Restoration of bone flow following fracture and reaming in rat femora

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In rats, bilateral closed femoral fracture was produced after intramedullary reaming to 1.6 mm on the left side and 2.0 mm on the right side. The fractures were fixed with 1.6 and 2.0 mm steel pins. Radioactive microspheres were used to determine bone blood flow at 30 min, 1 day, 3 days and 9 days after fracture. 8 rats were used to estimate normal bone blood flow, and an additional 8 rats to examine the vascular effects of fracture only.

Following fracture, total bone blood flow was reduced to about 50 percent and cortical flow to about 40 percent of that in intact bones. Fracture and reaming to 1.6 mm reduced total bone flow to 40 percent and reaming to 2.0 mm reduced the total bone flow to approximately one third of normal flow. Cortical flow decreased to about one third and one quarter in the 2 groups. On Day 1, total flow was practically normalized in both groups. Cortical flow in the 1.6 mm group was about equal to that of intact bones, while it was about one third of normal flow in the 2.0-mm group, and significantly less than the 1.6-mm group. On Day 3, total bone flow was more than double that of intact bones and cortical flow 3 times greater in both groups. Flow continued to increase to Day 9 when a threefold increase in total bone blood flow and approximately a fivefold increase in cortical flow were found. On Day 9, a separate callus area was defined and flow measurement revealed a rich vascularized callus in both groups, but no differences between the groups were found.

Following fracture, neither moderate nor aggressive intramedullary reaming seem to create any further impairment in bone flow. Following fracture and reaming, blood flow is rapidly restored, however, extensive reaming results in a delayed restoration of cortical bone blood flow. After 9 days, rich vascularized callus areas were found irrespective of the initial degree of reaming.

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Fractures of long bones are usually operated on by intramedullary reaming and nailing. Following a fracture, significant decreases in bone blood flow have been observed (Grundnes and Reikerås 1992). Reaming and nailing are supposed to impair further the medullary and endosteal circulations, and the effect of modest versus extensive reaming on bone flow in this situation is unknown. In a previous study on intact rat femora, bone blood flow was measured 30 min following different degrees of intramedullary reaming (Grundnes and Reikerås 1993a). Reaming to a diameter smaller than the medullary diameter had no significant impact on bone flow, while flow was significantly reduced when reaming was more aggressive. Furthermore, we have shown that fractures treated by modest reaming and loose-fit nails were superior in bending moment, bending rigidity and fracture energy at 4, 8, and 12 weeks compared to those treated by extensive reaming and tight-fit nails. However, bone blood flow was not different in the 2 groups (Grundnes et al. 1994). In the present study we have examined the restoration of femoral bone blood flow following fracture and different degrees of reaming for intramedullary stabilization.

Material and methods

A total of 56 male Wistar rats (Møllegård Avlslaboratorium, Eiby, Denmark) weighing 350 (309–374) g were used. Under intraperitoneal anesthesia (pentobarbital 5 mg/100g body weight), the trochanteric areas were exposed on both sides. From the trochanteric groove the medullary cavity was penetrated by an awl, and with steel burrs mounted on an electrical drill successively reamed to a diameter of 1.6 mm on the left side and 2.0 mm on the right side. Closed bilateral fractures were produced by first driving an awl percutaneously through the bone at the midshaft of the femur to weaken it and to avoid communition of the fracture.

Both bones were then manually broken. The fractures were standardized to the distal end of the trochanteric rim, which could easily be felt under the skin. Closed medullary pin insertion was performed by closed reduction with one hand while driving a steel pin mounted on an electrical drill from the trochanteric area through the fragments. On the left side, a 1.6 mm steel pin was used for fixation and on the right side, a 2.0 mm steel pin. Fixation was achieved without roentgenographic control.

8 rats were used to determine normal bone blood flow. In these rats both trochanters were exposed. However, no bone intervention was done. In an additional 8 rats, bone blood flow was estimated 30 min after closed bilateral femoral fractures. Both trochanteric areas were exposed prior to fracture. For bone blood flow measurements following reaming and fracture, 10 rats each were randomly assigned to be included in one of the groups: 30 min, 1, 3, and 9 days after fracture.

Radioactive microspheres (New England Nuclear, Boston, MA, U.S.A.) labeled with 85-Strontium of 15.5 ± 0.1 μm diameter were used for blood flow measurements, and each injection consisted of 750,000 spheres suspended in 0.9 percent saline. To obtain a homogeneous solution, the spheres were shaken on a whirl mixer for 2 min prior to injection. A heparinized polyethylene catheter (PE-50) was introduced via the carotid artery and placed in the aortic root for injection of microspheres. The microspheres were injected over a period of 30 sec, and the catheter was then flushed with 0.5 mL saline. The caudal artery was cannulated with a heparinized polyethylene catheter (PE-10) and connected to a Harvard infusion-withdrawal pump for reference sampling. The flow rate in the reference organ was set at a rate of 195 μL/min. Withdrawal started 15 sec prior to injection of the microspheres and continued for 30 sec after the injection was finished. In the 30 min group, blood flow measurement started 30 min after the operation.

1 rat in the 9 days group died during instrumentation. After the animals were killed in a CO_2 chamber, both hind limbs were dissected, soft tissues removed from the femurs and the bones were then wiped dry and weighed. For blood flow estimations, the bones were placed in counting vials and together with the reference samples counted in a Packard Auto Gamma Scintillation Spectrometer. Specimens were counted for 5 min, which gave a counting error of less than 1 percent. After the total bone flow was calculated, a diaphyseal segment of cortical bone proximal and distal to the fracture was separated. The medullary cavity was rinsed out and the bone segments were weighed and counted together with their reference samples for cortical flow estimations. The callus area was left undisturbed, except for the 9 days group in which a well-developed callus was found around the fracture site, which permitted a separate callus flow estimation in this group.

Data are presented as medians and 25–75 percentiles. For statistical evaluation, the Mann-Whitney U-test was used when comparisons between the intact and fractured groups were made, and for comparisons between each time interval. The Kruskal-Wallis test was used for comparisons between fractured bones and the 2 different groups of intramedullary reaming, the Mann-Whitney U-test was used when statistical differences were found. For comparisons within the groups at each time interval, the paired Wilcoxon sign-rank test was used. $P \leq 0.05$ was considered significant.

Results

30 min following fracture, total bone blood flow was reduced to about 50 percent of control flow and cortical blood flow to 40 percent (Table 1). Fracture and reaming to a diameter of 1.6 mm reduced total blood flow to about 40 percent of normal flow (P 0.022) and cortical flow to approximately one third (P 0.025). Following fracture and intramedullary reaming to 2.0 mm, total bone blood flow was approximately one third of control flow (P 0.014), and cortical bone flow one quarter of that in intact bones (P 0.014). Both total bone and cortical bone flows were insignificantly affected when reaming was performed after fracture, either reaming was done to a diameter of 1.6 mm or 2.0 mm. There were no differences in total bone or cortical bone flows between the two degrees of reaming.

Between 30 min on Days 0 and 1, total bone flow increased by 2.5 times in the 1.6-mm reamed bones (P 0.012), approximately doubled in the 2.0-mm reamed bones (P 0.022), and was practically equal to normal bone flow in both groups. There were no dif-

Table 1.Total bone and cortical bone blood flow (mL/min × 100g^{-1}) in control and fractured bones 30 min after fracture. Median (25–75 percentile)

Bone	Total bone flow	Cortical bone flow
Intact (n 16)	25 (18–27)	8.0 (3.2–9.5)
P-value	0.05	0.05
Fractured (n 16)	13 (5.8–19)	3.1 (2.1–4.5)

Table 2. Total bone blood flow (mL/min × 100g^{-1}) in fractured bones with different degrees of reaming at 30 min, 1, 3, and 9 days after fracture. Medians and 25–75 percentiles

Fractured and reamed	1.6 mm	*P*-value	2.0 mm
30 min (n 10)	10 8.8–15	0.2	8.7 7.6–13
1 day (n 10)	25 20–30	0.1	18 13–29
3 days (n 10)	56 41–63	0.1	57 42–76
9 days (n 9)	71 34–130	0.7	82 37–104

Table 4. Cortical blood flow (mL/min × 100g^{-1}) in fractured bones with different degrees of reaming at 30 min, 1, 3, and 9 days after fracture. Medians and 25–75 percentiles

Fractured and reamed	1.6 mm	*P*-value	2.0 mm
30 min (n 10)	2.4 1.7–4.5	0.3	2.0 1.6–2.7
1 day (n 10)	8.1 5.6–12	0.008	2.5 1.6–5.6
3 days (n 10)	24 20–33	0.8	24 14–32
9 days (n 9)	38 21–72	0.7	45 37–83

ferences in total bone flow between the 2 degrees of reaming on Day 1. Between Days 1 and 3, significant increases in total bone flow were found in the 1.6-mm group (*P* 0.013) and in the 2.0-mm group (*P* 0.002). On Day 3 total bone flow increased to more than twice that of normal blood flow in both groups (*P* 0.023). On Day 9, total bone flow was approximately three times the flow in intact bones (*P* 0.026 and *P* 0.013). However, there was no significant increase in total bone flow between Days 3 and 9 (*P* 0.248). No differences in total bone flow were found between the 2 groups at the different time intervals (Table 2).

Cortical flow in the 1.6-mm reamed bones significantly increased from Days 0 to 1 (*P* 0.011). In the 2.0 mm group, no increase was seen between Days 0 and 1 (*P* 0.408), and cortical flow was only 30 percent of that in the 1.6-mm group and 32 percent of normal cortical flow (Table 3). Between Days 1 and 3, significant increases in cortical bone blood flow were found in both groups (*P* 0.002), and at that time cortical blood flow had increased by approximately 3 times the control flow in the two groups (*P* 0.001). No differences were found between the 2 groups. On Day 9, cortical flow in the 1.6-mm group was not different from that on Day 3 (*P* 0.328), while cortical flow in the 2.0 mm group significantly increased between Days 3 and 9 (*P* 0.043). However, there were no differences between the 2 groups (Table 4).

Table 5. Callus blood flow (mL/min × 100g^{-1}) and callus weight (g) of healing bones 9 days after fracture and different degrees of reaming. Medians and 25–75 percentiles

	1.6 mm	*P*-value	2.0 mm
Callus flow	93 45–150	0.3	87 49–118
Callus weight	0.16 0.12–0.19	0.5	0.12 0.10–0.21

At 9 days, rich vascularized callus areas were found in both groups, but not different from cortical bone flow was present. Callus blood flow and callus weight were about the same in the 2 groups (Table 5).

Table 3. Separate data on total and cortical bone blood flows (mL/min × 100g^{-1}) with bone and specimen weights (g) for the 1 day rats

Specimen No.	Total bone 1.6 mm		Total bone 2.0 mm		Cortical bone 1.6 mm		Cortical bone 2.0 mm	
	Flow	Weight	Flow	Weight	Flow	Weight	Flow	Weight
1	10	0.83	17	0.80	14	0.16	4.9	0.14
2	30	0.90	22	0.83	12	0.21	2.0	0.20
3	45	0.80	29	0.71	11	0.16	6.6	0.16
4	7.2	0.86	6.3	0.83	4.6	0.18	1.3	0.20
5	26	0.81	31	0.81	7.8	0.20	7.8	0.16
6	20	0.75	19	0.81	5.3	0.14	1.5	0.18
7	29	0.76	30	0.76	5.6	0.19	6.4	0.16
8	23	0.76	13	0.83	8.0	0.19	2.3	0.17
9	37	0.81	7.8	0.75	14	0.16	1.6	0.15
10	23	0.85	17	0.89	8.2	0.21	2.7	0.20

Discussion

In the present study we found that fracture of the femoral bone in rats caused a significant reduction in total bone and cortical bone blood flows. Additional trauma to the bone vascular system in the form of reaming did not further significantly impair bone circulation at the acute stage. However, an important finding was a significantly faster restoration of cortical bone blood flow in the group which was modestly reamed. On the basis of dimensional data from previous studies in rats of comparable size, the two different dimensions were selected (Grundnes and Reikerås 1993a).

The use of the microsphere method for bone flow analysis is well established (Buckberg et al. 1971, Heyman et al. 1977, Flaim et al. 1984, Li et al. 1989). In small animals, such as rats, the number of spheres in each specimen may be too low to obtain accurate results. Li et al. (1989) found the error to be less than 10 percent when the number of microspheres per sample was more than 150. In our study no actual counts per sample were done, however, it can be calculated that of the injected number of 750,000 spheres each femur receives about 2,600 spheres, and the number of spheres in the specimens used for normal cortical flow analysis was about 430. Thus, in ischemic cortical bone at 30 min and in the 2.0 mm bones on Day 1, the number of spheres may be critically low and the hazard of not detecting small differences exists.

Several authors have outlined the importance of the blood supply in promoting osteogenetic cell differentiation (Trueta 1963, Whiteside and Lesker 1978, Simmons 1985). The prime function of the vascular response after a fracture has been attributed to the supply of nutrients necessary for the repair (Holden 1972, Trueta 1974, Kessler et al. 1986). Several factors important in promoting bone growth are thought to be derived from the vascular system (Simmons 1985, Hulth 1989). Furthermore, the presence of a blood supply directs these mesenchymal stem cells toward osteogenic development, whereas in the absence of blood vessels, chondrogenesis predominates. This is due to the phenomenon that low oxygen tension favors chondrogenesis (McKibbin 1978).

In a previous study we found that fracture healing was prolonged if extensive reaming was done. However, at 4 weeks after fracture, blood flow and callus formation were of the same magnitude in modest and extensively reamed bones (Grundnes et al. 1994). The inflammation phase may be most critical for the reparative phase of fracture healing. If serious impairment of the inflammation phase occurs, tissue healing may be compromised (Simmons 1985). The induction and proliferation of undifferentiated periosteal callus tissue is the first critical step in fracture healing by external callus. These periods of periosteal callus formation are finite (Cruess and Dumont 1975). In rats, the first occurrence of external callus has been observed from Day 2. After 8–10 days, growth is arrested, while waiting for the maturation of the cartilage (Henricson et al. 1987). Consequently, removal of the fracture hematoma some days after fracture will be detrimental to fracture healing, indicating that the first steps in callus formation are crucial (Grundnes and Reikerås 1993b). Thus, prolonged ischemia or hypoperfusion to cortical bone surrounding the fracture site in the first few days after fracture may cause sub-optimal conditions for the critical steps in organizing the fracture hematoma.

Various results have been obtained concerning the effects of intramedullary reaming on bone blood flow (Trueta and Cavadias 1955, Pfitzer et al. 1979, Indrekvam et al. 1992). In intact rat bones, total blood flow has reached normal values within 1 week after intramedullary reaming (Indrekvam et al. 1992). On the other hand, fractures are accompanied by perfusion disturbances of varying degrees, followed by a significant hyperemic response (Kessler et al 1986). Anastomosis between the periosteal and endosteal systems in the cortex have been demonstrated and, depending on the physiological conditions, blood can flow in either direction. Strachan et al. (1990) demonstrated a reversal of the normal centrifugal blood supply to the cortex to a centripetal pattern when the medullary circulation was occluded. In a fracture, it is not clear how long this situation persists because there is strong evidence that it may not be long until endosteal flow is re-established (Rhinelander 1974). Rhinelander (1974) also showed that smaller nails were less damaging to the endosteal blood supply and permitted a more rapid revascularization than reamed tight-fitting nails. Others have found no impact on restoration of flow due to the presence of an implant (Kessler et al. 1986, Indrekvam et al. 1992). An unexpected finding in the present study was that both total bone and cortical bone blood flows were restored 24 h after fracture and modest reaming to 1.6 mm. Since revascularization can hardly be responsible within this time interval, these results most probably are due to a reversal of flow, indicating the relative importance of the periosteal system even at the early stage of healing, as suggested by others (Macnab and De Haas 1974). On the other hand, if revascularization were due to the periosteal contribution, cortical flow should have been about equal in both groups. However, aggessive reaming demolishes the endosteal cortex. Apart from the circulatory interruption produced by intramedullary reaming and nailing, the production of a high medullary pressure and

occlusion of vessels by fat emboli and dust particles has been reported (Trueta and Cavadias 1955, Danckwardt-Lilliestrøm et al. 1970, Kessler et al. 1986). In the extensively reamed bones, we used 2.0 mm tight-fit nails which may be more effective in blocking the endosteal circulation, thus obstructing efferent flow in a reversed flow pattern. Similar effects have been observed after plate application (Perren et al. 1988).

Angiogenesis can be stimulated by so-called angiogenetic growth factors, and hypoxic gradients seem to be essential for the maintenance of angiogenesis in healing tissue (Knighton et al. 1983). Angiogenesis may be controlled by macrophages which produce angiogenetic factors under hypoxic conditions. Fracture callus and the medullary cavity during external callus formation show low oxygen tension (McKibbin 1978). We found increases in bone flow as early as 3 days after fracture. At this stage, the source of blood supply is extraosseous, regeneration of medullary arteries across the fracture site takes place within weeks (Rhinelander 1974). On Day 9, a firm definable callus was present in all fractures and flow studies showed rich vascularization in the callus areas. At the acute stage, angiogenesis due to hypoxic gradients may dominate. However, the hyperemic response seen after the first week must be due to other factors, such as increased metabolic demands in the healing callus. Flow was increased not only at the fracture site, but throughout the cortex. Our results confirm those of other studies on fracture healing (Rhinelander 1974, Wallace et al. 1991). The increased perfusion in this phase of healing would seem to be more than adequate for delivery of nutrients to the healing tissue. Furthermore, our results indicate that the external callus receives its major vascular supply from the periosteal and extraosseous tissues at the early stage of healing.

Except for the 9 days group, we used a mid-diaphyseal segment including the fracture area to estimate cortical bone flow. Thus, these specimens contained a mixture of cortical and callus flows. Separate blood flow determinations in the healing callus might be informative at the acute stage when cortical flow was marginal. However, at this stage the callus area was small and the possibility of obtaining inaccurate results due to a low number of spheres in each specimen exists (Li et al. 1989).

In conclusion, the present study indicates that fracture significantly reduces total bone and cortical bone blood flows. Neither moderate nor aggressive reaming following fractures seems to create any further impairments in bone flow. Both total and cortical flows are restored within Day 1, when modest reaming is performed, while cortical flow in extensively reamed bones is significantly reduced at this time. On Day 3, both total and cortical flows are restored, irrespective of the degree of reaming, and on Day 9, richly vascularized callus areas are found in both groups.

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