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BONE BLOOD FLOW IN CONSCIOUS DOGS AT REST AND DURING EXERCISE

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Using the microsphere technique bone blood flow was measured in different anatomical and functional regions in long bones in conscious dogs. The measurements were performed during physical exercise upon a treadmill, and the bone blood flow values were obtained as prework resting values after 1 and 2 hours of exercise and after 1 hour of rest.

The perfusion rates increased 50 per cent from 1.6 to 2.5 ml · 100 g tissue^{-1} · min^{-1} in the femoral and tibial cortical bones during work. In the cancellous bone of the femoral head an increase from 12.6 to 20.6 ml · 100 g tissue^{-1} · min^{-1} was found. Equal flow responses were determined in the fat-filled tibia-condylar and femoral supracondylar bone. The increase took place after 2 hours' exercise, but nonstatistically verified increased perfusion was found after 1 hour's work.

The alternation in bone blood flow suggests that bone has a capability of physical vasodilatation during muscular work but the flow response is slow and therefore the vasodilatation seems mediated by a metabolically induced stimulus.

Key words: bone blood flow; hyperaemia; microcirculation; microspheres

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Most tissue in the body have the ability to regulate the perfusion in a manner which secures optimal nutrition for the tissues. In skeletal muscle this functional hyperaemia is profound and the increase in the perfusion is more than 10 fold during exercise. It is unknown to what extent bone has the ability to increase its perfusion during muscular activity and normal limb use.

Paradis et al. (1975) and McInnis et al. (1980) have demonstrated increased flow during fracture repair. Gross et al. (1979) have found increased vascular resistance but unaltered perfusion rates during moderate exercise of short duration in dogs. Differences in perfusion rates in different regions in bone have been demonstrated (Tøndevold & Eliasen 1982a, b).

The purpose of this study is to measure the perfusion in different regions of long bones at rest and after 1 and 2 hours of exercise. Furthermore the purpose was to investigate if the functional hyperaemia observed in working muscles could occur in bone tissue.

MATERIAL AND METHODS

Surgical and experimental procedures

Six adult, mature mongrel dogs (weight approximately 20–40 kg) with closed epiphyseal lines were used in the study. The dogs were trained to run a treadmill for at least 2 hours at a speed of 6 kilometers per hour and 10–13° inclination.

Two to three days before the experiment the dogs were put under general anaesthesia (Immobilon, vet®, Pharmacia) and under sterile conditions a midline incision was made on the ventral side of the neck. Both carotid arteries were isolated and a pig tail catheter no. 8 F was inserted in the left ventricle in a place where

ECG was normal without arrhythmias. Using X-ray fluoroscopy a polyethylene catheter was introduced via the other carotid artery down to the abdominal aorta at the level of L_1–L_2. The catheters were sealed with Heparin Leo® (5000 IE/ml) and the wound closed with interrupted nylon sutures. The dogs were allowed at least 2 days' recovery after this operation.

The exercise experiment consisted of four different periods: first a rest period, followed by two consecutive 1-hour periods in which the dogs ran upon the treadmill, and finally a rest of at least 45 min before the dogs were sacrificed. On the day of the experiment the correct position of the catheters was controlled by pressure curves, and the dogs were placed upon the treadmill to obtain prework resting values of the perfusion. Fifteen million microspheres were injected into the left ventricle and the reference samples were obtained from the abdominal aorta by means of the polyethylene catheter connected to a suction pump (Sage, USA) at a speed of 2.4 ml/min^{-1} for at least 3 min. The heparinised reference sample was transferred to plastic vials for gamma radiation counting.

After the resting values were obtained the dogs ran for the 2 periods of 1 hour each, and during the run microspheres were injected into the left ventricle after 1 and 2 hours' exercise. The blood pressure and the heart rate were measured prior to and after the injection of the spheres. After the exercise periods the dogs rested at least 45 min on the treadmill to obtain resting flow levels after the work, and a last injection of spheres was given.

After the last injection and collection of the last reference sample the dogs were sacrifice using a lethal dose of mebumal sodium intracardially.

The hind limb bones were excised and all soft tissue including the periosteum was removed. Biopsies were taken from the bones with the aid of a saw according to different functional areas in the bones (Figure 1).

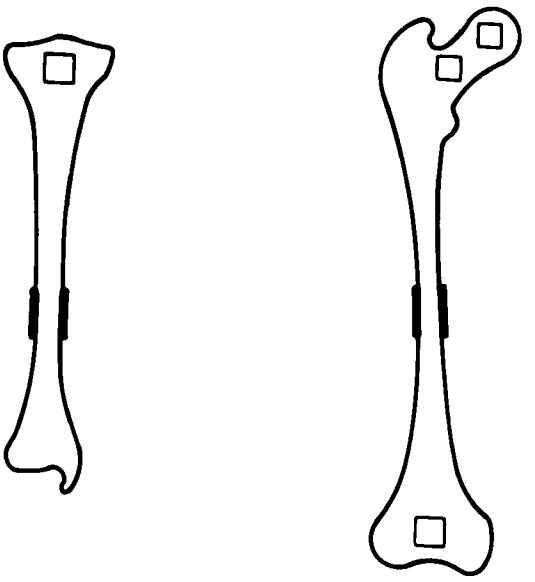

Figure 1. The anatomical location of the biopsies taken from the tibia (left) and femur (right).

Microspheres and counting procedure

New England Nuclear (NEN-TRAC) tracer microspheres (15μ) were used in the study. The microspheres were labelled with ^{46}Scandium, ^{51}Cromium, ^{141}Cerium and ^{103}Ruthenium. A single injection dose contained 15 × 10^6 microspheres and carried an activity of approximately 300 μCi. The spheres were suspended in 0.9 per cent saline and 0.01 per cent Tween 80 was added.

Prior to the injection the vials containing the microspheres were agitated on a Whirlimixer and immediately injected via the pig tail catheter into the left ventricle. The biopsies were placed in preweighed plastic vials for counting of gamma activity in a well type counter (Modified Searle 1195). The isotopes were separated in this four-channel system according to the different levels of their photopeaks. The activity in an individual sample was corrected for cross talk, physical decay and background during the counting procedure.

Statistics

In each period the mean value and standard error of the mean were calculated at each anatomical site. The differences between the individual observations were analyzed in a two-way analysis of variance. A modified t-test was performed to determine statistical differences (Armitage 1974). The level of significance was chosen as 95 per cent ($P < 0.05$).

Calculations

The blood flow rate of each sample was determined from the formula

$$\text{Blood flow rate} = \frac{\text{Activity (100 g tissue)}^{-1} \cdot \text{reference pump flow}}{\text{Total activity of reference sample}}$$

RESULTS

The flow rates obtained at rest showed a distribution with low cortical bone blood flow rate, 1.6 ml · 100 g tissue^{-1} · min^{-1}. After 1 hour of physical exercise the flow rate increased to 2.1 ml · 100 g tissue^{-1} · min^{-1} (not significantly different). After 2 hours of running the cortical flow rate in tibia and femur increased to 2.5 ml · 100 g tissue^{-1} · min^{-1}, which is significantly different (P

Table 1. The flows in ml · 100 g tissue^{-1} · min^{-1} for different regions in long bones in the hind limb are listed. The mean and standard error of the mean are given. Values different from the prework resting values are marked with an asterisk

Anatomical location of biopsy	Blood flow rate at rest	Blood flow rate after 1 hour's work	Blood flow rate after 2 hours' work	Blood flow rate after termination of work
Femoral head $N=12$	12.6±1.1	13.7±1.5	20.1±3.2*	17.7±2.0
Femoral neck $N=12$	27.3±3.1	23.2±2.6	36.9±6.2	34.8±3.4
Cortical bone $N=12$	1.6±0.2	2.1±0.2	2.5±0.1*	2.6±0.3*
Supracondylar femoral bone N=24	6.3±0.8	7.2±0.7	10.5±2.3*	11.2±1.6*
Tibial condylar bone $N=12$	2.6±0.5	3.6±1.0*	3.7±0.8*	3.5±0.7*

< 0.05) from the resting value. This increase is around 60 per cent and it was maintained during the postexercise 1 hour resting period. The flow rate in this period was determined to 2.6 ml · 100 g tissue^{-1} · min^{-1}. In cancellous bone from the femoral neck the perfusion was rather high with a resting value of 27.3 ml · 100 g tissue^{-1} · min^{-1}. During physical activity the perfusion increased to 36.9 ml · 100 g tissue^{-1} · min^{-1} in the second of the working periods, but this is not significantly different from the resting value due to considerable variation ($P < 0.05$). The flow rate obtained in the femoral head showed intermediate flow rates with a resting value of 12.6 ml · 100 g tissue^{-1} · min^{-1}, This flow increased to 20.1 ml · 100 g tissue^{-1} · min^{-1} during the second work period. These flow rates are statistically different ($P < 0.05$).

In the supracondylar fat-filled marrow the resting value of 6.3 ml · 100 g tissue^{-1} · min^{-1} increased to 10.5 ml · 100 g tissue^{-1} · min^{-1} ($P < 0.05$). In tibia-condylar bone marrow the flow increased from 2.1 ml · 100 g tissue^{-1} · min^{-1} to 3.7 ml · 100 g tissue^{-1} · min^{-1} ($P < 0.05$) in the second working period.

Obviously, 1 hour of moderate heavy work did not consistently affect the flow rate in bone (Table 1). However, during the second hour of work all regions investigated except the femoral neck showed a statistically verified increase in flow.

DISCUSSION

The resting flow rates determined in this study are in accordance with earlier results obtained on anaesthetized dogs (Tøndevold & Eliasen 1982a, b) as well as in conscious dogs (Gross et al. 1979, Morris & Kelly 1980). The resting flow rate in canine bones is approximately 1–3 ml · 100 g tissue^{-1} · min^{-1} in cortical bone and 30–40 ml · 100 g tissue^{-1} · min^{-1} in the red cancellous bone of the femoral neck.

The microsphere technique used in this study has been employed by different authors and the validity discussed (Lunde & Michelsen 1970, Okubo et al. 1979, Gross et al. 1979, Tøndevold & Eliasen 1982a, b).

The use of this technique in flow studies and flow determinations is well established. Other techniques do not have the capacity to make flow determinations possible without reservations in this multiple inlet/multiple outlet system. As the only method available at present it makes multiple measurement possible in conscious animals, and it is atraumatic to the animals. Injection of 15 · 10^6 microspheres into the left ventricle in a 20 kg dog does not cause any detectable discomfort to the animal. Since the number of capillaries which are embolized are at a magnitude of 0.01 per cent (Hales 1974) the physiological effect of this event seems negligible.

Anatomical studies performed by Brookes (1971) and Rhinelander (1968) demonstrate the connection between the vessels in the cortical bone and the vessels in the adjoining soft tissue, particularly muscle-tendon-bone insertions. Shim et al. (1972) measured the bone blood flow with the aid of medullary pressure measurements during stimulation of the great motor nerves. The results are difficult to interprete but the medullary pressure as well as the venous pressure are affected by the contraction in the surrounding muscles.

These anatomical and physiological studies may indicate variation in bone perfusion during muscle contraction, although not the extent to which the net flow to the bones is changed during physical work.

Paradis et al. (1975) and McInnis et al. (1980) have demonstrated hyperaemia during fracture repair. In clinical work it is known that some slow healing fractures only heal when the muscular activity is restored in the adjacent muscular tissue. Whether this is due to an osteogenic stimulus from the axial loading or to increased perfusion is at present unknown. All this indicates that bone vessels might regulate their perfusion in order to meet different requirements from the bones during repair and function. Gross et al. (1979) have demonstrated that hypoxaemia and hypotension produce vasoconstriction in bones. Their findings are in agreement with medullary pressure measurements made by Tøndevold et al. (1979a, b). Gross et al. also found that stimulation of the sympathetic nervous system causes vasoconstriction in bones. Medullary measurements performed by Stein et al. (1958) and Herzig & Root (1959) have shown an identical physiological reaction. During exercise Gross et al. (1979) found increased vascular resistance in bone but unaltered perfusion rates. They calculated the vascular resistance from the perfusion rate and the arterial blood pressure. It is well known that arterial blood pressure increases during exercise. To what extent this affects the driving pressure in the bone microcirculation is not known. Valderrama & Trueta (1965) have shown that muscular contraction increases intraosseous pressure. They have also shown that the venous pressure increased considerably during muscular contraction. This biological event was confirmed by Michelsen (1967). The driving pressure from the arterial to the venous side of the circulation depends upon the arterial as well as the venous pressure. The venous counterpressure will increase during contraction, and change the arterial-venous pressure gradient in an unpredictable manner. If Gross et al. (1979) did not take this into consideration their conclusions are less valuable. We have therefore avoided calculations where the arterial-venous pressure gradient was used, as driving pressure is difficult to measure during muscular work.

With one exception there was no increase in the perfusion during the first hour of exercise in our study. In the second hour of moderately heavy exercise a marked increase in bone perfusion took place. This functional hyperaemia was found in all areas of the bones, except in the red cancellous bone in the femoral neck. At rest the perfusion in the femoral neck is quite brisk. It should, however, be mentioned that all dogs showed an increase in this region, but as the increase is subject to a considerable interindividual variation, the increases found were non-significant.

The hyperaemia was sustained during the post-exercise period. Whether this is due to repayment or redistribution of the blood in the limbs after exercise is undetermined.

Conclusions

1. The bone blood flow rates increase during prolonged exercise by at least 50 per cent, except in the highly perfused cancellous bone.
2. More than 1 hour of moderate exercise was necessary to achieve hyperaemia in a tubular bone in a working limb.
3. The hyperaemia continued after cessation of work and was maintained at a high level at least for 1 hour.

REFERENCES

Armitage, P. (1974) *Statistical methods in medical research.* p. 217. Blackwell Scientific Publications, Oxford.

Brookes, M. (1971) *Blood supply of bone.* p. 118. Butterworths, London.

Gross, P. M., Heistad, D. D. & Marcus, M. L. (1979) Neurohumeral regulation of blood flow to bones and marrow. *Am. J. Physiol.* **237**, H 440–H 448.

Hales, J. R. S. (1974) Radioactive microsphere techniques for studies ofof the circulation. *Clin. Exp. Pharmacol. Physiol.*, Suppl. 1, 31–46.

Herzig, E. & Root, W. S. (1959) Relation of sympathetic nervous system to blood pressure of bone marrow. *Am. J. Physiol.* **196**, 1053–1056.

Lunde, P. & Michelsen, K. (1970) Determination of cortical blood flow in rabbit femur by radioactive microspheres. *Acta Physiol. Scand.* **80**, 39–44.

McInnis, J. C., Robb, R. A. & Kelly, P. J. (1980) The relationship of bone blood flow, bone tracer deposition and endosteal new bone formation. *J. Lab. Clin. Med.* **96**, 511–522.

Michelsen, K. (1967) Pressure relationships in the bone marrow vascular bed. *Acta Physiol. Scand.* **71**, 16–29.

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

Okubo, M., Kinoshita, T., Yukimura, T., Abe, Y. & Shimazu, A. (1979) Experimental study of measurement of regional blood flow in the adult mongrel dogs using radioactive microspheres. *Clin. Orthop.* **138**, 263–270.

Paradis, G. L. & Kelly, P. J. (1975) Blood flow and mineral deposition in canine tibial fractures. *J. Bone Joint Surg.* **57-A**, 220–226.

Rhinelander, F. W. (1968) The normal microcirculation of diaphyseal cortex and its response to fracture. *J. Bone Joint Surg.* **50-A**, 784–800.

Shim, S. S., Hawk, H. E. & Yu, W. Y. (1972) The relationships between blood flow and marrow cavity pressure of bone. *Surg. Gynecol. Obstet.* **135**, 353–360.

Stein, A. H., Morgan, H. C. & Porras, R. F. (1958) The effect of pressor and depressor drugs on intermedullary bone marrow pressure. *J. Bone Joint Surg.* **40-A**, 1103–1110.

Tøndevold, E. & Eliasen, P. (1982a) Blood flow rates in canine cortical and cancellous bone measured with $^{99}Tc^{m}$-labelled human albumin microspheres. *Acta Orthop. Scand.* **53**, 7–11.

Tøndevold, E. & Eliasen, P. (1982b) Regional vascular volumes and dynamic haematocrit compared to regional perfusion in canine cancellous and cortical bone. *Acta Orthop. Scand.* **53**, 197–203.

Tøndevold, E., Eriksen, J. & Jansen, E. (1979a) Observations on long bone medullary pressure in relation to mean arterial blood pressure in the anaesthetized dog. *Acta Orthop. Scand.* **50**, 527–531.

Tøndevold, E., Eriksen, J. & Jansen, E. (1979b) Observations on long bone medullary pressures in relation to arterial PO_2, PCO_2 and pH in the anaesthetized dog. *Acta Orthop. Scand.* **50**, 645–651.

Valderrama, J. F. De & Trueta, J. (1965) The effect of muscle action on the intra-osseous circulation. *J. Path. Bacteriol.* **89**, 1979–1986.

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