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Variations in fibre composition of the gastrocnemius muscle in rats subjected to speed training

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Summary. Thirty-six adult Wistar rats were divided into three groups. One group was used as a control, and the other two underwent different training programmes in which greater relevance was attached to the intensity of exercise than to its duration. Samples of the red and mixed portions of m. gastrocnemius (*caput lateralis*) were stained with m-ATPase to determine the percentage of type I, IIA and IIB fibres, and with NADH-TR in order to quantify variations in the percentage of low staining intensity (FG) fibres.

The most notable results obtained were: a) the ratio of type I type II fibres remained unchanged; b) the proportion of IIA fibres increased, while that of IIB fibres decreased correspondingly; c) FG fibres, which were virtually absent from the red portion, recorded a clear decrease which was more marked, and occurred more rapidly, than in IIB fibres. These differences were all statistically significant in the mixed portion of the muscle. Adaptative changes in fibre composition in the red portion were less marked.

Key words: Muscle fibres types, Histochemistry, Exercise

Introduction

The increased functional demand associated with exercise forces the muscle cell to undergo certain adaptations which tend to be primarily biochemical and structural. Moreover, the characteristic extreme plasticity of the skeletal muscle (Pette, 1980) not only enables fibres to undergo structural transformations, but also gives rise to changes at molecular level involving contractile proteins (Howald, 1982).

The considerable variation in fibre composition from one individual to another depending on its physical constitution (Saltin et al., 1977; López-Rivero et al., 1989) indicates the activity or type of exercise for which the individual is best suited. A great deal of research has been carried out, both in myology and in sports medicine, into the possible modification of fibre composition patterns as a result of training. The changes occurring in muscle fibre following training depend largely on the type of muscle involved and the type of training to which its is subjected (running, swimming, prolonged standing, etc). Another key factor in fibre adaptability is the intensity and duration of exercise. It is these two parameters (intensity/duration) which have traditionally been varied in the development of training programmes (Herbison and Gordon, 1973; Jaweed et al., 1974; Green et al., 1984). Indeed, the principal difference between endurance-exercise and speed-exercise schedules lies in the combination of these parameters. The borderline between the two occurs at the point where maximum aerobic capacity (Vo2max) is exceeded, giving way to the use of anaerobic pathways in speed training (Sherperd and Gollnick, 1976; Sullivan and Armstrong, 1978).

Many researchers have developed endurance training programmes involving a high degree of aerobic work (Jaweed et al., 1974; Ingjer, 1979; Salmons and Henrikkson, 1981; Green et al., 1984), but few studies have dealt with the effects of speed training on fibre composition (Jansson et al., 1978; Roberts et al., 1982). In the exercise schedules implemented in this study, the intensity of exercise was considered more relevant than its duration. Variations in fibre composition were analysed in the gastrocnemius muscle, which is involved in flexion of the limb and thus plays a major role in locomotion.

Materials and methods

Thirty-six white Wistar rats of both sexes were used

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for this experiment. They were housed in the same conditions, with an *ad libitum* supply of water and feed (commercial pellets). After one week of acclimatisation, rats were divided into three groups, each consisting of twelve animals (six males, six females). All animals were slaughtered at 20 weeks old, and had a mean body weight of 237 ± 29 g (females) and 354 ± 27 g (males).

Group I (control) animals were not subjected to any exercise schedule during the experiment. The other two groups underwent different running programmes using a motor driven treadmill set at a slope of 0°. Group II animals ran on the treadmill for five one-minute periods every day, resting for one minute after each exercise. The speed was increased progressively from 35 m/min in the first week to 55 m/min in the fourth week. Group III rats ran on the treadmill for four one-minute periods per day, resting for one minute between exercises. The speed was set at 55 m/min at the start of the experiment and was not modified over the four weeks that the experiment lasted. Both groups thus ran a total of 4500 metres, in 100 minutes in Group II and 80 minutes in Group III.

At the end of the training programmes, all animals were sacrificed by inhalation of chloroform. Samples of the *caput lateralis* of m. gastrocnemius were taken from the right limb, at a point half-way along the muscle belly.

Samples were frozen according to the ultra-rapid method proposed by Dubowitz and Brooke (1973), and serial sections 10 μm thick were cut in a cryostat at -20° C. Sections were stained with myofibrillar adenosine triphosphatase (m-ATPase) at pH 9.4 after acid preincubation at pH 4.4 (Brooke and Kaiser, 1970) in order to evaluate cell contractile capacity (Bárany, 1967). Oxidative capacity was assessed using nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) (Novikoff et al., 1961).

Muscle fibres were classified into types I (dark), IIA (light) and IIB (intermediate), according to their m-ATPase activity (Brooke and Kaiser, 1970) (Fig. 1a). On sections stained with NADH-TR, only fibres of low staining intensity —FG fibres (Peter et al., 1972)— were counted (Fig. 1b). Once the three muscle portions of the *caput lateralis* had been identified (Armstrong and Phelps, 1984), the red and mixed portions were selected for quantitative study, the percentage of each fibre type being calculated by counting a minimum of 500 fibres. Care was taken to exclude fibres in areas bordering the white portion of the muscle.

Quantitative results are expressed as the mean (± standard deviation, Sd). Differences between groups were evaluated by means of Snedecor's F test and Tukey's studentized range test. Discriminant analysis —pairwise squared generalised distances between groups (SAS Institute Inc., 1982)— enabled us to assess values for type I, IIA and IIB fibres in each group.

Results

Preliminary analyses revealed no statistical difference in fibre composition between sexes, so that in subsequent analyses males and females were considered together.

The percentages (mean ± Sd) obtained for each fibre type in each of the groups are shown in Table 1 for the red portion of m. gastrocnemius and in Table 2 for the mixed portion. These tables also show the results of the analysis of variance carried out between groups for each fibre type. The data is represented in schematic form in Figs. 2, 3.

No significant modification was found in the proportion of type I fibres with respect to other fibre types. However, a marked increase in the proportion of IIA fibres was accompanied by a parallel decrease in the proportion of IIB fibres. The proportion of low oxidative

Table 1. Results of the analysis of variance performed in order to determine inter-group differences in the red gastrocnemius muscle (mean ± Sd).

VARIABLE	ATPase			NADH-TR
GROUPS	% Type I	% Type IIA	% Type IIB	% Type FG
Group I	42.1 ± 3.5	25.0 ± 1.6	32.9 ± 2.2	0.0 ± 0.0
Group II	43.4 ± 1.7	26.1 ± 2.4	30.5 ± 2.8	0.0 ± 0.0
Group III	43.5 ± 2.5	27.9 ± 2.6 *	28.6 ± 2.9 *	0.0 ± 0.0

* P < 0.05 with respect to grupo I.

Table 2. Results of the analysis of variance performed in order to determine inter-group differences in the mixed gastrocnemius muscle (mean ± Sd).

VARIABLE	ATPase			NADH-TR
GROUPS	% Type I	% Type IIA	% Type IIB	% Type FG
Group I	11.7 ± 1.7	19.6 ± 1.9	68.7 ± 2.2	42.0 ± 2.1
Group II	12.0 ± 1.8	22.0 ± 1.8 *	66.0 ± 2.7	37.6 ± 1.3 *
Group III	11.1 ± 1.9	27.2 ± 1.7 *+	61.7 ± 2.5 *+	33.1 ± 1.4 *+

* P < 0.05 with respect to grupo I.
+ P < 0.05 with respect to grupo II.

Table 3. Discriminant analysis. Generalized squared distances.

	GROUPS	Group II	Group III
Red	Group I	2.86	4.30
	Group II	—	0.69
Mixed	Group I	10.69	56.25
	Group II	—	20.54

capacity (FG) fibres also decreased with training. Analysis of these data from a multivariate viewpoint (Table 3) showed that variations were more marked in Group III animals, which were subjected to more intensive training.

Variations in fibre composition were more acute in the mixed portion of m. gastrocnemius than in the red portion.

Discussion

Each of the three portions which make up the *caput lateralis* of m. gastrocnemius has a different fibre composition (Sullivan and Armstrong, 1978; Armstrong and Phelps, 1984). The red portion in this study had a high proportion of type I (slow-twitch) fibres (42.1%) and showed a complete absence of low oxidative capacity fibres (0% FG), endowing this muscle portion with a high degree of fatigue resistance. The mixed portion —adjacent to the red portion and separated from it by a fibrous septum— contained only a small proportion of type I fibres (11.7%) and a high percentage of IIB fibres (68.7%). The white portion, which was the largest, contained only IIB fibres.

The absence of changes in the I:II ratio coincides with the findings reported by Andersen and Henriksson (1977) and Salmons and Henriksson (1981), although the exercise schedules in their experiments involved a large degree of aerobic work (endurance training). In the present experiment, greater emphasis was placed on the intensity of training programmes than on their duration; Group III animals

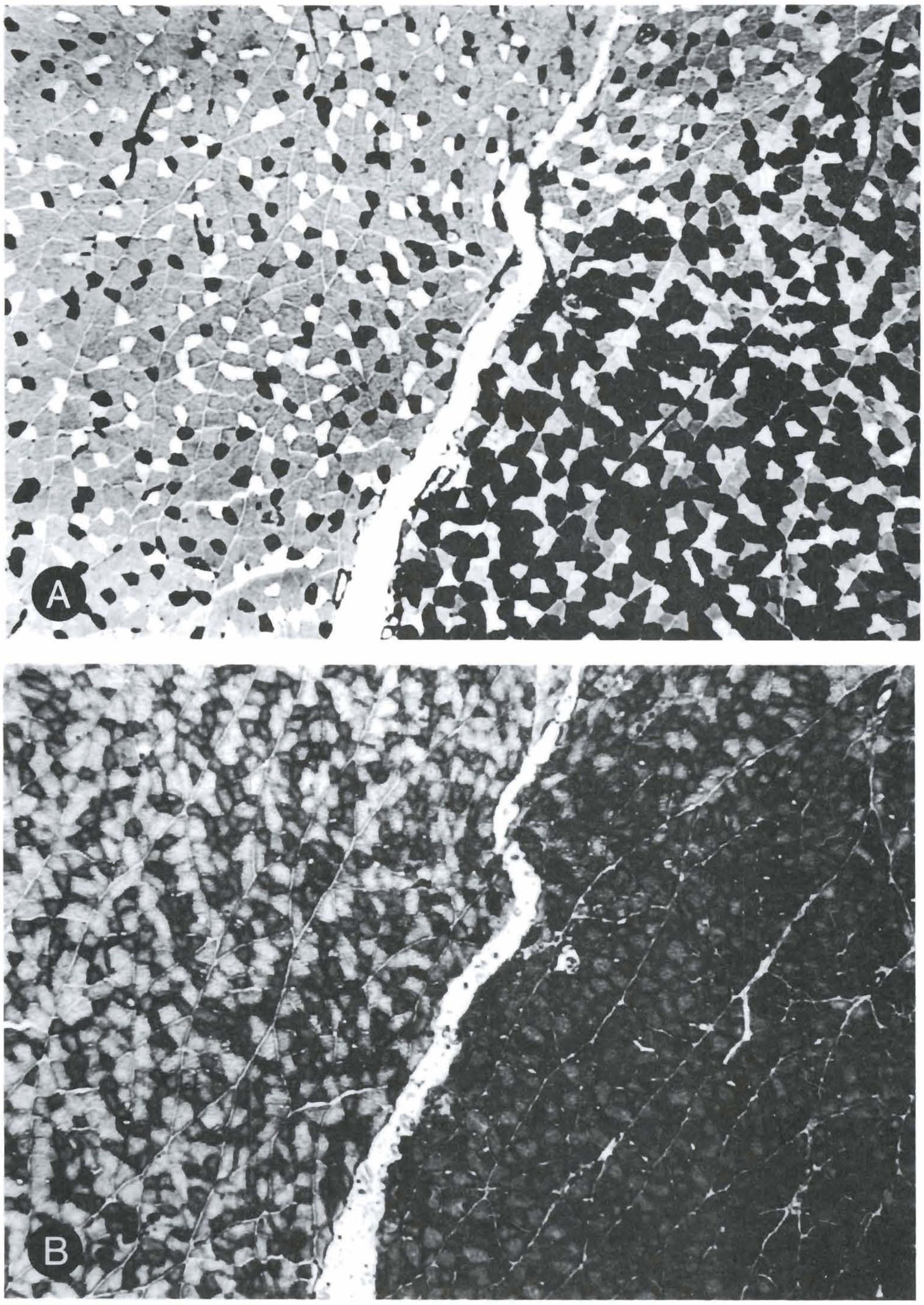

Fig. 1. Serial micrographs showing fibre type identification in red and mixed gastrocnemius muscle. × 25. **A.** Myosin adenosin triphosphatase activity (m-ATPase after acid preincubation, pH 4.4). **B.** Nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR).

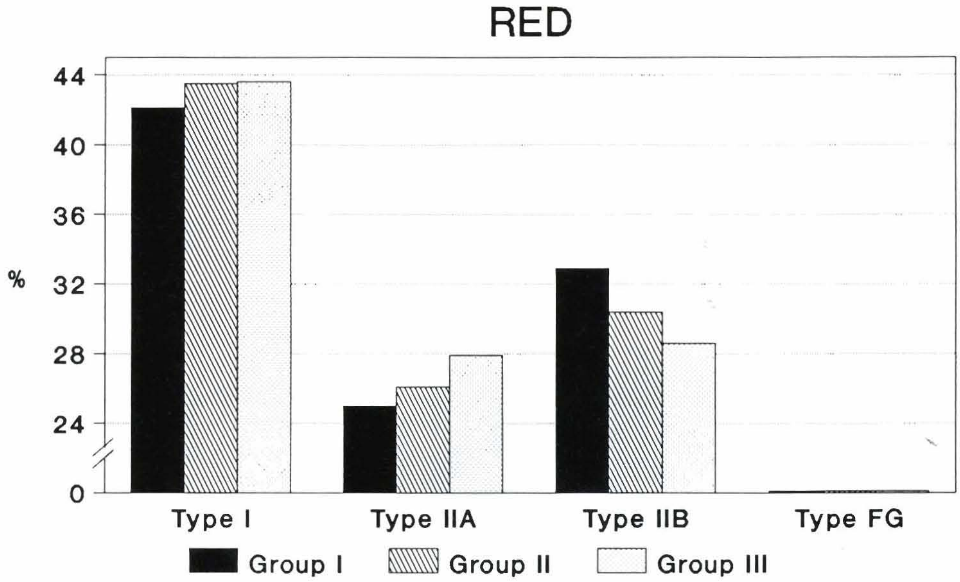

Fig. 2. Histogram showing mean percentages of each fibre type in red gastrocnemius muscle in every group.

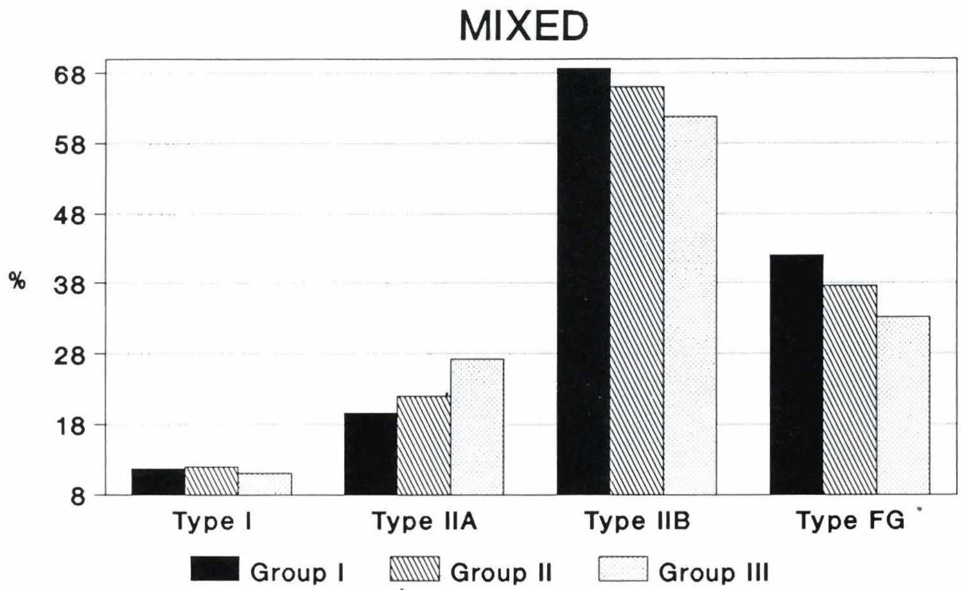

Fig. 3. Histogram showing mean percentages of each fibre type in mixed gastrocnemius muscle in every group.

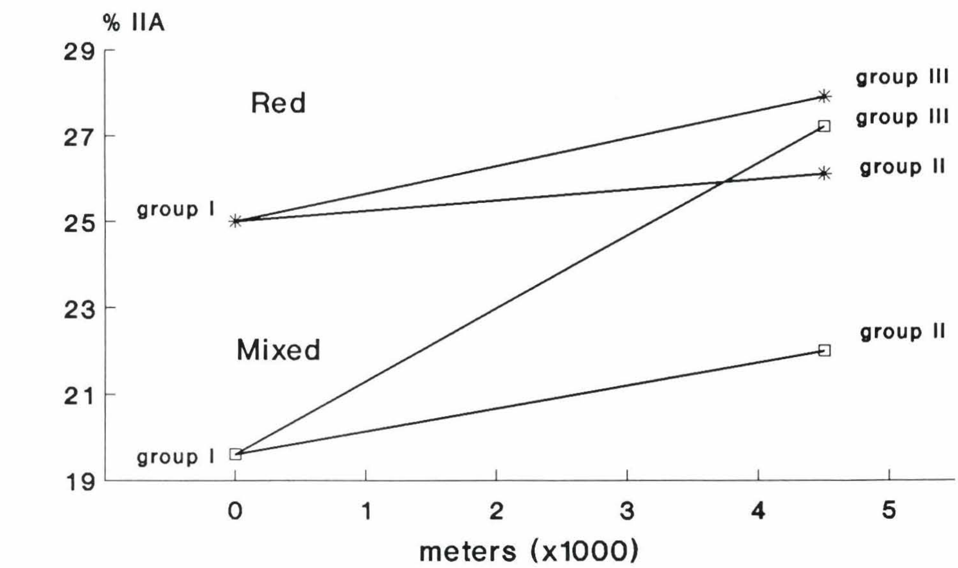

Fig. 4. Graph showing the IIA fibre type percentage variations in red and mixed gastrocnemius muscle in each group in relation to the whole distance run by each group.

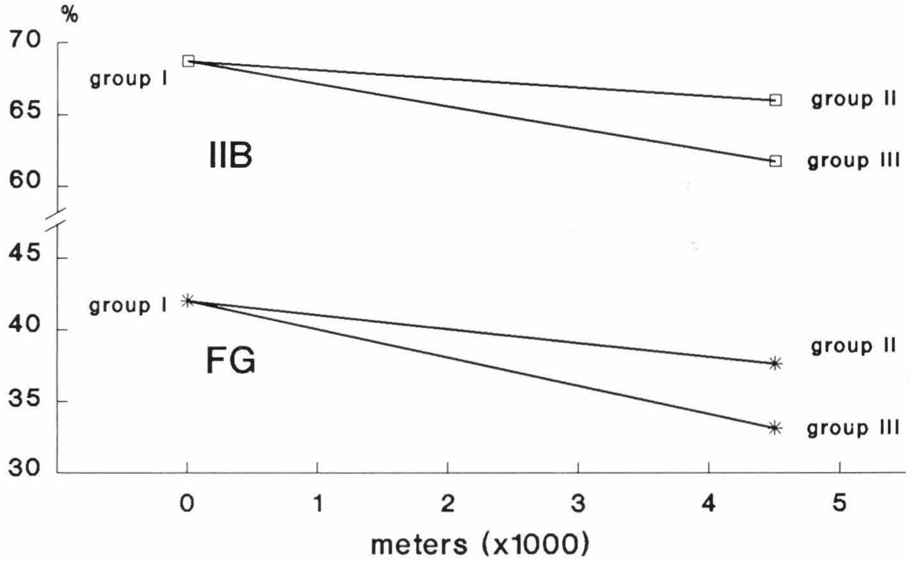

Fig. 5. Graph showing the IIB and FG fibre type percentage variations in mixed gastrocnemius muscle of each group in relation to the whole running time by each group.

exceeded maximum aerobic capacity by 100% throughout the experiment (Shepherd and Gollnick, 1976). Nevertheless, Jansson et al. (1978) and Green et al. (1984) have reported an increase in the proportion of type I fibres in extreme endurance training programmes involving strenuous effort over a long period of time. The results obtained in the present study show that the ratio of fast-twich to slow-twitch fibres remains remarkably stable, which bears out the theory postulated by Komi et al. (1977) and Szentkuty and Schlegel (1985) that this ratio is largely determined by genetic factors.

The results obtained in both the red and mixed portions of m. gastrocnemius suggest a transformation of IIB fibres into IIA fibres, a widely-accepted phenomenon of adaptation to aerobic exercises (Ingjer, 1979; Green et al., 1984). The present data, however, conflict with findings reported by Jansson et al. (1978) suggesting that in exercises involving anaerobic work IIA fibres would be transformed into IIB fibres better suited to strenuous effort over a short period of time (glycolytic metabolism). The increase in IIA fibres in the present study indicates that the muscles are more fatigue-resistant (Brooke and Kaiser, 1970).

In the red portion (Fig. 4), this increase in IIA fibres was only significant in Group III (2.9%), whereas in the case of the mixed portion the increase was significant both in Group II (2.4%) and in Group III (7.6%). Additionally, a statistical difference was noted between Group II and Group III in the mixed portion. These results suggest, for both muscle portions, that the specifically anaerobic training schedule undergone by Group III animals gave rise to greater fibre adaptation than that undergone by Group II animals. Comparison of the two muscle portions studied reveals that the changes in fibre composition are more acute in the mixed portion, and that in both experimental groups the increase in the proportion of IIA fibres occurs more rapidly in the mixed than in the red portion. It may thus be concluded that the increase in IIA fibres and the corresponding decrease in IIB fibres differs from one portion to another; i.e. after identical training, fibre adaptation in one portion of m. gastrocnemius differs from that of another portion. This difference may be linked to the functional purpose of each muscle portion: the abundance of type I fibres in the red portion, together with the fact that they are larger than IIA and IIB fibres (Armstrong and Phelps, 1984; Morales-López, 1989) suggests greater participation in statico-postural functions (Burke, 1981), making the red portion less dynamic than the adjacent mixed portion.

Virtually no low oxidative capacity (FG) fibres were found in the red portion of m. gastrocnemius, a finding which prevented us from analysing enzyme changes in the fibres. It must, however, be assumed that any increase in oxidative capacity in this portion is negligible, since training represents a smaller stimulus for muscles which are already adapted to highly repetitive activities (Edgerton et al., 1969; Tamaki, 1987), as is the case with this muscle portion whose main function is statico-postural.

In the mixed portion (Fig. 5), the proportion of FG fibres fell significantly after training (4% in Group II and 8.9% in Group III). This overall increase in oxidative activity is to be expected, since, irrespective of changes in contractile potential, any muscle exercise leads to the acquisition of at least moderate oxidative capacity (FOG) and a corresponding loss of FG fibres (Howald, 1982; Green et al., 1983). The statistical difference between changes in fibre composition in Groups II and III clearly suggest that specific speed training schedules give rise to greater fibre adaptation. Comparison of this data with findings from previous studies (Morales-López, 1989) of training programmes involving aerobic work (endurance), reveals that the increase recorded in oxidative capacity during the present study was smaller in relation to the total distance covered. This would suggest that endurance training leads to a greater increase in mitochondrial oxidative enzymes than speed training, and that other metabolic pathways not dealt with here may be involved in the enzyme adaptation occurring in muscle fibres as a result of speed training.

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