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Immobilization and retraining of cruciate ligaments in the rat

Fifty male rats, aged 40 days, were assigned to one of five groups to examine the influence of immobilization and retraining on the strength and elastic stiffness of the anterior and posterior cruciate ligaments. Following the experimental treatments, the anterior ligament from the right leg and the posterior ligament from the left leg were tested in tension at a separation speed of 50 mm/min.

After 4 weeks of cast immobilization, the separation force of the anterior ligament declined by one fourth and elastic stiffness of both ligaments declined by one fourth and one third, respectively. No changes occurred in the separation force of the posterior ligament.

Following a 6-week retraining swimming program, the separation force and elastic stiffness values had returned to control levels. Additionally, there was a transition from avulsion type failures in the immobilized groups of animals to insertion site and intraligament failures in animals subjected to rehabilitative swimming exercise. This was indicative of a return in the strength of the osseous component of the bone-ligament-bone complex following immobilization.

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Following severe knee ligament injury, regardless of whether surgical repair has been attempted, some form of immobilization and support appears necessary to prevent further injury and to allow healing to occur. Despite the current proliferation of research into methods of surgical repair and/or reconstruction of ligaments, there has been little attempt to determine the effects of immobilization and remobilization alone on the mechanical properties of ligaments.

In one of the few studies to have examined these aspects, Noyes et al. (1974) showed that the strength and elastic stiffness of the anterior cruciate ligament in monkeys was reduced following 8 weeks of cast immobilization and that a remobilization period of up to 12 months was necessary for these values to return to normal. Although training has been shown to increase the strength of ligaments (Cabaud et al. 1980, Tipton et al. 1975, Viidik 1968, Zuckerman & Stull 1969), few investigators have attempted to examine whether or not a graded retraining program will reduce the time needed for the ligaments to return to their normal strength following immobilization (Cabaud et al. 1979, Vailas et al. 1981). These latter two studies only examined this problem following the surgical repair of ligaments. Hence, the response of ligamentous tissue, alone, to immobilization and retraining is still poorly understood. Because there is a danger that premature remobilization, especially using a weight-bearing activity, such as running, will compromise the previously weakened ligament, we have chosen to examine swimming exercise because of its non-weight-bearing nature.

Knowledge of the effect of immobilization on the cruciate ligaments is limited to the anterior ligament, and no studies have examined the effects of immobilization and retraining on more than one knee ligament concurrently. Hence, justification exists for examining whether the response of the ligaments within the joint to such treatments is a general alteration in their mechanical properties; or, whether changes will be specific to individual ligaments. Therefore, we examined the effects of a period of immobilization – followed by a graded rehabilitation program of swimming – on the mechanical properties of both the anterior and posterior cruciate ligaments in rats.

Methods

Fifty male Wistar rats were randomly assigned to one of five groups as follows: 1) control – 4 weeks; 2) immobilized – 4 weeks; 3) control – 10 weeks; 4) immobilized – 4 weeks; retrained swimming – 6 weeks; 5) control – 4 weeks; swimming – 6 weeks.

The rats were 40 days of age at the commencement of the program, with body weights ranging from 77 to 150 grams. Animal care was in accordance with the procedures established by the Universities Federation of Animal Welfare (1972).

Casts were applied to the hind limbs of the rats in immobilized groups under anesthesia; they were applied to both limbs to enable examination of both cruciate ligaments from the same animal. The knee joint was fixed in a position as close as possible to its normal resting angle of approximately 87 degrees of flexion (Booth & Kelso 1973). The hip joint was also placed in a cast in the normal resting position, except for slight abduction. The ankle joint was not fixed in order to alleviate possible problems arising from venous occlusion. In addition, strips of plaster were also applied to the dorsal aspect of the body, superior to the tail and around the abdomen, connecting the casts of both limbs. A small brace fastened between the knees contributed significantly to the stabilization of the cast and complete immobilization of the knee. The casts were inspected daily to check wear and hygiene. On the average, the casts were replaced every 9 days to compensate for wear and atrophy of the hind limb muscles.

Two groups of 10 rats, Groups 4 and 5, were exercised for 6 weeks in a graded swimming program. These groups were subsequently reduced to 9 rats each owing to attrition. The first 2 weeks of the program were for swimming acclimatization. This allowed time for the remobilization of the stiffened joints of the immobilized animals and conditioning of the experimental animals to swimming in a confined space, which improves the reliability of swimming times (McArdle & Montoye, 1966). During the acclimatization period, the swimming time, load, and depth of water were gradually increased until the rats could swim for 1 hour in water 60-cm deep with a weight equivalent to 1 per cent of their body weight attached to the tail. The temperature of the water during swimming was maintained at 35°±1° C in accordance with procedures established by Dawson & Horvath (1970).

The duration of each training session was 1 hour. The intensity of the swimming effort was progressively elevated by loading the animals with lead weights, increasing from 1 per cent of body weight in the third week to 3 per cent in the final 2 weeks. Every fourth day was a rest day. Animals were killed and all extraneous soft tissues, except the anterior cruciate ligament, were removed from the right leg. Similarly, the left leg was dissected to leave only the posterior ligament intact. The tibia and femur were then secured in special grips designed to closely support the eiphyses of the femur and tibia. This lessened the likelihood of failures occurring at the epiphyseal plate. The ligaments were tested in tension, within 20 minutes of the animals death, on an Instron tensile testing machine at a strain rate of 50 mm/min. The failure sites of the specimens were classified by macroscopic observation using a dissecting stereomicroscope (Olympus) into one of the following categories: intraligament failure, tibial or femoral insertion site failure, and tibial or femoral avulsion failure (Figure 1).

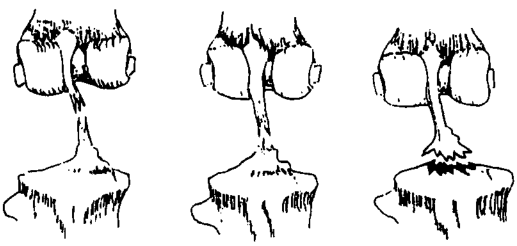

Figure 1. The failure mode was classified as intraligament rupture, insertion site rupture, or avulsion fracture.

Values for the maximum separation force (strength) and elastic stiffness for both the ligaments were determined. Separate statistical analyses were performed on each of these ligaments. Within these groups, data obtained from animals aged 68 and 110 days were treated separately. We performed *t*-tests for independent groups using data recorded for animals aged 68 days. One-way analysis of variance was performed on data recorded for the 110-day-old groups. Differences were reported as significant at $P<0.05$ level of confidence.

Results

Following the immobilization period, the mean body weight of Group 2 (155±6 g) was less ($P<0.05$) than that of the control Group 1 (241±7 g). By the end of the training program there was no difference between the mean body weights of Groups 4 (309±7 g) and 5 (320±10 g), the body weights of which were lower ($P<0.05$) than their Group 3 controls (357±11 g).

Immobilization resulted in a reduction ($P<0.01$) in the strength of the anterior cruciate ligament, but no change occurred in the posterior ligament (Table 1). In this same age group, the elastic stiffness of ligaments from immobilized animals was reduced ($P<0.05$) by 25 per cent and

Table 1. Mean (SEM) values for the separation force and elastic stiffness of the anterior (A) and posterior (P) cruciate ligaments

Group	Ligament	N	Separation force (N)	Elastic stiffness (N. mm^{-1})
2	A	10	32 (1.48)[a]	58 (5.92)[a]
	P	10	22 (1.93)	31 (3.84)[a]
1	A	10	44 (2.38)	78 (4.48)
	P	10	25 (2.16)	50 (5.37)
4	A	9	56 (3.30)	96 (8.08)
	P	9	31 (2.70)	47 (5.08)
5	A	9	60 (2.29)	134 (21.95)
	P	9	30 (2.47)	61 (4.17)
3	A	9	62 (3.24)	127 (20.00)
	P	9	30 (2.65)	57 (4.36)

[a] Different from control ($P<0.05$, *t*-test for independent groups).

38 per cent in the anterior and posterior ligaments, respectively (Table 1).

There were no differences between Groups 3–5 for separation force or elastic stiffness in either cruciate ligament (Table 1). The majority of failures for the anterior ligament occurred at the tibia, with 53 per cent at the tibial insertion site and 42 per cent as tibial avulsion failures. Conversely, two-thirds of posterior failures occurred at the femoral insertion site (Table 2).

Discussion

The decrease in strength and elastic stiffness of the anterior cruciate ligament following immobilization in our study supports the findings of previous investigations (Noyes et al. 1974, Noyes 1977). This period of immobilization, however, had limited effects on the strength of the posterior ligament, the only difference being a decrease in its elastic stiffness. It is likely that this ligament was less sensitive to immobilization due to its different anatomic function, which supports the notion that connective tissues respond quite specifically to alterations in their customary loading environment.

Although the strength of the posterior ligament did not decrease, the finding of a reduction in elastic stiffness supports Tipton et al. (1967), who suggested that these parameters are distinct entities involving different mechanisms of response from within the ligament. No chemical analyses were done in our study; however, decreases in ligament mass (Woo et al. 1982) and collagen concentration (Vailas et al. 1981) have been correlated with depressed strength of ligaments following immobilization. The decreased elastic stiffness levels in our study are similar to the findings of Amiel et al. (1982) for the lateral collateral ligament of rabbits immobilized for 9 weeks. Amiel et al. (1982) found that these changes were accompanied by increased collagen turnover (measured in the medial collateral ligament), but, interestingly, there was no alteration in the cross-sectional area of the ligament. They concluded that the reduced stiffness of the collateral ligament was due to changes in the ligament substance itself, rather than due to simple atrophy. This change was related to the less mature collagen fiber being more pliable and hence possessing a lower degree of stiffness.

In contrast to earlier investigations (Cabaud et al. 1980a, Tipton et al. 1975, Viidik 1968, Zuckerman & Stull 1969), positive mechanical effects did not occur in Group 5 when compared with its Group 3 control. Such differences may be due to inconsistency in the species, ligament tested, and exercise protocols. Although it has been shown previously (Baker & Horvath 1964) that rats swimming with a 3 per cent load have increased oxygen uptake, the training stimulus may not have

Table 2. Failure frequency and sites of anterior (A) and posterior (P) cruciate ligaments

Failure site	Treatment Groups									
	1		2		3		4		5	
	A	P	A	P	A	P	A	P	A	P
Intraligament					1			6		
Tibial insertion site	7		3	2	2		8	2	5	
Tibial avulsion	3		7	4	6	1	1		3	
Femoral insertion site		10		2		8		1		9
Femoral avulsion				2					1	

been of sufficient intensity to elicit a training response. The return to normal control values for strength and stiffness by Group 4, however, suggests that the training stimulus was sufficient in this case. Unfortunately, this cannot be compared with previous investigations of reconditioning (Noyes et al. 1974, Noyes 1977, Cabaud et al. 1980b, Vailas et al. 1981) due to the diversity of experimental procedures.

In the immobilized animals, there was a trend towards tibial avulsion failures, whereas the control specimens failed predominantly at the tibial insertion site for the anterior ligament and femoral insertion site for the posterior ligament. These findings support those of Laros et al. (1971), who reported increased tibial avulsion failures of the collateral ligaments in dogs following immobilization. This was accompanied by marked subperiosteal bone resorption and profound morphologic alterations, particularly at the tibial insertion site of the ligament. Noyes (1977) found similar changes at the femoral insertion site of the anterior cruciate ligament in primates, which resulted in an increased incidence of femoral avulsion failures. Comparison of the failure mode of Groups 2 and 4 showed that retraining resulted in a transition from avulsion type failures in the immobilized group to a predominance of tibial insertion site failures in the anterior ligament and intraligament failures in the posterior ligament. This was indicative of a return in strength of the osseous component of the bone-ligament-bone complex following retraining as previously demonstrated by Noyes (1977).

Our study has shown that a period of immobilization had deleterious effects on the strength and stiffness of the anterior cruciate ligament and stiffness of the posterior ligament in rats. These changes were considered specific adaptations by each ligament in response to changes in their normal loading environments. Secondly, the study has indicated that the relatively short and carefully graded swimming program was an efficient means of regaining lost strength and elastic stiffness in the previously immobilized ligaments. However, from a clinical viewpoint, it would be anticipated that the duration of the rehabilitative program required to overcome the adverse effects of immobilization alone, as shown in our study, is much shorter than that required to overcome the combined effects of surgery and subsequent immobilization. Further study is required to examine the influence of differing periods of immobilization on all four knee ligaments and the effects of differing modes, intensities, and durations of exercise in remediating these changes.

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