Blood Flow Distribution in Hind Limb Bones and Joint Cartilage from Young Growing Pigs

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ABSTRACT

Blood flow distribution was studied in hind limb bones and joint cartilage from four ten week old and four 20 week old pigs using [141]Cemicrospheres. In general, blood flow rate was greater in the epiphyseal and metaphyseal cancellous bone than in the cortical bone and joint cartilage. In the epiphyseal cancellous bone, blood flow rate was three to tenfold greater ($P<0.01$) in the surface 2 mm layer than in the remaining deeper bone in all femoral andd tibial samples examined. In the joint cartilage, blood flow rate in the femoral condyle was greater ($P<0.05$) than in the proximal femur, patella, central tarsus and metatarsus in ten week old pigs, and was greater ($P<0.05$) in the femoral condyle than in the patella and metatarsus in 20 week old pigs. A significant ($P<0.05$) age-associated decrease in the rate of blood flow was observed in the femoral, patellar and metatarsal cartilage. Within the femoral condyle, no differences in blood flow were found between areas associated with relatively high versus relatively low incidences of osteochondrosis.

Key words: Bone, cartilage, blood flow, micropsheres, osteochondrosis, pig.

RÉSUMÉ

Cette expérience consistait à étudier la distribution de l'apport sanguin dans les os et les cartilages articulaires des membres postérieurs, chez quatre porcs âgés de dix semaines et chez quatre autres âgés de 20 semaines, à l'aide de microsphères radioactives de Ce^{141}. En général, cet apport sanguin s'avéra plus important dans le tissu spongieux épiphysaire et métaphysaire que dans le cortex osseux et les cartilages articulaires. Dans le tissu spongieux épiphysaire, il se révéla de trois à dix fois plus grand ($P<0,01$), au sein d'une couche superficielle de 2 mm d'épaisseur que dans le tissu osseux plus profond, dans tous les échantillons fémoraux et tibiaux examinés à cette fin. Quant aux cartilages articulaires, l'apport sanguin dans le condyle fémoral s'avéra plus grand ($P<0,05$) que dans le fémur proximal, la rotule, la partie centrale du tarse et du métatarse, chez les porcs âgés de dix semaines; il se révéla aussi plus important ($P<0,05$) dans le condyle fémoral que dans la rotule et le métatarse, chez les porcs âgés de 20 semaines. À mesure que les porcs avançaient en âge, l'apport sanguin subit une diminution appréciable ($P<0,05$), dans le tissu cartilagineux fémoral, rotulien et métatarsien. À l'intérieur du condyle fémoral, on ne constata aucune différence entre l'apport sanguin des endroits plus ou moins sujets à l'ostéochondrose.

Mots clés: os, cartilage, apport sanguin, microsphères, ostéochondrose, porc.

INTRODUCTION

Regulation of blood flow is an important mechanism in controlling the rate of uptake and release of nutrients and waste products in tissues. There have been a number of reports concerning the blood flow in bones from laboratory animals (1,2,3,4,5) but little is known about blood flow in joint cartilage. There is also little information available concerning blood flow in bone and joint cartilage in young growing pigs. Such knowledge is important to understanding metabolism in normal and pathological skeletal tissues including osteochondrotic bone and cartilage from pigs.

The radioactive microsphere technique is a useful method to measure tissue blood flow distribution, and its validity has been reported (6,7,8). The present study was undertaken to measure blood flow distribution in hind limb bone and joint cartilage from ten and 20 week old pigs using the radioactive microsphere technique.

MATERIALS AND METHODS

Four ten week old and four 20 week old crossbred (Yorkshire x Landrace) pigs with normal locomotory ability, weighing an average of 23.3 kg and 79.0 kg, respectively, were used. These animals were anesthetized with halothane. A polyethylene catheter for injection of radioactive microspheres was inserted through the left

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carotid artery until it reached the left ventricle. A second polyethylene catheter for withdrawing blood was inserted through the right carotid artery and placed in the aorta approximately 2 cm cranial to the renal artery. Approximately 20 h later, microspheres were injected into each animal while it was in the standing position.

Radioactive ^{141}Ce-microspheres (New England Nuclear), 15±3 μm in diameter, suspended in 10% dextran solution were used. Each injection contained approximately 7.6 x 10^6 microspheres in 2.1 g for a ten week old pig, and 3.2 x 10^7 microspheres in 6.1 g for a 20 week old pig. After injection, the catheter was flushed with a small volume of saline. A reference blood sample for calculating blood flow rate was collected through the blood sampling catheter by means of a Harvard infusion withdrawal pump (Model 600-950V), which withdrew blood at a constant rate beginning 10 sec before injection and continuing for 60 sec after completion of injection. Animals were then sacrificed by mechanical stunning and exsanguination.

Samples of cancellous and cortical bone and joint cartilage were dissected from both the left and right hind limbs. Sampling sites in the femur, tibia, and metatarsal bones are shown in Fig. 1. Each of the epiphyseal bone samples from the proximal and distal femur and from the proximal tibia were divided with a scalpel into two samples; the surface layer consisting of approximately the first 2 mm and the remaining epiphyseal bone. Both the medial and lateral condyles of the femurs were divided into four regions; cranio-medial, cranio-lateral, caudal-medial and caudal-lateral regions.

The amount of radioactivity in blood and tissue samples was measured using a Model 8000 Beckman gamma counter. Tissue blood flow (X) in mL/min/100 g of wet tissue was calculated using the formula X = C_t/C_b x V x 100 g, where C_t is the count per gram of tissue sample, C_b is the count per gram in the reference blood sample, and V is the rate of withdrawal of the reference blood sample (mL/min). The volume of blood was calculated by using a density value of 1.05 g/mL. Student's

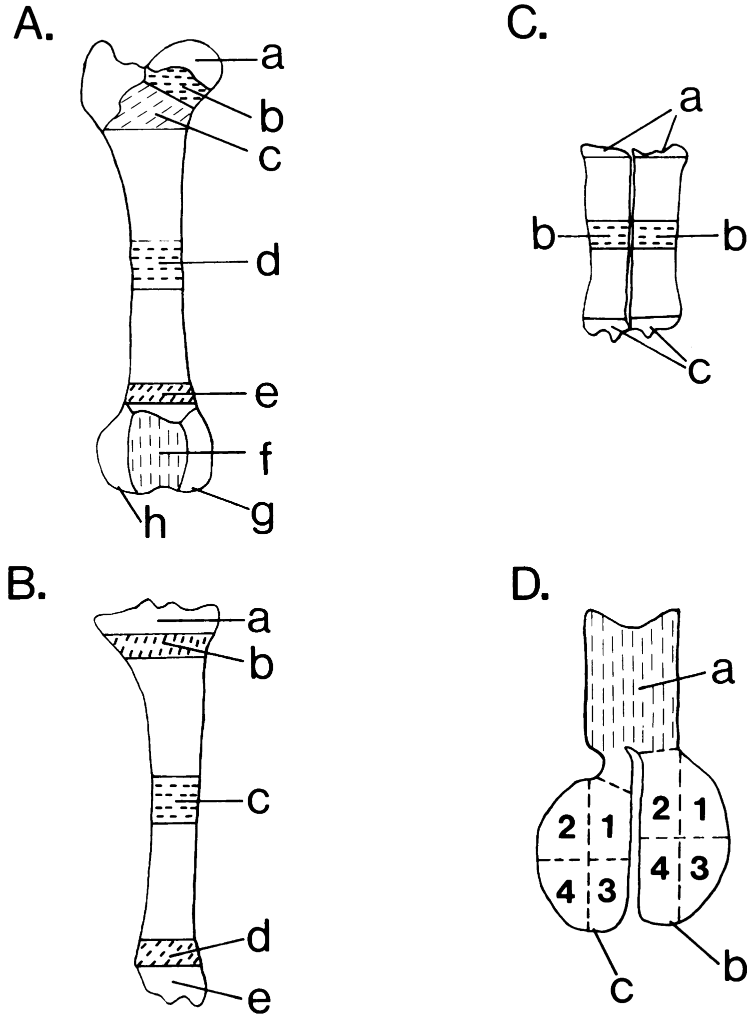

Fig. 1. Tissue sampling sites in the femur, tibia and metatarsus.
A. Femur:
- **a) proximal epiphysis**
- **b) proximal metaphysis I**
- **c) proximal metaphysis II**
- **d) diaphysis**
- **e) distal metaphysis**
- **f) trochlea**
- **g) medial condyle**
- **h) lateral condyle**

B. Tibia:
- **a) proximal epiphysis**
- **b) proximal metaphysis**
- **c) diaphysis**
- **d) distal metaphysis**
- **e) distal epiphysis**

C. Metatarsus: The third and fourth metatarsi were used.
- **a) proximal epiphysis**
- **b) diaphysis**
- **c) distal epiphysis**

D. Joint cartilage surface of the medial and lateral femoral condyles; each condyle was divided into four regions:
- **1) cranio-medial**
- **2) cranio-lateral**
- **3) caudal-medial**
- **4) caudal-lateral**

Samples of epiphyseal cancellous bone, including the surface 2 mm layer and the remaining portion, and those of joint cartilage, were collected from each of the following regions:
- **a) trochlea**
- **b) medial condyle**
- **c) lateral condyle**

t-test was used to detect a significant difference between means.

RESULTS

Postmortem examination of joints indicated that three of the 20 week old pigs had relatively mild bilateral osteochondrotic lesions while no lesions were found in the joints from the ten week old pigs. The condition was manifested as disturbed endochondral ossification in the craniolateral region of the medial femoral condyle (region 2 in Fig. 1D). In each case, a small portion of cartilage, approximately 2 to 3 mm in diameter and 1 mm in depth, protruded into the subchondral bone. All other bone and joint cartilage samples appeared normal.

In the femoral condyles the rate of blood flow to the four different regions studied within each of the surface and deep epiphyseal cancellous bone and joint cartilage samples was not significantly (P>0.05) different (data not shown). Similarly, no differences (P>0.05) were found in the rate of blood flow to tissues from either the medial or lateral femoral condyles. Thus, for comparing femoral condyle blood flow values between different tissues and between the ten week and 20 week old pigs, the values for the four regions in both the medial and lateral condyles from both the left and right legs were pooled.

Blood flow rates in pooled right and left hind limb bone and joint cartilage samples are shown in Tables I and II, respectively. In general, blood flow rate was greater in the epiphyseal and metaphyseal cancellous bone than in cortical bone and joint cartilage. Blood flow rate was similar (P>0.05) between the cortical bones and their joint cartilage with the exception of metatarsal flow rate, which was greater (P<0.05) for cortical bones in both the ten and 20 week old animals.

In the epiphyseal cancellous bone, blood flow rate was much greater (P<0.01) in the surface 2 mm layer than in the remaining bone in all samples examined. Blood flow rate was relatively similar (P>0.05) in cancellous bone from the femur, patella, tibia, tarsus and metatarsus and in cortical bone from the femur, tibia and metatarsus.

The effect of age on bone blood flow rate was determined by comparing mean flow rates in the samples obtained from the ten and 20 week old pigs (Table I). There was no significant (P>0.05) age effect on the rate of blood flow in any of the epiphyseal and metaphyseal cancel-

TABLE I. Blood Flow Rates in Hind Limb Bone from Ten and 20 Week Old Pigs[a]

Tissue[b]	Age of Pigs: 10 weeks	20 weeks	Age Effect
Epiphyseal cancellous bone, surface layer (2 mm thick)			
Proximal femur	78.5 ± 22.3[c]	65.8 ± 7.1	NS[d]
Distal femur (trochlea)	84.9 ± 13.4	52.6 ± 10.2	NS
Distal femur (condyles)	97.7 ± 14.8	85.8 ± 12.3	NS
Proximal tibia	44.4 ± 7.6	69.4 ± 10.8	NS
Epiphyseal cancellous bone, remaining portion			
Proximal femur	10.1 ± 1.0	18.9 ± 3.5	NS
Distal femur (trochlea)	12.1 ± 1.0	12.7 ± 2.3	NS
Distal femur (condyles)	9.5 ± 1.1	12.8 ± 2.1	NS
Proximal tibia	9.7 ± 1.2	17.7 ± 3.7	NS
Other cancellous bone[e]			
Patella	14.2 ± 1.2	16.9 ± 2.9	NS
Distal tibia	17.0 ± 2.2	15.6 ± 2.5	NS
Central tarsus	12.7 ± 0.9	11.2 ± 2.2	NS
Metatarsus	9.6 ± 0.2	10.5 ± 2.2	NS
Metaphyseal cancellous bone[f]			
Proximal femur I	21.2 ± 4.8	20.6 ± 3.6	NS
Proximal femur II	15.0 ± 1.5	20.6 ± 2.5	NS
Distal femur	11.0 ± 1.8	16.7 ± 2.9	NS
Proximal tibia	12.5 ± 2.0	16.1 ± 2.3	NS
Distal tibia	21.0 ± 7.1	22.3 ± 5.4	NS
Diaphyseal cortical bone[g]			
Femur	12.8 ± 1.0	7.1 ± 1.0	P<0.05
Tibia	8.9 ± 1.0	7.9 ± 1.3	NS
Metatarsus	9.9 ± 1.4	4.7 ± 0.8	P<0.05

[a]Mean of right and left legs. Values for femoral condyles were calculated by combining values from the four regions sampled as shown in Fig. 1D
[b]Tissue sampling sites are shown in Fig. 1
[c]Mean value (mL/min/100 g tissue) ± standard error
[d]NS = no significant (P>0.05) effect
[e]Bone containing surface layer. For metatarsal samples, both proximal and distal epiphyses were combined
[f]Growth plate (physis) not included
[g]Free from marrow, cancellous bone and periosteum. Diaphyses from both the third and fourth metatarsi were combined

TABLE II. Rate of Blood Flow to Hind Limb Cartilage from Ten and 20 Week Old Pigs[a]

Joint Cartilage[b]	Age of Pigs: 10 weeks	20 weeks	Age Effect
Proximal femur	5.0 ± 0.4[c]	3.2 ± 0.3	P<0.05
Distal femur (trochlea)	7.7 ± 1.0	3.3 ± 0.7	P<0.05
Distal femur (condyles)	13.1 ± 1.7	5.5 ± 0.7	P<0.05
Patella	5.8 ± 0.7	2.7 ± 0.4	P<0.05
Tibia	6.8 ± 1.1	4.6 ± 1.2	NS[d]
Central tarsus	3.7 ± 0.3	3.0 ± 0.9	NS
Metatarsus	4.7 ± 0.7	2.0 ± 0.7	P<0.05

[a]Mean of right and left legs. Values for femoral condyles were calculated by combining values from the four regions sampled as shown in Fig. 1D
[b]Tibial and metatarsal samples contained cartilage from both the proximal and distal ends of each bone
[c]Mean value (mL/min/100 g tissue) ± standard error
[d]NS = no significant (*P*>0.05) effect

lous bone samples. However, in cortical bone samples from the femur and metatarsus, the rate of blood flow was approximately 100% greater (<0.05) in the ten week than in the 20 week old pigs. The rate of blood flow in cortical bone samples from the tibia was similar in the ten week and 20 week old animals.

The rate of blood flow to the joint cartilage was considerably lower than that associated with the underlying bone (Tables I and II). The greatest rate of blood flow in joint cartilage (13.1 mL/min/100 g tissue) was measured in cartilage from the femoral condyles obtained from the ten week old pigs. This value was significantly greater ($P<0.05$) than the rates measured in joint cartilage obtained from the proximal femur, patella, central tarsus, and metatarsus from pigs of the same age. In the 20 week old pigs, the blood flow to the femoral condyles was reduced ($P<0.05$) to 5.5 mL/min/100 g tissue, but was still significantly ($P<0.05$) greater than that measured in joint cartilage from the patella and metatarsus of pigs of the same age. In general, the rate of blood flow to joint cartilage was greater in the ten week old than in the 20 week old pigs. In cartilage from the tibia and central tarsus this decrease did not reach significance ($P>0.05$) at the level tested.

DISCUSSION

A minimum of 384 microspheres must be present in each tissue sample counted to achieve a distribution variability within 10% of the mean distribution at the 95% confidence level (6). In the present experiment, the number of microspheres in each tissue sample reported in Tables I and II was estimated to be greater than 384 in all samples of bone and femoral condylar cartilage, but lower in the other cartilage samples. The lower number is due to both a smaller tissue size and a lower blood flow rate.

Bone blood flow data have been published for laboratory animals such as dogs (1,2,5) and rabbits (3), but relatively few researchers have measured blood flow in growing farm animals. In a study of growing mongrel dogs, Morris and Kelly (2) reported that the rate of blood flow calculated for the pooled cancellous and cortical bones from the femur, tibia and ulna was 15.4 and 7.0 mL/min/100 g, respectively. In the present study, the mean flow rates calculated for the femoral and tibial bones were 17.6 and 21.3 mL/min/100 g in cancellous bone and 10.9 and 7.5 mL/min/100 g in cortical bone from ten and 20 week old pigs, respectively. These findings reveal that the greater blood flow rate observed in the cancellous than in the cortical bones is consistent between these species, and that the blood flow rates in bones from growing pigs are similar to those in bones from growing dogs.

Immature joint cartilage has minute channels known as cartilage canals which contain blood vessels involved not only in nutrient transport but also in the process of endochondral ossification (9). Little is known about the rate of blood flow in joint cartilage. In this study, the rate of flow measured in the relatively immature femoral, tibial, and metatarsal joint cartilage ranged from 3.7 to 13.1 mL/min/100 g in ten week old pigs, and from 2.0 to 5.5 mL/min/100 g in 20 week old pigs. The blood flow rate was greatest ($P<0.05$) in the femoral condylar cartilage. In view of the disappearance of cartilage canals with maturation of animals, it is suggested that femoral condylar cartilage may mature more slowly than cartilage found at other sites in the hind limb. Whether a slower rate of maturity is related to the higher incidence of osteochondrotic lesions in the femoral condyles of pigs (10,11,12,13) is unknown.

Osteochondrosis is a problem in swine. Previous studies suggest that most market weight pigs have osteochondrotic lesions (12,13). Thus, the high incidence of the lesions observed in the medial femoral condyle from 20 week old pigs in this study is not surprising. Osteochondrosis is frequently attributed to circulatory disturbances in growing bone (14), but little evidence for this suggestion has been provided so far. Blood flow was measured in four different regions of the medial and lateral condyles of the femur. These regions included those associated with relatively high (e.g. cranio-lateral region of the medial condyle) and relatively low (e.g. caudal-lateral region of the lateral condyle) incidences of osteochondrosis. There were no differences in blood flow to cartilage or subchondral bone among these different regions from either the medial or lateral femoral condyles. Also, blood flow to cartilage and subchondral bone with osteochondrotic lesions did not appear to differ from blood flow to normal cartilage and subchondral bone. It is possible that circulatory disturbances might have occurred in minute areas of the tissue, which could not be detected by the present technique. Further research is required in this regard.

Blood flow in the subchondral region is of interest as this region is involved in endochondral ossification of growing pigs. Thus, we divided epiphyseal bone into two regions; surface layer (approximately 2 mm) and the remaining epiphysis to compare blood flow rate between these regions. The results indicate that blood flow rate is much greater ($P<0.01$) in the surface 2 mm layer than in the remaining deeper portions of epiphyseal bone of the femur and tibia from all pigs studied. Since microspheres with a diameter 15 μm are trapped in capillaries (15), the observation suggests that capillary density is greater in the subchondral region of these epiphyses, probably to maintain normal endochondral ossification. Little information is available on subchondral vascular distribution.

In the present study, blood flow rate in the femoral and metatarsal compact bones was lower in the 20 week old than in the ten week old pigs. This finding is similar to that of Morris and Kelly (2), who suggested that the decrease with age is due to higher rates of bone remodelling and of appositional bone growth in younger animals. The decreased blood flow rate observed in the cartilage from 20 week old pigs herein is consistent with our histological observations of joint cartilage from growing pigs (16,17), which revealed a decrease in vascularity with age in both humeral and femoral cartilage with a concomitant decrease in cartilage thickness and chondrocyte density. Possibly thicker cartilage from younger animals requires greater blood flow rate to deliver nutrients (18) in addition to those delivered through synovial fluid by diffusion.

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