Acta Orthop Scand 57, 204–208, 1986

Effects of exercise on bone morphology

Vascular channels studied in the rat tibia

14 male rats were divided into exercise and control groups to examine the effect of a 1-month exercise program on the vascular morphology of the tibial diaphysis. Following the exercise program the number of haversian canals per mm^2 increased in the middle third of the cortex, but not in the outer or inner zone. No change was observed in the number of nonhaversian canals. The size of the nonhaversian canals increased following the training program, which was not evident in the haversian canals.

Mark R. Forwood
Anthony W. Parker

Department of Anatomy
University of Queensland,
St Lucia, Australia 4067

Long bones are responsive to physiologic changes that occur during exercise. During physical activity, for example, increases in bone perfusion (Tøndevold 1983, Tøndevold & Bülow 1983) and vascular resistance (Gross et al. 1979) have been observed. Despite these observations, there is a paucity of research relating physiologic events with detailed morphologic adaptations resulting from physical activity.

Parker (1977) found an increase in the number of haversian and nonhaversian canals in the femoral diaphysis of rats following an 8-week swimming program. Likewise, Antonov (1980) demonstrated an increase in both the diameter and the number of haversian canals in the femoral diaphysis of rats following 500 hours of daily swimming training for 100 or 200 minutes. In Antonov's study, the magnitude of changes was proportional to the intensity of the exercise, which correlates with Nilsson & Westlin's (1972) observations on the relationship between exercise intensity and bone density in athletes.

We have examined the effects of weight-bearing exercise on the morphology of the bone vascular system in the rat.

Materials and methods

14 Male Wistar rats, aged 22 days, and weighing 45 ± 5 g, were randomly divided into exercise and control groups. The rats were housed in individual cages and fed a standard laboratory stock feed and watered ad libitum. The temperature of the laboratory was maintained at 20 ± 1°C, and a diurnal light control provided a 12-hour light and dark period, respectively.

The exercise program began immediately with treadmill training (Key et al. 1984) and swimming acclimatization. The rats swam individually in a large tank divided into 20 × 16 cm compartments with depths of 60 cm. The temperature of the water was maintained at 35 ± 1°C, in accordance with procedures established by Dawson & Horvath (1970). The training program began at 35 days of age and continued until the rats were 63 days old. At the end of the acclimatization period, the body weights had doubled, with the exercise rats weighing 10 per cent less than the controls. By the end of the training period, the exercise rats weighed 208 ± 7 g and the control rats 250 ± 8 g. The running and swimming schedules were previously designed to provide a progressive increase in the exercise load throughout the 4-week period (Oakes et al. 1980, Table 1).

Using prediction equations relating running speed to oxygen uptake (Brooks & White 1978) and body weight to maximum oxygen uptake (MVO_2) (Pica & Brooks 1982), we estimated that our animals were exercising at approximately 80 per cent of their MVO_2 throughout the duration of the training program. This exercise schedule has increased the heart to body weight ratio of male Wistar rats (Oakes et al. 1980), demonstrating the effectiveness of the exercise stress.

At the completion of the training program, the rats were killed; the right hind limb was dissected from the body; and all extraneous tissues were removed from the tibia, which was immediately placed in Gooding & Stewart's decalcifying solution (Culling 1963) for 48 h. After decalcification, the tibia was sectioned transversely at the distal margin of the ti-

Table 1. Exercise program

Weeks	1	2	3	4
Treadmill intensity	16 m.min^{-1}	19 m.min^{-1}	26 m.min^{-1}	26 m.min^{-1} 10% gradient
Swimming intensity	1% BW	2% BW	3% BW	3% BW

BW = Body-weight load attached to tail.

bial tuberosity and double embedded in celloidin and paraffin wax. Next, 10 μ thick sections were cut transversely and stained with toluidine blue. Variability due to deviation from the tibial tuberosity was tested by cutting serial 10 μ sections from 50 μ proximal to 50 μ distal of the distal margin. One-way analysis of variance (ANOVA) for repeated measurements demonstrated no difference in the number of haversian or nonhaversian canals within this range.

To analyze the vascularity of the tibia, a montage of the tibial cortex was prepared from photomicrographs. The cortex was then divided into equal thirds, and the number of haversian and nonhaversian canals were measured in the outer, middle, and inner thirds of the cortex. Counting was performed as "blind trials", so that the group to which each section belonged was unknown. A test-retest method for reliability showed that counting was accurate with a significant Pearson correlation coefficient ($r_{40} = +0.96$, $P < 0.01$). Vascular canals were classified as haversian or nonhaversian on the basis of parameters described by Singh & Gunberg (1970): That is, haversian canals were defined as a cylinder of osteocyte-bearing lamellae with a central canal; and nonhaversian canals were distinguished by the lack of cement lines or other sharply defined borders, and by the absence of circumferential lamellae (Figure 1). The area of each zone of the cortex was measured on a digitizing table to obtain the number of canals per mm^2.

Because most of the canals approximated an ellipsoid, the maximum and the minimum diameters of the canal were measured using an eyepiece micrometer in a binocular microscope to calculate the area of each measured canal.

Because of inhomogeneity of variance, the nonparametric Mann-Whitney U test was employed to test differences in the number of nonhaversian canals.

Table 2. Effect of exercise on the mean (S.D.) number of haversian canals per mm^2 in the tibial cortex of rat

	Zones of cortex		
	Inner	Middle	Outer
Control	3.0 (4.5)	3.8 (3.5)	1.2 (2.0)
Exercise	2.4 (1.3)	9.0 (2.0)[a]	2.1 (2.5)

[a] $P < 0.05$

Table 3. Effect of exercise on the mean (S.D.) size (μ^2) of haversian and nonhaversian canals in the tibial cortex of rat

	Group	N	Size
Haversian	Control	30	568 (374)
	Exercise	38	561 (306)
Nonhaversian	Control	144	623 (203)
	Exercise	151	740 (431)[a]

[a] $P<0.004$.

Results

Typical of rat bone (Singh & Gunberg 1970), the number of nonhaversian canals was considerably larger than haversian canals ($P < 0.01$). Following the exercise program, the number of haversian canals per mm^2 increased in the middle third of the cortex (Table 2). This change was not evident in the other two zones

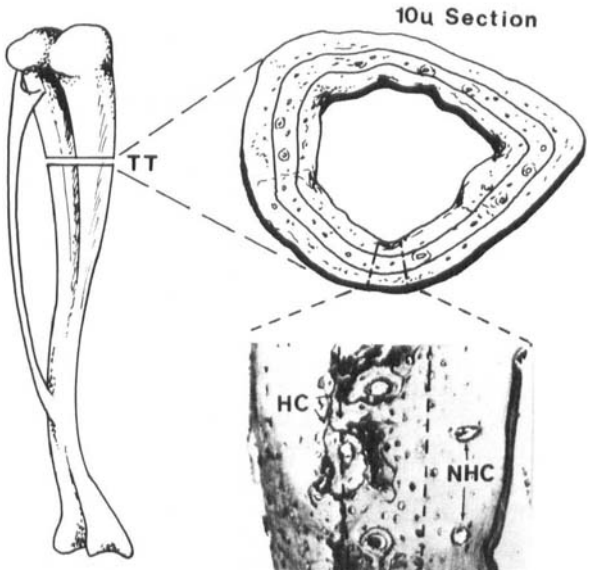

Figure 1. Schematic diagram showing the selection of histologic sections, HC = Haversian Canal, NHC = Nonhaversian Canal, TT = Tibial Tuberosity.

or in nonhaversian canals. The average area of nonhaversian canals had increased after the exercise program (Table 3); this was not the case for haversian canals.

In addition, Bartlett's test for inhomogeneity of variance showed that the variance in the number of nonhaversian canals in the control group was higher than in the exercise group in the outer ($P < 0.001$) but not in the middle or inner zones of the cortex ($P > 0.05$).

Discussion

During physical activity perfusion of long bones may increase by 50 to 75 per cent of their resting value, the hyperemia of which can be maintained for up to an hour after the exercise (Tøndevold 1983). The increase in the number of haversian canals and the size of nonhaversian canals found here may represent a response to altered physiologic conditions associated with exercise. Blood vessels, for example, readily adapt structurally in response to sustained functional changes, particularly those related to chronic pressure alterations or changes in the nutritional demands of the tissue (Folkow 1983).

In addition to the physiologic consequences of increased stress concentrations, the differences in radial distribution of haversian and nonhaversian canals and the increase in size without an increase in number of nonhaversian canals indicate important mechanical considerations. Under conditions of increased loading, there are two options available by which a crack serious enough to eventually lead to failure can be checked. First, the bone substance itself can undergo a change through remodelling – thereby increasing fracture toughness. And secondly, a crack can be deflected by rearrangement of the bone in areas of increased stress concentration.

Additionally, Carter & Hayes (1976) found a negative correlation between the fatigue life of bone and haversian remodelling. The alteration in size, as opposed to numbers, of nonhaversian canals (the predominant system in rat bone) may reflect a requirement for an increase in the fatigue resistance of bone under conditions of cyclic loading.

This finding is in some disagreement with that of Parker (1977) who found that the number of nonhaversian canals in the femur of rats had increased after 8 weeks of swimming. Swimming exercise, which negates the effects of gravity, would not be expected to produce mechanical stress and strain levels of the same magnitude or frequency as those produced by running. Although muscle tension alone can cause changes in bone (Valderrama & Trueta 1965), increased fatigue resistance may be more desirable under conditions of cyclic and repetitive loading, experienced in weightbearing exercise.

The large variance in the number of nonhaversian canals in the outer zone of the cortex of control animals is an interesting observation. It lends support to Carter's (1982) hypothesis that for a fairly broad range of normal activities there is little stimulus of bone remodelling. A wide variance would be expected, therefore, in a given morphologic analysis. However, if high levels of loading are experienced, Carter (1982) suggests that remodelling is produced, with growing bone being the most sensitive to changes. Not only were the animals in our study exercised during a growth period, but the largest variance was noted in the outer zone, furthest from the longitudinal neutral axis, and thus subjected to the highest stress levels.

It is apparent that exercise can modify bone vascular morphology. The extent of remodelling that occurs appears to be governed by the type and intensity of exercise. Although physiologic conditions have been implicated in such changes, the evidence presented supports the conjecture that vascular canals have important mechanical functions in determining the fatigue resistance of bone.

Acknowledgements

We would like to thank Mr. C. Nguyen for his assistance in caring for the laboratory animals and Miss L. Bell for advice on histologic procedures.

References

Antonov, S. (1980) Effect of daily and interval dynamic loading on appositional growth and vascularisation of diaphysis in rat femoral bones. *Arkhiv. Anatomii Gistologii I Embriologii.* **78**, 19–24.

Brooks, G. & White, T. (1978) Determination of metabolic heart responses of rats to treadmill exercise. *J. Appl. Physiol. Environ. Exercise Physiol.* **45**, 1009–1015.

Carter, D. (1982) The relationship between in-vivo strains and cortical bone remodelling. *CRC Crit. Rev. Biomed. Eng.* **8**, 1–28.

Carter, D., Hayes, W. & Schurman, D. (1976) Fatigue life of compact bone-II: Effects of microstructure and density. *J. Biomech.* **9**, 211–218.

Culling, C. (1963) *Handbook of histopathological techniques.* 2nd ed., Butterworth, London.

Dawson, C. & Horvath, S. (1970) Swimming in small laboratory animals. *Med. Sci. Sports* **2**, 51–78.

Folkow, B. (1983) Structural autoregulation. The local adaptation of vascular beds to chronic changes in pressure. In: *Development of the vascular system,* CIBA Foundation Symposium 100, Pitman, London.

Gross, P., Heistad, D. & Marcus, M. (1979) Neurohumeral regulation of blood flow in response to exercise. *Am. J. Physiol.* **237**, 440–448.

Key, B., Parker, A. & Giorgi, P. (1984) Endurance exercise does not modify nerve fibre morphology in the rat soleus nerve. *Brain Research.* **297**, 137–144.

Kiiskinen, A. & Suominen, H. (1979) Blood circulation of long bones in trained growing rats and mice. *Europ. J. Appl. Physiol.* **34**, 303–309.

Martin, R. & Burr, D. (1982) A hypothetical mechanism for the stimulation of osteonal remodelling by fatigue damage. *J. Biomech.* **15**, 137–139.

Nilsson, B. & Westlin, N. (1972) Bone density in athletes. *Clin. Orthop.* **77**, 179–183.

Oakes, B., Parker, A. & Norman, J. (1980) Changes in collagen fibre populations in young rat cruciate ligaments in response to a one months intensive exercise program. (Proc.) *Human Adaptation.* (Eds. Russo, P. & Gass, G.), Cumberland College of Health Sciences, Sydney.

Parker, A. (1977) Changes in the vascularity of the femoral cortex of the rat following exercise. (Abst.) *J. Anat.* **124**, 525.

Pica, A. & Brooks, G. (1982) Effects of training and age on the VO_{2max} in laboratory rats. *Med. Sci. Sports Exercise* **14**, 249–252.

Singh, L. & Gunberg, D. (1970) Quantitative histology of changes with age in the rat bone cortex. *J. Morphol.* **133**, 241–252.

Tøndevold, E. (1983) Haemodynamics of long bones. An experimental study in dogs. *Acta Orthop. Scand.* **54**, Suppl. 205, 1–38.

Tøndevold, E. & Bülow, J. (1983) Bone blood flow in conscious dogs at rest and during exercise. *Acta Orthop. Scand.* **54**, 53–57.

Valderrama, J. & Trueta, J. (1965) The effect of muscle action on the intra-osseous circulation. *J. Path. Bacteriol.* **89**, 1879–1986.