

Effect of intermittent stretch on immobilised muscle

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SUMMARY When muscle is immobilised in a shortened position there is a reduction in fibre length and an increase in the proportion of connective tissue. This results in reduced muscle compliance and a loss of range of joint motion. Experiments have been carried out to determine whether short periods of stretch are effective in preventing these changes. The soleus muscle of the mouse was immobilised in a shortened position for a period of 10 days by means of a plaster cast. Every two days the cast was removed and the muscle passively stretched for a 15 minute period. It was found that this treatment prevented the connective tissue changes but did not prevent the reduction in muscle fibre length, which in itself resulted in considerable loss of range of motion.

Key words: connective tissue, sarcomere number, compliance.

Subjecting of a muscle to periods of immobilisation in a shortened position results in reduced compliance—that is, the muscle becomes more resistant to passive stretch.^{1 2} This is partly owing to a loss of serial sarcomeres along the length of the muscle fibres³ but also to changes in the intramuscular connective tissue framework which constitutes the parallel elastic component of the muscle; the ratio of collagen (which is the major component of connective tissue) to muscle fibre tissue increases during periods of immobilisation in the shortened position.⁴ A reduction in muscle compliance following immobilisation would be expected to affect mobility after not only limb casting but also periods of bed rest in the presence of joint disease. One of the aims of this study was, therefore, to determine to what extent changes in connective tissue content and serial sarcomere number in an immobilised ankle extensor muscle affect the range of motion of the ankle joint.

Maintenance of a muscle in a stretched position throughout the period of immobilisation prevents the connective tissue changes.⁵ When one muscle group is immobilised in a stretched position, however, its antagonists are placed in a shortened position resulting in deleterious changes in these muscles. Experiments have therefore been carried out to determine whether short periods of stretch

are effective in preventing the changes in muscle connective tissue and fibre length and thus maintaining range of joint motion.

Materials and methods

Mice of the strain S/Hy were divided into two groups each containing five animals. In one group the animals were anaesthetised with pentobarbital sodium, and one hind limb of each animal was immobilised by means of plaster of Paris bandage with the ankle extended (soleus muscle shortened). In a second group immobilisation was carried out as in the first group with the exception that every second day the cast was removed under anaesthetic and the ankle held in full flexion for 15 minutes by means of a piece of tape. After this the cast was then reapplied with the ankle again in extension. After a 10 day period of immobilisation or immobilisation combined with intermittent stretch the animals were killed and, using a goniometer, the range of movement of the ankle measured both before and, in the case of half of each of the two groups, after section of the tendons of the extensor muscles.

CONNECTIVE TISSUE ANALYSIS

The soleus muscles from the animals in which the tendons had been severed were removed, supported on a piece of liver, frozen in isopentane precooled in liquid nitrogen, and sectioned on a cryostat at 10 μm . Sections were stained with picro-sirius red.⁶ This stains the connective tissue of the epimysium,

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perimysium, and endomysium a bright red, which, under a green optical filter, contrasts well with the pale muscle fibres. Sections were examined under a Leitz microscope to which was attached a high resolution video camera. The video information was fed into a digitising interface (Computec diplomat) connected to a peripheral slot of a microcomputer (Apple II), which converted the video image into computer graphic image with two selectable grey levels.⁷ A control slide was used to adjust the video image by means of a threshold facility so that there was a good correspondence between the optical and video images. No further adjustments were made while readings of slides from all experimental and control muscles for a given group were taken. Sections were scanned using a small area (approximately 0.02 mm²) and readings taken of the connective tissue content. Five experimental and five contralateral control muscles were scanned for each group. Approximately 10 readings were taken from each muscle. Results, expressed as the percentage muscle area stained, were compared using a one way analysis of variance.

SARCOMERE NUMBER MEASUREMENTS

Experimental and control muscles from the remaining animals of each group were used for determining sarcomere number. The soleus muscles were exposed, the hind limbs then removed and fixed to pieces of cork with the ankle as flexed as possible before fixation in 2.5% glutaraldehyde. The soleus muscles were then removed, placed in 30% HNO₃ for two days, and stored in glycerol.⁸ Single fibres were teased out and mounted in glycerol jelly. A projection microscope was used to count the number of sarcomeres along the length of each fibre. Measurements were made of five fibres from each muscle and results compared using a *t* test.

Results

RANGE OF ANKLE MOVEMENT

In muscles which were immobilised in the shortened position the range of ankle movement was greatly

reduced (Fig. 1, Table 1) and the ankle could not easily be flexed to less than 97°, whereas in control limbs the foot could be placed along side the lower limb bone. After section of the tendons of the ankle extensors the range of ankle movement in the limbs which had been immobilised was greatly increased but was still significantly less than in the control limbs.

In the animals that had been subjected to short periods of stretch the range of ankle movement was much greater than in the immobilised only group. There was still a significant reduction compared with the controls, however (Fig. 1). This meant that towards the end of the experiment it was not possible to flex the ankle fully during the period of stretch. After section of the tendons there was no difference between experimental and control limbs.

CONNECTIVE TISSUE CONTENT

In immobilised muscles there was a significant increase in the proportion of connective tissue to muscle fibre tissue. In muscles which had undergone

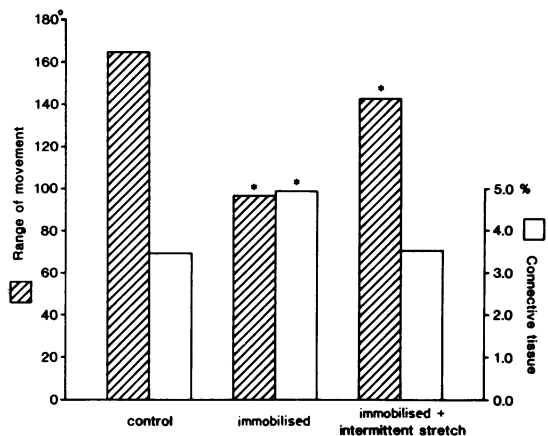


Fig. 1 Connective tissue content of the soleus muscle and range of ankle motion after immobilisation and immobilisation combined with intermittent stretch. *Significantly different from control value ($p < 0.01$).

Table 1 Effect of short periods of stretch on the loss of range of joint movement, muscle connective tissue, and fibre length in immobilised muscle

	Control	Immobilised (shortened)		Immobilised + intermittent stretch
Connective tissue (% area)	3.4 (0.1)	4.9 (0.4)*	(*)	3.5 (0.9)
Serial sarcomere number	2276 (48)	1824 (92)*	(NS)	1987 (80)*
Range of ankle movement (ROM) (deg)	163.2 (0.8)	96.4 (5.6)*	(*)	143.2 (2.4)*
ROM after section of extensor tendons (deg)	163.7 (1.1)	150.1 (3.9)*	(*)	162 (2.9)

Results are expressed as mean (SE).

Significantly different from control value ($p < 0.01$); () significant difference between the two experimental groups ($p < 0.01$).

intermittent stretch connective tissue content was normal (Fig. 1, Table 1).

SARCOMERE NUMBER

In immobilised muscles sarcomere number was considerably reduced compared with control muscles (Table 1). There was no significant difference between muscles which had undergone short periods of stretch and muscles which had been immobilised in a shortened position throughout the experimental period.

Conclusion

The results for sarcomere number and connective tissue proportions are in agreement with earlier work on both rabbit and mouse muscle, which showed that immobilisation of a muscle in a shortened position results in a reduction of serial sarcomeres and an increase in proportion of intramuscular connective tissue.⁴ Measurements of the range of ankle movement made in this study show that these changes result in a considerable loss of joint flexibility, though the fact that joint movement is not completely restored after tenotomy of the extensor muscles confirms that some of the stiffness is due to changes in the joint itself.⁹

Intermittent stretch was shown to prevent the accumulation of connective tissue; the range of ankle movement, however, was still reduced. This would appear to be owing to the reduction in serial sarcomeres in the extensor muscle fibres. Tenotomy of these muscles restored normal joint flexibility. Thus periods of passive stretch of only 15 minutes every other day both maintained normal muscle connective tissue proportions and prevented at least some of the changes which occur in the tissues surrounding the joint.

These experiments show that the connective tissue changes which occur in immobilised muscle and which affect joint movement can be prevented by a simple, short regimen of passive stretching

exercise. It is not yet known whether the results are due to the mechanical effects of stretch or to neural activation of the muscle in the stretched position.¹⁰ The results also show the importance of changes in muscle length in the restriction of movement following periods of immobilisation. These changes were not prevented by the regimen used. Work is currently under way to determine whether longer or more frequent periods of stretch will prevent loss of serial sarcomeres. This information would provide the basis for the development of exercise regimens which would not only prevent the connective tissue changes but also maintain muscle fibre length in conditions where joint rest is necessary.

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