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EFFECT OF PREVIOUS EXERCISE ON FRACTURE HEALING: A BIOCHEMICAL STUDY WITH MICE

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Morphological, physical and metabolic changes occur in connective tissue during adaptation to physical exercise. Prolonged physical training has been reported to thicken tendons and ligaments in growing rabbits (Ingelmark 1945), in young mice (Ingelmark 1948) and in dogs (Tipton et al. 1970). Alterations in the elasticity of rabbit tendons have also been found (Viidik 1967, 1968), suggesting that the number of crosslinks is decreased by training (Viidik 1972). A direct relationship between ligament strength and the degree of mobilization of the knee joint has also been reported (Adam 1966). Muscle and bone hypertrophy is found in rats exercised by running (Saville & White 1969). The level of physical activity affects the turnover of collagen in long bones and achilles tendons of mice (Heikkinen & Vuori 1972) and the mineral and organic bone turnover in swine (Anderson et al. 1971).

It is not known whether prior exercise affects tissue regeneration after an injury. The present study was designed to establish the effects of previous training on the healing of experimentally produced fractures in mice.

MATERIALS AND METHODS

A total of 150 two-month-old mice of the NMRI-strain, obtained from Ylä-Mankkaan tila, Mankkaa, Finland were divided into two groups. Mice in group 1 were exercised daily on a treadmill with a progressively increased running program: 30 minutes per day during the first week, 60 minutes per day during the second week and 2 × 60 minute periods per day during the third week at a speed of 30 cm/s. Group 2 was kept under normal laboratory conditions. The animals were fed pelletted mouse diet (Manufactured by Hankkija, Finland) and fresh milk was available *ad libitum*.

After the exercise period bilateral closed tibial fractures were produced manually

Table 1. Dry weights (mg) and contents of hexosamine (μg), hydroxyproline (μg) and nitrogen (μg) of the 5–14-day-old fracture calluses in the exercised and control mice. Statistical significances of the differences were calculated by the *t*-test.

Days after fracture	Animal group	Dry weight	Hexosamines	Hydroxyproline	Nitrogen
5	exercised	6.0±0.4 (23)	60.0± 7.9 (8)	75.7± 6.9 (8)	654.1± 60.3 (8)
	control	7.3±0.3 (29)	105.1± 8.2 (10)	111.9± 6.1 (10)	1207.2± 81.1 (10)
	signif.	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
7	exercised	7.4±0.5 (24)	193.6±14.8 (8)	133.9± 8.2 (15)	645.7± 60.7 (15)
	control	7.5±0.3 (29)	193.3± 6.4 (18)	136.7± 9.1 (19)	713.6± 38.5 (19)
	signif.	NS	NS	NS	NS
10	exercised	13.2±0.7 (12)	433.2±28.7 (8)	323.7±20.6 (8)	1113.9± 70.4 (8)
	control	15.4±0.9 (20)	551.3±73.2 (10)	342.3±34.0 (10)	1357.2±148.8 (10)
	signif.	NS	NS	NS	NS
14	exercised	21.0±1.00 (34)	289.9±30.5 (19)	383.1±23.1 (17)	1246.2±138.6 (17)
	control	22.3±0.8 (42)	305.8±20.4 (19)	355.6±14.4 (22)	1531.6± 60.3 (22)
	signif.	NS	NS	NS	NS

Note – the means, their standard errors, the number of determinations and statistical significances of the differences are given. (NS: Non-significant).

in both exercised and control mice. The animals were killed at 5, 7, 10 and 14 days and the calluses were prepared and analyzed as described by Penttinen et al. (1972 a).

To measure the rate of protein accumulation into the calluses [^{3}H-L]-proline (TRA 82, Radiochemical Centre, Amersham, England) 1 μCi/g, was injected intraperitoneally into mice 5 and 10 days after the fracture and the animals were sacrificed 1, 4, 8 and 24 hours later.

Following lyophilization for 24 hours and drying in a vacuum desiccator to constant weight (dw) the calluses were weighed. The samples were hydrolyzed in 2 N HCL at 103° for 16 hours. Part of each hydrolyzate was removed for the determination of hexosamines. Additional hydrochloric acid was added to the samples to a level of 6 N and the hydrolysis continued at 130° for 3 hours. The hydrolyzates were evaporated dry on a boiling water bath, the residues dissolved in distilled water and used for the determinations.

Hexosamines were determined according to Blix's (1948) modification of the Elson-Morgan method after removing interfering chromogens with a Dowex-1×50 cation exchange resin column (Boas 1953). *Hydroxyproline* was determined according to Woessner (1961) and *nitrogen* after combustion of samples in 8 N sulphuric acid according to Minari & Zilversmit (1963).

Calcium was determined with an atomic absorption spectrophotometer (Unicam SP-90, Unicam Instrument Ltd, Cambridge, England) in a 1 per cent solution of

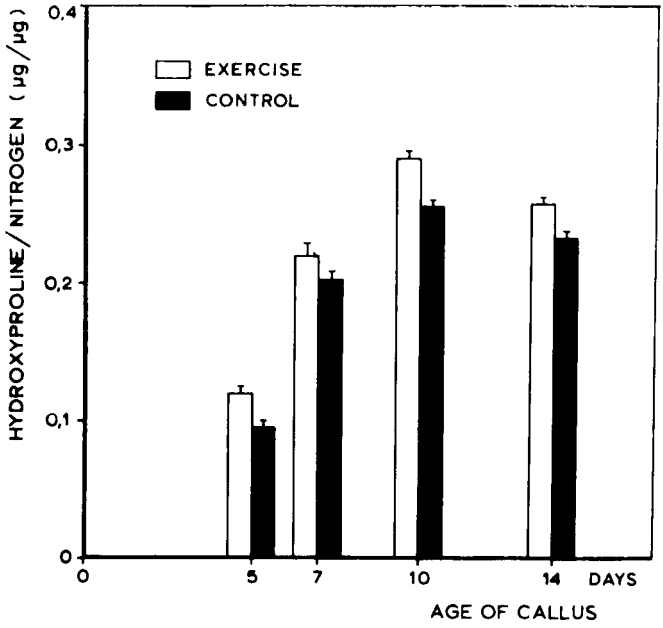

Figure 1. Effect of previous exercise on the ratio of hydroxyproline to nitrogen in tibial fracture calluses of mice. All values are means of 8–10 calluses. Each vertical bar represents the standard error of the mean. Statistical significances of the differences were calculated by the analyses of variances.

lanthanum chloride in 25 per cent HCl. The *DNA and RNA-ribose* content of the calluses was determined by the method of Schmidt & Thannhäuser (1945) with some modifications (Penttinen et al. 1972 c). For the measurement of total radioactivity an aliquot of 200 µl was transferred to a counting vial and 10 ml of a "phosphor" solution (6 ml of methycellosolve and 4 ml of POP-POPOP solution) was added. The radioactivity of hydroxyproline was determined according to the method of Prockop & Udenfriend (1961) as modified by Juva & Prockop (1966).

The statistical significances of the results were calculated by the analyses of variances or by the *t*-test.

RESULTS

Little difference was noted in the dry weight of the calluses from the two groups (Table 1). Tissue samples from the control mice contained more hexosamines, hydroxyproline and total nitrogen at 5 days after the fracture. Between the 7th–14th days the control calluses contained more nitrogen but approximately the same amount of hydroxyproline

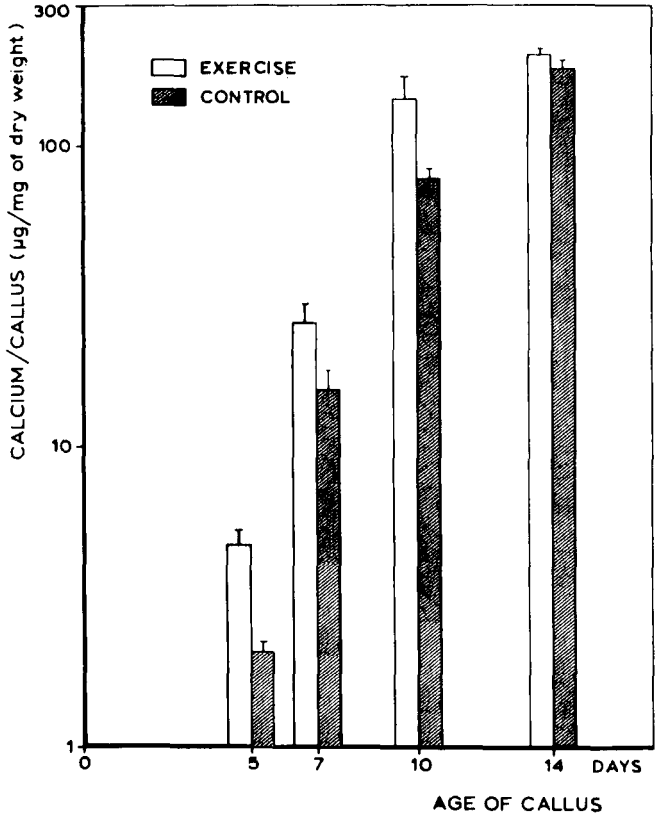

Figure 2. Effect of previous exercise on the amount of calcium in tibial fracture calluses of mice. All values are means of 14–16 calluses. Each vertical bar represents the standard error of the mean. Statistical significances of the differences were calculated by the analyses of variances.

(Table 1). The ratio of hydroxyproline to nitrogen (Figure 1) averaged 20.8 per cent higher in the calluses of the exercise animals ($P < 0.01$).

On the 5th and 7th days the contents of calcium were 123.8 per cent and 67.7 per cent larger ($P < 0.001$), respectively, in the calluses of the exercised mice (Figure 2). The incorporation of proline to collagen hydroxyproline on the 5th day was higher in the calluses of the exercised animals but on the 10th day lower incorporation rates were observed (Figure 3). The amounts of RNA-ribose were higher on the 5th and 7th days and the amounts of DNA on the 10th day lower in the calluses of the exercised group (Table 2). The ratio of RNA-ribose to

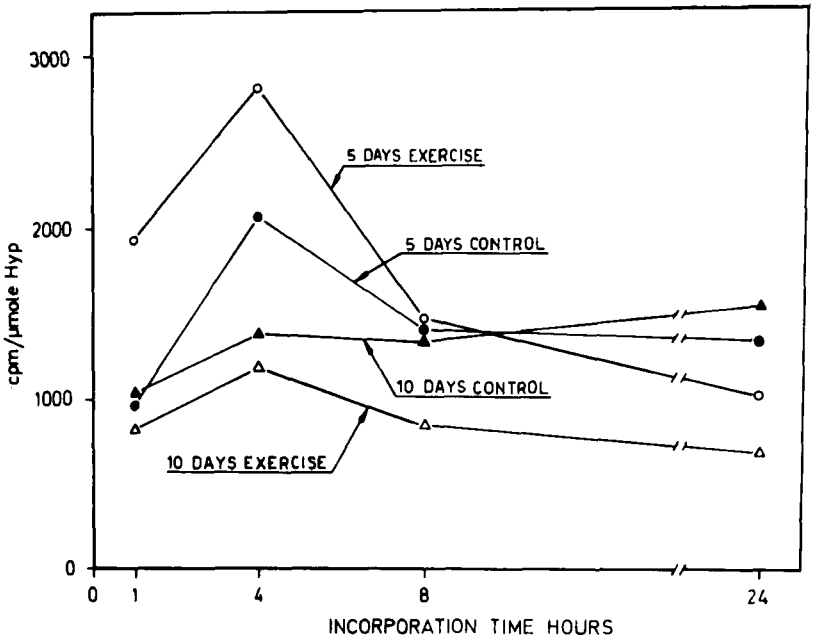

Figure 3. Effect of previous exercise on the incorporation of ^{3}H-proline into collagen ^{3}H-hydroxyproline in vivo *in the 5- and 10-day fracture calluses.*

DNA was on the average 34 per cent higher in the calluses of the exercised animals (Table 2).

In addition, the ratio of calcium to hydroxyproline was higher in the calluses of the exercised animals over a period 5–10 days after the fracture.

DISCUSSION

The present chemical data suggest that previous exercise accelerates the sequence of fracture healing in mice. Callus tissue develops faster as judged from the increased proportion of collagen to the total proteins (Figure 1), increased proline incorporation rates (Figure 3) and the concentrations of calcium (Figure 2) in the exercised callus. The effects of exercise are most marked on the 5th and 7th days after the fracture which in the rat corresponds to the proliferation of cartilaginous components in the callus (Penttinen 1972 c). Exercised animals produced tissue which turned to bone more rapidly than the callus in the control mice. Interestingly, the size and mass of callus tissue was not increased

in the exercised group. The chemical properties of the repairing tissue are therefore important to the final result.

Table 2. The contents of RNA-ribose (μg/callus) and DNA (μg/callus) and the ratio of RNA-ribose to DNA in the 5–14-day-old fracture calluses in the exercised and control mice. Statistical significances of the differences were calculated by the *t*-test.

Days after fracture	Animal group	RNA	DNA	RNA-ribose/DNA
5	exercised	76.1±4.5 (8)	26.0± 1.1 (8)	2.93
	control	61.4±4.8 (10)	27.8± 2.6 (8)	2.21
	signif.	$P < 0.05$	NS	
7	exercised	82.5±2.8 (7)	33.8± 4.0 (7)	2.44
	control	75.5±2.9 (10)	41.2± 5.7 (9)	1.83
	signif.	$P < 0.05$	NS	
10	exercised	138.0±7.2 (8)	190.1±12.8 (8)	0.73
	control	150.8±7.2 (10)	320.5±13.1 (8)	0.47
	signif.	NS	$P < 0.001$	
14	exercised	93.7±6.3 (17)	142.0±13.1 (8)	0.66
	control	104.3±6.4 (19)	118.1±10.7 (10)	0.55
	signif.	NS	$P < 0.02$	

Note – The means, their standard errors, the number of determinations and statistical significance of the differences are given. (NS: Non-significant).

Mechanisms which lead to altered capacity for bone healing in exercised mice are not known. Numerous factors have been claimed to promote the regeneration of bone and some of them may have a correlation to physical activity. These include secretion or administration of growth hormone (Nichols et al. 1968, Hsu & Robinson 1969, Misol et al. 1971, Koskinen et al. 1971), or thyroid hormones (Tarsoly et al. 1965, Koskinen 1967, Ewald & Tachdijan 1967, Ziegler & Delling 1972), and application of local electric currents (Cieszynski 1967, Becker & Murray 1967). The plasma concentration of growth hormone is reported to increase during exercise (Roth et al. 1964, Hunter et al. 1965).

Physical training increases the a-v-oxygen difference in skeletal muscles (Saltin et al. 1964). Hyperbaric oxygenation of rats is known to increase the callus size, collagen content and mineralization (Coulson et al. 1966, Yablon & Cruess 1969, Penttinen et al. 1972 a). On the

other hand, hypoxia due to decreased atmospheric air pressure retards all parameters of the healing process (Penttinen et al. 1972 b). It seems tempting to speculate that training facilitates the circulation in bone and callus and the transport function of the callus cells.

The increased ^{3}H-proline incorporation rates and the RNA-ribose/DNA-ratio suggest that the metabolism of callus cells is increased in the exercised animals. This is supported by recent data on increased activities of some key enzymes of the Krebs' cycle and glycolysis in tendon and bone cells after an exercise period similar to that used in this study (Heikkinen et al. unpublished results).

SUMMARY

We have studied the effect of prior physical exercise on the healing of leg fractures in mice. Male mice were exercised for 3 weeks on a treadmill or kept under standard laboratory conditions. After the training period, tibias were fractured in both test and control animals. Mice were sacrificed at 5, 7, 10 and 14 days after the fracture and the reparative callus tissue was isolated and analyzed. The ratio of hydroxyproline to nitrogen in the calluses was on the average 21 per cent higher in the exercised mice. The rate of synthesis of collagen and other proteins was greater at 5 days in the calluses of the exercised mice while calcium levels were strikingly higher. The results suggest that fracture healing is hastened by previous physical exercise.

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