Effect of physical activity on muscle and bone blood flow after fracture

Exercise and tenotomy studied in rats

Oliver Grundnes and Olav Reikerås

In male Wistar rats, a transverse osteotomy of the midshaft of the left femur was performed. The rats were allocated to three groups: 1) one that underwent a 4-week training program 4 weeks after the osteotomy, 2) one that had a tenotomy of the left Achilles tendon to prevent weight bearing, and 3) one that had normal function and activity. Eight weeks after the osteotomy, total bone, proximal diaphyseal, callus, and muscle blood flows were measured using the microsphere technique. Initial and final body weights, bone weight, and callus production were also recorded. There were no differences in bone or muscle blood flow between the three groups. An increase in total bone and muscle blood flows was seen on the osteotomized side. In weight-bearing rats, the callus area was more vascular as compared with the diaphyseal bone. No correlation between callus mass and callus flow was found. Our results support the concept that blood supply is mandatory for fracture healing; however, other factors seem to be decisive for the rate of healing.

Department of Orthopedics, Institute of Clinical Medicine, University Hospital, N-9012 Tromsø, Norway

The current concept of fracture healing is mainly based on two variables: stability and blood supply. In experimentally fractured bones, normal function and full weight bearing accelerate bone healing (Sarmiento et al. 1977, Terjesen and Svenningsen 1986, 1988).

Our present study was designed to evaluate the effect of different degrees of activity and weight bearing on muscle and bone blood flows in a fractured long bone.

Material and methods

The study was performed on 30 male Wistar rats (Møllegård Avlslaboratorium, Eiby, Denmark) weighing 326 (313–340) g. The rats were assigned to either an exercise, a nonweight-bearing, or a control group. After intraperitoneal anesthesia (fentanyl-fluanisone, 0.1 mL/100 g body weight), the left femur was exposed between the lateral vastus and hamstring muscles. A partial transverse osteotomy at the midshaft of the bone was performed with a fine-toothed circular saw blade mounted on an electric drill, and then the bone was manually broken. A periosteal elevator was passed underneath the middle part of the femur, as is done for protection of the soft tissues when performing osteotomies. The medullary cavity was successively reamed (using steel burrs mounted on the electric drill and starting from the osteotomy site—producing proximal and distal fragments) to a diameter of 1.5 mm. The burred residue was not rinsed out. A 1.6-mm-diameter steel pin was used for intramedullary nailing, and finally the wound was closed in two layers.

In the nonweight-bearing group, the ipsilateral Achilles tendon was cut just proximal to its insertion site. Thus, although these rats were permitted full mobility of the limb, weight bearing was impossible. To prevent the Achilles tendon from regaining its function, a new tenotomy was performed 4 weeks after the first operation. The animals in the control and exercise groups resumed partial weight bearing a few days postoperatively, and full weight bearing was regained after approximately 2 weeks. Tenotomized rats moved their operated on limb with ease; however, weight bearing was insufficient throughout the experimental period, as was intended

Four weeks after the osteotomies, the exercise group started their training program in a dark room on a treadmill with a 10 percent set inclination. Both the treadmill speed and the duration of training were gradually increased until the rats were running 2 h/day at a speed of 20 m/min.

Eight weeks after the osteotomy, bone and muscle blood flows were measured using the microsphere technique (Morris and Kelly 1980). A polyethylene catheter (PE-50) was led through the carotid artery and placed in the aortic root for injection. Radiolabeled microspheres (New England Nuclear, ^{141}Ce) of 15 ± 0.05 µm diameter were used, and each injection consisted of 350,000 spheres suspended in 0.9 percent saline. The distal artery was cannulated with a polyethylene catheter (PE-10) and connected to a Harvard infusion-withdrawal pump for reference sampling at a rate of 195 mL/min. The superficial layers of the vastus lateralis were used to measure muscle blood flow. This muscle consists of mixed red and white fibers, which minimize the bias concerning diversion of the blood flow between red and white muscle fibers after exercise.

Finally, the animals were killed, and the left and right femurs were dissected free from all the soft tissue and then dried and weighed. The frontal and transverse diameters of the callus mass were measured with a sliding caliper, and the quantity of the callus was expressed as the cross-sectional area, assuming it to be an ellipse. The bones were placed in counting vials together with the reference samples and counted in a Pacard Auto-Gamma Scintillation Spectrometer. Total bone flow was first determined, after which a proximal diaphyseal part and the callus area were separated for fractional blood flow estimates.

For statistical evaluations, we used the nonparametric Kruskal-Wallis test, comparing several means. To evaluate differences between the two limbs, we used the Wilcoxon rank-sum test. $P < 0.05$ was considered significant. Data are presented with medians and 25–75 percentiles.

Results

The weight gain in the exercise group was 152 (132–172) g, which was 31 percent less than the increase found in the control group ($P < 0.05$).

No differences in total bone blood flow were found between the three groups. The total fractured bone blood flow was increased in all three groups (Table 1). Further, no differences were found in the fractional bone blood flow between the three groups. In the exercise and control groups, the blood flow was increased in the callus area when compared with the diaphyseal bone, whereas the difference in the nonweight-bearing group was not significant (Table 2).

The external callus area was 61 (53–82) mm^2 in the exercise group, 79 (53–93) mm^2 in the nonweight-bearing group, and 80 (71–106) mm^2 in control group. No statistical differences were found. Regression analysis showed no correlation between callus area and callus blood flow ($r = 0.1612$).

Table 1. Total bone and muscle blood flow (mL/min×100 g^{-1}) in the exercise, nonweight-bearing, and control groups 8 weeks after osteotomy. Median (25–75 percentiles)

	Fractured side	*P*-value	Nonfractured side
Bone blood flow			
Exercise group	29 (20–43)	< 0.005	14 (6.8–27)
Nonweight-bearing group	24 (13–58)	< 0.005	11 (5.0–26)
Control group	27 (12-32)	< 0.005	8.9 (7.0–20)
Muscle blood flow			
Exercise group	14 (9.8–26)	NS	9.7 (5.6–23)
Nonweight-bearing group	10 (9.9–19)	NS	17 (10–38)
Control group	8 (6.6–15)	NS	6.6 (3.2–17)

Table 2. Fractional bone blood flow mL/min × 100 g^{-1} in the exercise, nonweight-bearing, and control groups 8 weeks after osteotomy. Median (25–75 percentiles)

	Proximal diaphysis	*P*-value	Callus area
Exercise group	27 (17–34)	< 0.005	72 (36–88)
Nonweight-bearing group	29 (18–41)	0.09	50 (21–87)
Control group	26 (14–37)	< 0.005	43 (27–58)

Muscle blood flow did not differ in any group (Table 1). The flow ratio between the fractured side and the control side showed a 17 percent increase in muscle blood flow to the operated on side in the nonweight-bearing group, and 28 percent and 20 percent, respectively, in the exercise and control groups ($P = 0.05$).

Discussion

The lower weight gain in the exercise group was deemed sufficient to confirm the effects of the training program (Forwood and Parker 1986, Sommer 1987).

The present method using radioactive microspheres for bone-blood flow measurements is well established (Morris and Kelly 1980, Tøndevold and Bülow 1983, Li et al. 1989). In our study, the osteotomies were pinned after reaming the medullary cavity, with destruction of the intramedullary vessels and part of the corticalis (Dankwardt-Lilieström et al. 1969). This induced damage stimulates periosteal osteogenesis, as is reflected in the increased total bone blood flow. Sim et al. (1970)—who studied the relationship of bone remodeling, oxygen consumption, and blood flow in bone—found close adjustments of osseous blood flow with remodeling activity as measured with ^{85}Sr clearance. The neovascularization after a fracture, as described by Rhinelander (1974), is considered to be of vital importance in fracture healing; however, to what extent this response is necessary is a matter of debate (Hulth 1989). The massive increase in callus flow found in our study reflects the increased metabolic demands, which are due to the ongoing healing process in the bridging callus. Our results show that bone blood flow increases during fracture healing, but bone blood flow is not the limiting factor as regards healing rate.

Early mobilization after a fracture has been shown to accelerate the fracture-healing process (Terjesen and Svenningsen 1986, 1988). Tøndevold and Bülow (1983) found, with exception to highly perfused cancellous bone, an increase in bone blood flow rates during prolonged exercise of at least 50 percent. We found a twofold increase in resting blood flow to the fractured bone. According to our results, the physiologic stimulus initiating vasodilatation after a fracture exceeds, by far, the response mediated by increased muscular work. The blood vessels that already have overtaxed their dilating capacity in a fractured bone seem to be incapable of meeting any further demands.

In experimental fractures, the musculature constitutes the basis for vessel sprouting into the fracture exudate, and treatment of severe fractures demonstrates the importance of soft-tissue coverage (Göthman et al. 1961, Edwards et al. 1983). Nather et al. (1990) showed that in nonvascularized diaphyseal transplants, the bones isolated from their vascular muscle bed were impaired in terms of fracture healing. The substantial increase in muscle blood flow found in our study supports the concept that the surrounding musculature plays an active role in the fracture-healing process. However, there seems to be a preexisting, or inherent, healing capacity irrespective of the degree of muscular function.

References

Danckwardt-Lillieström G. Reaming of the medullary cavity and its effect on diaphyseal bone. A fluorochromic, microangiographic and histologic study on the rabbit tibia and dog femur. *Acta Orthop Scand* (Suppl 128) 1969.

Edwards C C. Staged reconstruction of complex open tibial fractures using Hoffmann external fixation: Clinical decisions and dilemmas. *Clin Orthop* 1983; 178: 130–61.

Forwood M R, Parker A W. Effects of exercise on bone morphology. Vascular channels studied in the rat tibia. *Acta Orthop Scand* 1986; 57 (3): 204–7.

Göthman L. Arterial changes in experimental fractures of the monkey's tibia treated with intramedullary nailing. A microangiographic study. *Acta Chir Scand* 1961; 121: 56–66.

Hulth A. Current concepts of fracture healing. *Clin Orthop* 1989; 249: 265–84.

Li G, Bronk J T, Kelly P J. Canine bone blood flow estimated with microspheres. *J Orthop Res* 1989; 7 (1): 61–7.

Morris M A, Kelly P J. Use of tracer microspheres to measure bone blood flow in conscious dogs. *Calcif Tissue Int* 1980; 32 (1): 69–76.

Nather A, Balasubramaniam P, Bose K. Healing of nonvascularised diaphyseal bone transplants. *J Bone Joint Surg* (Br) 1990; 72: 830–4.

Rhinelander F W. Tibial blood supply in relation to fracture healing. *Clin Orthop* 1974; 105 (0): 34–81.

Sarmiento A, Schaeffer J F, Beckerman L, Latta L L, Enis J E. Fracture healing in rat femora as affected by functional weight bearing. *J Bone Joint Surg* (Am) 1977; 59 (3): 369–75.

Sim F H, Kelly P J. Relationship of bone remodeling, oxygen consumption, and blood flow in bone. *J Bone Joint Surg* (Am) 1970; 52 (7): 1377–89.

Sommer H M. The biomechanical and metabolic effects of a running regime on the Achilles tendon in the rat. *Int Orthop* 1987; 11 (1): 71–5.

Terjesen T, Svenningsen S. Function promotes fracture healing. Plate fixed osteotomies studies in rabbits. *Acta Orthop Scand* 1986; 57 (6): 523–5.

Terjesen T, Svenningsen S. The effects of function and fixation stiffness on experimental bone healing. *Acta Orthop Scand* 1988; 59 (6): 712–5.

Tøndevold E, Bülow J. Bone blood flow in conscious dogs at rest and during exercise. *Acta Orthop Scand* 1983; 54 (1): 53–7.