damage (split fibres, central nuclei) were rare (on average 0·5–0·6%) and equally frequent in all experimental groups.

9. However, in continuously trained compared to sedentary animals, EDL muscles showed typical signs of endurance training, i.e. lower tetanic force, elevated twitch time-to-peak values, and increase in fast oxidative (type II B) fibres. A slight increase in the proportion of type I fibres was also noticed: $3·1 \pm 1·8\%$ ($n = 3$) *versus* $1·2 \pm 0·3\%$ ($n = 3$, $P < 0·05$).

10. It is concluded that intermittent wheel-running causes an increase in cross-sectional area and muscle force in soleus in spite of repeated muscle damage and the occurrence of split fibres. It is speculated that the vulnerability of soleus in wheel-running might depend on the adaptability of the spinal motor patterns. Collateral axonal sprouting is discussed as a possible mechanism underlying the simultaneous increase in the proportion of type I fibres.

INTRODUCTION

It is well documented that motor activity can cause muscle fibre damage in both man and animals (for recent publications see Armstrong, Ogilvie & Schwane, 1983; Hikida, Staron, Hagerman, Sherman & Costill, 1983; Kuipers, Drukker, Frederik, Geurten & van Kranenburg, 1983; Fridén, 1984; Jones, Newham, Round & Tolfree, 1986; Irintchev & Wernig, 1987). Under normal conditions such injuries, with and without muscle fibre necrosis, seem to be easily compensated for by muscle fibre regeneration and cell repair, since after recovery from acute muscle weakness and soreness, muscle function is not apparently further impaired (Byrnes, Clarkson, White, Hsieh, Frykman & Maughan, 1985). However, little is known about the consequences of repeated exercise-induced muscle damage in man occurring during a lifespan and controlled animal experiments have not, to our knowledge, been performed.

It was observed recently that soleus and tibialis anterior, but not extensor digitorum longus (EDL), muscles of mice suffer focal muscle fibre damage at the onset of voluntary wheel-running; with continuation of exercise, muscle fibre repair occurs within days and no further muscle damage can be detected although some split muscle fibres remain (Irintchev & Wernig, 1987). The present experiments were performed to study in detail first, whether and after what duration of rest muscles can suffer fresh running-induced damage and second, to study the effects of repeated short running periods, and thus repeated damage, *versus* continuous running over several months, on muscle isometric contractile properties, fibre type composition and muscle structure.

Preliminary results of this work have been published previously (Wernig & Irintchev, 1989).

METHODS

Animals and training procedure

A total of forty-two male and female CBA/J mice purchased at an age of 10–12 weeks from Charles River Laboratories (Germany) were used in this study. One group of male mice was trained for a period of 5 months continuously, then exercise was discontinued for periods of 1, 2, 3, 4 and

5 weeks and resumed for 7 days (fifteen animals) or investigated immediately after rest periods of 0, 2 and 4–6 weeks (ten animals). Age-matched non-exercised animals served as controls (four animals). Another group consisted of female animals divided into three subgroups. One subgroup was trained continuously for 12 months (runners, $n = 4$), one served as untrained control (non-runners, $n = 4$) and one performed thirteen training periods each of 4 days followed by a rest period of 21–25 days over 12 months (intermittent runners, $n = 6$). Intermittent runners were investigated 3–5 weeks after the last running period; runners exercised until the acute experiment.

Wheel-running in this study was unforced. During training periods each cage was supplied with a running wheel to which the animal had free access; mice usually covered several kilometres per night with little or no activity during day periods. Oxygen consumption during the 12 h dark period was elevated by 25 and 54% in two runners compared to non-runners (Badke, 1988). Since running was unforced the daily amount did not remain constant. It typically ranged from 2–9 km, with little changes in some animals but substantial changes during training periods in others.

Trained animals and male control animals were housed individually in lucite cages; female control animals were kept together in a single standard cage. They had access to standard dry food (Altromin) and water *ad libitum*. Running activity in wheels was continuously recorded and the number of turns usually read daily. Animal laboratories were kept at constant temperature (23 ± 2 °C) with 12 h dark–light cycles including 15 min dim light at each change.

Isometric tension measurements

Under pentobarbitone anaesthesia (40 mg/kg) nerve–muscle preparations of soleus and EDL muscles were removed before animals were killed by dislocation of the cervical spinal column. Isolated preparations were mounted in chambers and superfused with aerated (95% O_2, 5% CO_2) Tyrode solution (125 mM-NaCl, 24 mM-$NaHCO_3$, 5·37 mM-KCl, 1 mM-$MgCl_2$, 1·8 mM-$CaCl_2$ and 5% glucose) kept at a constant temperature of $25 \pm 0{\cdot}5$ °C (cf. Segal & Faulkner, 1985). Direct muscle stimulation was performed via bath electrodes (silver wires) with pulses of 20–25 V and 0·5 ms duration. Prior to measurements, muscle length was adjusted to give maximal responses to single pulses. Tetanic stimulation was performed with trains of square pulses of 50 Hz for soleus (T_{50}) and 100 Hz for EDL (T_{100}) lasting for 2 s. Indirect muscle stimulation was via nerve suction electrodes with pulses of 3 × threshold amplitude (usually 3·5 V, 0·1 ms). To estimate the safety margin of transmission, nerves were stimulated in normal Tyrode solution and solution containing 0·6 or 1·2 μM-*d*-tubocurarine. Nerve index is defined as the relative force (in per cent) produced in the blocking solution compared to normal Tyrode solution. Other experimental details were as described previously (Badke, Irintchev & Wernig, 1989).

Histochemical and morphometric evaluations

Immediately after removal from the animal or after tension recording (see above) isolated muscles were frozen onto a piece of liver (fixed in formalin and thoroughly washed with running tap water followed by Tyrode solution) in isopentane pre-cooled with liquid nitrogen.

Frozen cross-sections (10 μM) from three positions in the endplate region, 300 μM apart, were cut in series and stained with 1% Toluidine Blue/1% Borax or tested for the following enzyme activities: succinate dehydrogenase (SDH; Nachlas, Tsou, DeSouza, Cheng & Seligman, 1957), glycogen phosphorylase (GP; Lojda, Gossrau & Schiebler, 1976), myofibrillar ATPase after acid (pH 4·5, ac-mATPase) and alkali (pH 10·3, alk-mATPase) pre-incubation (modified by Butler & Cosmos, 1981; Guth & Samaha, 1970) and acid phosphatase (AcPase; Lojda *et al.* 1976; with Naphthol AS-TR phosphate and para-rosaniline).

Acute muscle fibre damage

This was defined as the presence of pathological criteria in any of the levels of sectioning in at least two of the staining procedures performed. The following criteria were considered as pathological (Carpenter & Karpati, 1984; Irintchev & Wernig, 1987).

Toluidine Blue: pale fibres with featureless 'glass' appearance (necrosis, Plate 1*A*); small, diffusely basophilic muscle fibres, often containing central nuclei and appearing singly (Plate 1*C*) or in clusters (Plate 1*A*) at the place of necrotized fibre segments (regeneration); prominent peripheral basophilia (ribosomal proliferation after sublethal injury, Plate 1*A* and *C*); coarse inhomogeneous myofibrillar pattern (destruction of myofibrils, Plate 1*C* and *E*).

Enzyme reactions for SDH, GP, mATPase: complete or patchy disappearance of reaction

product (disturbed metabolism due to necrosis or sublethal injury, Plate 1*B*, see also Carlson & Faulkner, 1983; Maki, Korthals & Prockop, 1986); redistribution of SDH staining pattern (regeneration, Plate 1*D*).

Acid phosphatase: intracellularly localized enzyme-positive cells (phagocytosis); presence of AcPase-positive granules (lysosomes) in muscle fibres (Plate 1*F*). Normal muscle fibres are completely negative for AcPase with the Naphthol AS-TR phosphate/para-rosaniline method but injured fibres are often positive as a result of increased lysosomal activity (Vihko, Rantamäki & Salminen, 1978; Salminen & Vihko, 1980).

For quantification of acute muscle damage, one section stained for GP-activity was photographed at a primary magnification of ×600 using a video camera. A series of overlapping videoprints was made to reconstruct the whole muscle cross-section. This composite picture was used as a reference 'hard copy' on which the pathological alterations at all three levels were marked.

Chronic fibre damage

This was defined from the incidence of central muscle fibre nuclei and 'split fibres' in the absence of any of the above-mentioned characteristics for acute damage (Plate 2*A*). However, only isolated 'split fibres' could easily be recognized in a single section on the basis of complementarity of fragments, equal staining intensity of all profiles in a split fibre (e.g. mATPase), and size and shape approximating that of adjacent normal fibres (in Plate 2*A*, 'split fibre' marked by three arrows); in the case of adjacent split fibres (central part in Plate 2*A*) arbitrary borders have to be taken. This way, ignoring the possibility of new fibre formation, each small profile was considered as part of a split fibre. In a separate evaluation the total number of individual profiles on a cross-section was determined.

Number of fibres

Fibres in the soleus muscle were counted on videoprint montages of a cross-section through the endplate region stained for mATPase activity (acid pre-incubation, pH 4·5; magnification ×230–370). Where applicable, videoprints from adjacent sections were compared to ensure that damaged fibres or folded parts of sections were properly evaluated. Two separate counts were performed: in one, split fibres were defined as single fibres (see above), in the other each individual profile was counted separately. Unless defined differently, all calculations in this work are based on counting fibres, not profiles. No attempt was made in EDL to obtain a total fibre count.

Muscle fibre diameters

These were measured from videoprints of Toluidine Blue-stained sections using a digitizing tablet. The mean orthogonal diameter was determined as the mean of the longest axis and a short axis bisecting the longest at a right angle (modified method of Song, Shimada & Anderson, 1963; see Schmitt, 1976). All fibres in a cross-section were measured except mechanically damaged fibres or fibres in areas with any artifacts. In one evaluation, split fibres were evaluated as single fibres, in another each profile was measured separately (Fig. 1). With one exception (52% of the fibres in a sedentary soleus) 81–100% of the fibres could be evaluated in this way. Net cross-sectional area of a muscle was calculated, assuming circular shapes, from the mean fibre diameter in this muscle (see above) multiplied by the total number of fibres counted; this was done after verifying that diameters were normally distributed (Fig. 1, lower histogram).

Muscle fibre types

These were quantified on videoprints of a whole muscle cross-section stained for mATPase activity after acid pre-incubation (pH 4·5) for soleus and alkali pre-incubation (pH 10·3) for EDL. In soleus, the following fibre types were defined from ac-mATPase staining: type I (dark), type II (unstained) and type IIC (intermediate staining intensity, also after alkali pre-incubation). The vast majority of type II fibres in mouse soleus are highly oxidative and cross-react with monoclonal antibodies against myosin heavy chain IIA (Desypris & Parry, 1990). Atypically, however, they are darkly stained for mATPase after acid pre-incubation at pH 4·6 (authors' observations) and should thus be histochemically classified as type IIB or type IID/X (see below). To avoid possible misunderstandings we denote these fibres as type II without further classification. For EDL, the alk-mATPase appeared more suitable to identify type I (unstained), type IIA (intermediate intensity, FG fibres in the mouse), and type IIB (dark, FOG fibres). It should be noted that in the

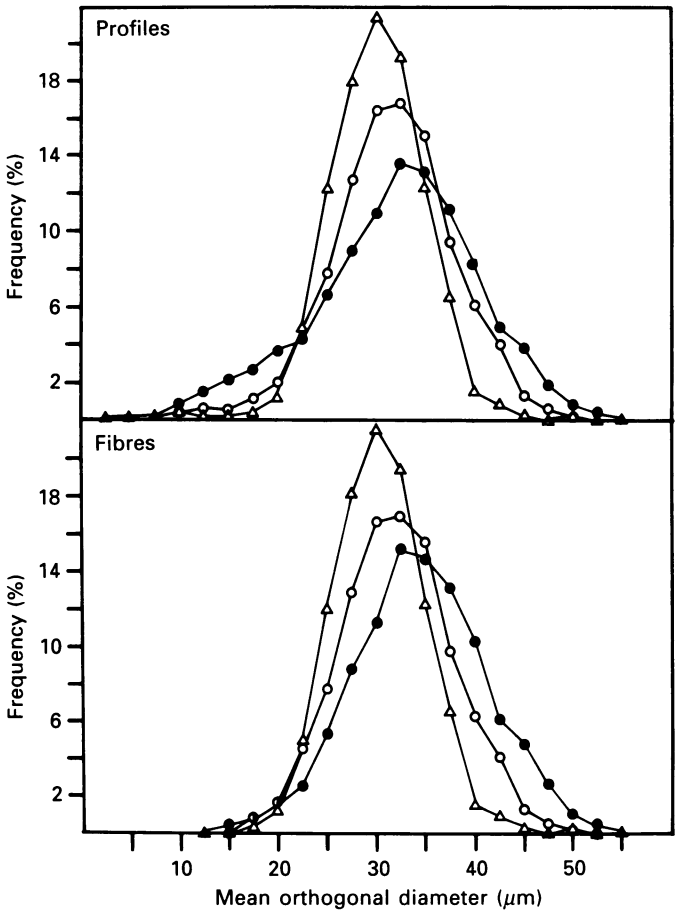

Fig. 1. Normalized frequency distributions of average orthogonal muscle fibre diameters obtained from frozen cross-sections of soleus muscles of intermittent (●), continuous (○) and non-runners (△). The numbers of muscles evaluated were six, three and three. Upper histogram: each muscle fibre profile was measured and counted individually. Lower histogram: all small profiles were assumed to be segments of a split fibre. Presumed split fibres were counted and measured as single muscle fibres. Expressed in this way the number of counts declined from 4491 to 3375 in intermittent runners; in continuous runners and non-runners numbers remained similar (declined from 2039 to 2002 and 1575 to 1563, respectively).

mouse, a new type of fast-oxidative fibre was recently described (type IIX, Schiaffino, Gorza, Satore, Saggin, Ansoni, Vianello, Gundersen & Lömo, 1989) which was, however, not considered here (see also Termin, Staron & Pette, 1989; for type IID fibres).

RESULTS

Exercise-induced damage following short rest periods

It was noticed previously that soleus, but not EDL muscles, suffer focal muscle fibre damage including necrosis at the onset of wheel-running, but no further damage occurs with continuation of running for several months (Irintchev & Wernig, 1987).

When running in wheels was stopped for periods of 1–5 weeks following 5 months of continuous exercise, and then resumed for 1 week, fresh damage occurred in soleus muscles: average 8·7 ± 3·5 % (mean ± S.D.) of the muscle fibres (Table 1). Plate 1*A–F* shows different signs of acute damage detectable. The amount of damage is not obviously correlated with the duration of the rest period (Table 1) or the amount of running during the following week of activity (total amount, or activity during the first or last day; data not shown). Control animals studied 0, 2 and 4–6 weeks after termination of a period of 5 months of continuous exercise, and age-matched unexercised animals had about 1 % muscle fibres displaying signs of acute damage (Table 1). These results indicate that animals adapted to wheel-running become vulnerable to acute muscle damage after short rest periods (see Discussion).

Split muscle fibres and central nuclei without signs of acute damage (Plate 2*A*) were previously observed several months after damage and repair of muscle fibres (Irintchev & Wernig, 1987). Split fibres probably originate from incomplete fusion of proliferated satellite cells after focal fibre damage at the very beginning of exercise or to a small degree, during normal activity in cages. Internalization of nuclei occurs in sublethally damaged or regenerated fibres and apparently persists for long periods thereafter (e.g. in regenerated fibres in the mdx-mutant mouse; Anderson, Bressler & Ovalle, 1988). Both split fibres and central nuclei were present in continuously trained muscles (1·5 ± 0·73 and 2·4 ± 1·0 %, Table 1) but to a negligible extent in unexercised animals (0·6 ± 0·2 %, $P < 0·05$, *t* test, Table 1).

Repeated short periods versus continuous wheel-running

It was shown above (and Table 1) that exercised animals are vulnerable to fresh damage in soleus upon onset of running after short rest periods. Another group of animals was therefore trained intermittently over a period of 12 months with 4 days wheel-running followed by 21–25 days of rest, to test if repeated muscle damage would alter muscle properties. The occurrence of repeated damage was confirmed in three animals which showed various degrees of acute damage following two, three and four running episodes (data not shown).

Histological and contractile properties of soleus muscles

Not unexpectedly, the percentage of muscle fibres showing signs of previous damage (split fibres, central nuclei) is highly elevated after 12 months of intermittent running (Plate 2*B* and *C*). Accordingly, a considerable proportion of small diameter profiles is present, raising the total numbers of profiles in complete muscle cross-sections to 768 ± 68 (mean ± S.D.; $n = 6$) compared to 676 ± 54 ($n = 3$) in continuous runners and 683 ± 33 ($n = 4$) in non-runners ($P < 0·05$, Table 2). Only when split fibres are counted as single muscle fibres, i.e. small fragments are considered as unfused parts of regenerated fibres, are their numbers similar to those of the other groups (Table 2) and the frequency distribution of diameters then becomes bell-shaped (Fig. 1). Thus the estimated occurrence of altered fibres was 12 ± 4·9 % ($n = 6$, Table 2) with twice as many split fibres as muscle fibres which only had central nuclei. Altered fibres were slightly but significantly more frequent in runners (3·4 ± 0·12 %; $n = 3$) than in non-runners (0·53 ± 0·17 %; $n = 4$; Table 2). Despite the unusual muscle architecture, tetanic and single twitch forces were larger in solei of intermittently trained animals than in unexercised animals ($P < 0·05$, Table 2). This

TABLE 1. Amount of damage (acute and chronic, for definitions see Methods) in soleus muscles after 5 months of continuous running, a rest period of 1–5 weeks followed by 7 days of wheel-running (A), 5 months of continuous running and rest periods of 0, 2 and 4–6 weeks (B), together with unexercised age-matched controls (C)

	Signs of damage		Number of animals (muscles)
	Acute	Chronic	
(A) Running after rest periods of (weeks)			
1	5·8 (3·5–8·8)	2·7 (0·1–5·5)	4 (4)
2	13·0 (3·7–34)	1·2 (0·0–4·4)	4 (4)
3	11·4 (0·0–23)	1·2 (0·0–2·4)	2 (2)
4	8·6 (3·7–13)	0·8 (0·0–1·9)	3 (3)
5	4·7 (1·7–7·6)	1·6 (1·4–1·9)	2 (2)
Average	8·7 ± 3·5*	1·5 ± 0·73	
(B) Weeks after exercise			
0	0·3 (0·0–0·7)	2·5 (1·1–4·1)	4 (5)
2	0·4 (0·0–0·9)	3·4 (0·9–6)	2 (2)
4–6	0·7 (0·0–2·2)	1·3 (0·6–2·8)	3 (3)
Average	0·47 ± 0·21	2·4 ± 1·0*	
(C) Unexercised controls			
	1·3 ± 1·1 (0·0–2·4)	0·6 ± 0·2 (0·4–0·9)	4 (7)

Numbers represent percentage of fibres per complete cross-section through soleus muscle cut in the endplate region; mean values and ranges are given for individual groups as well as S.D. for the overall groups.

* Significant difference as compared to unexercised controls ($P < 0·05$), t test.

TABLE 2. Physiological and histological parameters of soleus muscles of sedentary, continuously and intermittently exercised CBA/J mice

Physiological parameters	Sedentary	Continuous runners	Intermittent runners
Tetanic force P_0 (T_{50}, mN)	126 ± 26	146 ± 32	160 ± 29*
Twitch force P_t (mN)	32 ± 3·8	34 ± 4·8	40 ± 6·6*
Twitch–tetanus ratio	0·25 ± 0·03	0·23 ± 0·04	0·25 ± 0·02
Twitch time-to-peak (ms)	54 ± 5·4	50 ± 5·1†	56 ± 6·1
Twitch half-relaxation time (ms)	85 ± 11	81 ± 12	78 ± 10
Tetanic force per muscle cross-sectional area (N/cm^2)§	27·8 ± 3·6	28·5 ± 5·1	28·2 ± 2·9
Animal body weight (g)	27·7 ± 0·9	27·3 ± 2·2	27·3 ± 2·3
Number of animals (muscles)	4 (8)	4 (8)	6 (12)
Histological parameters			
Profiles per cross-section§	683 ± 33	676 ± 54	768 ± 68*
Muscle fibres per cross-section	674 ± 34	653 ± 50	661 ± 30
Net cross-sectional area (mm^2) (calculated)§	0·46 ± 0·031	0·53 ± 0·076	0·59 ± 0·069*
Altered fibres (%) (range)	0·53 ± 0·17 (0·30–0·65)	3·4 ± 0·12‡ (3·3–3·5)	12 ± 4·9** (8·1–19)
Fibre types (%)			
type I	75 ± 0·9	81 ± 5·6	90 ± 4·4**
type II	24 ± 1·1	19 ± 6·5†	8·7 ± 4·6*
type IIC	0·9 ± 0·4	0·6 ± 0·4	0·9 ± 0·9
Number of animals (muscles)	4 (7)	3 (5)	6 (11)

Mean number ± S.D. and some ranges are given. Symbols: *, ** = $P < 0·05$, $< 0·01$ compared with sedentary animals (t test). †, ‡ = $P < 0·05$, $< 0·01$ compared with intermittent runners (t test). § = 3, 3 and 6 muscles from as many animals.

increase is obviously due to an increase in cross-sectional area (Table 2) since specific force (tetanic force per cross-sectional muscle fibre area) remains unchanged. Times-to-peak of single twitches were shortest in continuously trained solei ($P < 0.05$, Table 2).

When screening for correlates it appeared that intermittent runners with larger body weight (and larger contractile force of soleus muscles) had more altered fibres; in the six animals studied, mean body weight was 29.7 ± 2.1 g in the three animals with the largest degree of damage and 25.3 ± 1.1 g S.D. in the others. Strikingly, the amount of running during the 4-day periods was not correlated with the amount of chronic damage ($r = -0.5$, n.s.).

Fibre-type distribution in soleus muscle

There is a clear increase in type I fibres in intermittently trained soleus muscles over unexercised muscles (Plate 2*B* and *C*, Table 2) while continuous runners are intermediate. It was noted previously that in soleus muscles fibre-type grouping occurs after wheel-running (Irintchev & Wernig, 1987). Thus it is possible that the increase in type I fibres is related to muscle damage and repair (see Discussion). No significant correlations were found, however, between the occurrence of type I fibres and the amount of running during the running episodes, the frequency of chronically altered fibres or body weight.

EDL muscles in continuously and intermittently trained mice

In contrast to soleus (and tibialis anterior), EDL muscles do not suffer injury during wheel-running (see Irintchev & Wernig, 1987); thus only changes consequent on the amount of exercise should be present. Typically, EDL is working against little load, thus performing mainly endurance training; accordingly there is in fact a clear decrease in contractile force and increase in time-to-peak values in EDL of

TABLE 3. Physiological and histological parameters of EDL muscles of sedentary, continuously and intermittently exercised CBA/J mice

Physiological parameters	Sedentary	Continuous runners	Intermittent runners
Tetanic force P_0 (T_{100}, mN)	333 ± 32	285 ± 32*	323 ± 46
Twitch force P_t (mN)	92 ± 12	87 ± 10	93 ± 18
Twitch time-to-peak (ms)	16 ± 1.0	19 ± 1.6**	18 ± 1.7
Twitch half-relaxation time (ms)	19 ± 3.9	23 ± 6.1	21 ± 3.8
Muscle weight (mg)	13 ± 1.4	13 ± 1.7	12 ± 1.3
Number of animals (muscles)	4 (8)	4 (8)	6 (10)
Histological parameters			
Altered fibres (%)	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.2
Fibre types (%)			
type IIA	54 ± 3.8	46 ± 4.1*	55 ± 3.5
type IIB	45 ± 4.0	51 ± 3.9*	44 ± 3.3
type I	1.2 ± 0.3	3.1 ± 1.8*	1.1 ± 0.2
Number of muscle fibres evaluated	745 ± 53	734 ± 100	761 ± 101
Number of animals (muscles)	3 (5)	3 (5)	6 (7)

* $P < 0.05$; ** $P < 0.01$ compared with sedentary animals (t test).

continuous runners while intermittent runners are not altered at all (Table 3). Fibre-type distributions in intermittent runners are strikingly similar to controls (Table 3). In continuous runners, however, a small but significant increase in type I and type IIB fibres at the cost of type IIA fibres is present; this can be considered as an increase in oxidative capacity in response to endurance exercise.

TABLE 4. Nerve block (nerve index) of soleus muscles of sedentary, continuously and intermittently trained mice for two concentrations of curare

	Nerve index (%)		
	Sedentary	Continuous runners	Intermittent runners
0·6 μM-*d*-tubocurarine			
Single twitch	91·2 ± 7·5 (4)	89·7 ± 6·0 (8)	93·9 ± 7·4 (11)
Tetanus, 2 s (50 Hz)	67·4 ± 8·7 (5)	72·4 ± 10·1 (8)	85·3 ± 9·7** † (11)
Tetanus, 0·2 s (100 Hz)	84·8 ± 11·1 (3)	87·3 ± 4·9 (6)	94·8 ± 2·7* † (9)
1·2 μM-*d*-tubocurarine			
Single twitch	42·2 ± 26·4 (3)	80·6 ± 11·7 (3)	85·8 ± 5·7** (5)
Tetanus, 2 s (50 Hz)	34·8 ± 40·7 (3)	51·0 ± 14·9 (3)	54·4 ± 5·8 (5)
Tetanus, 0·2 s (100 Hz)	24·6 ± 12·1 (2)	44·3 ± 14·5 (3)	64·4 ± 10·0** † (5)

Number of muscles tested is in parentheses.

* $P < 0{\cdot}05$ compared to sedentary animals (*t* test). ** $P < 0{\cdot}01$ compared to sedentary animals (*t* test). † $P < 0{\cdot}05$ compared to continuous runners (*t* test). ‡ $P < 0{\cdot}01$ compared to continuous runners (*t* test).

Other observations

It has been observed previously that prolonged elevated nerve activity causes changes in transmitter release in vertebrate neuromuscular junctions (Dorlöchter, Brinkers, Irintchev & Wernig, 1990; but see Hinz & Wernig, 1988). Resistance to curare block, commonly used as a measure of safety margin of transmission, was tested here and significant differences are found. While there is merely a trend towards higher resistance to block in continuously trained soleus muscle, the increase is significant for intermittent runners (Table 4). Thus for soleus, the experimental groups with higher proportions of type I fibres have higher resistances to block. Note that in mouse, resistance is generally higher in the predominantly slow soleus than in the fast EDL muscle (M. Dorlöchter, M. Brinkers, A. Irintchev and A. Wernig, unpublished).

DISCUSSION

Previous experiments revealed that in wheel-running, muscle fibre damage occurs at the onset of exercise but not thereafter. Therefore it is surprising to find acute muscle fibre damage in trained soleus muscles on resumption of running after rest periods as short as one week. In wheel-running, soleus and tibialis, but not EDL,

muscles may perform eccentric movements and show signs of damage while EDL does not (Irintchev & Wernig, 1987). Eccentric muscle contractions were found to cause muscle fibre damage in several species, including man (Armstrong *et al.* 1983; Fridén, Skafianos & Hargens, 1986; Jones *et al.* 1986). It is therefore possible that eccentric contractions are the cause of damage in the present experiment. The nature of the adaptation that prevents further damage with continuation of wheel-running is unknown but it evidently fails during intermittent exercise or after short rest periods. Improvements in the co-ordination of motor units during wheel-running could reduce the amount of eccentric contraction in muscle fibres of individual motor units, and deadaptation might occur within short rest periods due to return to the co-ordination patterns used on flat ground. Muscle pain, which in humans is a component of muscle soreness, might in mice be the sensory feedback necessary for adaptations to occur at the CNS level. During intermittent training, adaptations to wheel-running might not occur, since running periods are short (4 days) and followed by relatively long rest periods (21–25 days). The relatively long-lasting (several weeks) protective effect of a single exercise-induced injury in man (Byrnes *et al.* 1985)–contrasted with the vulnerability within a week after cessation of running in mice (present results)–might be due to the better learning and storage capacity of the human CNS.

The increase in type I fibres in solei of intermittent runners clearly suggests that changes also occur at muscle fibre level; it is not clear, however, whether these can be considered as an adaptation or as a mere byproduct of other events. At present it is not known whether type I fibres are less vulnerable to running-induced damage than type II fibres. It is unlikely that elevated motoneurone activity caused an increase in proportion of type I fibres in soleus, for example via fibre transformation (e.g. Salmons & Sréter, 1976); if this were the case, continuous runners should show larger, not smaller, changes than intermittent runners. The increase in type I fibres is evidently related to the amount and frequency of muscle damage. In EDL muscles, which lack signs of damage, fibre type changes are absent in intermittent runners whereas typical effects of endurance training are present in continuous runners (see below).

It is likely, therefore, that the increase in type I fibres in soleus is a direct consequence of muscle damage and repair, and adaptations to prevent further damage might occur at the CNS level.

Possible mechanisms for an increase in the proportion of type I fibres in repeatedly damaged soleus

The increase in the proportion of type I fibres in soleus of intermittently running mice appears to be related to muscle fibre damage. Two different mechanisms could account for such an occurrence.

It is conceivable that regenerated cells predominantly express acid stable myosin ATPase, i.e. type I fibre characteristics owing to incorporation of new nuclei from fused satellite cells. In this case one might expect a mosaic distribution of different ATPase along the length of muscle fibres since fibre damage occurs focally (Irintchev & Wernig, 1987); this possibility was not ruled out in the present experiments and in other circumstances has indeed been observed (Staron & Pette, 1987). However,

split muscle fibres expressing type II fibre characteristics have also been observed following muscle injury (Irintchev & Wernig, 1987), which renders this notion less likely.

A more likely mechanism is axonal sprouting and new synapse formation. Recent observations show axonal sprouting to be a physiological process which is enhanced in pathological situations, in particular when accompanied by muscle fibre inactivity (for a review see Wernig & Herrera, 1986). In the case of focal muscle fibre damage and repair, sprouting factors originating from proliferating satellite cells or muscle fibre segments temporarily or permanently disconnected from the endplate-bearing, and thus action potential-generating, parts might cause enhanced collateral and terminal sprouting.

The observed increase in type I fibres would suggest a higher sprouting capacity of type I motoneurones, which might in addition overrule other activity patterns to cause transformation into type I fibres (for further discussions of this hypothesis see Salmons & Sréter, 1976; Pette & Vrbová, 1985; Wernig & Herrera, 1986). Interestingly, tonic nerve stimulation via implanted electrodes which causes an increase in type I fibres seems to involve some muscle fibre necrosis and repair (see Maier, Gambke & Pette, 1986). Also after enforced treadmill running (Müller, 1974) increase in type I fibres in soleus was accompanied by accumulation of split fibres suggesting muscle damage in the course of training. In contrast, during transformation of denervated muscle from slow to fast, embryonic myosin chains could be detected only near the site of electrode implantation (Gorza, Gundersen, Lömo, Schiaffino & Westgaard, 1988) indicating that satellite cell proliferation was not involved in fibre transformation.

Muscle damage and contractile force

Soleus muscles of intermittently running animals contain considerable numbers of 'split muscle fibres'. This seeming disadvantage is obviously more than balanced by the marked muscle hypertrophy. Since tetanic force was less in continuously running animals, although the actual duration of training was longer by a factor of 5–6, force is not increased by more running. Possibly, therefore, multiple episodes of satellite cell proliferation which also take place in subtotally damaged fibres (Teräväinen, 1970; Darr & Schultz, 1987) lead to increased cross-sectional area and thus contractile forces.

There have been reports of muscle fibre hyperplasia as a consequence of elevated motor activity (e.g. Gonyea, Sale, Gonyea & Mikesky, 1986). Also in the present experiments, the number of individual muscle fibre profiles recognizable on single cross-sections was markedly elevated; however, when adjacent fragments are counted as single fibres, which admittedly includes some arbitrary decision and ignores the possible existence of small fibre branches (Schmalbruch, 1984), the number of muscle fibres becomes similar to that in control animals.

Training effects in soleus and EDL muscles in continuous runners

In EDL muscles which do not show running-induced damage, contractile force and contraction times are not altered in intermittently trained animals, but are typically changed in continuous runners. A similar concomitant decline in force and increase

in time-to-peak of the single twitch observed here may be found after substantial electrical stimulation of innervated fast muscle (Salmons & Vrbová, 1969; Kernell, Eerbeek, Verhey & Donselaar, 1987). At the same time a significant increase in fast-oxidative fibres (possibly including type IIX fibres, Schiaffino *et al.* 1989) was noticed which is the most typical outcome of endurance training (Edström & Grimby, 1986). The occurrence of additional type I fibres in EDL is possibly caused by a corresponding change in activity pattern of one or two type II motoneurones, or the type I motoneurone(s) present in the muscle extended their motor unit size e.g. by hyperinnervating muscle fibres (for further discussion see Wernig & Herrera, 1986). The moderate increase in type I fibres in some of the continuously trained solei might have similar origins to those discussed above for EDL but it is more likely to be connected with fibre damage occurring at the very onset of running.

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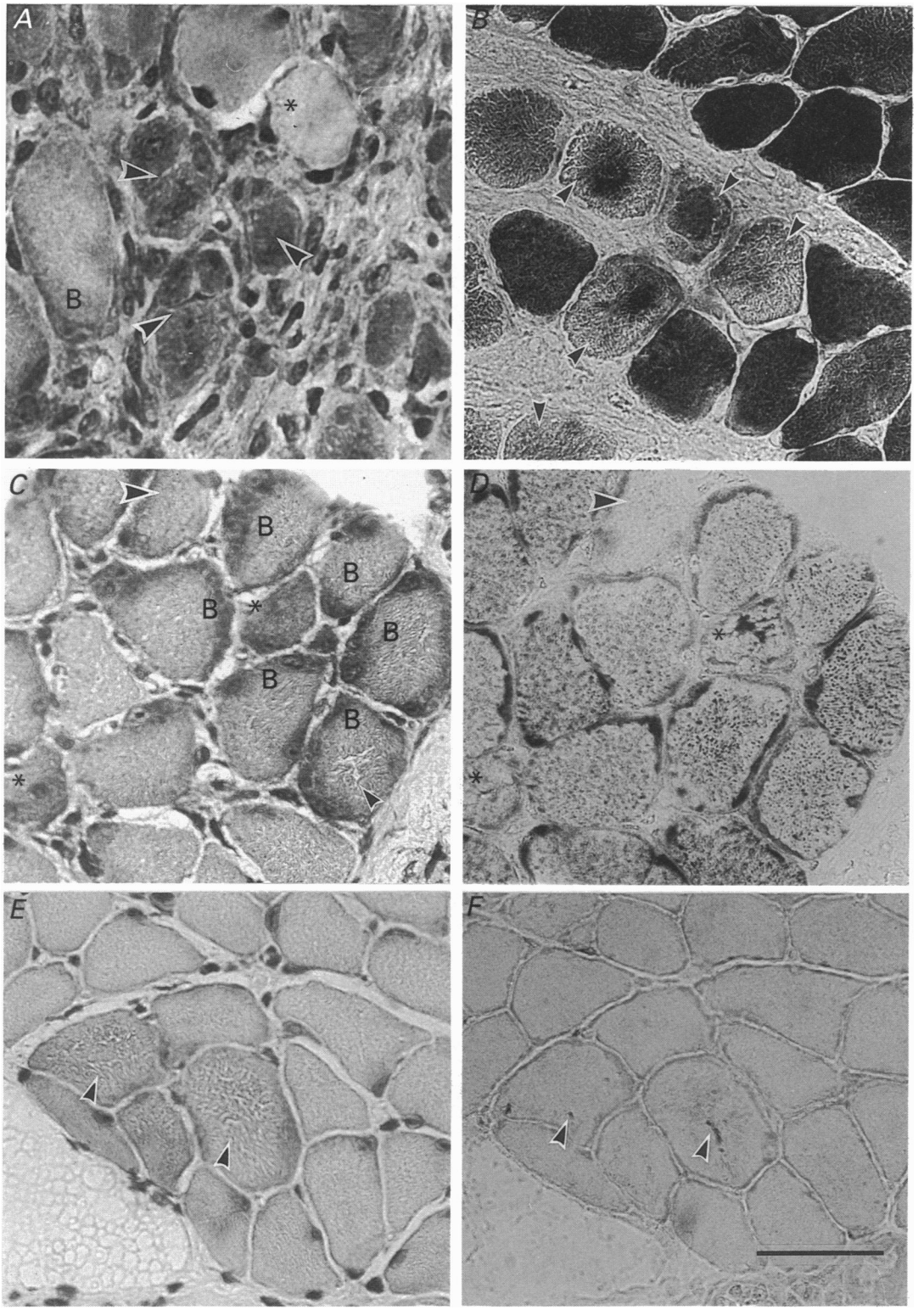
EXPLANATION OF PLATES

PLATE 1

Signs of acute muscle damage in cryostat cross-sections (10 μm) from short-term exercised (1 week) mouse soleus muscle. Toluidine Blue staining (*A*, *C* and *E*) reveals a necrotic pale fibre with 'glass' appearance (asterisk in *A*), single (asterisks in *C*) and clusters (arrow-heads in *A*) of small, darkly stained regenerating fibres, inhomogeneous coarse myofibrillar pattern (small arrow-heads in *C* and *E*), prominent peripheral basophilia (fibres marked with B in *A* and *C*). Note numerous infiltrated cells surrounding muscle fibres in *A*. Reaction for glycogen phosphorylase (*B*) shows muscle fibres with inhomogeneous loss of activity (arrow-heads in *B*) and normally stained fibres in the upper right corner. Sections stained for succinate dehydrogenase (*D*, section adjacent to *C*), regenerating fibres (asterisks in *C* and *D*) appear lightly stained but clumped reaction product is often seen (upper fibre marked with asterisk); complete loss of activity is seen in one muscle fibre (large arrow-heads in *C* and *D*). Acid phosphatase activity, undetectable in normal fibres, is visualized in some affected fibres (arrow-heads in *E* and *F*) indicating enhanced lysosomal activity. Scale bar in *F* indicates 30 μm for *A* and 50 μm for *B–F*.

PLATE 2

Cross-sections from soleus muscle stained with Toluidine Blue (*A*) and for myofibrillar ATPase activity after acid pre-incubation, pH 4·5 (*B* and *C*). *A*, concomitant acute and chronic signs of muscle injury in a mouse exercised for 5 months, followed by 1 week rest and 1 week exercise. In the central part, several small fibre profiles without signs of acute damage, representing fragments of split muscle fibres, are seen. Isolated split fibres are easily recognized in a single section (arrows), but in the case of adjacent split fibres (central part of *A*) arbitrary borders have to be taken. Some otherwise normal fibres have central nuclei (asterisks). Most split fibres and fibres with central nuclei do not display any sign of acute injury (compare Plate 1) and are a consequence of damage at the very onset of exercise. A regenerating fibre (small arrow-head) and a fibre with prominent peripheral basophilia and inhomogeneous coarse myofibrillar pattern (large arrow-head) provide evidence of acute damage after renewal of exercise. *B* and *C*, distribution of type I (dark) and type II (white) fibres in a sedentary control (*B*) and an intermittently exercised (*C*) soleus muscle; the intermittently exercised soleus contains very few type II fibres and numerous split fibres (upper part). In *C* several intermediately staining (type IIC) fibres can be seen. Scale bar indicates 100 μm for *A* and 300 μm for *B* and *C*.



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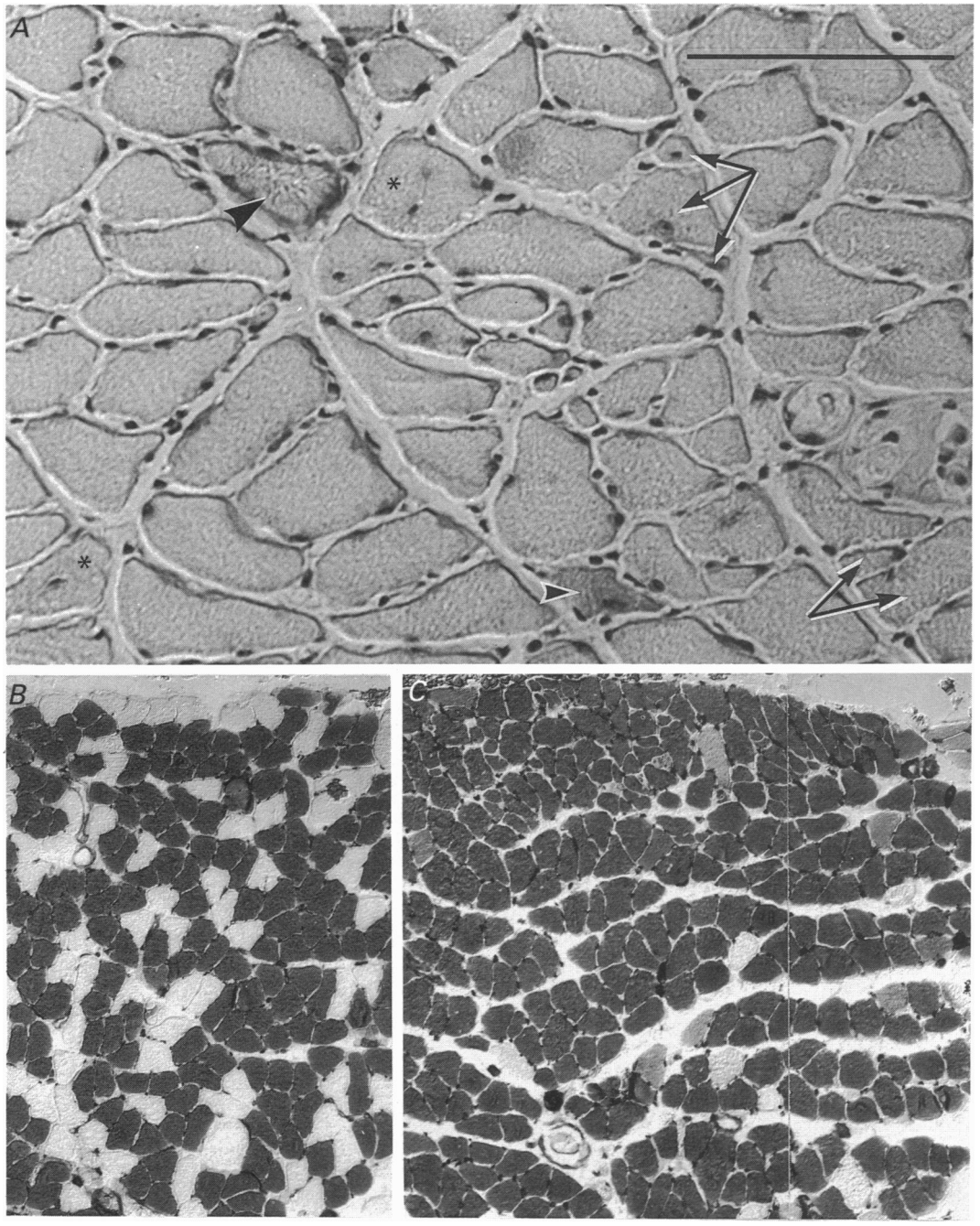
A
B
C