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Haemodynamic changes in the rat femur and tibia following femoral vein ligation

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ABSTRACT

Interference with venous outflow from a limb to stimulate fracture repair and bone growth has a long history, and its beneficial effects have been confirmed by many experimental and clinical studies. With the development of fracture fixation systems, the therapeutic use of circulatory intervention became redundant. Recently, a venous tourniquet effect has been cited to explain the enhancement of bone healing observed after fracture fixation with the Aircast system. As bone appears to be altered by venous stasis, it is important to characterise the vascular perturbations leading to these changes. Previous studies have often given conflicting results. This study investigated the short and long term haemodynamic effects of femoral vein ligation. Changes in blood flow rate and blood volume in the distal femur and proximal tibia of the rat were examined at 6 h and 1, 3 and 7 d following unilateral femoral vein ligation, and at 8 and 16 wk. Blood flows and volumes were generally reduced in the ligated limb 6 h after femoral vein ligation. This initial depression was followed by a relative increase (comparing the ligated limb with the contralateral unoperated bone) in blood volume during the 1st week. A significant relative reduction in epiphyseal vascular space was observed after 16 wk. A sustained reduction of arterial input to whole femora and tibiae was present in the ligated limb throughout the investigation although, for the cancellous knee joint epiphysis of the tibia, a localised relative increase in flow was apparent during the 1st week, again comparing the ligated limb with the nonligated contralateral bone. There was a relative weight gain in the ligated femora and tibiae, compared with the contralateral bones, increasing from 8 to 16 wk; bone lengths also showed some increase.

Key words: Bone blood flow; bone blood volume; venous stasis.

INTRODUCTION

It has been appreciated for many years that venous stasis is able to stimulate bone growth and accelerate the healing of fractures. Ambroise Paré (*c.* 1509–1590) is credited with the first clinical use of venous engorgement as a therapeutic aid in the treatment of delayed fracture healing. The technique was 'rediscovered' in the late 19th century and advocated for treatment of difficult fractures, and for limb lengthening in growing children. These early clinical experiences of venous stasis were comprehensively reviewed by Pearce & Morton (1930), who also obtained the first experimental confirmation that venous ligation stimulated fracture repair in dogs.

Modern experimental studies have shown that the results of a venous impediment on intact bone may be variable. In growing dogs, venous constriction has produced an increase in bone diameter and weight, although lengthening was less constantly achieved (Hutchison & Bordeaux, 1954; Colt & Iger, 1963). Other studies have claimed that bone lengthening does not occur as a result of venous ligation (Keck & Kelly, 1965), although an increase in periosteal bone apposition has been confirmed in the same model (Lilley & Kelly, 1970). Singh & Brookes (1971), however, have shown in the rat that mean weight and length of femora and tibiae from a limb subjected to femoral vein ligation continued to increase up to 24 wk postligation. Other studies in the rat have

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Table 1. *Blood flow and volume to whole femora and tibiae*

	L (ligated)	S.D.	<*P*>	R (nonligated)	S.D.	L/R
Whole bones: blood flow (ml min^{-1} 100 g^{-1})						
Femur						
6 h	25.7	9.9	0.03**	29.2	12.4	0.88
1 d	45.7	15.3	0.3	48.1	15.1	0.95
3 d	35	14	0.3	36.7	12	0.95
7 d	31.5	7.7	0.16	32.78	9.1	0.96
8 wk	33.56	7.1	0.12	35.05	5.6	0.95
16 wk	30.93	6.2	0.19	32.44	7.1	0.95
Tibia						
6 h	23.2	10.8	0.095*	28.8	13.2	0.8
1 d	44.05	14.9	0.049**	47.4	15.6	0.93
3 d	31.8	14.2	0.006**	35.1	14.5	0.91
7 d	26.43	9.9	0.05**	28.46	9.8	0.93
8 wk	35.57	6.53	0.0003**	39.01	6.2	0.91
16 wk	28.59	6.11	0.003**	31.93	6.43	0.89
Whole bones: blood volume (ml 100 g^{-1})						
Femur						
6 h	0.91	0.29	0.002**	1.18	0.46	0.77
1 d	1.68	0.37	0.1	1.54	0.38	1.09
3 d	1.29	0.17	0.01**	1.07	0.16	1.21
7 d	1.31	0.26	0.07*	1.09	0.15	1.2
8 wk	1.24	0.26	0.65	1.22	0.21	1.01
16 wk	1.08	0.15	0.09*	1.15	0.2	0.93
Tibia						
6 h	0.92	0.21	0.02**	1.15	0.42	0.8
1 d	1.46	0.2	0.17	1.59	0.21	0.92
3 d	1.21	0.31	0.63	1.16	0.11	1.04
7 d	1.15	0.09	0.23	1.08	0.18	1.06
8 wk	1.25	0.21	0.66	1.27	0.21	0.98
16 wk	1.17	0.18	0.35	1.27	0.15	0.92

* $P < 0.1$; ** $P < 0.05$.

suggested that femoral vein ligation produces sclerotic changes in the knee joint epiphyses; these changes are characterised by coarsening of cancellous bone, thickening of the subarticular lamella and subchondral plate, thinning of articular cartilage, and an increase in the relative thickness of the calcified zone (Brookes, 1966; Brookes & Helal, 1968; Revell & Brookes, 1991).

As these changes result from a disturbance of the osseous circulation, it is not surprising that many studies have attempted to characterise the haemodynamic sequelae of femoral vein ligation. McPherson et al. (1961) and Shaw (1963) used heat loss from a heated thermocouple to obtain a measure of blood flow in the cat femur. They described a marked and instant increase in marrow flow rate and pressure following occlusion of the femoral vein and when a venous tourniquet was applied. These results were not confirmed by White & Stein (1965), who employed a radioisotope technique to determine blood flow rate in the rabbit tibia. Build-up curves were obtained after ^{51}Cr radiochromate labelled erythrocytes were allowed to enter a previously tourniquet-isolated lower limb. The authors were aware of the problem of reactive hyperaemia resulting from prolonged tourniquet use, and recorded a 43% reduction in measured tibial blood flow following femoral vein ligation.

Although fracture fixation devices have made venous interventions for bone healing largely redundant, it is of interest that venous obstruction has recently been cited as the stimulus for fracture repair enhancement when the Aircast system of fracture management was used (Dale et al. 1989). It was therefore decided to reexamine the haemodynamic changes caused by femoral vein ligation in the femur and tibia of the rat, both immediately following the ligation, and for periods up to 16 wk.

MATERIALS AND METHODS

Male Wistar rats, 8–11 wk old, were used. After being anaesthetised (intramuscular Hypnorm and intraperitoneal diazepam), the left femoral neurovascular bundle was exposed close to its emergence from beneath the inguinal ligament. The femoral vein was

Table 2. *Blood flow and volumes to epiphysis*

	L (ligated)	S.D.	<*P*>	R (nonligated)	S.D.	L/R
Epiphyses: blood flow (ml min^{-1} 100 g^{-1})						
Femur						
6 h	29.3	10.8	0.03**	31.9	11.8	0.92
1 d	52.2	11	0.58	53.5	9.1	0.98
3 d	45	14.8	0.86	44.6	9.6	1.01
7 d	43.6	15	0.77	42.7	12.9	1.02
8 wk	42.8	5.5	0.003**	48.4	7.6	0.88
16 wk	44.85	5.7	0.1*	50.5	9.9	0.89
Tibia						
6 h	26.6	10.4	0.002**	32.3	12.9	0.82
1 d	68.3	11.2	0.04**	62	7.6	1.1
3 d	49.5	12.9	0.49	47.8	13	1.04
7 d	43.3	17	0.08*	39.5	12.9	1.1
8 wk	48.2	7	0.08*	52.8	7.8	0.9
16 wk	45.1	9.6	0.001**	55.8	8.3	0.81
Epiphyses: blood volume (ml 100 g^{-1})						
Femur						
6 h	0.78	0.26	0.09*	0.92	0.42	0.85
1 d	1.23	0.39	0.58	1.13	0.27	1.09
3 d	1.06	0.2	0.02**	0.86	0.1	1.23
7 d	1.03	0.28	0.46	0.96	0.18	1.07
8 wk	0.84	0.14	0.96	0.86	0.13	0.98
16 wk	0.71	0.08	0.02**	0.94	0.32	0.76
Tibia						
6 h	0.76	0.24	0.9	0.74	0.28	1.03
1 d	1.17	0.27	0.24	1.04	0.14	1.13
3 d	0.91	0.2	0.09*	0.77	0.13	1.18
7 d	0.99	0.14	0.68	1.01	0.22	0.98
8 wk	0.82	0.11	0.1*	0.9	0.13	0.91
16 wk	0.8	0.13	0.003**	1.04	0.17	0.77

* $P < 0.1$; ** $P < 0.05$.

separated from the artery. Two 3/0 silk ligatures were tied around the femoral vein approximately 5–7 mm apart. The vein was sectioned between these ligatures, the cut ends separating widely (> 1 cm) due to the natural elastic construction of the vein. The wound was closed with 2/0 silk sutures and the animal allowed to recover. On the contralateral side, the right femoral neurovascular bundle was similarly exposed but the vein was not separated from the femoral artery, or ligated. There was no evident distress caused by the procedure, and normal locomotion was exhibited upon recovery in all cases. Groups of 10 animals were terminated at 6 h, 1, 3 and 7 d, and 8 and 16 wk after the procedure. Measurement of blood flow rate and blood volume in the femur and tibia were made at these times.

Haemodynamic measurements

Bone blood volume was measured by haemodilution, using ^{51}Cr radiochromate labelled red blood cells and blood flow rates by arteriolar blockade using ^{59}Fe radioferrous sulphate labelled cationic exchange resin particles. Both procedures were combined in each rat to yield simultaneous volume and flow data. The methods have been described in detail elsewhere (Brookes, 1965; Revell & Brookes, 1993) and only an outline description will be given.

Under general anaesthesia, packed ^{51}Cr labelled erythrocytes were injected into the left jugular vein and allowed to mix in the circulation for 10 min. At the conclusion of this period a volumetric sample of mixed tail blood was taken, together with a haematocrit sample. A catheter was then inserted into the left ventricle via the right carotid artery. Another catheter was inserted into the left carotid artery, and connected to a withdrawal pump. ^{59}Fe labelled resin particles (> 10^6) were injected into the left ventricle and the syringe flushed with saline. Simultaneously the pump removed a fixed volume of blood at a known rate. Subsequently the animal was terminated by a barbiturate injection into the intracardiac catheter, the bones removed and their weight and lengths recorded. The contained ^{51}Cr and ^{59}Fe activities were then determined, together with blood sample activity, in a 2-channel counter. The whole bone samples were then

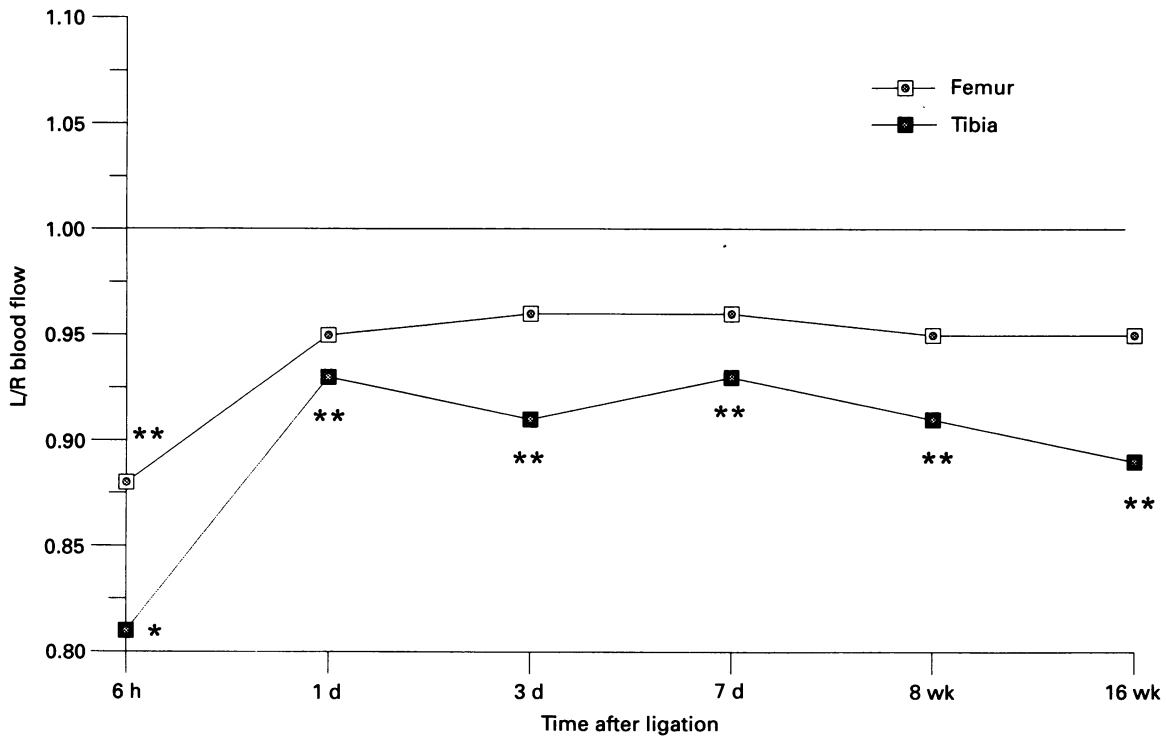

Fig. 1. Whole femora and tibiae: L/R blood flow. * $P < 0.1$; ** $P < 0.05$.

separated into inferior femoral epiphyses and metaphyses, superior tibial epiphyses and metaphyses, together with midshaft samples of femoral and tibial diaphyses. These regional samples were weighed and their isotope activity determined.

Blood flow was determined by correlating tracer activity from a locality with an unknown flow, with the activity from an area with a known flow rate. In practice, the isotope count contingent upon the known flow rate was established by withdrawing blood from the left carotid artery at a fixed rate. Blood volume was calculated by correlating ^{51}Cr activity in bone samples with the activity of a known volume of mixed tail blood. Both flow rates and volumes were standardised for a bone weight of 100 g. All calculations were performed using a UNISTAT statistical spreadsheet.

RESULTS

The results of this investigation for whole bones and for femoral and tibial epiphyses are summarised in Tables 1 and 2. Statistical comparisons have been made using the parametric match paired t test and significance levels shown, as well as left/right ratios obtained from these values. In general, statements made concerning the effects of femoral vein ligation are made with reference to the value obtained from the contralateral nonligated limb. It will be remembered that the femoral vein on the left side has been ligated in every case.

Six hours after venous ligation the results in general show a relative vascular depression on the ligated side. The blood flows and volumes in whole femora and tibiae and parts thereof were, in general, significantly reduced ($P < 0.05$). There were exceptions: reductions in blood flow to the whole tibia ($P < 0.1$), and tibial and femoral epiphyseal blood volumes ($P < 0.9$; $P < 0.1$ respectively) were not significant at the $P < 0.05$ level (Tables 1, 2).

Apart from the 6 h values ($P < 0.1$), flows to whole tibiae (Table 1) were significantly reduced in ligated limbs ($P < 0.05$), compared with the unoperated contralateral control for the whole period of investigation. In femora, the 6 h value in ligated limbs was significantly reduced ($P < 0.05$) but the flow reductions found at later times were not significant ($P > 0.05$). The left/right ratios plotted against time for whole femoral and tibial blood flows (Fig. 1) show an obvious tendency towards a flow reduction in both bones, but significance in the femur might have been obscured by high standard deviations and low sample numbers (n = 10). If the assumption is made that no specific time-related changes occur during the 1st week, from d 1 to d 7, it is statistically legitimate to pool data for d 1, 3, and 7 in order to increase sample size, and obtain a '1st week' mean value (Table 3). The combined data show on this basis that both

Table 3. *Combined '1st week' blood flow and volume data*

	L (ligated)	S.D.	<P>	R (nonligated)	S.D.	L/R
Combined '1st week' data (1, 3 and 7 d postligation)						
Bone blood flow (ml min^{-1} 100 g^{-1})						
Femur	37.33	12.8	0.047**	39.08	12.7	0.95
Tibia	34.28	13.6	0.0001**	37.1	14.1	0.92
Femoral epiphyses	46.8	13.6	0.97	46.76	11.4	1
Tibial epiphyses	53.26	17.3	0.006**	49.36	14.6	1.08
Femoral metaphyses	65.23	18.4	0.3	68.32	18.3	0.95
Tibial metaphyses	68	21.5	0.7	69.24	27.8	0.98
Femoral diaphyses	17.9	4.4	0.8	17.76	4.3	1.01
Tibial diaphyses	15.8	4.8	0.7	16.15	4.9	0.98
Bone blood volume (ml 100 g^{-1})						
Femur	1.43	0.3	0.0006**	1.25	0.3	1.15
Tibia	1.32	0.3	0.07*	1.24	0.2	1.07
Femoral epiphyses	1.11	0.3	0.09*	0.98	0.2	1.13
Tibial epiphyses	1.02	0.2	0.1*	0.94	0.2	1.09
Femoral metaphyses	1.93	0.5	0.003**	1.64	0.5	1.18
Tibial metaphyses	2.11	0.5	0.14	1.96	0.6	1.07
Femoral diaphyses	1.26	0.4	0.01**	1.09	0.4	1.15
Tibial diaphyses	1.11	0.3	0.6	1.07	0.2	1.03

* $P < 0.1$; ** $P < 0.05$.

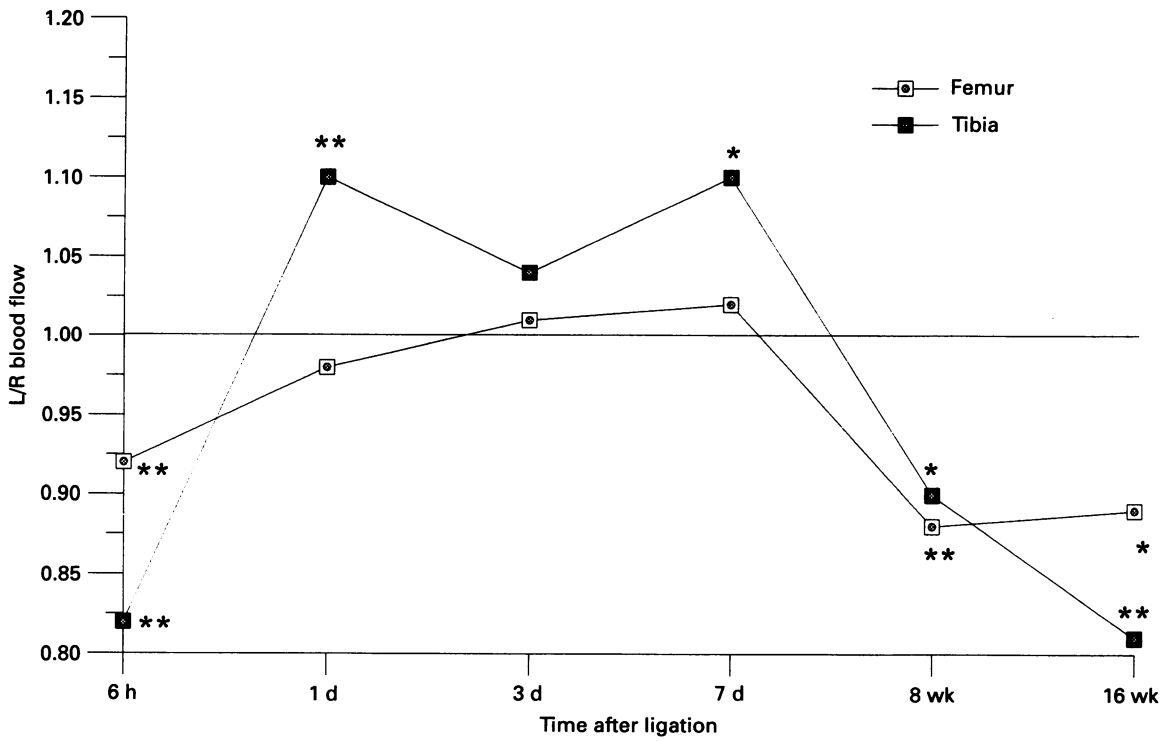

Fig. 2. Femoral and tibial epiphyses: L/R blood flow. * $P < 0.1$; ** $P < 0.05$.

femoral ($P < 0.05$) and tibial ($P < 0.0001$) flows were significantly reduced during the 1st week following venous ligation. The 6 h data for femur and tibia were not included in the combined '1st week' values because we take them to represent ischaemia following surgical intervention (see Discussion) and are not typical of the later response.

In the tibial epiphyses, relative flow (i.e. left/right comparisons) increased during the 1st week. At 8 and 16 wk postoperatively, however, relative flow rates in both ligated femoral and tibial epiphyses appeared to be reduced (Fig. 2). Metaphysical and diaphyseal blood flows were not altered by femoral vein ligation, and detailed results for these regions have therefore not been given.

At 1, 3 and 7 d the data showed a clear trend to a

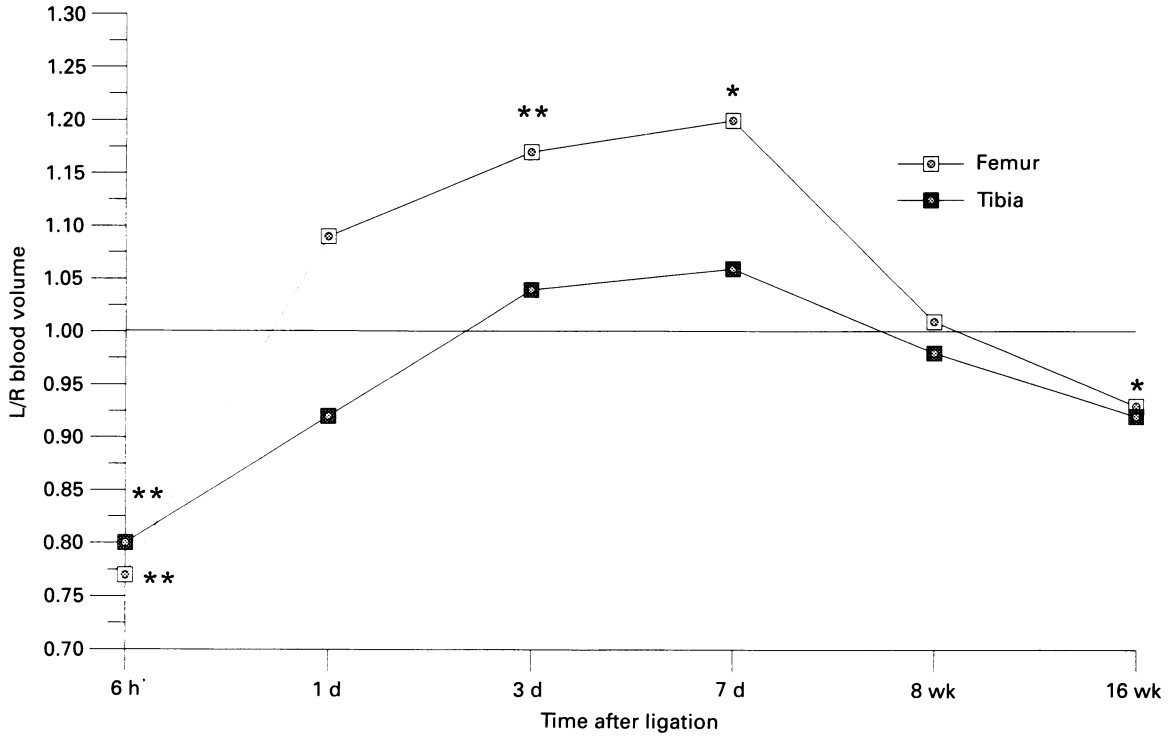

Fig. 3. Whole femora and tibiae: L/R blood volume. * $P < 0.1$; ** $P < 0.05$.

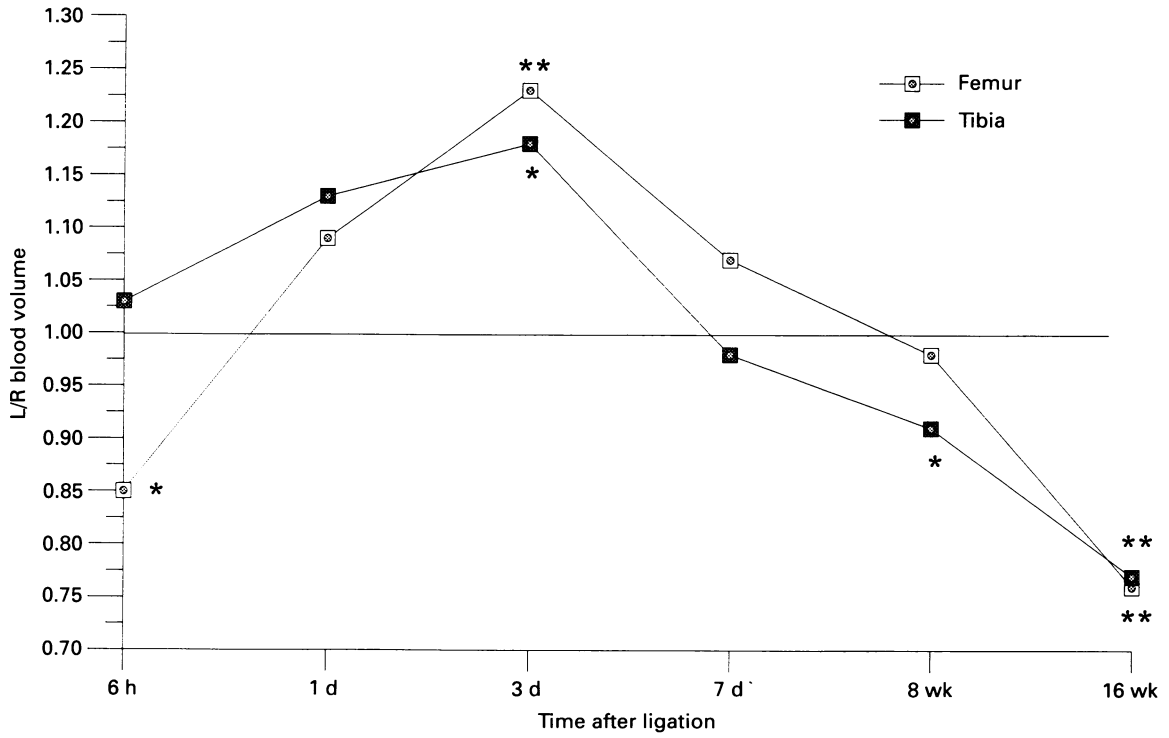

Fig. 4. Femoral and tibial epiphyses: L/R blood volume. * $P < 0.1$; ** $P < 0.05$.

relative elevated femoral blood volume in the ligated limb (Fig. 3), but only data for d 3 were significantly elevated ($P < 0.05$). Pooled data for 1, 3 and 7 d (Table 3) showed a significant increase in relative femoral blood volume following ligation ($P < 0.0001$) and suggest a parallel trend in the tibia ($P < 0.07$). This volume increase was absent at 8 and 16 wk. Tibial and femoral epiphyseal blood volumes were

elevated in ligated bones, compared with nonligated controls, in the 1st week following ligation, whilst at 16 wk, epiphyseal volumes in ligated bones were substantially below values for the contralateral normal. (Fig. 4). Ligated femoral metaphyseal blood volumes were significantly increased in the 1st week, although this was not maintained at 8 and 16 wk. There were no other haemodynamic changes resulting from venous ligation.

There was a significant ($P < 0.05$) relative weight gain in the ligated femora (+2.9% at 8 wk; +3.7% at 16 wk) and tibiae (+3.7% at 8 wk; +4.8% at 16 wk) compared with the contralateral bones. Relative bone lengths also showed a slight increase, but significant ($P < 0.05$) only at 16 wk following ligation (femur +1%; tibia +0.44%).

DISCUSSION

Haemodynamic investigations often give results which seem to be operationally dependant; it is important therefore that the methods selected are appropriate. Bone blood flow is measured here using arteriolar blockade, combined with a reference flow to estimate absolute values. The method is commonly known as the 'microsphere technique', although here we have used resin particles. An extensive validation has been given elsewhere for the use of this material, where it was established that the results obtained with resin particles are indistinguishable from those using microspheres. Sufficient numbers of appropriately sized resin particles must be injected, and procedures adopted to prevent leaching of isotope to plasma (Revell & Brookes, 1993). The red cell dilution method to determine blood volume in bone was first described by Brookes (1965). The use of chromium labelled red blood cells was described by Grey & Sterling (1950*a*) for measuring circulating blood volume in dogs and man (Grey & Sterling, 1950*b*; Sterling & Grey, 1950). The anionic hexavalent form ($Na_2{}^{51}CRO_4$) is highly specific for erythrocytes, and injected labelled cells retain their activity, without significant loss to plasma, for at least 24 h.

The absolute parameters measured for blood flow and volume are quite variable, both between different postoperative groups and within groups; this is a common observation in haemodynamic studies. However, investigations in our own laboratory have shown that left and right values of blood flow to femora and tibiae in normal rats do not differ significantly (Revell & Brookes, 1993). In this investigation differences were found between ligated and nonligated limbs, and statistically significant differences between the 2 conditions at the arbitrary $P < 0.05$ level have been recorded. Biological measurements of this nature, however, are prone to large variations, and in this case the sample size is small. Strict adherence to the $P < 0.05$ criteria may in these circumstances obscure genuine differences, and we have therefore also shown instances where $P < 0.1$, and believe this to be a useful additional indicator to identify possible biological change.

In Figure 1 the femoral flow decrease observed is consistently about 5%, at 5 different times. Each point in itself may not be statistically significant, but if the mean values at 1, 3, and 7 d are pooled, thus expanding the total population for comparison to 30 animals, then differences do achieve statistical significance. We have accepted that there is a trend towards a reduced femoral flow, which is not as great as found in the tibia ($P < 0.05$). By presenting data as left ligated/right nonligated ratios (Figs 1–4) it is easier to identify trends, which may not be apparent on inspection of tables.

The results show that blood flows and volumes were generally reduced, 6 h after femoral vein ligation, relative to the unoperated contralateral control. It is well known that trauma to the large arteries may cause spasm, which lasts for several hours. Usually such arterial spasm is related to trauma, and can be incurred by dissection of an artery during surgical procedures (Kinmonth, 1952; Kinmonth et al. 1956). In this investigation, although the neurovascular bundle was exposed on the right control limb, the femoral vein was not ligated, the femoral sheath was not torn, and the artery was not subjected to longitudinal stresses due to elastic recoil of the vein. All these additional factors, of course, are applied to the artery on the ligated side and, not surprisingly, in general the relative depression in values for flow and volume are lowest at this early postoperative stage. It is also known that following constriction of an artery, the contralateral artery also undergoes spasm, usually at the same level, but often involving proximal and distal structures (Jaya, 1958). This response is mediated by a reflex loop involving sympathetic nerves. It will be seen in the present investigation that absolute flow and volume values are lower, in both limbs, than other values reported at later stages, and presumably bilateral contraction of the arterial input has been stimulated by the ligation. In addition to arterial spasm affecting the early postoperative responses, it is also to be expected that ligation decreased the driving pressure, by reducing the arteriovenous pressure differential pushing blood through the bone.

The initial postoperative depression was followed by a relative increase in vascular volume during the 1st week following ligation (Figs 3, 4), clearly showing that venous congestion is present in the short term. Presumably, later collateral circulatory changes act to reduce this. There is a tendency for relative vascular volume to be depressed when measured in whole bones on the ligated side, by 16 wk; in epiphyseal cancellous bone of the knee joint, this reduction is also observed and is, in this case, statistically significant ($P < 0.05$). A sustained relative reduction of arterial input in the ligated side, to whole femora and tibiae was present during the whole period of the investigation. This diminished blood flow to the bone could also act as a compensatory mechanism to reduce congestion, and vasoconstrictive agents may be of importance here. The sustained reduction in blood flow eventually produces an ischaemic condition in the bones, with regard both to flow and volume.

In the cancellous bone of the knee joint, a localised increase in flow was apparent in the 1st week, comparing ligated with nonligated bone. It must be borne in mind that the rat knee joint epiphyses are compartmental isolates because of the persistence of growth cartilages. Vasoactive reflexes may act to produce vascular dilatation in the epiphysis in the short term, thereby adapting to the congestion; sympathetic vasodilator substances (Lundgaard et al. 1993), as well as vasoconstrictor agents (Lindblad et al. 1993), are known to be active in bone. The metaphysis and diaphysis are in continuity, and venous escape routes are present to overcome vascular congestion and increased blood volume and flow, which would otherwise develop. These escape routes are via the medullary sinuses, linking with metaphyseal and periosteal veins (Brookes, 1971). The presence of effective alternative venous drainage routes has been verified by perfusion studies (McPherson et al. 1961; Brookes & Singh, 1968).

Shim & Patterson (1967) showed qualitatively that femoral vein occlusion produced an increase in nutrient venous outflow. This was interpreted as an increase in venous congestion rather than an increase in flow rate; arterial supply, in the same experiment, showed a slight reduction. An increase in blood volume was also demonstrated by Brookes (1966) using ^{51}Cr radiochromate labelled red blood cell dilution. Following occlusion of the femoral, iliac and saphenous veins, the femoral and tibial knee joint epiphyseal bone showed a 36% and 43% relative increase in blood volume. The tibial metaphyseal blood volume was increased by 20%; femoral metaphyseal volume was unaltered.

Brookes & Singh (1972) occluded the femoral vein and examined blood volume changes in whole femora and tibiae compared with unoperated contralateral controls, for periods up to 24 wk. Surprisingly, they reported that the mean value of the circulating red blood cell volume was relatively lower at all times on the operated side, although the fall only achieved statistical significance at 7 d postligation. This, however, seems unlikely when the substantial venous congestion caused by ligation of the iliac and femoral vein is considered (Brookes, 1966).

Blood flow rate changes using arteriolar blockade with resin particles have also been examined by Singh & Brookes (1971) in whole bones, for periods up to 24 wk post femoral vein ligation. A significant fall in arterial supply was noted immediately after venous ligation but, unlike the present study, no further changes between the ligated and the contralateral normal limb were observed. Recent work, however, has corrected some of the problems identified with the earlier use of resin particles for measuring bone blood flow (Revell & Brookes, 1993) and the present technique permits flow measurement with increased resolution.

The significant reduction in epiphyseal vascular space, in the long term, could be related to the observed sclerosis in rat epiphyses previously described, following venous ligation (Brookes, 1966; Brookes & Helal, 1968; Revell & Brookes, 1991). Bone accretion is indicated here by the significant increase in weight found from 8 to 16 wk postligation. Brookes (1971) presented evidence that femoral vein ligation results in venous blood being shunted through knee joint cancellous bone, resulting in an environment with reduced pH and increased pCO_2. He suggested that this acid pH drift is the stimulus for the reported sclerotic changes in bone density and growth, and particularly for the normal morphogenesis of cortical compact bone. More recently, Annan et al. (1985) suggested a different mechanism. They observed in puppies that a proximally applied low-pressure tourniquet increased the vascular space in hindlimb bone, and more than doubled the standing venous pressure, measured 40 d after application. It was argued that increased venous pressure caused increased fluid loss from capillaries which, as bone is rigid, leads to an increased centrifugal flow of extravascular fluid. A computer simulation predicted that this increased fluid transfer created streaming potentials, and it is the change in electrical environment which leads to changes in bone turnover. These views are both supported by the present study, and they could, perhaps, be considered as comp-

lementary mechanisms; a change in pH may also be regarded as a redistribution of charge.

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REFERENCES

ANNAN I, BRONK J, AN K-N, KELLY PJ (1985) Stimulation of bone growth and remodeling by raised venous pressure: a proposed explanation. *Federation Proceedings* **44**, 1760.

BROOKES M (1965) Red cell volumes and vascular patterns in long bones. *Acta Anatomica* **62**, 35–52.

BROOKES M (1966) The vascular factor in osteoarthritis. *Surgery, Gynaecology and Obstetrics* **123**, 1255–1260.

BROOKES M (1971) *The Blood Supply of Bone*. Butterworth: London.

BROOKES M, HELAL B (1968) Primary osteoarthritis, venous engorgement and osteogenesis. *Journal of Bone and Joint Surgery* **50B**, 493–504.

BROOKES M, SINGH M (1972) Venous shunt in bone after ligation of the femoral vein. *Surgery, Gynaecology and Obstetrics* **135**, 85–88.

COLT JD, IGER M (1963) An attempt to stimulate bone growth by creating a venous stenosis. *Angiology* **14**, 584–587.

DALE PA, BRONK JT, KELLY PJ (1989) Fracture healing with an elevated venous pressure. *Transactions of the Orthopaedic Research Society* **35**, 590.

GREY SJ, STERLING K (1950*a*) The tagging of red cells and plasma proteins with radioactive chromium. *Journal of Clinical Investigation* **29**, 1604–1613.

GREY SJ, STERLING K (1950*b*) Determination of circulating red cell volume by radioactive chromium. *Science* **112**, 179.

HUTCHISON WJ, BORDEAUX BD (1954) The influence of stasis on bone growth. *Surgery, Gynaecology and Obstetrics* **99**, 413–420.

KECK SW, KELLY PJ (1965) The effect of venous stasis on intraosseous pressure and longitudinal bone growth in the dog. *Journal of Bone and Joint Surgery* **47A**, 539–544.

KINMONTH JB (1952) The physiology and relief of traumatic arterial spasm. *British Medical Journal* **1**, 59–64.

KINMONTH JB, HADFIELD GJ, CONNOLLY JE, LEE RH, AMOROSO EC (1956) Traumatic arterial spasm: its relief in man and monkeys. *British Journal of Surgery* **44**, 164–169.

JAYA Y (1958) Contralateral vasoconstriction in the hind limb of the rat and rabbit. *Clinical Science* **17**, 55–61.

LINDBLAD BE, NIELSEN LB, BJURHOLM A, BUNGER C, HENSEN ES (1993) Vasoconstrictive action of neuropeptide Y and norepinephrine in bone: a comparative study in the in-situ perfused porcine tibia. *Transactions of the European Orthopaedic Research Society* **3**, 31.

LUNDGAARD A, AALKJAER C, MULVANEY MJ, BJURHOLM A, HENSEN ES (1993) Calcitonin gene-related peptide, vasoactive intestinal peptide, and substance P induce relaxation of resistance arteries isolated from cancellous bone. *Transactions of the European Orthopaedic Research Society* **3**, 30.

LILLY AD, KELLY PJ (1970) Effects of venous ligation on bone remodelling in the canine tibia. *Journal of Bone and Joint Surgery* **52A**, 515–520.

MCPHERSON A, SCALES JT, GORDON LH (1961) A method of estimating qualitative changes of blood flow to bone. *Journal of Bone and Joint Surgery* **43B**, 791–799.

PEARCE HE, MORTON JJ (1930) The stimulation of bone growth by venous stasis. *Journal of Bone and Joint Surgery* **12**, 97–111.

REVELL WJ, BROOKES M (1991) The effect of a pulsed electromagnetic field on bone sclerosis. *Proceedings of the Bioelectrical Repair and Growth Society* **11**, 8.

REVELL WJ, BROOKES M (1993) Bone blood flow in the rat using arteriolar blockade: comparisons between labelled resin particles and microspheres. *Journal of Anatomy* **182**, 305–312.

SHAW NE (1963) Observations on the intramedullary blood flow and marrow pressure in bone. *Clinical Science* **24**, 311–318.

SHIM SS, PATTERSON FP (1967) A direct method of qualitative study of bone blood circulation. *Surgery, Gynaecology and Obstetrics* **125**, 261–268.

SINGH M, BROOKES M (1971) Bone growth and blood flow after experimental venous ligation. *Journal of Anatomy* **108**, 315–322.

STERLING K, GRAY SJ (1950) Determination of the circulating red cell volume in man by radioactive chromium. *Journal of Clinical Investigation* **29**, 1614–1619.

WHITE NB, STEIN AH (1965) Observations on the rate of blood flow in the rabbit's tibia following ligation of the femoral vein. *Surgery, Gynaecology and Obstetrics* **121**, 1081–1084.