THE EFFECT OF DEvascularisation UPON EARLY BONE HEALING IN DYNAMIC EXTERNAL FIXATION


From the Department of Orthopaedic Surgery, University of Edinburgh

We examined the effect of periosteal devascularisation upon the early healing of osteotomies of sheep tibiae held in an instrumented external fixation system with an axial stiffness of 240 N/mm. At 14 days, cortical blood flow measured by the microsphere technique was 19.3 ml/min/100g in the well-vascularised osteotomies, but only 1.7 ml/min/100g in the devascularised osteotomies, despite an increase in medullary flow (p < 0.0005). Delay in healing of the devascularised osteotomies was suggested by an in vivo monitoring system and confirmed by post-mortem mechanical testing. We suggest that the osteogenic stimulus of dynamic external fixation is dependent on the early restoration of cortical blood flow in devascularised fractures.

It has long been recognised that the healing of diaphyseal fractures is related to the severity of damage to the bone and soft tissues at the time of injury (Ellis 1958; Oestern and Tscherne 1984). The fracture configuration, the presence of infection, the blood supply of the fragments and the stability of fixation all have an effect upon the rate of fracture union (Court-Brown and Hughes 1982; Gustilo, Mendoza and Williams 1984).

The term 'biomechanical environment' has been used to describe the influence of fixation devices upon the histological pathways of fracture union (Chao et al 1989), but in a wider sense it may include the effect of biological variables implicit in the healing process, such as changes in oxygen tension (Heppenstall, Goodwin and Brighton 1976), blood flow (McCarthy and Hughes 1984), neural mechanisms (Aro, Eerola and Aho 1985), electrical potential (Law et al 1985), and osteo-inductive factors in the extracellular matrix (Reddi, Wientroub and Muthukumar 1987).

Investigators have attempted to define an 'optimal' biomechanical environment in terms of the direction and magnitude of loading which will stimulate osteogenesis.

In the isolated intact avian ulna, Rubin and Lanyon (1987) showed that bone mass may be maintained by as little as four cycles of loading per day, and a maximal osteogenic response was produced by 36 cycles per day, though this was dependent on the strain distribution within the bone.

Yamagishi and Yoshimura (1955) showed, in an externally-fixed osteotomy of the rabbit tibia, that moderate intermittent axial compression resulted in more rapid healing than did shear forces. Wolf et al (1981) used similar experiments to discover a threshold of cyclic axial loading, below which no demonstrable effect on healing occurred.

The introduction of the first clinical external fixator to allow axial loading of healing callus (De Bastiani, Aldegheri and Brivio 1984) has been followed by experimental and clinical attempts to control the amount of loading and consequent fragment displacement (Goodshep and Kenwright 1985; Kenwright and Goodship 1989). These have generated the concept of an optimal 'window' of axial fixation stiffness (Perren 1979).

Much of the experimental evidence for the beneficial effect of axial micromovement has relied on well-vascularised osteotomy models, whereas the clinical problems of delayed and nonunion are frequently associated with severe open fractures (grades II, III), in which there is extensive periosteal stripping (Court-Brown et al 1990).

The importance of the osteogenic stimulus of dynamic external fixation in fractures with a poor vascular supply is not known. Studies which have endeavoured to alter the afferent blood flow to healing fracture models, are not comparable because of differing
systems of fixation, affecting the mechanical environment (Olerud and Danckwardt-Lillieström 1971; Whiteside and Lesker 1978; Richards and Schemitsch 1989) and altering the blood flow to cortical bone at the fracture site (Smith, Bronk and Kelly 1990).

The objectives of this study were to examine quantitatively the effect of periosteal devascularisation on the healing of the osteotomised sheep tibia, held in an instrumented external fixation system which allowed measurement of axial fixator stiffness over a period of two weeks. This early stage was chosen as the time at which blood flow is at its peak (Paradis and Kelly 1975) and the capillary surface area is maximal from hypertrophy and regeneration (Hughes et al 1978). Bone mineral deposition, determined by uptake of the tracer technetium-99m methylene diphosphonate, has been found to increase significantly two weeks after osteotomy in the tibiae of dogs (McCarthy and Hughes 1984). At this stage blood flow to the healing callus appears to be largely independent of the endosteal supply (Strachan et al 1990), although the timing of re-establishment of normal centrifugal circulation, as conceived by Brookes et al (1961), has never been accurately defined.

**MATERIAL AND METHODS**

A pilot study was undertaken to design an experimental fixation system. A unilateral fixator was used, with stiffness allowing an axial load of 1000 N, to produce a combined deflection of less than 1 mm. The fixator bar was composed of one solid piece with integral strain-gauge transducers to measure the axial load and the bending moment in the plane of the pins.

From these measurements a modular fixator was devised (Fig. 1). It consists of a main body with two sets of three bone pins, connected by linear bearings (RSR-15M, THK Co, Tokyo, Japan) which allow movement only in the axis of the fixator. The pin-holding blocks are separated by a spring-transducer module which determines passive axial micromovement. The spring is constructed of room-temperature-vulcanising silicone (Cosmesil Silicone, Cosmedica, Cardiff, Wales) and an epoxy resin spacer; by varying the dimensional ratio of these components, a wide range of linear stiffness can be accurately prescribed. A transducer measures the axial load on the fixator bar; since the spring stiffness is known, the axial displacement at the osteotomy can be calculated.

Displacement at the osteotomy site due to pin bending was reduced by increasing the diameter of standard 110 mm self-tapping pins (Orthofix, Verona, Italy) from 6 to 10 mm, using a tapered stainless steel sheath. Pin loosening was controlled by regular tightening with a calibrated torque wrench (Torqueleader, MHH Engineering, Bramley, England) to greater than 2 N or 2.5 N for metaphyseal and diaphyseal bone respectively.

Direct measurement of loads on the osteotomised bone is very difficult if not impossible. It is assumed that the axial load on the tibia, due to the mass of the animal and to muscle activity, remains roughly proportional to the ground reaction force during normal walking. A measure of healing is given by the fracture stiffness index (FSI) calculated in the following way:

\[ \text{Fracture stiffness index} = \frac{\text{ground reaction force}}{\text{fixator axial load}} \]

As healing progresses, and the bone carries proportionately more load, the FSI should increase. The vertical component of the ground reaction force was measured using a force-plate mounted under the belt of a treadmill (Fig. 2). At a velocity of approximately 0.5 m/sec, readings were taken from between 50 and 100 consistent steps. Signals from both the fixator bar and the force-plate were simultaneously recorded at a frequency of 50 Hz using a microcomputer (BBC Model B, Acorn Computers, Cambridge, England).

**Experimental procedure.** Fourteen female blackface sheep, averaging three years of age and weighing 45 to
65 kg, walked on the instrumented treadmill, after several training sessions. Thereafter they were randomly allocated to one of two equal groups: standard (well-vascularised) or devascularised. Each sheep was then intubated under general anaesthesia (halothane/nitrous oxide), given 1.5 g intravenous cefuroxime, and the fixator applied to the anterior aspect of the tibia using a special jig. The distance of the bar from the bone was 60 mm. The mean (+ standard deviation) axial stiffness of fixation chosen for this study was 240 ± 5 N/mm, with stiffnesses in all other planes high enough to ensure that other deflections were insignificant. Pins were applied through stab incisions after pre-drilling using a 4.8 mm bit and saline irrigation.

In the seven sheep in the standard (well-vascularised) group, a longitudinal incision was made over the medial subcutaneous border of the right tibia. The soft tissues were carefully protected with minimal extra-periosteal disturbance. The periosteum was incised transversely at a level 65% of the length of the tibia distal from the stiffe joint. The external fixator having been applied as above, a transverse osteotomy was performed between the innermost pins with a Gigli saw, leaving a 2 mm gap.

A similar osteotomy was performed in the other seven sheep but in these it was followed by circumferential stripping and excision of the periosteum for 20 mm proximally and distally. A 40 mm sleeve of silicone (internal diameter 12.5 mm, wall thickness 1.25 mm; Mackay and Lynn, Edinburgh, Scotland), was placed over both fragments to prevent revascularisation of the underlying cortex from the surrounding soft tissues. Wound closure was performed in layers using an absorbable suture of 3/0 Dexon.

The animals were given cefuroxime 500 mg by intramuscular injection, for three days after operation. Weight-bearing was allowed from the day of operation and the osteotomy was monitored two, seven and 14 days postoperatively. Each animal was then anaesthetised and lateral radiographs of the leg taken. Through a midline incision in the neck, the right carotid artery was exposed and cannulated with a Hilal-65 aortic catheter, passed into the left ventricle using a pressure transducer for reference. A measured activity of Cobalt-57 labelled microspheres (diameter 15 ± 0.5μm, half-life 267 days; DuPont Inc, Stevenage, England) was injected into the left ventricle over 30 seconds. Sufficient was administered to achieve approximately 100 to 150 microspheres per sample, which according to the work of Li, Bronk and Kelly (1989) allows estimation with an error of less than 10%. A reference sample was collected from a second catheter in the right brachial artery, attached to a withdrawal pump (Harvard Apparatus, Edenbridge, England), at a fixed rate of 0.852 ml/min for two minutes.

Each sheep was killed with 10 ml saturated potassium chloride, delivered into the ventricle. The hindlimbs were disarticulated through the stiffe joints and all the soft tissues were carefully removed. Samples of muscle, and metatarsal cortical bone and marrow were taken from each side for blood flow estimation.

**Mechanical testing.** The ends of the osteotomised and the intact contralateral tibiae were mounted in cups using Wood's metal and each preparation was placed in a torsional testing machine. The fixators were removed from the osteotomised tibiae only after the bones were secured, to avoid accidental damage to the early callus. In the devascularised group, the silicone sleeve was incised longitudinally and removed without disturbing the medullary contents. As each pin was removed, a swab sample was taken from the pin track and sent for bacteriological culture. All bones were tested to failure within two hours of death. Measurements of applied torque and angular displacement were made, to permit calculation of maximum torque (torsional strength), torsional stiffness and energy absorbed to failure.

**Measurement of regional blood flows.** After mechanical testing, the metaphyses, with their cancellous bone and ligamentous attachments, were discarded to avoid over-estimation of cortical bone blood flow. The diaphysis was then stripped of periosteum and sectioned transversely at 1 cm intervals. Superficial periosteum and fibrous tissue were removed from the surface of the early callus. The sections were divided into cortical and medullary fractions which, together with muscle from both compartments at the level of the osteotomy and metatarsal samples were each counted for 300 seconds in a gamma scintillation counter (LKB-Wallac, Turku Oy, Finland). Blood flows were determined from the ratio of tissue to reference sample activity, which may be arranged to give the formula:

\[
\text{Flow (ml/min/100 g) = } \frac{\text{tissue activity} \times \text{withdrawal rate} \times 100}{\text{reference activity} \times \text{tissue mass}}
\]

Statistical significance within each group, between osteotomised and intact tibiae, was assessed using the paired Student's t-test. Comparison between groups was made using the unpaired t-test.

**RESULTS**

All sheep were weight-bearing on the first day after operation. Complications were few. Clinical pin-track infection occurred in three of the 84 pins while 48 yielded positive cultures of predominantly mixed Gram-negative organisms, in spite of antibiotic prophylaxis. The remaining 33 pins were sterile. One sheep in the standard group sustained a fracture through the lowermost pin hole on the first postoperative day, and was therefore excluded from further consideration.

Although there was no statistically significant difference between the groups during in vivo monitoring, the trend indicated delay in healing in the devascularised group. In both groups the ground reaction force and stance-phase duration (time the hoof was on the ground) were lowest on the second postoperative day and
increased progressively to more than 50% of the pre-operative level by the 14th day (Table I). As weight-bearing increased in both groups, the devascularised group demonstrated a greater displacement of the osteotomy (mean 1.57 mm ± 0.14). There was also a slower increase in the FSI in the devascularised group (Fig. 3).

There was no difference between the mean blood flow of medulla and cortex of the tibial diaphyses of the two groups, although there were statistically significant increases in flow when compared to the contralateral intact tibiae (Table II). Medullary diaphyseal flow demonstrated a great increase to over 20 ml/min/100g, and cortical diaphyseal flow increased by a factor of five to more than 8 ml/min/100g in both groups of osteotomised tibiae. At the osteotomy site, medullary flow again demonstrated significant increases in both groups com-

![Figure 3](image_url)

Fracture stiffness index at 14 days, expressed as mean (± standard error).

| Table I. In vivo monitoring data expressed as mean (± standard error) in vascularised and devascularised tibia at 14 days |
|---------------------------------|------------------|------------------|-------------------|
| Ground reaction force (Nm)      | Stance phase duration (s) | Osteotomy displacement (mm) |
| Standard                        | Devascularised    | Standard          | Devascularised    | Standard          | Devascularised    |
| Pre-operative                   | 234 (± 12)        | 243 (± 23)        | 0.62 (± 0.05)     | 0.66 (± 0.05)     | -                 | -                 |
| Day 2                           | 95 (± 16)         | 102 (± 19)        | 0.38 (± 0.05)     | 0.44 (± 0.03)     | 0.99 (± 0.12)     | 0.96 (± 0.10)     |
| Day 7                           | 147 (± 29)        | 164 (± 24)        | 0.47 (± 0.05)     | 0.48 (± 0.05)     | 1.08 (± 0.17)     | 1.44 (± 0.14)     |
| Day 14                          | 152 (± 24)        | 193 (± 20)        | 0.52 (± 0.04)     | 0.55 (± 0.04)     | 1.25 (± 0.21)     | 1.57 (± 0.14)     |

| Table II. Regional blood flows expressed as mean (± standard error) ml/min/100 g at 14 days |
|---------------------------------|------------------|------------------|------------------|
|                              | Cortex | Marrow | Cortex | Marrow | Anterior | Posterior |
| TIBIAL DIAPHYSIS               |        |        |        |        |          |           |
| Standard                       | Experimental | 8.22 ± 1.40 | 24.92 ± 4.15 | 0.60 ± 0.25 | 1.31 ± 0.43 | 3.35 ± 0.67 | 4.01 ± 0.79 |
| Control                        | 0.75 ± 0.14 | 0.65 ± 0.12 | 0.32 ± 0.09 | 0.87 ± 0.21 | 1.93 ± 0.19 | 2.27 ± 0.25 |
| Significance (p)               | < 0.0005 | < 0.0005 | NS    | NS    | < 0.05   | < 0.05    |
| Devascularised                 | Experimental | 8.40 ± 1.85 | 21.23 ± 3.04 | 0.53 ± 0.16 | 1.03 ± 0.21 | 2.98 ± 0.72 | 3.14 ± 0.75 |
| Control                        | 1.46 ± 0.65 | 0.78 ± 0.20 | 0.47 ± 0.21 | 0.60 ± 0.23 | 2.70 ± 1.03 | 2.57 ± 0.71 |
| Significance (p)               | < 0.0005 | < 0.0005 | NS    | NS    | NS       | NS        |

pared to an equivalent level in the non-osteotomised bones. Cortical flow at the osteotomy site, however, was significantly lower in the devascularised group (p < 0.0005), remaining at 1.70 ml/min/100g (± 0.58) compared to 19.30 ml/min/100g (± 3.38) in the standard group (Fig. 4).

Muscle blood flow was similar in both compartments in both limbs in the devascularised group. In the standard group, there appeared to be a 'steal phenomenon' in favour of the experimental limb, with significantly increased flow (p < 0.05) in both compartments in which there was no barrier to cortical revascularisation from the periosteum and adjacent muscle (Table II). Metatarsal flow showed no differences, suggesting that the increases in medullary and cortical flow were confined to the bone with the osteotomy.

**Mechanical properties.** Following removal of soft tissues, there was evidence of external callus enveloping the fragments in the standard group. After removal of the silicone sleeve in the devascularised group, there was little more than organising haematoma to be found lying in the osteotomy gap and medullary canal. Up to 90°
rotation, there was no measurable torsional resistance in any of the seven devascularised osteotomies (< 0.02 Nm). The well-vascularised osteotomies, by contrast, exhibited a consistent linear slope to the torque-angular deformation curve prior to ‘failure’ (Fig. 5) with a mean 1.63% of the torsional strength of the intact contralateral bones (Table III).

The angular displacement to failure of 86.62% of the non-osteotomised bones suggests the presence of ‘rigid’ tissue. The energy absorbed to failure of 1.52% reflects the proportionately low volume of this material. However, it appears to fail at an angle close to that of non-osteotomised bone, and therefore probably represents areas of mineral deposition or new-bone formation, even at a very early stage of healing.

**DISCUSSION**

Following an experimental osteotomy, the normal vascular response of bone is an increase in flow which peaks at approximately two weeks. Flow is increased not only at the site of the osteotomy, but throughout the cortex and marrow of the diaphysis (Rhinelander 1968). Our results in the standard osteotomies confirm this phenomenon, as did previous measurements in our laboratory using the canine model (Strachan et al 1990). The increased perfusion would seem to be more than adequate for delivery of nutrients to the healing tissue.

However, human tibial fractures are usually associated with some degree of damage to the soft tissues surrounding the bone, even in closed fractures (Oestern and Tcherne 1984). Muscle has been shown to provide an important collateral source of blood to cortical bone
in both clinical (Byrd, Cierny and Tebbetts 1981) and experimental studies (Richards and Schemitsch 1989). Interference with perfusion of muscle may result in delayed union in tibial fractures (Court-Brown and McQueen 1987). Whiteside and Lesker (1978) demonstrated mechanical evidence of delayed union in rabbit osteotomies associated with muscle trauma.

When the medullary vessels have been divided by osteotomy or a displaced fracture, the periosteal circulation, augmented by vascular anastomoses from adjacent muscle, assumes dominance and results in centripetally-directed flow through the cortex (Rhinelander et al 1968; MacNab and de Haas 1974). This mechanism is clearly at risk in the event of significant muscle injury. The devascularised model used in our experiments demonstrates the vulnerability of cortical blood flow when the periosteum is circumferentially destroyed, and angiogenesis from muscle is prevented. Although such an extreme vascular insult is unusual in clinical practice, this study demonstrates the importance of an extra-osseous blood supply in the early stages of healing.

Medullary flow, although increased at the osteotomy site, did not appear to provide more than basal levels of cortical perfusion in the devascularised model used in this study. Mechanical testing revealed no evidence of medullary callus whereas, in the standard group, there was 'rigid' tissue in a well-vascularised external callus. In experiments on callus distraction in the rabbit, Kojimoto et al (1988) gave histological evidence of medullary callus formation at ten days after osteotomy, but we were not able to confirm this in the sheep model.

In vivo monitoring provided encouraging results at this early stage of healing and gave clearer evidence than radiographs of the delay in healing in the devascularised group. This was confirmed by mechanical testing. Further experiments at a later stage of healing are required but recent clinical studies have demonstrated the usefulness of similar techniques (Cunningham et al 1988). In vivo monitoring depends not only on the integrity of connections within the fixator assembly, but perhaps more importantly on a firm link between the pins and the bone, where plastic deformation due to pin bending may occur under axial loads (Klip and Bosma 1978). Because resistance to bending is a function of the fourth power of the pin radius, increasing the pin width significantly increases its stiffness (Kempson and Campbell 1981) and the validity of in vivo monitoring.

Our experiments suggest that in the early stage of fracture healing, enhancement by axial loading of the callus may depend fundamentally on the musculoperiosteal vascular reserve. The fixation system selected for this study relies on passive transmission of axial loads through the osteotomy site which will vary slightly from step-to-step and between different animals in the same group. Other methods of achieving dynamic external fixation include 'elastic' fixation which is reliant upon the pins bending under load, and active controlled displacements using mechanical actuators (Aro and Chao 1990). The calculated initial displacements in this study are consistent with those found to stimulate osteogenesis in the sheep tibia by Goodship and Kenwright (1985).

It has been suggested that with rigid external fixation, the clinical outcome of severe open tibial fractures is independent of the device chosen (Court-Brown et al 1990), but reflects the degree of devascularisation of the fractured bone. Clearly further attempts to quantify the vascular and mechanical parameters are required. Our experiments indicate that in early healing, the establishment of an optimal 'biological' environment, to revascularise cortical bone, for example, by muscle coverage, is necessary to maximise the osteogenic potential of dynamic external fixation.

This study was funded by a grant from Action Research for the Crippled Child.

The authors wish to thank Mr R. H. Fleming and Mrs B. C. Wyatt for their invaluable technical assistance.

Although none of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article, benefits have been or will be received but are directed solely to a research fund, foundation, educational institution, or other non-profit institution with which one or more of the authors is associated.

REFERENCES


THE JOURNAL OF BONE AND JOINT SURGERY


