BJR

RESEARCH

Intraosseous pressure during loading and with vascular occlusion in an animal model

M. Beverly,
S. Mellon,
J. A. Kennedy,
D. W. Murray

RPMS, Hammersmith Hospital, London, United Kingdom

Objectives

We studied subchondral intraosseous pressure (IOP) in an animal model during loading, and with vascular occlusion. We explored bone compartmentalization by saline injection.

Materials and Methods

Needles were placed in the femoral condyle and proximal tibia of five anaesthetized rabbits and connected to pressure recorders. The limb was loaded with and without proximal vascular occlusion. An additional subject had simultaneous triple recordings at the femoral head, femoral condyle and proximal tibia. In a further subject, saline injections at three sites were carried out in turn.

Results

Loading alone caused a rise in subchondral IOP from 11.7 mmHg (SD 7.1) to 17.9 mmHg (SD 8.1; $p < 0.0002$). During arterial occlusion, IOP fell to 5.3 mmHg (SD 4.1), then with loading there was a small rise to 7.6 mmHg (SD 4.5; $p < 0.002$). During venous occlusion, IOP rose to 20.2 mmHg (SD 5.8), and with loading there was a further rise to 26.3 mmHg (SD 6.3; $p < 0.003$). The effects were present at three different sites along the limb simultaneously. Saline injections showed pressure transmitted throughout the length of the femur but not across the knee joint.

Conclusion

This is the first study to report changes in IOP *in vivo* during loading and with combinations of vascular occlusion and loading. Intraosseous pressure is not a constant. It is reduced during proximal arterial occlusion and increased with proximal venous occlusion. Whatever the perfusion state, *in vivo* load is transferred partly by hydraulic pressure. We propose that joints act as hydraulic pressure barriers. An understanding of subchondral physiology may be important in understanding osteoarthritis and other bone diseases.

Cite this article: *Bone Joint Res* 2018;7:511–516.

Keywords: Intraosseous, Pressure, Weight bearing, Subchondral, Physiology

Introduction

As the musculoskeletal system is clearly designed for weight bearing, studies of intraosseous pressure (IOP) should be undertaken, not only under static conditions, but also under conditions of loading or during activity *in vivo*.[1,2] Denham, as well as others,[3-5] have calculated that a considerable force, perhaps five or more times body weight, is transmitted through weight bearing joints in use. Previous authors[6-8] have explored the possibility that bone might be hydraulically strengthened, but those tests used non-physiological techniques such as grease-saturated dry bones, or bone plugs in mechanical testing jigs.

A raised IOP has been associated with bone pain in osteoarthritis and osteonecrosis, and in vascular diseases such as osteonecrosis and Perthes' disease of the hip in children. Embolic bone diseases, for example sickle cell and caisson, and storage diseases, such as Gaucher's, have been reported to have a raised IOP.[9] Alcohol, corticosteroid use and diabetes have also been associated with a raised IOP.[10] Osteonecrosis incidence varies across cultures, but is said to affect 10 000 to 20 000 new patients a year in the

■ M. Beverly, FRCS (Orth), Consultant Orthopaedic Surgeon and DPhil Student,
■ S. Mellon, BSc (Hons), PhD, Research Fellow,
■ J. A. Kennedy, MBBS MRCS, Clinical Research Fellow and DPhil Student,
■ D. W. Murray, MD, FRCS (Orth), Professor of Orthopaedic Surgery and Consultant Orthopaedic Surgeon, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Botnar Research Centre, Nuffield Orthopaedic Centre, Oxford, UK.

Correspondence should be sent to M. Beverly; email: michael.beverly@btinternet.com

doi: 10.1302/2046-3758.78.BJR-2017-0343.R2

Bone Joint Res 2018;7:511–516.

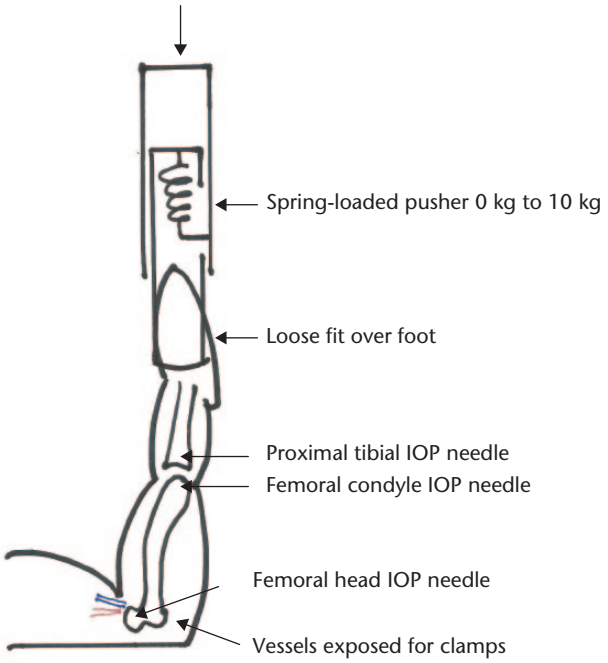

Fig. 1

Diagram showing experimental set up. The spring-loaded pusher covers the foot and prevents flexion and extension while applying a longitudinal one body weight load (4520 g to 5400 g; IOP, intraosseous pressure).

United States. Worldwide, osteoarthritis affects the majority of the older population.[11]

Since Clopton Havers (1657 to 1702)[12] first described canals in cortical bone, the anatomy of the blood supply in bone has been widely investigated. Trueta and Caladias[13] and Brookes and Revell[14] used a barium injection with decalcification. Others used microsphere[15] and isotope[16] studies. Experimental models of osteonecrosis or Perthes' disease have been developed by obliterating the blood supply, by cryotherapy and by using steroids.[17] Ficat[18] developed a means of classifying and treating osteonecrosis, but did not consider the possible effect of weight bearing on IOP. The physiological relationship between subchondral IOP, loading and blood supply in normal healthy bone has received little attention.

The factors that usually control IOP physiology in normal bone, and whether IOP might change during ordinary activity, remain largely unknown. In 1964, Azuma[19] reported fluctuating IOP *in vivo* at rest. More recently, a falling IOP has been noted in exsanguination studies.[20] It is possible that loading itself might change IOP in the subchondral bone below the cartilage.

In concurrent work using the same animal model, we found that at rest, in addition to a wave form synchronous with the arterial pulse, there is often an underlying wave synchronous with respiration. Intraosseous pressure also reflected other circulatory changes, for example those due to anaesthetic drugs. We found that in our model, IOP was not significantly affected by gender, weight, needle size or site of measurement. Technique affects IOP measurement with a prolonged recovery after saline injection but rapid recovery after aspiration. We confirmed that IOP is proportional to systemic blood pressure, and that whatever the initial or basal IOP value, the associated pulse pressure correlates well with IOP.[21]

Our hypothesis is that loading forces might be transmitted by hydraulic pressure through bone fat and marrow from the subchondral region to the trabeculae, and on to the cortical shaft during weight bearing. We therefore studied the effect of physiological loads on subchondral IOP. We then studied the effects combined with vascular occlusion. We also assessed possible compartmentalization within bones by saline injections.

Materials and Methods

Intraosseous pressure was measured experimentally in the subchondral bone of the femoral condyle and proximal tibia of five anaesthetized adult New Zealand White rabbits (two male, three female; Royal Postgraduate Medical School, Home Office licence ELA 24/4994). Induction of anaesthesia was by IV fentanyl (Sublimaze) 2 ml of 0.05 mg/ml solution, depending on the size of animal, with a top-up IV infusion of diazepam (Valium) 0.5 ml of 5 mg/ml solution, alternating with fentanyl (0.5 ml to 1.0 ml), given slowly on an approximately half-hourly basis.

The femoral vessels were exposed at the inguinal ligament. Sheathed 'bulldog' vascular clips could then be applied as required to the proximal femoral artery or vein. A 23G saline-filled venesection needle was pushed into the subchondral bone at the femoral condyle, or proximal tibia, percutaneously by rocking the needle through a 5° to 10° arc along the line of the bevel of the needle. Femoral head needle insertion was by an anterior approach dissection and direct puncture through the capsule.

A heparinized saline-filled line was connected from the intraosseous needle to a pressure transducer (Bell and Howell, Wheeling, Illinois or Druck PDCR75, Druck & Temperatur, Leitenberger, Germany) and to a four-channel chart recorder (Lectromed MX4P- 31, Jersey, United Kingdom). The transducers were calibrated on a 0 mmHg to 100 mmHg scale. Intraosseous pressure recordings were made at each site. For each set of recordings, the pressure transducers were zeroed to air, calibrated and kept level with the site measured.

Loading was carried out using a reversed spring pusher. A weighing spring and its calibration scale were mounted in concentric tubes, one end attached to the inside of each tube such that longitudinal compression of a set force could be applied. The inner tube contained and controlled the foot, preventing collapse into a flexed posture. The tube prevented movement of the hind limb and avoided direct squeezing of limb muscle. The subject was supine, with the leg extended. A steady load of one body weight (between 4520 g and 5400 g) was applied down the limb as in Figure 1. The loading recordings were carried out with the leg in an extended

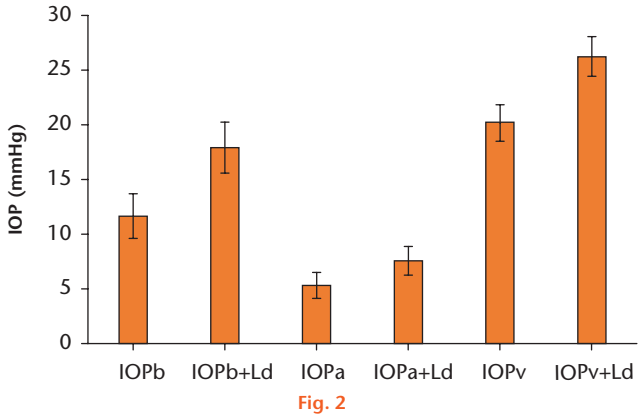

Fig. 2

Graph showing the basal intraosseous pressure (IOP) during perfusion at rest (IOPb), an increase in IOP with loading by one body weight (IOPb+Ld) $p < 0.0002$; IOP during proximal arterial occlusion (IOPa) and with loading during arterial occlusion (IOPa+Ld) $p < 0.002$ and IOP during venous occlusion (IOPv) and loading during venous occlusion (IOPv+Ld) $p < 0.003$; (all p-values *t*-test). Error bars are standard error of the mean.

position and the pusher in place, without movement of the limb.

A series of experiments was undertaken to investigate the following:

1. Loading and vascular occlusion. IOP recordings from five femoral condyles and seven proximal tibias in five subjects were obtained.

- The effect on basal IOP (IOPb) of a load of one body weight (IOPb+Ld) was measured.
- The effect on IOP of arterial occlusion (IOPa), then loading of one body weight (IOPa+Ld), was measured.
- The effect on IOP of venous occlusion (IOPv), then loading by one body weight (IOPv+Ld), was measured.

2. Simultaneous triple recording. An additional experiment was undertaken in one subject in which IOP was recorded simultaneously at the femoral head, femoral condyle and proximal tibia during vascular occlusion and loading. This required placement of an additional needle in the femoral head.

3. Compartmentalization. A further experiment was carried out in one subject to assess the qualitative effect on IOP of saline bolus injection at the femoral head, femoral condyle and proximal tibia in turn, while recording at the other sites.

Statistical analysis. Experimental duration varied between 30 minutes and two hours. Basal IOP (IOPb) was measured on three occasions (early, middle and late) for each site during each experiment, and the readings were averaged. The occlusion and loading experiments lasted for several minutes and IOP responses were recorded only once. Results were expressed as means, SDs and ranges. The Student's *t*-test was used to determine if there were significant differences. Each subject was used as its own control, and paired tests for differences were used.

Results

1a. The effect on IOP of loading by one body weight. We compared the basal IOP at the femoral condyle and proximal tibia. There was a wide range of values but no significant difference ($p = 0.159$, *t*-test) between them. The tibial and femoral results were therefore pooled.

When a load of one body weight was applied longitudinally without moving the limb, the IOPb (mean 11.7 mmHg, SD 7.1 mmHg) rose to IOPb+Ld (17.9 mmHg, SD 8.1 mmHg; $n = 12$, $p < 0.0002$, *t*-test), as in Figures 2 and 3 and Table I.

1b. The effect of arterial occlusion then loading. When a clip was applied to the proximal femoral artery, there was a fall in IOP (IOPb mean 11.7 mmHg, SD 7.1) to IOPa (mean 5.3 mmHg, SD 4.1; $n = 12$, $p < 0.005$, *t*-test). The fall in IOP was proportional to the initial or basal IOPb (Pearson correlation 0.81). Loading the limb under these conditions caused a small rise in IOPa to IOPa+Ld (mean 7.6 mmHg, SD 4.5; $n = 12$, $p < 0.002$, *t*-test) as in Figures 2 and 4 and Table I.

1c. The effect of venous occlusion and loading. When the femoral vein was clamped, there was a rise in IOPb (mean 11.7 mmHg, SD 7.1) to IOPv (20.2 mmHg, SD 5.8; $n = 12$, $p < 0.0001$, *t*-test). The rise in IOP was inversely proportional to the starting IOPb (Pearson correlation -0.57). The addition of one body weight load caused a further rise in pressure to IOPv+Ld (26.3 mmHg, SD 6.3; $n = 12$ $p< 0.003$, *t*-test) as in Figures 2 and 4 and Table I.

The difference between IOPv+Ld and IOPa+Ld was significant ($n = 12$, $p < 0.0001$, *t*-test).

2. Simultaneous recordings at different sites. When simultaneous records were made in one subject at the femoral head, femoral condyle and proximal tibia, the effect of loading was to raise IOP at all three sites simultaneously, as in Figure 3. With vascular occlusion and

Table I. Values for intraosseous pressure (IOP) (mmHg) at 12 sites among five subjects. Columns are for basal IOP at rest (IOPb), with only loading (IOPb)+Ld (difference $p < 0.0002$), proximal arterial occlusion (IOPa), arterial occlusion with load (IOPa)+Ld (difference $p < 0.002$), venous (IOPv) occlusion and venous occlusion and load (IOPv)+Ld (difference $p < 0.003$; all *t*-test)

n = 12 for all	IOPb mmHg	IOPb+Ld mmHg	IOPa mmHg	IOPa+Ld mmHg	IOPv mmHg	IOPv+Ld mmHg
Mean	11.7	17.9	5.3	7.5	20.2	26.3
sd	7.1	8.1	4.1	4.5	5.8	6.3
Range	5 to 27	7 to 30	2 to 17	4 to 18	14 to 35	16 to 34

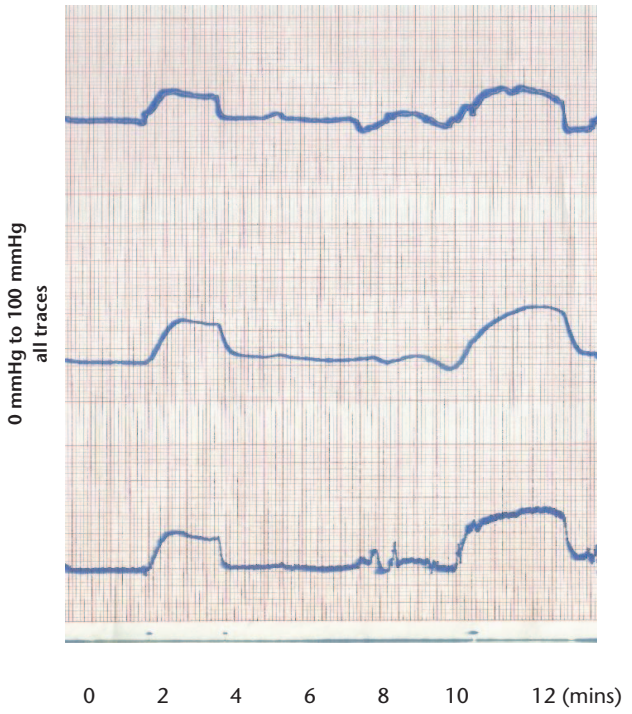

Fig. 3

Graph showing the effect of loading alone with one body weight. Upper trace - femoral head, middle trace - femoral condyle and lower trace - proximal tibia. All traces shows basal intraosseous pressure, loading by one body weight for two minutes, rest for six minutes and load for three minutes. Vertical scale 0 mmHg to 100 mmHg on all traces, trace speed 12.5 mm/min.

loading, the effects were seen to be present at all three sites simultaneously, as demonstrated in Figure 4.

3. Saline injection at different sites. Saline 0.5 ml was injected into the femoral head, femoral condyle and proximal tibia in turn. Pressure was not recorded at the injection site itself, but pressure was recorded at the other two IOP needles. There was a pressure rise in the femoral condyle when the femoral head was injected, and vice versa. Neither caused a rise in proximal tibial pressure. Proximal tibial injection caused no rise across the knee joint in the femur, as shown in Figure 5.

Discussion

We can find no previous references to direct IOP measurement during physiological loading, still less with and without vascular occlusion.[22] Although individual measurements of IOP vary considerably, as other authors have noted,[23] we have shown that subchondral IOP is increased by physical load, providing the bone is perfused. The effect of loading during venous occlusion is more marked, and IOP may then even exceed the perfusion pressure. The pressure change is less marked during arterial occlusion. Subchondral bone appears to be acting as a perfused tissue, in an enclosed space, with additional IOP rises during weight bearing.

The finding that loading results in a marked increase in IOP suggests that there is transfer of force by hydraulic pressure from a slightly flexible subchondral plate through the perfused soft or semiliquid marrow fat contained between the trabeculae and onwards, to the rigid cortical shaft. It therefore suggests that not all of the load is transmitted through the trabeculae, but that a substantial proportion is transmitted by hydraulic pressure. This hydraulic load transfer would also be effective at dissipating the energy associated with impact loading, as proposed by Simkin,[24] and would reduce the risk of trabecular fracture.

Other authors who experimented with grease-saturated dry bones, or cancellous bone discs in compression jigs, concluded that load in bone was not transferred by hydraulic pressure.[7,8] We believe the reason why their conclusion was different from ours is that their bone was not perfused, and there was no pressure within the bone before load was applied. In our experiments, when the bone was perfused and there was normal IOP or elevated IOP resulting from venous inclusion, mechanical loading increased the IOP, substantially demonstrating transmission of load by hydraulic pressure. With arterial occlusion and a very low IOP, loading caused only a small increase in pressure, suggesting that only a small amount of load was transmitted hydraulically. Therefore, when there was no resting IOP, as in the grease experiments, we would expect no hydraulic load transmission. As a result, these non-physiological experiments support our conclusion that, in the normal physiological situation when bone is perfused, load is transmitted by hydraulic pressure.

With loading, the IOP rises, sometimes to above-perfusion pressure. It is therefore likely that there are circulatory adaptations to withstand these high pressures. Burkhardt[25] described histological features which we believe could protect the delicate subchondral capillaries and fat cells while they operate at rest with ordinary perfusion pressures, while at the same time allowing higher pressures to be transmitted through the tissues during weight bearing. These include ‘choke’ cells at 90° capillary branching points, and apparent valves or muscular cuffs at exit veins. In addition, there may be adaptations in vessels in the subchondral plane adjacent to the cortex where they exit bone to limit venous outflow under load to maintain IOP for load transmission.

The effects are seen simultaneously at different points in the limb as shown in Figures 3 and 4. The question arises as to the compartmentalization of bones or separation of one from another by joints. Our injection study demonstrated that while the load bearing pressure change is found throughout the bony weight bearing chain as shown in Figure 5, each bone forms a relatively enclosed and separate compartment. We found that pressure could be transmitted from one end of the bone to the other. As pressure did not cross the knee joint in either an antegrade or retrograde manner, as shown in Figure 5, we conclude that joints form a hydraulic barrier to pressure transmission. We can find no previous

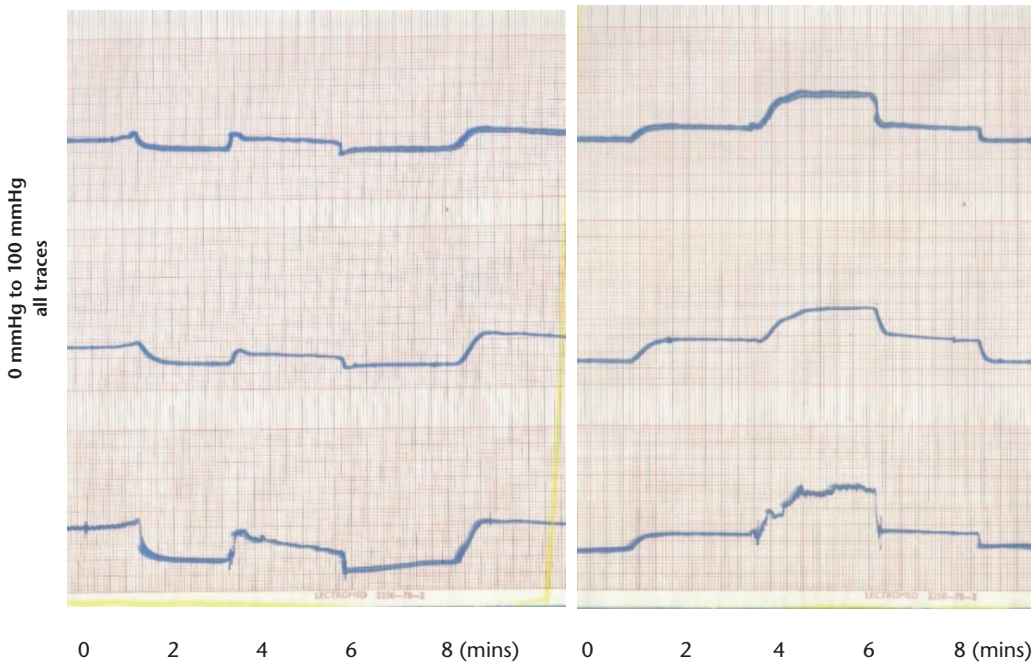

Fig. 4

Graphs showing the upper trace from the femoral head, middle trace femoral condyle and lower trace proximal tibia. From left to right, the traces show the effect of arterial occlusion, arterial occlusion with loading, load removal and arterial clamp removal. The second part shows changes at the same sites after venous occlusion, venous occlusion with loading, removal of load and removal of the occluding clamp. The intraosseous pressure changes are seen to be similar at all three sites and simultaneous. Vertical scale 0 mmHg to 100 mmHg on all traces, trace speed 12.5 mm/min.

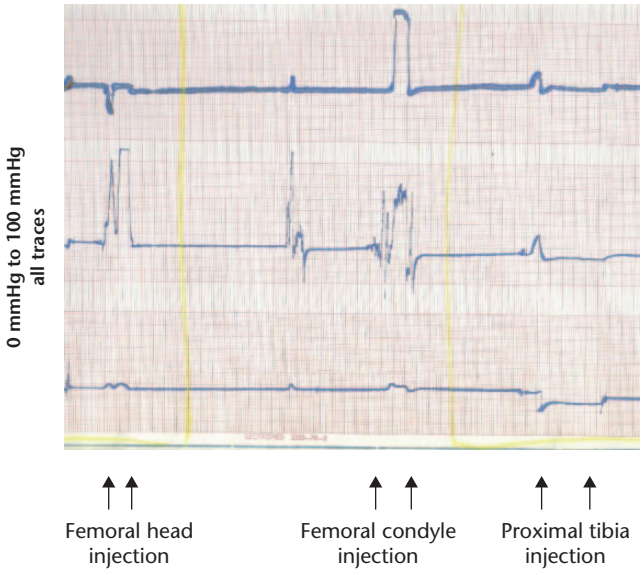

Fig. 5

Graph showing the upper trace from the femoral head, middle trace femoral condyle and lower trace proximal tibia. Left: injection (between the arrows) at the femoral head caused a rise in intraosseous pressure (IOP) at the femoral condyle, but not the proximal tibia. Centre: injection at the femoral condyle caused a rise in IOP at the femoral head, but not the proximal tibia (some artefactual shake is seen on the femoral condyle trace during injection). Right: injection at the proximal tibia had no effect on femoral head or femoral condyle IOP. Vertical scale 0 mmHg to 100 mmHg on all traces, trace speed 12.5 mm/min.

reference to suggest that load bearing increases IOP, which is transmitted through the bone, or that joints act as pressure transmission barriers. We note that intraosseous saline injection continues to be used clinically for resuscitation.[26,27] We injected relatively small volumes (0.5 ml) but consider that virtually any saline injection into the delicate bone vascular tree is potentially damaging. There is now some evidence that larger volumes of intraosseous saline injection may be harmful.[28]

The clinical relevance of our work is that it shows that IOP is not a constant, as previously supposed, but fluctuates *in vivo* with local perfusion conditions at the needle tip. Load bearing causes a rise in IOP, with hydraulic pressure transmitted through subchondral bone, rather than the trabeculae alone taking the full load. Subchondral bone is not an inert region with a fixed IOP, but a slightly flexible structure which transfers load partly by hydraulic pressure through the soft or semifluid bone marrow to the cortical shaft or diaphysis. This potentially opens a new field of subchondral perfusion physiology. There are likely to be circulatory modifications to allow the relatively delicate subchondral capillaries and fat cells to cope with fluctuating high pressures during load bearing. Once the normal physiology of subchondral perfusion and load transmission is more clearly understood, a better appreciation of the vascular contribution to the pathology of bone diseases, such as osteonecrosis and arthritis, may follow. Future work should explore this vasculo-mechanical model of joint physiology.

There are limitations in this work. The animals were not identical in terms of age or weight. Needle placements

could never be identical. Anaesthesia without specialized equipment in these subjects is recognized to be brittle. As the duration of each experiment varied, an average value for IOPb from early, middle and late in each subject was used. The vascular occlusion and loading values were recorded once only at the steady state achieved within 30 seconds of applying the clamp or load. For the main study of loading, we had five subjects with a total of 12 IOP recordings, from the femoral condyle and the proximal tibial. Although there was a wide range of values for IOP at both sites, we found no statistical difference between the femoral condyle and the proximal tibial sites, thus we used data from both. This reflects the clinical situation where IOP may be measured at virtually any site.

In conclusion, our work demonstrates that IOP is not a constant, but is reduced during proximal arterial occlusion, and increased with proximal venous occlusion. Loading increases IOP, whatever the perfusion state. With loading, force is transferred through fluid marrow and fat constrained within the bone. Load is thereby transmitted partly by means of hydraulic pressure from the subchondral plate to the diaphyseal shaft. In addition, we suggest that each bone represents a relatively enclosed space, through which pressure is transmitted, and that joints act as hydraulic barriers to pressure transmission.

References

1. **Afoke NY, Byers PD, Hutton WC.** Contact pressures in the human hip joint. *J Bone Joint Surg [Br]* 1987;69-B:536-541.
2. **Day WH, Swanson SA, Freeman MA.** Contact pressures in the loaded human cadaver hip. *J Bone Joint Surg [Br]* 1975;57-B:302-313.
3. **Denham RA.** Hip mechanics. *J Bone Joint Surg [Br]* 1959;41-B:550-557.
4. **D'Lima DD, Fregly BJ, Patil S, Steklov N, Colwell CW Jr.** Knee joint forces: prediction, measurement, and significance. *Proc Inst Mech Eng H* 2012;226:95-102.
5. **Klenerman L, Swanson SA, Freeman MA.** Mechanical and physiological properties of bone. *Proc R Soc Med* 1967;60:850-854.
6. **Kafka V.** On hydraulic strengthening of bones. *Biorheology* 1983;20:789-793.
7. **Swanson SA, Freeman MA.** Is bone hydraulically strengthened? *Med Biol Eng* 1966;4:433-438.
8. **Bryant JD.** The effect of impact on the marrow pressure of long bones in vitro. *J Biomech* 1983;16:659-665.
9. **Mukisi MM, Bashoun K, Burny F.** Sickle-cell hip necrosis and intraosseous pressure. *Orthop Traumatol Surg Res* 2009;95:134-138.
10. **Zizic TM, Marcoux C, Hungerford DS, Stevens MB.** The early diagnosis of ischemic necrosis of bone. *Arthritis Rheum* 1986;29:1177-1186.
11. **Allen KD, Golightly YM.** State of the evidence. *Curr Opin Rheumatol* 2015;27:276-283.
12. **Havers C.** *Osteologia Nova, or some New Observations of the Bones, and the Parts belonging to them, with the manner of their Accretion and Nutrition.* London: Samuel Smith, 1691.
13. **Trueta J, Caladias AX.** Study of Blood Supply of Long Bones. *Surg Gynecol Obstet* 1964;118:485-498.
14. **Brookes M, Revell WJ.** *Blood Supply of Bone: Scientific Aspects.* London: Springer-Verlag, 1998.
15. **Trotman NM, Kelly WD.** The effect of sympathectomy on blood flow to bone. *JAMA* 1963;183:121-122.
16. **Hughes SPF, McCarthy ID.** The Regulation of Blood-Flow in Bone. *Nato Adv Sci Inst Se* 1993;247:57-62.
17. **Motomura G, Yamamoto T, Irisa T, et al.** Dose effects of corticosteroids on the development of osteonecrosis in rabbits. *J Rheumatol* 2008;35:2395-2399.
18. **Ficat RP.** Idiopathic bone necrosis of the femoral head. Early diagnosis and treatment. *J Bone Joint Surg [Br]* 1985;67-B:3-9.
19. **Azuma H.** Intraosseous Pressure as a Measure of Hemodynamic Changes in Bone Marrow. *Angiology* 1964;15:396-406.
20. **Frascone RJ, Salzman JG, Adams AB, et al.** Evaluation of intraosseous pressure in a hypovolemic animal model. *J Surg Res* 2015;193:383-390.
21. **Beverly M, Murray D.** Factors affecting intraosseous pressure measurement. *J Orthop Surg Res* 2018;13:187.
22. **Wilkes CH, Visscher MB.** Some physiological aspects of bone marrow pressure. *J Bone Joint Surg [Am]* 1975;57-A:49-57.
23. **Salzman JG, Loken NM, Wewerka SS, et al.** Intraosseous Pressure Monitoring in Healthy Volunteers. *Prehosp Emerg Care* 2017;21:567-574.
24. **Simkin PA.** Marrow fat may distribute the energy of impact loading throughout subchondral bone. *Rheumatology (Oxford)* 2018;57:414-418.
25. **Burkhardt R.** *Bone Marrow and Bone Tissue: Color Atlas of Clinical Histopathology.* Berlin, New York: Springer-Verlag, 1971.
26. **Buck ML, Wiggins BS, Sesler JM.** Intraosseous drug administration in children and adults during cardiopulmonary resuscitation. *Ann Pharmacother* 2007;41:1679-1686.
27. **Joanne G, Stephen P, Susan S.** Intraosseous vascular access in critically ill adults - a review of the literature. *Nurs Crit Care* 2016;21:167-177.
28. **Taylor CC, Clarke NM.** Amputation and intraosseous access in infants. *BMJ* 2011;342:d2778.

Funding Statement

- A grant to support this work was made by The Wellcome Trust (12425/1.5/SC/wj) to the Royal Postgraduate Medical School, Hammersmith Hospital, London.

Acknowledgements

- The authors wish to thank the late Dr Joseph Pflug for his valuable assistance to MB in the early stages of this work at the Hammersmith Hospital, and Mrs Barbara Marks for her help in putting together the manuscript.

Author Contributions

- M. Beverly: Study conception, design, data acquisition, Analysis and interpretation, critical revision for intellectual content, final approval.
- S. Mellon: Analysis and interpretation, critical revision for intellectual content, final approval.
- J. A. Kennedy: Analysis and interpretation, critical revision for intellectual content, final approval.
- D. W. Murray: Analysis and interpretation, critical revision for intellectual content, final approval.

Conflict of Interest Statement

- None declared