Tibial Bone Healing:
Experiments with External Fixation and Intramedullary Nailing

Thesis by

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List of publications

I External Fixation Compared to Intramedullary Nailing of Tibial Fractures in the Rat *(Sigurdsen UE, Reikeras O, Utvag SE. Acta Orthopaedica, 2009 Jun;80(3):375–9)*

II The Influence of Compression on the Healing of Experimental Tibial Fractures (Sigurdsen UE, Reikeras O, Utvag SE. Injury, in press; DOI: 10.1016/j.injury.2010.08.018)

III Conversion from External Fixation to Definitive Intramedullary Nailing in Experimental Tibial Fractures *(Sigurdsen U, Reikeras O, Utvag SE. Journal of Investigative Surgery, 2010 Jun;23(3):142–8)*

IV The Effect of Timing of Conversion from External Fixation to Secondary Intramedullary Nailing in Experimental Tibial Fractures *(Sigurdsen U, Reikeras O, Utvag SE. Journal of Orthopaedic Research, in press; DOI: 10.1002/jor.21182)*

V Correlations Between Strength and Quantitative Computed Tomography Measured Callus Mineralization in Experimental Tibial Fractures *(Sigurdsen U, Reikeras O, Hoiseth A, Utvag SE, Clinical Biomechanics, in press; DOI:10:1016/j.clinbiomech.2010.09.004)*

Abbreviations, technical terms and definitions

Adult respiratory distress syndrome	A syndrome characterized by progressive life-threatening respiratory insufficiency in the absence of known lung diseases, usually following a systemic insult such as surgery or major trauma.
ARDS	Adult respiratory distress syndrome.
Beam hardening	The change of the spectral distribution of polychromatic radiation when passing through matter.**
Biomechanics	The mechanical laws and the action of forces in living structures.
BMD	Bone mineral density.
Bone	A specialized connective tissue that is the main constituent of the skeleton. The principle cellular component of bone is comprised of osteoblasts; osteocytes; and osteoclasts, while fibrillar collagens and hydroxyapatite crystals form the bone matrix.
Bone density	The amount of mineral per square centimeter of bone. This is the definition used in clinical practice. Actual bone density would be expressed in grams per milliliter. It is most frequently measured by x-ray absorptiometry or tomography, x-ray computed. Bone density is an important predictor for osteoporosis.
Bone strength	The load that causes the bone to fail [unit: newton (N) or pound-force (lbf)].*
Calibration	Measurement for determining the individual detector channel sensitivity for each detector element of a CT system.**
Computed tomography	Tomography using x-ray transmission and a computer algorithm to reconstruct the image.
CT	Computed tomography.
CT number	The final result of the CT measurement and is given in Hounsfield units.**
Display window	Freely selectable range within the CT number scale displayed on the monitor screen and making use of the full range of brightness levels of the display unit; usually the display window is defined according to its window width and the window center; all pixels of the image matrix with a CT number above the window center plus one half of the width are displayed as white, while those below the center minus one half of the window width are displayed as black.**

Dual-energy x-ray absorptiometry	A non-invasive method for assessing body composition. It is based on the differential absorption of x-rays (or gamma rays) by different tissues such as bone, fat and other soft tissues. The source of (x-ray or gamma-ray) photon beam is generated either from radioisotopes such as gadolinium 153, iodine 125, or americum 241 which emit gamma rays in the appropriate range; or from an x-ray tube which produces x-rays in the desired range. It is primarily used for quantification of bone mineral content, especially for the diagnosis of osteoporosis, and also in measuring bone mineralisation.
DXA	Dual-energy x-ray absorptiometry.
EF	External fixation.
Elastic modulus	Also called modulus of elasticity; Numerical expression indicating the measure of stiffness in a material. It is defined by the ratio of stress in a unit area of substance to the resulting deformation (strain). This allows the behavior of a material under load (such as bone) to be calculated.
Elasticity	Elasticity is the "stiffness" of the material.* Resistance and recovery from distortion of shape.
External fixator	External device which hold wires or pins that are placed through one or both cortices of bone in order to hold the position of a fracture in proper alignment. These devices allow easy access to wounds, adjustment during the course of healing, and more functional use of the limbs involved.
Fibula	The bone of the lower leg lateral to and smaller than the tibia. In proportion to its length, it is the most slender of the long bones.
Fracture	Breaks in bones.
Fracture energy	Also called toughness, work to fracture and deformation energy. The amount of work done by the deforming load.*
Fracture fixation	The use of metallic devices inserted into or through bone to hold a fracture in a set position and alignment while it heals.
HA	Hydroxyapatites.
Hounsfield unit	Unit of the CT number scale; the Hounsfield unit expresses the relative deviation of the measured linear attenuation coefficient from that of pure water, multiplied by 1000.

HU	Hounsfield unit.
Hydroxyapatites	A group of compounds with the general formula $M_{10}(PO_4)_6(OH)_2$, where M is barium, strontium, or calcium. The compounds are the principal mineral in phosphorite deposits, biological tissue, human bones, and teeth. They are also used as an anticaking agent and polymer catalysts.***
IMN	Intramedullary nailing.
Internal fixator	Internal device used in osteosynthesis to hold the position of the fracture in proper alignment. By applying the principles of biomedical engineering, the surgeon uses metal plates, nails, rods, etc., for the correction of skeletal defects.
Intramedullary nailing	A type of internal fixators where the device is a bone nail.
Load	Load is a general term describing the application of force and/or moment to a structure.*
Magnetic resonance imaging	Non-invasive method of demonstrating internal anatomy based on the principle that atomic nuclei in a strong magnetic field absorb pulses of radiofrequency energy and emit them as radiowaves which can be reconstructed into computerized images. The concept includes proton spin tomography techniques.
Micro-computed tomography	X-ray computerized tomography with resolution in the micrometre range.
MRI	Magnetic resonance imaging.
Partial volume artefact	Artefact caused by severe inhomogeneities of the materials within the beam of the corresponding attenuation measurement (e.g. bone and air).**
Phantom	Object to test or evaluate the imaging quality of a CT scanner.**
Pixel	Abbreviation of picture element.**
QCT	Quantitative computed tomography.
Quantitative computed tomography	Clinical examinations with the purpose of quantitatively measuring geometrical, density, functional or other tissue or organ parameters.**
Radiography	Examination of any part of the body for diagnostic purposes by means of x-rays or gamma rays, recording the image on a sensitized surface (such as photographic film).
Region of interest	Subset of pixels which lie within an arbitrary (circular, rectangular

	etc.) geometrical shape at a freely selectable position within a 2D image.**
ROI	Region of interest.
Stiffness	Stiffness is the resistance offered by a structure when it is subjected to external loads.*
Strain	Strain (normal and shear) is the ratio of the change in length to the original length in a structure. It is specific to a point and a direction in the structure.*
Stress	Stress (normal and shear) is the force per unit area in a structure. It is specific to a point and a direction in the structure (unit: Pascal (Pa) or newtons per square meter (N/m^2)).*
Tibia	The second longest bone of the skeleton. It is located on the medial side of the lower leg, articulating with the fibula laterally, the talus distally, and the femur proximally.
vBMD	Volumetric bone mineral density.
Volumetric bone mineral density	Bone density.
Voxel	Synonym for volume element, for two-dimensional CT images the voxel volume is defined by the width of the side of the pixels and the slice width.**
Wolff's law	The principle that every change in the form and the function of a bone or in the function of the bone alone, leads to changes in its internal architecture and in its external form [Julius Wolff (1836-1902)].
X-ray absorption	Basic physical ability of a material to absorb x-rays and transform their energy into other forms of energy, such as visible light, heat or fluorescence; in diagnostic imaging this process is dominated by Compton scatter and photoelectric absorption.**
X-ray attenuation	The physical law which quantitatively describes the attenuation of the incident x-ray intensity, $I_{0,}$ when passing through a homogenous object of thickness, d, and linear attenuation coefficient, μ.**
X-ray tube	Source of x-rays for nearly all CT systems; the x-ray tube consists of an anode and a cathode enclosed in an appropriate vacuum vessel.**
μCT	Micro computed tomography.

All definitions from the U.S. National Library of Medicine's controlled vocabulary, MeSH, except when marked with *, from Panjabi and White 'Biomechanics in the Musculoskeletal System' (Churchill Livingstone 2001); **, from Kalender 'Computed Tomography' (Publicis Corporate Publishing 2005); or ***, from The American Heritage Stedman's Medical Dictionary (Houghton Mifflin Company 2002).

1 Introduction

Tibial diaphyseal fracture healing is a complicated concept. This thesis addresses issues concerning the fundamental knowledge of bone healing of tibial shaft fractures (herein the term ‘tibial fractures’ refers to tibial diaphyseal, or shaft, fractures). Everyone has probably experienced a bone fracture personally or among close family or friends. According to a UK survey, fractures of the tibial shaft represent around 2% of hospital-treated fractures, with an average patient age of 40 years [47]. Tibial fractures display a typical bimodal age distribution curve, being more common in young (usually males) and old (usually females) subjects. Tibial fractures range from closed, undisplaced fractures that can be successfully treated with a cast and orthosis, to open high-energy fractures with severe bone and soft-tissue damage that require complex surgical treatment, which can be followed by numerous complications and often a poor outcome [31,46,183,259]. Even though recent bone and fracture research has focused intensely on biomechanical [107], pharmaceutical [133], genetic [72] and molecular-biological [53] enhancement of fracture healing, these approaches have rarely been applied in the clinic. The role of the orthopaedic surgeon is still limited to preparing and supporting the built-in repair processes of the body by preventing deformity and avoiding impairment of fracture healing. To help to explain the purpose of the study and the questions raised within it, bone, bone healing and tibial fracture treatment and evaluation are briefly introduced below.

1.1 Bone

Bone is a highly specialized support tissue that is characterized by its rigidity and hardness, and it is the main component of the skeleton. Its tensile strength nearly equals that of cast iron, but it is three times lighter and ten times more flexible [26]. It has four main functions: providing mechanical support, permitting locomotion, providing protection and acting as a metabolic reservoir [128,134,224]. Its main components are the supporting cells (osteoblasts and osteocytes), a non-mineral matrix of collagen and glycosaminoglycans (osteoid), inorganic mineral salts deposited within the matrix and remodelling cells (osteoblasts and osteoclasts). Bone is constantly being remodelled in response to changing demands (via mechanical stress) and to maintain its structure. The remodelling process is coordinated by osteoclasts (which erode formed bone) and osteoblasts (which synthesize new osteoid). Moreover, bone is remodelled during the normal repair of a fracture. The deposition of mineral salts in the osteoid gives bone its characteristic rigidity and functional strength [225]. The main salt constituent is a crystalline complex of calcium and phosphate hydroxides called hydroxyapatites (HA) ($Ca_{10}(PO_4)_6(OH)_2$).

The collagen of the osteoid is the marker of the two histological (microscopic) types of bone: woven and lamellar. When osteoblasts produce collagen rapidly and with a lack of order, they form woven bone, which is biomechanically weak. Woven bone is present initially in all fetal bones, in the callus formed early after fracture, and in Paget's disease. Osteoblasts can form parallel sheets of collagen (lamellae), and the organized, lamellar bone is biomechanically strong. Virtually all bone in the healthy adult is of the lamellar type [225]. The human body includes five macroscopic types of bone: long bone (e.g. the tibia, which is the focus of this thesis), short bone (e.g. the scaphoid), flat bone (e.g. the scapula), irregular bone (e.g. the vertebrae) and sesamoid bone (small bones within tendons) [127]. The outer zone of most bones is called the cortical zone, or the cortex. The inner region in the middle portion of long bones (the middle portion is called the diaphysis or shaft) includes the yellow bone marrow, and the trabecular or spongy region is present at the epiphyseal ends, with a trabecular meshwork and red bone marrow.

1.2 Fracture healing

Fracture healing is a unique biological event or process in which a broken bone fully recovers [60]. This event is not fully understood and is so complicated that it is usually divided into different processes for teaching purposes. The three most important subprocesses are (1) inflammation, (2) repair and (3) remodelling.

The immediate response at a fracture site is a fracture haematoma that is crucial to the subsequent repair process. Experiments have shown that its removal by the surgeon impairs healing and leads to a weaker bone [85,86]. An inflammatory injury response is also induced, which lasts for several days.

The first repair step of the fracture haematoma involves osteoclastic resorption of dead bone tissue on the bony fragments. Osteogenesis is initiated by cells appearing in the granulation tissue that replaces the haematoma [26]. Bone forms in two ways. In the central regions of the bone, a soft callus is formed by chondrocytes that produce cartilage, calcium and calcification-promoting enzymes, in which bone subsequently and gradually replaces cartilage (enchondral ossification). Vascularization and neovascularization are essential for bone healing, and it has been shown that there is a vascular invasion in these areas of enchondral ossification [156]. Secondly, a hard callus is formed in the peripheral outer regions, in the periosteal area, where bone forms directly without the cartilage stage (intramembranous ossification) [59].

The remodelling phase involves the activation of remodelling units comprising osteoclasts and osteoblasts. Woven bone and unnecessary callus are resorbed by osteoclasts and chondroclasts, and osteoblasts produce bone with the above-mentioned characteristic Haversian, lamellar, strong structure [60]. The overall purpose of the bone healing process is to consolidate the fragments and remodel them back into sound bone. The ability of bone tissue to recover fully from injury (regeneration) is fundamentally different from that of skin tissue wounds, for example, which involve the formation of inferior scar tissue.

These subprocesses occur concurrently. Although the molecular mechanism underlying fracture healing is not fully understood, several of the involved growth factors and cytokines have been identified [60]. Even though fracture healing is a process that is considered mainly to affect the fracture site, it has been documented that it leads to significant changes in other parts of the affected bone and in other bones in the body, which are probably changes other than those predicted by Wolff's law [67,129,246]. In addition, other concurrent severe injuries to the patient, such as head injuries, must be considered when choosing a specific treatment for a tibial fracture, for example [78].

The outcome of the repair process depends on four mechanobiological factors: the fracture itself, gap conditions, blood supply and the biomechanical fracture environment [42,193,265]. For instance, a comminuted fracture heals more slowly than a simple oblique fracture, and bone healing is hampered if the gap exceeds a critical size. Animal studies have demonstrated that moderate soft-tissue trauma only temporarily impairs bone healing [41]. However, host comorbidity and severe soft-tissue damage affect the blood supply, and they must be assessed since they can substantially influence the healing of a fracture [241,243] and the optimal choice of treatment [239].

Four biomechanical stages of fracture healing have been described based on the results of torsional testing of the bone [258]: (stage 1) bone failure at the original fracture site; (stage 2) bone failure at the fracture site despite the characteristics of the failure indicating a high-stiffness, hard-tissue pattern; (stage 3) bone failure partly at the original fracture site and partly at previously intact bone with a high-stiffness, hard-tissue pattern; and (stage 4) no failure at the original fracture site, indicating that the new tissue that has formed at the fracture site at least replicates the mechanical properties of the uninjured tissue.

1.2.1 Biomechanical fracture milieu and stimuli

The effect of the biomechanical fracture milieu on fracture repair has been studied in numerous animal experiments. Both enhancing and impairing effects have been found, often when using technically complicated set-ups [75,95,132,150,192]. There is abundant evidence that fracture healing is influenced by mechanical loading [3,32,107,193,265]. Even though the subprocesses in bone healing are robust, they are sensitive to movement, stress and spatial relationships [4].

The timing, magnitude and direction of the biomechanical fracture stimuli exert crucial effects on the healing process. More specifically, limited interfragmentary movement (micromotion) in the early phase has a demonstrated positive effect on callus formation and may increase biomechanical stability, whereas the same movement during the late phase inhibits union, especially in fractures with relatively flexible fixation [80,107,124]. Seventeen minutes of daily cyclic load in sheep tibial fractures with external fixation (EF), starting 1 week post-operatively, was found to increase callus formation, fracture and torsional stiffness, and fracture gap bridging with more-mature bone tissue [81]. However, very early fracture loading [75] and early full weight-bearing of fractures with relatively flexible fixation [10] reportedly impair fracture healing, while the effect of early dynamization of externally fixed fractures is more questionable [4,40,56,146]. In one dog study, a group of axially dynamized externally fixed fractures had healed similarly to a non-dynamized group at 13 weeks post-operatively [83]. The combination of temporary axial distraction and compression from post-operative days 7 to 19 in sheep tibial diaphyseal osteotomies increased both fracture stiffness and callus formation [38]. Animal experiments have shown that the optimal axial interfragmentary movement seems to be within the range 0.2–1.0 mm [43]. Qualitative analyses have suggested that especially shear movements may impair the healing process [265]. Shear movements can substantially delay experimental bone healing with reduced callus formation [6,66,207], and are generally considered unfavourable in clinical fracture treatment.

Experimental fracture healing research is no longer limited to biomechanical or simple pharmacological set-ups. The number of research results and progress reports related to translation research has recently grown substantially, and such research is now considered of significant importance to basic and clinical research [61]. Bone healing research now comprises genetics, immunology, molecular-biology, and advanced mathematics and computational models based on physics principles [53,70,117,130,214,222,226]. The fracture problems and technological advances both necessitate and facilitate interdisciplinary research teams [35,37,108].

1.2.2 Primary and secondary bone healing

Bone healing and fracture treatment are obligatorily intertwined, since the choice of fracture treatment provides the framework for bone healing. The different surgical techniques employed provide different biomechanical stability and stiffness as well as different complication profiles. Two of the most important fracture treatment principles are providing apposition and aligning bone fragments, and subsequently a certain level of stability to support bone tissue healing [39]. It was documented over half a century ago that fracture healing is influenced by the mechanical fracture milieu [122,265], but the optimal combination of biomechanical factors for bone healing remain unclear.

Two distinctly different morphological fracture healing patterns have been identified as being connected to biomechanical factors: primary and secondary bone healing. The common case of bone fragments being aligned and relatively moderate interfragmentary movements usually produces the pattern of secondary bone healing, which comprises a combination of intramembranous and endochondral ossification characterized by the formation of a visible external callus on an x-ray [43,81,193,217,221,264]. Such development of immature bone reduces the relative movement between the fragments, with the increased stability resulting in cortical bridging [170,198].

Primary bone healing occurs in the less-common situation of the internally rigidly fixed fracture with minimal or no fracture gap and little or no interfragmentary movement [60]. The main process in this case is remodelling by osteoclasts and osteoblasts units. The second histological subprocess of healing is absent, and an external callus is not evident on an x-ray. It was demonstrated early on that such absolute rigidity could be achieved with internal compression, for example, without degradation or necrosis of bone at contact areas, since bone can withstand a substantial amount of stress for a long time without complications [193].

These different healing patterns have been explained by Perren's interfragmentary strain theory, which proposes that the type of tissue formed in bone healing (fibrous, cartilage or bone) is dictated by the actual strain imposed and the ability of the tissue to tolerate this strain [193]. This was commented on by Carter *et al.*, who hypothesized that the type of mechanical stress also dictated what type of tissue is formed at a fracture site [28]. Consistent with this, the bone–implant stiffness has been shown to exert significant effects on healing in animal experiments. Destabilization of the external-fixator stiffness exerts healing effects similar to dynamization [2]. A change to a more flexible nail in rat femora after 30 days resulted in greater callus production but reduced the ultimate bending load [244]. Greater callus and higher stiffness were also observed with increased fixator frame stiffness in externally fixed fractures in sheep [82].

1.2.3 Non-union

An intuitive assumption is that bone healing will be hampered and may even fail if reduced stability results in excessive movement between the bony fragments. Even though most fractures at different sites of the skeleton normally heal within 3 to 4 months, delayed union or, in the worst case, non-union may result for several reasons, including tobacco use, poor metabolic and nutritional status, or excessive interfragmentary movement [26,44,168]. Excessive mechanical manipulation of a fracture during healing has been used as an experiments model of non-union [240]. Infection, which is especially frequent in open fractures, can also disturb bone healing so as to result in delayed union or even non-union [109,180].

Treatments of fracture non-union that were previously impossible have now become possible. If primary union fails, the orthopaedic surgeon often manages to create a bony union after one to three revision procedures using exchange nailing or plating with or without bone grafting, but this represents a formidable challenge [50,110]. No generally accepted definition of union exists [44,73,161], but the Weber-Cech classification is widely applied to non-unions as a basis for selecting the most appropriate surgical treatment plan [112].

1.3 Tibial fracture treatment in the clinic

The tibia is the second largest of the 206 bones in the human body. It is part of the appendicular skeleton and it is located on the medial side of the lower leg, articulating with the fibula laterally, the talus distally and the femur proximally. It is a long bone characterized by a middle part called the shaft, or diaphysis. Of special interest to the healing of tibial fractures are the organization of the surrounding soft tissue and the lack of a soft-tissue envelope on the anteromedial side of the bone. As explained above (see Section 1.1), the fracture healing process is dependent on many factors, including the characteristics of the surrounding soft tissue, and a tibial fracture can therefore be among the most difficult types to treat.

Classifying tibial fractures is not a purely academic procedure – accurate fracture classification is generally necessary for a correct diagnosis and for the development and standardization of the best treatment and accurate determination of the prognosis. As for other bones, numerous fracture classifications have been proposed. The OTA (Orthopaedic Trauma Association) classification of tibial diaphysis fractures is presented in Figure 1 [162]. In 1976, Gustilo and Anderson [88] classified open fractures into three types: (type I) an open fracture with a wound shorter than 1 cm and clean; (type II) an open fracture with a laceration longer than 1 cm but without extensive soft-tissue damage, flaps or avulsions; and (type III) an open segmental fracture, an open fracture with extensive soft-tissue damage, traumatic amputation, gunshot injury or farm injury, or any open fracture with accompanying vascular injury that requires repair. The main difference between types II and III is they reflect low- and high-energy injuries, respectively. In 1984 [89], type III was further subdivided into three subtypes: (type IIIA) adequate soft-tissue coverage of a fractured bone despite extensive soft-tissue laceration or flaps, or high-energy trauma irrespective of the size of the wound; (type IIIB) extensive soft-tissue loss with periosteal stripping and bone exposure (this is usually associated with massive contamination); and (type IIIC) open fracture associated with arterial injury requiring repair. As mentioned above (see Section 1.2), open fractures have a worse prognosis than closed fractures, and adequate attention to the soft tissues is therefore essential to obtaining a satisfactory outcome [69]. Soft-tissue injuries have been classified by Tscherne and Oestern [236], among others.

Many different surgical techniques have been promoted in recent decades [27,45], but there is still considerable controversy regarding the optimal method of skeletal stabilization in open tibial fractures [113]. Surgical techniques may include internal fixation by screws, plating or intramedullary nailing (IMN) with or without reaming [177], or EF implemented by different arrangements of pins and frames. Severe comminution of bone or bone segment defects may additionally require the use of bone graft techniques. Plate fixation has been associated with implant failures [74], non-unions and deep-infection rates as high as 35%, and requires a long period of non-weight-bearing [12]. Often the surgeon has to choose between stabilizing the tibial fracture by IMN or by EF [102,106,182].

1.3.1 External fixation

The external fixator was first presented as a concept in the mid-19th century [248]. Several different pin-and-frame configurations and constructions with different biomechanical qualities have been used and promoted [57,167,219]. The stiffness of the bone–implant system has a documented effect on fracture healing [34,264], and the surgeon's competence and knowledge of (especially) biomechanics and principles for successful application significantly affect the outcome of EF treatment [79]. EF has been promoted numerous times in clinical studies since its refinement and improvement in 1938 by R. Hoffmann [14,33,119,120,140,145,247], and tibial fractures are now the main area of application of EF. In cases with an unstable fracture and severe soft-tissue damage with an increased risk of infection**,** the use of EF provides advantageous soft-tissue management, relatively stable fixation without additional soft-tissue stripping, early range of motion of both the knee and ankle, and unique adaptability to diverse fracture patterns [22,237]. This has recently led to EF being called the gold-standard treatment for open tibial shaft fractures [101].

The disadvantages of EF are unfavourable cosmesis, frequent pin-tract problems (e.g. infection), the potential for neurovascular injury during pin insertion, pin loosening and the potential for fracture through a pin tract [84,195]. In addition, the reduced compliance of many patients combined with the long time to achieve union may interfere with care of the pin tract and fixator durability [49]. In temporary initial EF, pin-site granulation and the possibility of pin-tract infection are arguments in favour and against secondary IMN, respectively [163,257]. Whilst planned early conversion to locked IMN is regarded to be a safe treatment [154], reconstructive secondary nailing[1] has strict contraindications [233].

The main target of (temporary) EF, which has recently been advocated by several publications on damage-control surgery [96,173,197,205], is to identify severely damaged patients (borderline and unstable) and postpone traumatic definitive surgery for 4–6 days to lower the risk of life-threatening complications, such as ARDS (adult respiratory distress syndrome) [186,188]. Even though the additional complications of IMN in major trauma cases have proved difficult to reproduce and investigate [260], a recent trauma-bank study in the United States indicated that delayed internal fixation of femoral fractures in multisystem-trauma patients reduced mortality by approximately 50% [171]. The use of EF in long-bone leg fractures of such patients seems to be non-traumatic, effective, time-saving and safe as an initial fracture treatment, and research data suggest that EF significantly reduces pulmonary complications [92,178,189–191,228]. Many questions remain unanswered concerning the definitive treatment for a temporary externally fixed fracture [54]. However, a secondary conversion to IMN after a short period when adequate soft-tissue coverage is acquired is often the definitive treatment of choice [1,19,51,216,267].

1.3.2 Intramedullary nailing

Intramedullary implantation of the classical Küntscher nail after reaming provides good stability against bending and shear forces perpendicular to its long axis, but this method is rather inefficient against torque and is unable to prevent axial shortening. Improvements in nail design [15,99,223], such as the use of locking, provide better stability to torsional and axial loading [21,48,123]. The clinical advantages of interlocked intramedullary fixation include high patient acceptance, favourable cosmesis, access for soft-tissue care, secure control of alignment and rotation, early mobilization and the potential for biomechanically safe early weight-bearing [138,139,229]. IMN has become the standard of treatment for closed, unstable, but otherwise uncomplicated femoral and tibial shaft fractures [126,263].

Frequent arguments against IMN of open tibial fracture are based on the potential for the spread of infection throughout the medullary canal and the further disruption of intramedullary bone circulation, especially when reaming is performed [136,194,208]. Anterior knee pain has also been reported following nail insertion. The reaming of the long-bone marrow canal can lead to heat-induced cortical damage (thermal necrosis) [13] and has significant unwanted effects on the physiology of fracture healing, especially the coagulation system and pulmonary permeability [97]. The tibia bears weight without having the extensive soft-tissue envelope of the femur, which makes it more vulnerable to infection that may lead to delayed union or non-union. However, the impact of these factors has lessened with the development of the unreamed locked intramedullary nail [36,206,268] and the documented efficacy of early bone grafting and muscle flap coverage when needed

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[1] Reconstructive secondary nailing is indicated in patients with EF for an extended period with delayed union, malunion or non-union.

[234,235]. Although still debated [68], clinicians now seem to prefer unreamed tibial nails to reamed ones in open tibial fractures [71,113,114,121,144,213].

Despite the complications, many recent authors have stated that the locked intramedullary nail has become the standard treatment for open tibial fractures, since IMN appears to be associated with a higher bone union rate and a shorter time to full weight-bearing compared to EF [17,113,232,261].

1.4 Experimental fracture enhancement

Several experimental protocols for potential mechanical and biological fracture enhancement have been investigated in fracture research. In experiments, researchers can control and manipulate the variables either *in vivo* or *ex vivo*, with the study object being either human or non-human.

Constantly compressed healing dog tibial osteotomies showed the same maximum torque but increased stiffness compared to non-compressed osteotomies [91]. Osteotomies in rabbits exposed to cyclic loading performed better than those with constant compression, but only temporarily [185,262]. Biological enhancements include autografts, allografts, calcium ceramics, the use of demineralized bone matrix, platelets, growth factors such as bone morphogenic proteins, parathyroid hormone and bone-marrow injection [100,252], of which demineralized bone matrix is the most commonly used in the clinic today [111]. Growth factors are osteoinductive and promote fracture healing [142]. Even though animal experiments have demonstrated that the local application of growth factor does not alter the normal long-term healing process [209], pharmacological substances are not routinely used for fracture repair enhancement in the clinic.

1.5 Evaluation of fracture healing

Usually the most important biomechanical parameter that needs to be restored after a fracture is the bone strength. The gold standard for evaluating mechanical fracture healing is mechanical testing of the bone to failure. Of course, such mechanical testing is not an option in the clinic, with instead the orthopaedic surgeon having to rely on surrogate parameters to monitor the fracture healing. The ageing populations of Japan, Europe and North America with osteoporosis together with economic developments in South East Asia, South America and Africa imply that more fractures will need to be treated in the future. Osteoporosis and diabetes are considered to complicate fracture treatment [166,181], while the surgeon often faces pressure to remove patient restrictions and fracture fixation implants early. These factors increase the need for accurate non-invasive fracture monitoring both in the clinic and in experimental research.

In intact bone, quantitative x-ray-based imaging techniques such as single-photon absorptiometry, dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) provide densitometric measurements that have been experimentally demonstrated to be both accurate and strongly associated with bone strength [157] and the risk of fracture [176]. The callus calcium content at the fracture site has been shown to be correlated with mechanical strength in histological studies [196]. Whilst it is possible to measure calcium levels using x-ray-based imaging techniques [5,200], it is also known that bone strength depends on more than the raw amounts of radiopaque bone minerals, such as bone geometric and microstructural properties [11] as well as the properties of the surrounding soft tissue [175]. In addition, quantitative magnetic resonance imaging (MRI) has demonstrated promising results in non-invasive assessments of cortical and trabecular bone [255]. Of these, DXA is commonly used in clinical practice to identify at-risk patients who may be treated with bone-strengthening medications. Even ultrasound imaging has been used for evaluating fracture healing, although not quantitatively [203]. There has been intense technology-driven research activity in the area of bone healing in recent decades [77], but none of the findings so far have altered traditional methods of fracture-healing evaluations applied in clinics.

1.5.1 Mechanical testing

The gold standard for evaluating long-bone fracture healing is mechanical testing of a fracture to failure by bending or torsion. Various types of bending testing can be employed, such as three-point, four-point or cantilever type. Mechanical testing to failure by compression, tension, bending or torsion provides exact measurements of biomechanical properties. In biomechanics, an important distinction is made between structural and material properties.[2]

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[2] The distinction between structural and material properties can be illustrated by considering two different human long bones. Assuming that human bone tissue exhibits constant material biomechanical properties (bone tissue is in fact viscoelastic, which means that it changes properties under different conditions, but we will ignore this for now), different structures with obvious different sizes and configurations (e.g. tibia or phalanges – a bone of a finger or toe) constructed from this material may have different structural biomechanical properties. In another words, even though both the tibia and the phalanges are made from the same material (bone), they have different strength and stiffness due to the wider cortex and a larger diameter of the tibia.

Amongst the most important biomechanical structural parameters in bone healing research are the ultimate load, stiffness, and fracture energy or work to fracture. These parameters can be measured by bending a bone to failure. The corresponding parameter in a torsional test is called the ultimate torsional load or torque. The classic load–deformation diagram is essential for calculating structural biomechanical properties [184]. Figure 2 demonstrates the load–deformation curve of an arbitrary and intact rat tibia tested with cantilever bending to failure using a previously described test set-up [65]. This curve gives information about (a) the bone strength or maximum bending load (y value of the maximum point of the curve), (b) the stiffness of the bone (the slope of the tangent of the curve) and (c) the fracture energy (the area below the curve), which is the energy absorbed by the bone before a fracture or irreversible deformation occurs. In the following, 'bone strength' implies the ultimate cantilever load (except where stated otherwise).

Stiffness has been proposed as both a surrogate for and a definition of healing [204], but its clinical relevance is low. As mentioned above (see Section 1.5), the clinically valuable biomechanical factor for the patient and the surgeon is ensuring a high ultimate load, which is the ability of the bone to resist high loads without failure or irreversible deformation. Ethical considerations make it impossible to measure this ultimate bending strength in the clinic, which has led to fracture evaluations being performed routinely in animal and cadaveric experiments.

In biomechanics, the rigidity or elastic modulus (modulus of elasticity) corresponds to the structural property of stiffness. Elasticity is the 'stiffness' of the material [184]. Calculating biomechanical material properties relies on the use of a stress–strain diagram. Converting a load–deformation curve into a stress–strain curve requires knowledge of and attention to the size and shape of the chosen specimen and the type of test (compression, tension, bending or torsion) applied. Stress is the internal reaction that is equal in magnitude but opposite in direction to the applied, external force or load [58]. The term 'strain' is used to describe the displacement or deformation of the bone under the influence of an applied force.

1.5.2 Clinical testing – patient examination, history and x-rays

Evaluations of clinical fracture healing by orthopaedic surgeons has remained largely unchanged since the discovery of x-rays in 1895 by Wilhelm Conrad Roentgen (1845–1923). A few important parameters are considered: time from fracture and fracture treatment, patient examination with manual manipulation, patient history, and the acquisition of standard two-plane x-ray images (front and side projections). The bone strength is restored by the occurrence of a clinically verified bony union, which the surgeon can verify from a mechanically stable fracture site and the patient being pain-free. Even though the results of quantitative, photometric assessments of standard x-ray images have been shown to be correlated with the rigidity in EF-treated segmental diaphyseal defects [231], x-ray-based investigations only allow qualitative clinical follow-ups of fracture healing. Bony union can be clinical or radiographical. Radiographically verified bony union is usually indicated in plain radiographs by trabecular bony meshwork and cortical bone crossing the fracture site. Clinically verified bony union usually precedes radiographical union [26].

1.5.3 Dual-energy x-ray absorptiometry

DXA analyses use radiographic attenuation to calculate bone mineralization. It is both quantitative and more sensitive to mineralization changes than is the standard x-ray. It can quantify the bone mineral content, the amount of mineral in a bone or part of a bone (in grams), and bone mineral density (BMD) (in milligrams per square centimetre) on the basis of the bone area. The accuracy and precision of DXA are very high, though some factors need to be considered carefully, such as the requirement for careful positioning of the scanned object [151]. DXA does not measure the true BMD, since it measures relative to bone area rather than bone volume. However, this examination has such a well-documented high level of precision and predictive ability of fracture risk when applied to intact bones [159] that it is well established in clinical practice and is the most widely used examination of densitometry in the clinic.

Whilst several studies have found statistical correlations between DXA measurements and biomechanical properties in callus measurements [20,160], and DXA provides an accurate method of quantifying the changes in BMD that occur during fracture healing [29], there is currently little support for the use of DXA in clinical evaluations of fracture healing [30].

1.5.4 Computed tomography

Tomography is a word derived from two Greek words: tómos (a cut or section) and graphos (something drawn or written, or one who draws or writes). The theoretical idea of reconstructing the distribution of the material properties from an object layer was reported by the Austrian mathematician Johann Radon in 1917 [199], whose name is preserved in the term ‘Radon transform’. Evaluating computed tomography (CT) images is an everyday exercise in clinical orthopaedics. The data obtained in a typical CT scan are usually presented as a collection of images (slices) from a clinically interesting site, perpendicular to one or several anatomically important axis (e.g. coronal, sagittal or transverse images), or as a three-dimensional (3D) reconstruction of one or many anatomically important structures.

The additional value of QCT over CT can be explained by providing a brief introduction to the physics underlying CT. A CT system directly measures the x-ray attenuation, μ. Attenuation, P, is defined as the natural logarithm of the ratio of primary intensity, I0, to attenuated intensity, I [116]:

$$P = \ln(I0 / I)$$

A CT system measures I and I0. The distribution of the attenuation coefficient within the scanned object can be defined as $\mu = f(x, y, z)$. Furthermore, the attenuation can be expressed as an integral of the attenuation coefficients along the ray path or line along which an x-ray beam travels. This problem can then be viewed as finding N^2 unknown values in an N×N matrix, and solved by solving the N_x independent equations that arise from the attenuations measured along the different projection scans, usually in an iterative manner. This algebraic reconstruction technique is valid if the product of the number of projections and data points is larger than the number of unknown attenuation coefficients. In other words, many different x-ray images taken through the object from different angles is required to measure and calculate the spatial distribution of the attenuation coefficients within the scanned object in order to construct the digital CT images.

The CT image consists of two-dimensional (2D) pixels or 3D voxels. Every pixel or voxel is represented by a value called the CT value or CT number, whose unit of measurement is the Hounsfield unit (HU)[3]. The relationship between the CT value, tissue attenuation coefficient μ_T and HU can be expressed as

$$\text{CT value} = (\mu_T - \mu_{water}) / \mu_{water} \times 1000 \text{ HU}$$

The attenuation coefficient itself is not particularly useful since its absolute value is very dependent on the radiation energy of the system. The Hounsfield scale is based on the values for water and air: pure water and water-like tissue are given a value of 0 HU, while air has a CT value of –1000 HU. Lung and fat tissue, which have relatively low mass densities, exhibit negative CT values. As mentioned above, the attenuation coefficient is dependent on both the mass density and effective atomic number. The CT value is normally converted into an HA density by simultaneously scanning phantom materials [212] with known HA densities and subsequent linear transformation, which is considered a robust technique [174].

CT provides unsurpassed accuracy and visual demonstration of bone tissue. In the clinic, 3D reconstructions and construction of slices in any plane are used for fracture configuration outlining and qualitative assessments of fracture healing. However, the use of CT in clinical research is restricted by the associated radiation dosage, which prevents its routine use despite it being able to detect defects in the callus not seen on an x-ray [25].

CT provides both direct visualization of calcium tissue and the quantification of calcium, whereas MRI only indirectly visualizes calcium as a signal void. Thus, the use of CT has not diminished following the introduction and widespread availability of MRI. Multislice CT, also called volume CT, provides increased geometric resolution, shorter scan times and, most importantly, superior slice reconstruction possibilites. Both CT (especially QCT) and MRI can provide high-precision quantitative results with many applications [76,149]. For example, QCT can discriminate well between intact and previously fractured wrists [211]. Moreover, the large differences in CT densities between calcified bone and soft tissue mean that QCT can be performed with low radiation doses.

QCT further provides for the accurate assessment of true volumetric bone density [87,201], geometric measurements [24,137,166] and tissue differentiation – especially between calcium tissues, fat and other soft tissues, and quantification of bone density – based on the segmentation of CT values [116]. Studies have documented that compared to DXA, CT provides more accurate measurements of densitometry and stronger associations between cortical, metaphyseal [152] and trabecular bone scans and biomechanical properties [172]. Microarchitectural features of bone [11] have until recently been considered to be unattainable by image analysis methods, but statistical correlations between CT values and relevant parameters have recently been discovered [230], consistent with the strong covariation between biomechanical properties and microstructural properties such as the osteon area, osteon density, porosity and interstitial area in intact bone [238]. For example, the cross-links in collagen do not affect densitometric

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[3] The HU and the Hounsfield scale are named after the inventor of CT, Godfrey N. Hounsfield, who was awarded the 1979 Nobel Prize in Medicine.

measurements, but they play an independent role in bone strength [256]. Cadaveric studies and animal experiments have confirmed that interesting statistically significant associations between QCT and biomechanical properties exist also in metastatic bones [118] and bone grafts [202], and that QCT can be combined with finite element models to accurately predict internal bone stress under different loading scenarios [227]. Dynamic bone mineralization occurs in healing fractures, and QCT can detect small changes in bone mineralization, which has been statistically correlated to qualitative image assessments of healing in distal radius fractures [153]. Moreover, although the results are diverse [75,108], interesting investigations on the correlations between fracture-site measurements by DXA and QCT and its derivatives and biomechanical properties have already been reported [9,16,20,30,52,160,210,215,220].

Secondary bone healing (the most common form of diaphyseal healing in the clinic) is characterized by the dynamic healing of bone tissue with callus formation, callus resorption and cortical remodelling, and QCT could theoretically be used to monitor fracture healing by segmenting the obtained images into bone tissue categories with different degrees of mineralization, and studying them separately with regards to correlations with biomechanical parameters.

2 Purpose of the present study

The main purpose of the present study was to increase knowledge on bone healing in tibial fractures treated by EF and IMN by using an experimental rat model evaluated by DXA, micro-computed tomography (micro-CT) and mechanical cantilever testing. More specifically, five experiments were performed with the following aims:

I To compare bone healing in tibial diaphyseal fractures treated with EF and IMN.

II To evaluate the effect of early compression on bone healing in externally fixed tibial diaphyseal fractures.

III To study bone healing in tibial diaphyseal fractures treated with secondary small- and large-diameter intramedullary nails after initial temporary EF.

IV To study the effect of timing on bone healing in secondary IMN of tibial diaphyseal fractures after initial temporary EF.

V To study the statistical correlation between bone strength and segmented QCT data in internally and externally fixed tibial diaphyseal fractures.

3 Summary of publications

Publication I: External Fixation Compared to Intramedullary Nailing of Tibial Fractures in the Rat

Forty male rats were subjected to a standardized tibial shaft osteotomy and were randomly assigned to two treatment groups: EF (*N*=20) or IMN (*N*=20). Half of the animals in each treatment group were evaluated at 30 days, with the remaining half evaluated at 60 days; the evaluations included x-ray, DXA and mechanical cantilever testing. Radiographically, both treatment groups showed signs of fracture healing with gradual bridging of the fracture line, while in the IMN group the visible collar of the callus appeared increased peripherally, which was indicative of periosteal healing. At 30 days, densitometric and mechanical properties were similar in the two treatment groups. However, at 60 days the bone strength was greater, the callus was larger and the bone mineral content in the callus segment was higher in IMN fractures than in EF fractures. Tibial shaft fractures showed similar healing patterns in the early phase of fracture healing in the rats treated with EF and IMN, while at the time of healing both densitometric and mechanical properties were better in IMN than in EF. Clinical findings indicate that bone healing after human tibial fractures may be better in IMN than in EF.

Publication II: The Influence of Compression on the Healing of Experimental Tibial Fractures

Sixty male rats received a standardized tibial shaft osteotomy stabilized with a unilateral external fixator with zero interfragmentary distance and were randomly assigned to the compression (N=20), control (N=20) or distraction (N=20) group. From days 4 to 14 the external fixator in either tightened (compression group) or loosened (distraction group) once daily to gradually induce a total axial displacement of the external fixator pin-clamps of 1.25 mm. The control group received a sham manipulation. Evaluations at 30 and 60 days included x-ray, DXA, QCT and mechanical cantilever testing. Compared with the controls, compression did not enhance fracture healing in terms of mineralization, bending strength or stiffness at the time of union. Compared with the distraction group, the compression and control groups exhibited improved healing in terms of mechanical strength and stiffness, and more-mature callus mineralization.

Publication III: Conversion from External Fixation to Definitive Intramedullary Nailing in Experimental Tibial Fractures

Thirty male rats were subjected to a standardized tibial shaft osteotomy initially stabilized with EF. On day 7 they were assigned to the control group (group A, *N*=10) or to conversion to secondary nailing with small-diameter (group B, *N*=10) or large-diameter (group C, *N*=10) nails. The evaluation at 60 days included radiography, DXA and mechanical cantilever testing. All fractures healed radiographically with bridging of the fracture line and visible callus formation. Mineralization and callus formation (measured as the DXA parameters BMD and callus area) were significantly greater in Group B than in the other two groups. Group B also tended to have mechanically stronger bones with higher fracture energy compared to the other two groups. We found that converting lower leg fractures in rats from EF to IMN did not significantly improve bone healing, supporting continuation of EF as an acceptable fracture management option.

Publication IV: The Effect of Timing of Conversion from External Fixation to Secondary Intramedullary Nailing in Experimental Tibial Fractures

Forty male rats received a standardized tibial shaft osteotomy and EF, and were then randomly assigned to conversion to IMN at 7 (group A, *N*=10), 14 (group B, *N*=10) or 30 (group C, *N*=10) days after the initial fixation. Group D (*N*=10) served as a control group without conversion. The evaluation at 60 days included x-ray, DXA and mechanical catilever testing. The bone mineral content and callus area were significantly greater in Group A than in the control group, while mechanical bending strength and stiffness were significantly lower in Groups B and C than in groups A and D. The timing of the conversion procedure had a significant effect on fracture healing: an early conversion procedure did not improve healing compared to control, but was advantageous compared to late conversion (at 2 or 4 weeks), with higher mineralization and superior biomechanical properties.

Publication V: Correlations Between Strength and Quantitative Computed Tomography Measured Callus Mineralization in Experimental Tibial Fractures

Forty male rats were subject to a standardized tibial shaft osteotomy and initially stabilized either with IMN (*N*=20) or unilateral EF (*N*=20). Evaluations at 30 and 60 days included radiography, QCT and a mechanical cantilever test. A narrow and wide region of interest (ROI) of the tibia (1.25 and 3.75 mm long, respectively) at the fracture site was reconstructed and segmented with a voxel-based technique into soft callus, hard callus and cortical bone. The volumetric BMD (vBMD) was also calculated. Regardless of the fixation method, the study groups were characterized by pronounced soft- and hard-callus formation in the early phase. The volume of cortical bone and fracture cantilever bending strength were significantly increased at 60 days, but callus formation was significantly decreased compared to at 30 days. None of the QCT parameters demonstrated clinically valuable strength predicting abilities. However, the amount of cortical bone and the vBMD value measured by QCT at the fracture site were correlated positively and significantly with strength in the IMN group in the early phase of healing.

4 General discussion

4.1 Methodological considerations

This section considers issues concerning the rat model, the surgical procedures and the fracture evaluation methods.

4.1.1 The rat model

Adult male Wistar rats (Møllegårds Avlslaboratorium, Ejby, Denmark) were used in the experiments and given standardized care. Wistar rats are an albino strain of the wild brown rat (*Rattus norvegicus*). They are widely accessible and docile. The animals were housed in rodent cages with a lid holding a hinged water bottle divider and separate food area. Two rats in each cage received a standard rodent diet (RM3(E)M Special Diets Services, Witham, United Kingdom). The light cycle was 12 h/12 h. All experiments conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes, and the number of animals was minimized by performing statistical power analysis before commencing experiments. The animals studied for publication I were subject to the correlation studies and CT scans described in publication V.

Even though the rat is by far the most popular animal to use in fracture studies [179], differences must be considered and caution must be exercised both when animal experiments are designed and when experimental animal study results and conclusions are interpreted. The size, histological bone organization, locomotive stresses and anatomical relationships to the neighbouring fibula bone differ between the rat and human tibia. In our experiments, the rats resumed apparent normal locomotion with full weight-bearing within a few days post-operatively. The rat has a fibula, like humans and unlike some other mammals such as goats and sheep, where only a remnant of the head may be found.

In humans, the tibia articulates with the fibular bone laterally both proximally and distally. However, the tibia and fibula of the rat fuse distally over a distance of several millimetres and hence constitute a more rigid structure [93] (see Figure 3). Even though the epiphyseal plate closes very late in the rat, an adult Wistar rat tibia is around 42 mm long [93,250], whereas the adult human tibia in males is almost ten times longer, at around

390 mm [55]. The cortical bone of Wistar rats probably reaches maturity at an age of about 14 weeks, with the ultimate torsional load and modulus of rigidity being reached at that age [63]. Microscopically, the rat bone is similar to that of humans, but there are fewer and smaller Haversian systems [98], and these systems are scattered near the endosteal surface. Small avascular and acellular areas are present throughout the cortical bone [218]. These differences in the Haversian lamellar structure between human and rat bones might result in species-specific reactions. However, the basic remodelling processes are the same in rats and humans [249], and the deformation and biological repair of long bones are fairly constant across species [148], resulting in the rat being widely recognized as a suitable model for skeletal research relevant to humans.

Early studies indicated that leg fractures in the adult rat regain mechanical properties similar to those of intact bone at 60 days [62]. In our study, the fracture strengths at 60 days in the IMN and EF groups were 85% and 54% of that of intact bone. Since we also wanted information about the healing process in the early phase, we evaluated differences in mechanical properties and mineralization at 30 and 60 days.

4.1.2 Animal surgery

The surgical procedures performed in the experiments were (A) open osteotomy, (B) primary external fracture fixation by mounting an external fixator, (C) primary internal fracture fixation by unreamed, unlocked IMN, and (D) secondary IMN. Procedure A was always followed by either procedure B or C and completed during the same surgical session. Procedure D was performed for publications III and IV as a secondary procedure at 7, 14 or 30 days after the initial surgery, and it included the use of a percutaneous technique to remove the temporary external fixator prior to nail insertion.

The open osteotomy was performed by exposing the left tibia through an anterior incision from the tuberositas tibia and in a distal direction. The muscles on the medial and lateral aspects of the tibia were carefully elevated from the tibia, and the anterior two-thirds of the tibia was cut at the level of the anterior ridge using a fine-toothed circular saw blade mounted on an electric drill. The remaining one-third was then manually broken, leaving the fibula intact. This experimental model combines saw osteotomy and the induction of traumatic fracture by open surgery. The results can therefore not be applied directly to either closed fractures or high-energy open fractures in the clinic.

The aluminium/steel external fixator used in the experiments has been described previously by Mark *et al.* [155], and was refined somewhat by our research group in collaboration with the mechanical workshop at the University of Oslo (see Figures 4 and 5). Four steel pins (with a diameter of 1.0 mm) were inserted: two proximal and two distal to the fracture. The core drill holes in the tibia were 0.8 mm in diameter, and the fixator offset – the free length of the pins between the rat's anterolateral tibial surface and the inner side of the fixator bar – was 6 mm. The external fixator weighed 6.5 g and its position enabled free movements of the ankle and knee joints. The perioperative alignment and accurate fracture reduction with zero interfragmentary distance were verified both visually and manually. No perioperative or post-operative x-ray examination was performed.

The intramedullary nails were inserted from the proximal side into the bone-marrow cavity through the anterior tip of the tibial plateau to the distal tibiofibular junction, with the knee in a flexed position (see Figure 6). The nails were cut flush to the bony surface at the insertion side. The nails were not reamed or locked. The medial and posterior segments were left attached to the bone. Experimental studies have shown that the rotational stability provided by an intact fibula favours healing [103,131]. The fibula is often fractured in human tibial fractures, and this is compensated for by interlocking the nail. All fibulas were left intact in our study to ensure rotational stability.

The compressive and distractive stimuli used for publication II were applied via manual manipulation of the external fixator from days 4 to 14. Once daily during this early 10-day period, the tubular fixator steel screws connecting the pin clamps were tightened (compressive stimuli) or loosened (distractive stimuli) with a standardized tool, resulting in a daily screw rotation of 90 degrees corresponding to a displacement of the pin clamps of 0.125 mm; the total displacement was thus 10×0.125 mm = 1.25 mm. The control group received similar sham manipulations. The fixator was not manipulated after day 14. The operation wound was closed in two layers using absorbable sutures. A layer of transparent film dressing was then sprayed onto the sutured wound.

The surgical procedures involving the rat tibia in our experiments were performed with an aseptic technique and experienced few or no post-operative complications such as infections. Prophylactic antibiotics were not included in the surgical or post-operative care routines. This indicates differences in the functions of the immune systems between the research animals and human patients. Other mechanobiological and soft-tissue factors relevant to bone healing may also be different in the rat.

On the other hand, strong scientific conclusions could be drawn by standardizing important parameters such as the level and configuration of the osteotomy or fracture, age and health of individuals, independency of compliance and applying the gold standard for evaluating fracture healing and mechanical testing.

Mounting a 6.5-g four-pin unilateral external fixator on a 42-mm-long rat tibia is obviously more challenging than the corresponding clinical surgical procedures required for the human tibia. Even though precise reduction and stable fixation were verified both manually and visually perioperatively, as they are in the clinic, the small dimensions of the rat tibia naturally raise several concerns about the surgical accuracy and its influence on the results. To avoid the surgical experience and condition exerting systematic effects on the results, the animals were operated only during the daytime and they were randomly assigned to the animal surgeons. Complications such as drop foot, pin loosening and extramedullary positioning of the nail led to exclusion from the study. Two rats were excluded from publication I: one due to pin-tract infection and the other due to fixator loosening. For each of publications III and IV, one rat was excluded due to pin-tract infection and two rats due to extramedullary positioning of the nail. A few superficial infections/self-inflicted bite wounds were treated immediately with effective surgical debridement and skin closure.

Half of the animals in each study group were killed by an intraperitoneal injection of pentobarbital at 30 days, and the other half at 60 days. Even though the age of the rat influences healing [64], previous studies have shown that leg fractures regain mechanical properties similar to intact bone within 8 weeks [62,242]. The tibias were immediately dissected free and examined visually. The external fixator clamps (but not its pins) and intramedullary nails were carefully removed after obtaining x-ray images and before DXA, CT and mechanical testing. The bones were kept frozen at –80 degrees Celsius between dissection and radiological, DXA, CT and mechanical evaluation.

4.1.3 Imaging and densitometric evaluation

X-ray images in all experiments were obtained on a standard clinical digital system (Axiom Aristos, Siemens, München, Germany). The x-ray tube settings were 46 kV, 1.0 mAs and a focus-to-film (source-to-image-receptor) distance of 115 cm. All x-ray images were interpreted by the surgeons to confirm bone healing, but no inter- or intraobserver validation of the interpretation was performed.

DXA measurements for publications I–IV were performed using a densitometer system for research animals (Piximus, Lunar, Madison, WI). The x-ray tube voltage was 80 kV, current was 400 μA, focal spot size was 0.25 mm × 0.25 mm and focal spot-to-image-receptor distance was 32 cm. The callus area, BMD and bone mineral content were automatically calculated by the accompanying software from a standardized-size ROI at the fracture site of 21×43 pixels, which corresponds to around 0.30 cm^2 and included a longitudinal tibial segment of 3.75 mm. The dissected tibias were placed in the same orientation and position on the scan table after a daily calibration of the system, but minor variations in position might have occurred due to variations in the pins of the external fixator (note that the ROI itself did not include any external fixator pins).

QCT for publications II, III and V was performed by micro-CT scans and reconstruction of 2D and 3D images. The micro-CT system (Micro CAT II, Imtek – now Siemens) scan settings were 300 steps with 200 degrees of rotation, and the x-ray camera detector size was 2048×2048 with a bin factor of 2. The exposure time was 500 ms and the voxels were cubes with a side length of 50.7 μm. A micro-CT system was calibrated in an air scan that was performed daily prior to bone scanning. A bone tissue phantom was scanned simultaneously in every bone examination. The CT images were not used for qualitative fracture healing analysis. The images were reconstructed from scan data obtained from a narrow ROI near the fracture site and a wide ROI encompassing the callus region of the fracture: 1.25 mm (25 slices) and 3.75 mm (75 slices), respectively (see Figure 7). No external fixator pins were included in the ROI. The standard software beam-hardening error correction of the manufacturer was activated. Scan data were analysed with a commercially available reconstruction and visualization software package (Amira v4.1, Mercury Computer Systems, Mérignac Cedex, France).

A Lucite phantom with HA densities equal to 50, 250 and 750 mg/cm^3 was scanned simultaneously, and HUs were linearly converted into HA densities [141]. There is no clearly defined consensus on density thresholds between soft and hard calluses and cortical bone [8,40,135,172,251], since QCT is currently predominantly applied to calculate the density of intact bone and to predict its relative fracture risk. The voxels were segmented into four categories: the exterior, soft tissues and fat (<171 mg/cm^3), soft callus (171–540 mg/cm^3), hard callus (540–1200 mg/cm^3) and cortical bone (>1200 mg/cm^3) (see Figures 8 and 9). These threshold values were selected based upon previous experiments and careful visual examination of the CT images with a standard bone window and level [143,200].

Other threshold values could also be justified, but more investigations of segmenting QCT-measured callus formation are needed to determine definitive values.

Beam hardening is a known source of error in CT. In brief, the polychromatic x-ray beam is altered as it travels through an object. When travelling through low-attenuation tissue, part of the the x-ray beam spectrum is absorbed and the beam is hardened, which results in lower attenuation in the corresponding areas on images, and vice versa for high-attenuation tissue. No segmentation between high-attenuation tissue was applied. The consequences of this effect on the segmented data were therefore minimal. Any small beam-hardening artefact would probably have affected all study groups in a similar manner and hence can hardly explain intergroup differences.

The partial volume effect is another regularly debated feature of CT. In short, the CT value, or the voxel CT number, is a measurement of the radiopacity of the scanned tissue. Due to the relative low resolution of CT, a reconstructed 3D CT image represents an interpolated, approximate reflection of the true amount and distribution of mineral density of the scanned subject. This is commonly referred to as the partial volume effect [7]. Even though the geometrical resolution is better than for that of DXA, CT has limitations in visualizing certain features of fractured bones, such as their microstructure [11]. QCT measurement of radiopaque minerals is still crude, and at best provides an imprecise outline of bone repair and remodelling processes. The implications of averaging the mineralization of the tissue within the voxel volume are unknown. However, as for beam hardening there is no reason to believe that the partial volume effect would have been differed between the study groups.

CT imaging also has limitations in the clinic. The radiation dosage needs to be considered. CT scanning of bone tissue will adversely affect healing processes even though CT is generally considered to be a non-invasive procedure in clinical medicine. Moreover, in cases with internal fixation devices, CT imaging will be affected by interference and inaccuracies in quantitative results. Complicated computational techniques are utilized to compensate for such interference.

4.1.4 Mechanical testing

The tibias were ultimately placed between gauze pads that had been moistened with 0.9% saline before a cantilever test was performed. This was important to avoid changes in the biomechanical properties of bone due to variations in moisture content, since dry bone is more brittle. Even though there were minor differences in the time for which the bone samples had been at room temperature before testing, the bones were tested in a random order to minimize any resulting systematic effect. The used universal testing machine had a servohydraulic mechanical linear drive actuator with 100 mm of total vertical displacement and a maximum axial tension loading capacity of 250 N (MTS 858 Mini Bionix, MTS Systems, Eden Prairie, MN). The set-up included a cantilever test that was designed to test the fracture site, as described previously [65]. A standard control program set the vertical travel speed to 160 mm/min. The data file was then converted into a classic load–deformation curve, and values for basic biomechanical structural properties – such as the ultimate load, stiffness and energy to fracture – were obtained using a mathematical software package (Origin v 7.5, OriginLab, Northampton, MA) [184].

The ultimate torsional and bending loads are significantly associated with callus stiffness [158], and they are themselves the most important and interesting biomechanical parameters in the clinic. For example, a high leg stiffness is of little value to the patient if the load required for irreversible deformation is low.

The advantage of a fracture-site-specific test situation is that this measures the maximum strength of the bone at the fracture site. In a torsional test, where the entire bone is stressed torsionally, the bone will fracture at the weakest point. If the fracture site has healed biomechanically to stage 4 (see Section 1.2), it may be stronger than the fractured point. In that case a torsion measurement will determine the weakest point of the bone rather than measuring the strength at the fracture site. This is why torsional tests are usually applied to bone segments and grafts. It is known that the holes drilled for pin insertion in EF weaken the bone, and so the cantilever test may be superior to a torsional test in the testing of the fracture-site strength in externally fixed bones. However, the strengths of the pin sites are also of interest.

4.2 Discussion of results

4.2.1 External fixation versus intramedullary nailing

Major soft-tissue injuries delay bone healing in underlying tibial fractures [89], and attention to soft-tissue handling is important both in the clinic and in experimental fractures. In our study, the length of the incision and the surgical manipulation of the soft tissue around the fracture site may have been slightly more extensive in the EF procedure than in

IMN, although we did endeavour to use similar soft-tissue dissection procedures in the surgical protocol. This difference may have influenced the periosteal circulation and inhibited healing to some extent, and may partly explain the lower amount of callus formation and the inferior mechanical-testing results in externally fixed tibias. However, the application of external fixators in clinical practice usually implies a percutaneous, soft-tissue-preserving procedure.

In the clinic, tibial shaft fractures are often associated with a fractured fibula, and the nails are often interlocked. As mentioned above (see Section 4.1.2), our experimental design did not include locking of the nails, but all fibulas were left intact for rotational stability. Klein *et al.* [131] observed in an experimental sheep model (where the fibula was absent) that fractures treated with locked unreamed nails were inferior to those treated with EF (evaluated both mechanically and histomorphometrically). This suggests that rotational stability plays a major role in the outcome of tibial fractures and that the effect of fracturing the fibula ought to be studied systematically. The effect of the fractured fibula has been studied in IMN [103], with the results indicating that the presence of both a fractured tibia and fibula impairs the early phase of fracture healing. The use of a third treatment group with, for example, a segmental fibula osteotomy, could help to isolate and identify the effect of the fibula. Our research group has now initiated such a study of the role of the fibula in EF.

The alignment and stability of the bony fragments are especially important surgical principles in fracture treatment. An increased interfragmentary gap or movement may result in malunion, delayed union or even non-union. Fracture fixation with absolute stiffness inhibits interfragmentary motion and is associated with primary healing and reduced external callus formation, as mentioned above (see Section 1.2.2). Flexible and semi-rigid fixation promotes motion at the fracture site in favour of secondary bone healing, with the characteristic development of a bridging periosteal callus until cortical healing occurs. While it is well accepted that interfragmentary motion influences callus formation and the healing of fractures in both IMN and EF, the optimal biomechanical conditions for the fracture healing process remain unclear [39,43,81,125,169,221].

The stiffness of the bone–implant construct has been shown to be important to the fracture healing process in both IMN and EF [245,264]. The IMN tibia–implant construct, with inferior bending stiffness and less protection from torsional and axial forces, was significantly more favourable for mineralization and for recovering mechanical properties in the fracture repair process compared to the EF construct in our model. The initial post-operative bending stiffness of the tibia–implant construct was significantly higher in the EF group than in the IMN group (89% and 17%, respectively, of the bending stiffness of intact tibias). While the external fixator shares the axial load, unlocked nails provide little protection from axial load. A side effect of the size and location of the external fixator device could be to cause the animal to use the fractured limb less due to irritation from the device and the changed pattern of movement. This was not systematically tested in our study, even though careful visual examination indicated the presence of full weight-bearing with apparent normal quadrupedal locomotion in both groups within a few days post-operatively.

Our results are consistent with IMN being the standard for the definitive clinical management of lower extremity long-bone fractures in humans over the last 2 decades, and often being associated with a higher bone union rate and a shorter time to full weight-bearing. Shannon *et al.* [213] compared 17 patients treated with EF to 13 patients treated with unreamed locked tibial nails. They experienced four local pin infections in the EF group and one deep infection in the unreamed-locked-nail group. That retrospective study of grade-III fractures of tibial diaphysis also found that the time to full weight-bearing was significantly shorter for the nailed fractures (22 weeks) than for the externally fixed fractures (37 weeks). Schandelmaier *et al.* [206] found similar results in a comparison of 32 grade-IIIb open tibial shaft fractures, where the time to full weight-bearing was significantly shorter among the 17 patients in the unreamed-nail group (11±4 weeks, mean±SD) than among the 15 patients in the EF group (20±11 weeks). The times to bony union, infection and non-union did not differ significantly between the groups. A significantly shorter time to full weight-bearing for IMN was also found by Braten *et al.* [23] in a prospective, randomized study of tibial fractures involving 78 patients distributed into groups treated with EF (N=41) and IMN (*N*=38). However, they excluded patients with significant soft-tissue problems (grade III).

A few other studies have found no significant differences in healing between EF and IMN. Trabulsy *et al.* [234] evaluated the results of EF in 28 patients and unreamed intramedullary nails in 17 patients with grade-IIIb fractures. Early bone grafting was employed in 43% of the patients, and free muscle flaps or local flaps were used in all patients. No significant difference in the complication rate or the time to union was noted between the two groups (IMN=40 weeks, EF=41 weeks). Local infections occurred in three patients (6%) and osteomyelitis in two (4%). However, no assessments of the distribution of fracture types, severity of soft-tissue damage or time to flap coverage were reported.

Tornetta *et al.* [232] investigated 29 grade-IIIb open tibial fractures in a prospective study, of which 14 were randomly assigned to EF and 15 to unreamed locking IMN. The motion was slightly better in the IMN group, but there were no significant differences in the times to partial weight-bearing and bony union. One deep infection and two pin-tract infections occurred in the EF group, and one deep infection was found in the IMN group. The authors considered locked, unreamed nailing to be the treatment of choice for grade-IIIb tibial fractures. However, that study included only a rather small number of patients. Henley *et al.* [94] also found that the choice of implant did not significantly affect the healing rates. In the prospective study of 174 grade-II, -IIIa and -IIIb tibial shaft fractures, the numbers of malalignments, subsequent procedures and local infections were significantly lower in the IMN group (N=104) than in the EF group (N=70). The complications occurred in fracture patterns with higher degrees of comminution or bone loss regardless of the method of treatment. The main factor influencing the speed of healing of the tibial fractures was the severity of soft-tissue injury. In that study the fracture severity tended to be higher in the EF group (p=0.051).

4.2.2 Compression and distraction of external fixation

Both the tight apposition of fragments and an intact fibula may explain the lack of shortening of bones exposed to compressive force and the densitometrically and biomechanically comparable results between those bones and the control bones. The mean group lengths support that performing an exact initial surgical reduction of the fracture fragments with a zero interfragmentary distance limited further interfragmentary movement driven by compressive forces. The static (control) group would still allow interfragmentary contact between the bone ends and interfragmentary compressive stresses during function, although possibly of a lower magnitude than in the compression group. In addition, the fibula was left intact in order to increase torsional stability and prevent detrimental shear forces and rotational malunion [6,103].

The increased compressive interfragmentary force in the bone–implant system in the compression group would be expected to increase the system stiffness. While the stiffness of external fixators has a documented effect on healing [264], the role of the increased stiffness on fracture healing in our experiments is unclear since a control group without compression with such increased stiffness was not included.

Interestingly, at 30 days the groups exhibited remarkably similar important biomechanical and densitometric properties. Mechanical stimulation via interfragmentary compressive forces did not alter the strength, stiffness or densitometric properties significantly at this time point, with only a significantly lower energy absorption before fracture being observed in the compression group. This corresponds to Perren's finding that bone can resist a high amount of compressive stress without developing necrosis or degradation [193]. Moreover, even though both BMD and vBMD were significantly higher in the compression and control groups, strength, stiffness and QCT-measured callus formation did not differ significantly at 30 days. This confirms that the 30-day time point represents the early phase of fracture healing, with a relatively large, immature and weak callus being present in all groups.

Both the densitometric and mechanical properties suggest the presence of ongoing remodelling activity in all groups in the late phase, and two distinct densitometric patterns were observed. Firstly, in the compression and control groups there was a characteristic decrease in 'immaturity' from days 30 to 60, in terms of the bone mineral content and callus area (DXA parameters) and soft- and hard-callus volume (QCT parameters), with a simultaneous increase in 'maturity' in terms of strength, stiffness and cortical-bone volume, and vBMD values close to those of intact tibias. This indicates that the healing of the bones in these two groups was near completion. It is also evident that the 2D DXA parameter BMD was a poor marker of this callus maturation in the compression and control groups. Secondly, in the distraction group, the pronounced callus that formed in the early phase was not reduced at 60 days. Even though the amount of cortical bone increased significantly from 30 to 60 days, there was still significantly less of it compared to that observed in the compression and control groups. The early mechanical distraction did induce a positive stimulatory effect, in terms of larger amounts of soft and hard callus at 60 days, but the reduced strength, stiffness, cortical bone and vBMD indicate a delayed callus maturation in this group.

The presence of significantly longer tibias in the distraction group indicates that the early distraction creates a fracture gap that is, in turn, at least partly responsible for the significantly weaker and more-immature callus at 60 days [39]. Moreover, the longer tibias in the distraction group suggest that the intact fibula does not significantly limit the lengthening. However, the strain caused by distraction and a reduced bone–implant stiffness due to the fracture gap may also be partly responsible for the reduced recovery of strength and the densitometric pattern in this group at 60 days post-osteotomy.

The increase in cortical bone of more than a threefold in the distraction group between 30 and 60 days indicated that the fracture gap had not yet fully mineralized. A 1.25-mm gap corresponds to around 5.5 mm^3 of cortical bone, which is more than the mean difference between the distraction group and both of the other groups at both 30 and 60 days. Also, there was a significant increase in strength but no significant reduction in stiffness in this group between days 30 and 60. Thus, there were no clear signs of failed healing. The results in the distraction group therefore probably reflect the characteristic delayed but robust healing process in distraction osteogenesis [104,105]. However, this process has the disadvantages of an extended healing time and the need to avoid a critical gap size. In short, given a longer healing time, the distracted bones would probably continue to remodel and consequently improve their mechanical properties. In this study, a slower maturation of the distracted callus segment was expected, and this provided a second reference for comparison.

This study was subject to several limitations. The interfragmentary movement and compressive strain were not measured continuously. However, there were no signs of pin or fixator loosening, which suggests that only minor interfragmentary motion occurred [38,39]. Moreover, the callus segment was not examined histologically. Such an examination would have necessitated a larger number of animals or the ability to perform non-destructive biomechanical evaluations of the bones. However, previous investigations of the distraction and compression regimens have not revealed significant differences in data from histological light-microscopy evaluations [38]. The difference in the ROIs for DXA (length of 3.75 mm) and QCT (length of 2.0 mm) measurements prevents a direct comparison. Given the mean bone lengths in the compression and static (control) groups, similar portions of the callus and cortical-bone ends were probably measured in the two groups. It is difficult to separate the effects of the gap and distractive stress in the distraction group without including another group with an initial distraction gap of 1.25 mm. The effect of distracted cortical bone could be avoided by selecting an ROI outside the distraction zone. However, the biomechanically interesting fracture site would then not be measured. Moreover, measuring mineralization in an ROI adjacent to the distraction zone what make it difficult to decide which ROI in the compression and control groups is the true corresponding ROI. These are interesting questions that our research group are already focusing on in experiments. The lengthening of the tibias in the distraction group may limit the application of the protocol. However, our aim was to investigate a fracture healing process exposed to axial compressive forces and compare this to static fixation and distraction.

4.2.3 Initial temporary external fixation and secondary intramedullary nailing

Bone healing in experimental tibial fractures with initial EF and secondary IMN was investigated for publications III and IV.

It is widely accepted that it is necessary to differentiate between polytrauma patients who can and cannot tolerate major surgical procedures [187,205,236]. Data suggest that early total fracture care should only be performed in patients with lower injury severity scores. Unstable patients and those in a critical condition should not undergo a prolonged surgical procedure, and therefore they should be treated with a damage-control approach by EF to prevent unexpected complications [188]. The efficacy of this approach has recently been confirmed in a prospective randomized clinical study [190]. The results clearly demonstrated that patients in a borderline (uncertain) condition did worse when initial definitive stabilization was performed, with the conclusion being that stable patients should undergo IMN of long-bone fractures while unstable patient should undergo a temporary approach using an external fixator, followed by secondary IMN. Another study found that about 40% of trauma patients who underwent major secondary reconstructive surgery within 3 days after admission developed multiple organ failure [254], and some authors have delayed extensive orthopaedic procedures until 72 hours after injury.

The optimal timing for secondary procedures needs to be determined in damage-control orthopaedics. The optimal timing of secondary fracture surgery is unclear. A large survey of 4314 patients found that patients who developed multiple organ failure received secondary surgery between days 2 and 4, whereas patients without organ failure were operated at between 6 and 8 days after the initial trauma [186]. Based on these studies, the current recommendation seems to be that the optimal approach for damage-control orthopaedics in polytrauma patients is initial stabilization of long-bone fracture by EF followed by IMN at about 1 week. This was the basis for our time point for the secondary IMN procedure. Furthermore, 7 days is in the early phase of healing even in the rat [62], and the initial inflammatory response to multisystem trauma often normalizes during the first week [96,189]. A soft-tissue neocallus has normally formed at 7 days [147]. After nailing the tibia, the fibula and the neocallus make the fracture segment relatively stable to torsion, with flexibility to bending and axial movements. Also, the conversion to a bone–

implant construction with lower stiffness implies larger fracture-site micromotion, which has been documented to promote callus formation in the early phase [80].

As explained in the Introduction, the rationale of a secondary conversion procedure is to enhance fracture repair. Goodship *et al.* demonstrated that early axial cyclic micromotion with a relatively high strain rate significantly increased the fracture mineralization, mechanical stiffness and maximum torsional load, whilst micromotion applied in the late phase reduced bone mineral and mechanical properties and had a detrimental effect on bone healing [80]. Increasing the interfragmentary motion substantially in the later phase of healing may increase callus formation in leg shaft fractures, but also reduce the quality of bone healing [244]. It has also been demonstrated that applying physiological dynamic axial compression to canine mid-tibial osteotomies treated with EF after 2 weeks did not alter bone formation or the maximum mechanical torque [4], whereas others have found significantly higher torsional stiffness and a tendency to higher maximum torque when an externally fixed fracture was dynamized after 1 week [146]. This indicates that early conversion to the less-stiff bone–implant system of the intramedullary nail may be beneficial in terms of enhancing bone healing. While it is obvious that simply changing to a less-stiff fracture fixation method during healing will not necessarily enhance fracture healing, the observed advantage of early conversion compared to later conversion is consistent with other reported experiments finding that only early mechanical fracture segment stimuli have a positive effect on bone healing [107]. However, recent advances in biomechanics and biomaterials have resulted in improvements of EF frames, and they can now remain in place for prolonged periods of time without degradation of the pin–bone surface [253]. Our results indicate that continuation of EF in some (possibly more stable) configuration may be an option.

Some surgeons have promoted initial and temporary EF followed by a definitive IMN later to avoid compromising an already damaged circulation. However, secondary IMN following EF can increase the risk of infection following later nailing**,** leading to possible malunion, delayed union or non-union [164]. Our comparison of EF and IMN for publication I revealed no significant difference in the initial healing between the two implant types at 30 days. However, in the late phase IMN led to superior bone healing compared to EF. This indicates that when EF has been used as the primary treatment, exchange IMN in the later phase may enhance the healing process.

The exact magnitude of the insult that the actual surgical conversion procedure represents is unknown, but it can be minimized by careful removal of the four pins and the external fixator together with minimally invasive antegrade non-reamed IMN. Even though patients in our small-diameter-nail group received an extra surgical conversion, their mineralization and callus formation were superior to those in the control group. This group also tended to have higher mechanical bending strength and fracture energy than both other groups.

The callus disturbance caused by the conversion procedure may partly explain why late conversion IMN led to an inferior bone healing process with respect to mineralization and biomechanical properties. The concurrent soft-tissue damage and its management are documented predictors of the outcome and need for reoperation of a fracture patient [90,266]. The additional manipulation of the fractured leg represented by the conversion procedure may have interfered with the hardening and maturation of the callus. More specifically, the physical removal of the four external fixator pins and insertion of an intramedullary nail through the fracture site may have influenced the biological fracture repair process in our experimental set-up, as it would in a clinical situation. Furthermore, penetrating the fracture site with the nail might have represented a greater stimulation to the early neocallus in the early-conversion group than in the more-mature callus in groups B and C. The conversion procedure or the change in bone–implant fixation in the late-conversion groups may have delayed callus maturation and remodelling or reinitiated early repair processes with soft-callus production, and this may partly explain the lower BMD and significant reduction in bending strength and rigidity observed in these groups compared to the control and early-conversion groups.

Our two types of nail correspond to the different standard options used in clinical trauma care, and our findings support the current practice involving conversion to the use of intramedullary, unreamed, loose-fitting nails as soon as soft-tissue problems are resolved and the inflammatory response levels permit removal of EF [51,54].

Our experimental set-up included a standardized diaphyseal tibial fracture with limited soft-tissue injury and an intact fibula, and hence it differs from the clinical high-energy-damage situation that often includes extensive soft-tissue damage. In this aspect our set-up is more representative of patients with closed fractures but who have an otherwise immunological unstable situation where damage-control orthopaedics is an option.

There is an inherent risk of deep infection when performing a secondary conversion to IMN in long-bone fractures. Musculoskeletal trauma is often complicated by a high risk of ischemia/reperfusion injuries and secondary infections, and the risk increases with the degree of soft-tissue injury and fracture wound contamination. Although favourable results have been reported when secondary nailing is delayed until after granulation of the pin sites [257], recent clinical studies indicate that this risk significantly increases in late conversion procedures (e.g. after 28 days) [18].

4.2.4 Correlations between strength and quantitative computed tomography

An analysis of statistical correlation between segmented QCT data and bending strength in fractured leg bones is presented in publication V. As stated above (see Section 1.5), from a clinical point of view the most important biomechanical parameter is the maximum (bending and torsional) load, which is how much load a patient's leg can resist before it fractures. In theory, a torsional test tests the weakest region of the bone, whereas the cantilever test can be designed to test a specific site, such as the fracture site [62]. Stiffness is another frequently used surrogate marker for maturation of fracture healing [204]. However, the fractured bones in our study exhibited stiffnesses higher than that of intact tibias already at 30 days, even though the bending strength was no more than 50% that of intact tibias.

Massive callus formation in the early phase was evident in both treatment groups. Callus formation increases with reduced stability of the bone–implant system [34]. We have already determined that bone repair involves numerous processes taking place more or less simultaneously, which can be divided into (1) the early, inflammatory response, (2) soft- and hard-callus formation through endochondral and intramembranous ossification (cartilage formation, calcification and removal), and (3) osteon remodelling, also called primary healing [59]. Moreover, remodelling is dependent on the provision of adequate stability, such as by the callus formed after fracture and/or fracture fixation devices. Furthermore, very rigidly fixed fractures exhibit bone repair with diminished callus formation, which is also called primary healing. Fixations of tibial diaphyseal fractures with absolute rigidity are rare in the clinic [46].

The presence of soft and hard calluses was not significantly correlated with bone strength. When all study groups were pooled (N=40), strength tended to be negatively correlated with the soft-callus volume. In a clinical setting, the visible periosteal callus on an x-ray is not a direct indicator of the fracture strength. **The formation of a callus is indicative of a** normal course of healing in a fracture with relatively flexible fixation after some time, and signals that remodelling is imminent or perhaps already advancing with bone strengthening. Our results indicate that even though a callus provides stability for the remodelling process, its biomechanical splinting effect is not a major contributor to the maximum bending strength of the bone. In theory, this complicated bone healing mineralization that results from overlapping formation and resorption processes of calcified tissue may be difficult to describe clearly with sum or average densitometric parameters such as bone mineral content, BMD or vBMD. Our experimental set-up failed to identify a clinically valuable QCT parameter of the bone strength, but this may have been due to threshold selection and issues concerning image resolution, and hence future studies might reveal QCT parameters that can predict the strength of a healing fracture.

Only the fracture-site QCT-measured cortical-bone volume in the IMN group at 30 days was correlated positively and significantly with strength. Almost half of the variability in the strength could be accounted for by variation in the fracture-site cortical-bone volume. At 60 days, cortical bone in both treatment groups exhibited similar properties to intact bone, which suggests that cortical bridging had already occurred. Interestingly, the strength was found to differ between treatment groups even when the cortical-bone volume did not. Cortical-bone remodelling with the formation of new, strong lamellar structures across the fracture gap is the main factor responsible for a restoration of the mechanical strength. Our results support the idea that the proximity of the ROI of QCT to the fracture influences the biomechanical importance of the measurement. It may be that the QCT-measured cortical-bone volume is sensitive to biomechanically important mineralization processes such as cortical bridging or osteon remodelling in this situation, when the ROI is close to the fracture line in the early phase of healing in fractures with relatively flexible fixation. The higher bone implant stiffness in the EF group may be at least partly responsible for a smaller amount of hard callus forming, which in turn probably reduces the stability for the cortical remodelling process and bone maturation. Subsequent differences in lamellar structure and microstructural properties may explain differences in bone strength and the relatively weak correlation between strength and cortical-bone volume.

The relatively weak correlation between measured cortical bone and strength may partly be explained by variation of the fracture configuration. The fracture homogeneity and standardization of our experimental model promotes systematic bone research. However, the ROI of QCT was aligned with the saw osteotomy perpendicular to the tibia, and not necessarily with the remaining one-third of the fracture – the manual manipulation after partial osteotomy to fracture the remaining third of the tibial bone may have led to a variety of fracture configurations. Figure 10 shows that the accumulation of measured cortical bone (as represented by the curve) had a minimum in the centre of the ROI. This point probably expresses the fracture line and supports that our ROIs are centred on it.

None of the measurements in the QCT study group with the wide ROI were correlated significantly with strength. When all groups were pooled (N=40), the presence of cortical bone in the wide ROI tended to be positively correlated with strength. The QCT-based measurements of callus area included a relative large proportion of unfractured (cortical) bone, which might have weakened the correlation with bone strength.

The calculated tissue volumes are based on the measured CT values of the voxels. Even high-resolution CT systems have limitations in visualizing bone microstructure [11]. In heterogeneous materials (e.g. fractured bone), the CT value corresponds to the average attenuation contributed by all materials and chemical elements within its boundaries, and this is commonly referred to as the partial volume effect [7,115]. The CT value thus reflects local radiopacity and is an approximation of the true amount and distribution of mineral density of the scanned subject. CT values are influenced by the properties of bone marrow in osteoporotic subjects [142,165]. The full implications of the use of a tomographic technique to produce the CT value are unknown, but the effects are unlikely to have affected the groups in the present study unequally.

5 Conclusions

The observations made in this study indicate that treating tibial diaphyseal fractures with EF seems to be as effective as IMN in recovering mechanical properties in the early phase of healing. However, at the time of healing IMN provides significantly greater callus maturation, as measured by the mineralization of the callus segment, and results in superior mechanical properties compared to EF.

We found that (1) early compression of externally fixed tibias did not enhance fracture healing in terms of mineralization of the fracture gap and mechanical characteristics at mid-term or at the time of union compared with statically fixed bones, and (2) both compression and static fixation techniques induced superior mechanical properties at 60 days and a more-mature callus mineralization compared to distraction.

In our experiments we found that (1) the continuation of EF reduced mineralization and callus formation of the fracture segment compared with early conversion to definitive small-diameter nails after 7 days and (2) the conversion after 7 days to small-diameter nails induced increased callus formation compared with both the use of large-diameter nails and continuation of EF, whilst (3) the mechanical characteristics did not differ significantly between definitive EF and conversion to IMN using nails with different diameters.

The results of our study indicate that the timing of the conversion from initial EF to IMN has a significant impact on bone healing. Our experiments support the clinical practice of early conversion as soon as the patient or local soft-tissue conditions permit since (1) early conversion to IMN induces an advantageous increase in mineralization and callus formation in the fracture segment, and (2) late conversion has a detrimental effect on the biomechanical properties of bending strength and rigidity, both compared to early conversion and to the use of an external fixator for definitive fracture management.

In conclusion, even though QCT was able to quantify the characteristic pattern of secondary healing with early callus formation and late callus resorption with bone remodelling, segmenting the QCT data into these different types of bone tissue did no increase the correlation with strength relative to using vBMD or any clinically usable predictor of bone strength. The two significant correlations found between strength and QCT-measured cortical bone and vBMD suggest that fracture-site QCT measurements are sensitive to biomechanically important fracture mineralization in the early phase of healing in fractures with relatively flexible fixation.

6 Perspectives

Our experimental findings cannot be directly extrapolated to human patients. However, the following implications for clinical treatment of tibial diaphyseal fractures can be suggested from our results: (1) IMN is useful as a standard treatment for unstable tibial fractures since it may promote callus formation and thereby improve biomechanical characteristics compared to the use of a more-rigid unilateral external fixator; (2) in fractures initially treated with a unilateral external fixator with adequate soft-tissue conditions, there exists an optimal time window (between 1 and 2 weeks after the initial surgery) in which a conversion to IMN from initial temporary EF provides superior biomechanical fracture healing with a low infection risk relative to later conversion; and (3) when the conversion to IMN cannot be performed early due to the patient having a severe polytrauma status or to the presence of damaged soft tissue surrounding the fracture segment, maintaining the EF until bony union in some (possibly more stable) configuration seems a viable option both to promote biomechanical fracture healing and to avoid deep infection.

Even though we did not identify any enhancement in our compression regimen, future studies should combine defined periods with simultaneous distraction and compression of the fracture gap in order to prevent limb lengthening or shortening. A clinically applicable combination of early compression and distraction could theoretically stimulate callus formation without creating a fracture gap and avoiding the induction of delayed healing or non-union, and potentially enhance both the mechanical properties and healing.

The results from a high-resolution micro-CT analysis of small animal bones cannot be directly transferred to and applied in the clinic. Still, we believe that our findings contain important and relevant information for the non-invasive evaluation and staging of bone repair, and indicate that further exploration of tissue threshold selection using imaging systems with higher resolutions and better fracture-line alignment of ROIs may increase the value of QCT. The future of fracture treatment research should include not only the development and clinical application of advanced surgical methods and the use of biologically active healing modifier molecules and materials, but also the corresponding development and clinical application of non-invasive methods for evaluating fracture healing. Even though computational models, mechanical monitoring and imaging modalities such as CT and MRI have shown substantial technological improvements and are widely applied, the ability to adequately and non-invasively evaluate fracture healing remains unsolved. Combining improved scanning resolution, lower radiation doses, superior image analysis techniques, physical material models, mathematical models and increased computational power has shown promising results, and may eventually solve this problem. Further validation and exploration of tissue threshold selection for segmentation and ROI configuration of QCT may be the first steps toward obtaining such a solution.

7 Figures

Figure 1. The OTA classification of tibial diaphyseal fractures (from [162]).

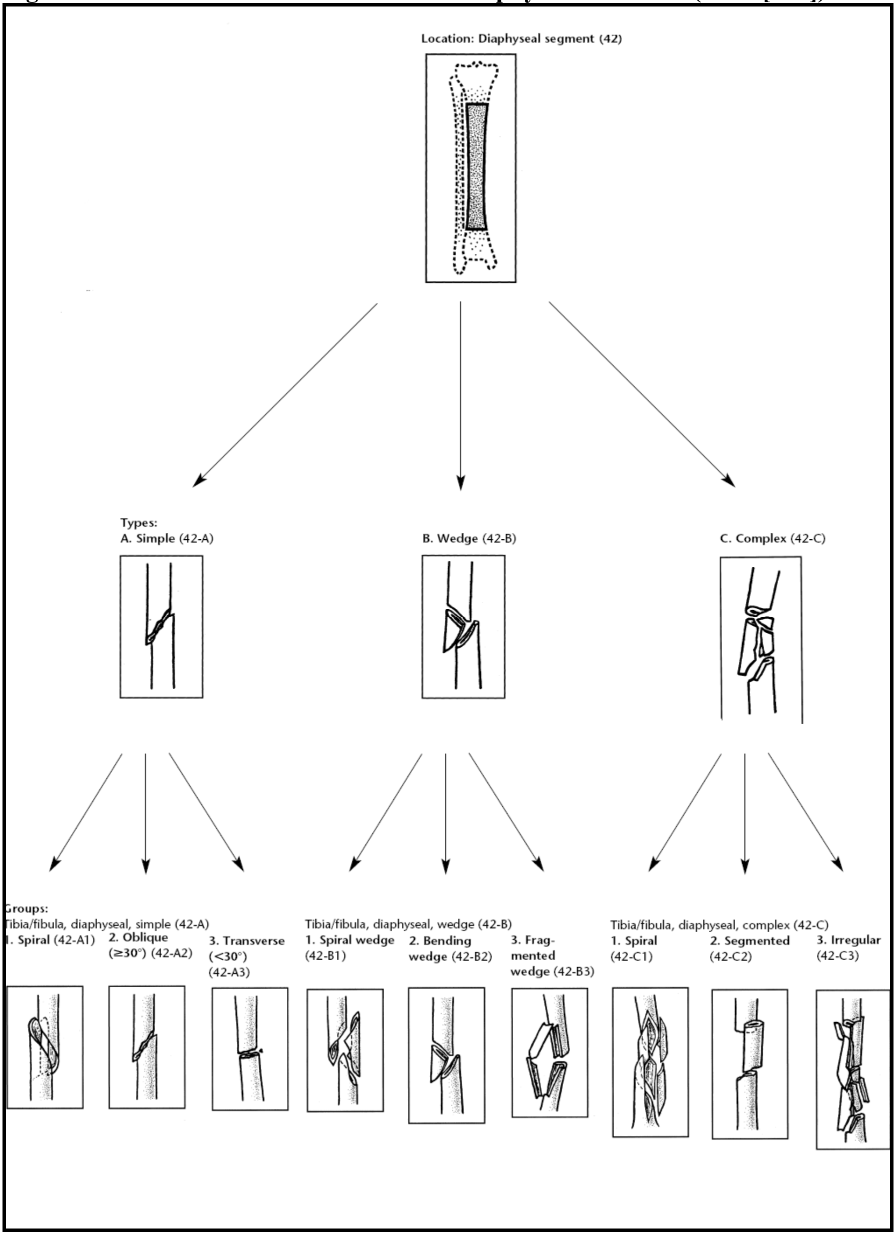


Figure 2. X-ray of an intact adult rat tibia.

Figure 3. Photograph of a rat tibia at 60 days after EF.

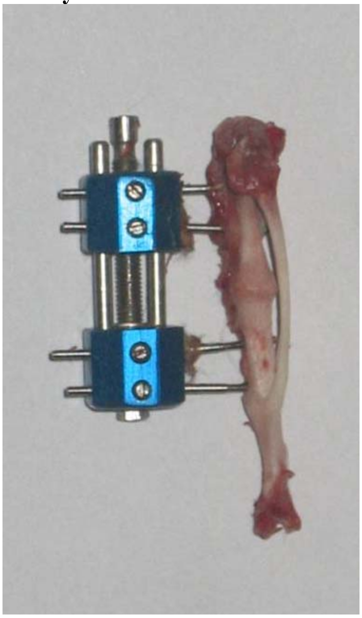


Figure 4. X-ray of a rat tibia at 60 days after EF.

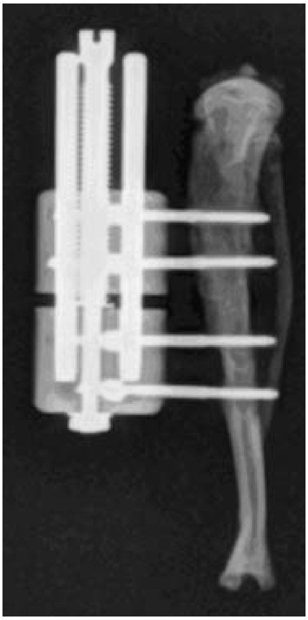


Figure 5. X-ray of a rat tibia at 60 days after IMN.

Figure 6. Load–deformation diagram from a cantilever bending test of an adult rat tibia tested to failure, showing the biomechanical structural properties of strength, stiffness and energy to fracture.

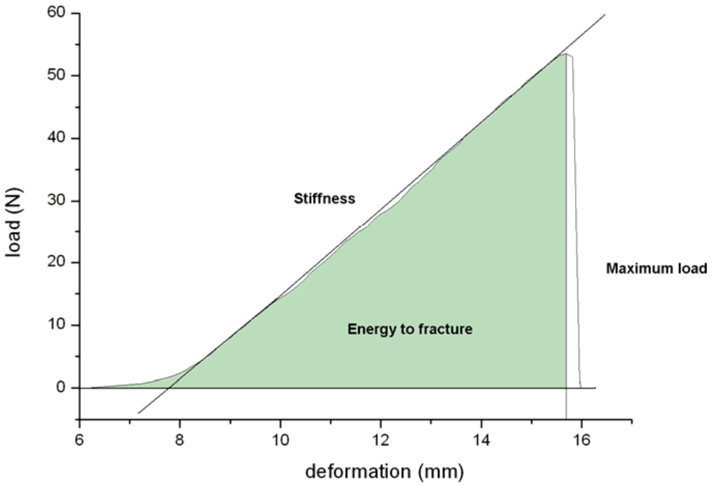


Figure 7. 3D CT reconstruction of a rat tibia at 60 days after initial treatment with IMN, showing the fracture line (A), the 1.25-mm-long narrow ROI (B), and the 3.75-mm-long wide ROI (C). The wide ROI is also shown enlarged. The fibula has been resected.

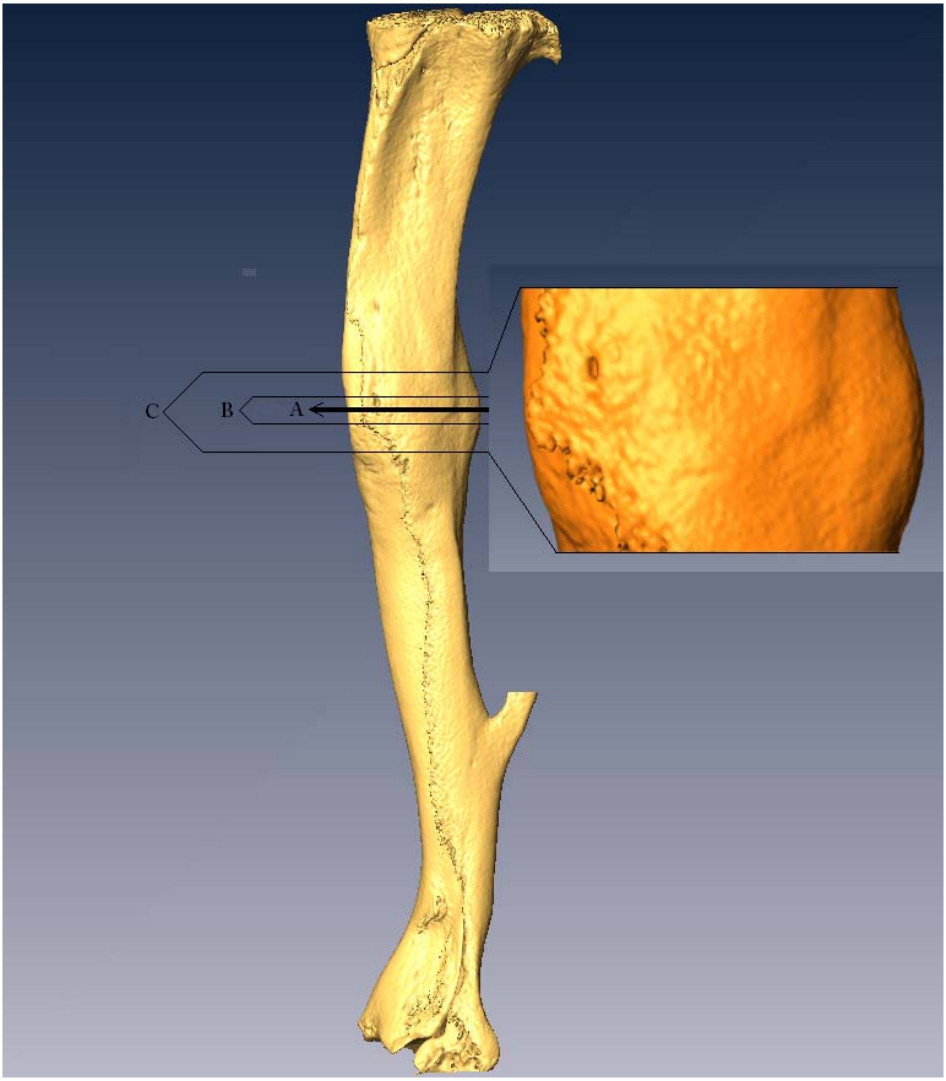


Figure 8. Normal and segmented cross-sectional CT images of the rat tibial diaphyseal fracture site. A) Normal cross-sectional image obtained after 60 days of EF, showing some periosteal and endosteal callus around the cortex. B) A segmentation of the image in panel A, where white = cortical bone, light grey = hard callus, dark grey = soft callus, and black = soft tissue or exterior. C) Normal cross-sectional image obtained after 30 days of IMN, showing some cortical bone and a massive periosteal callus. D) Segmentation of image C (colours are the same as in panel B).

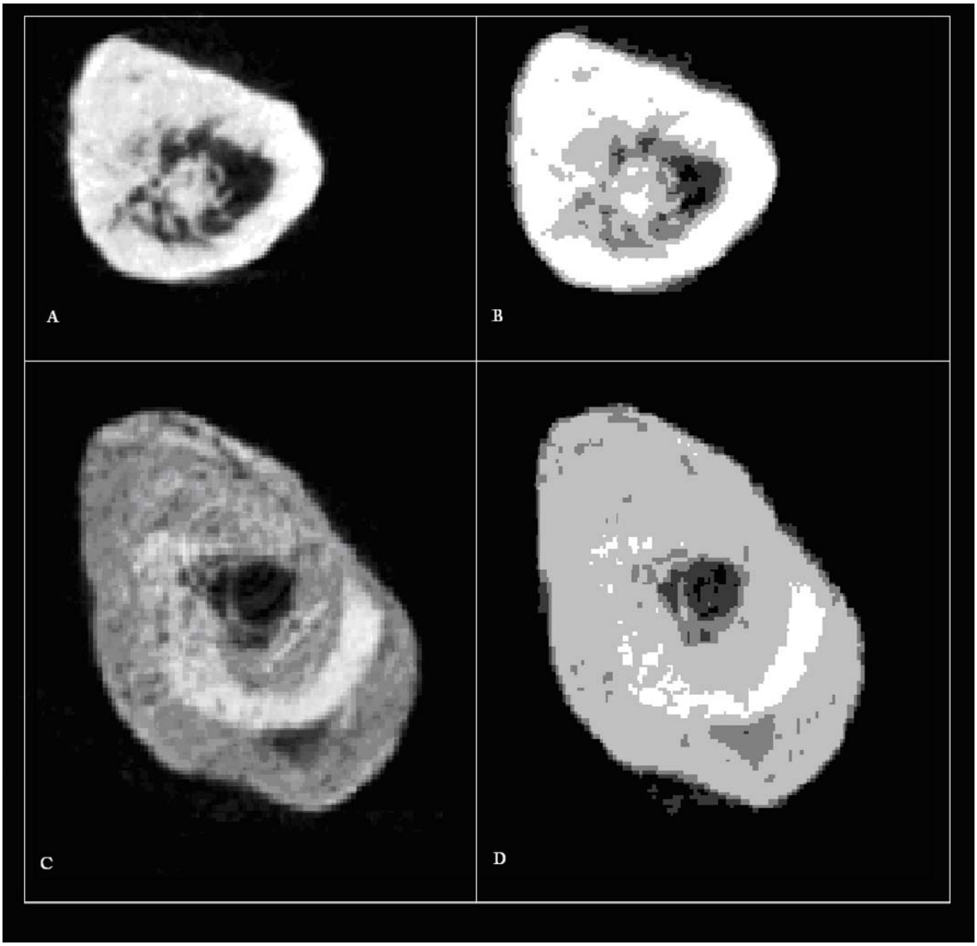


Figure 9. Slice-by-slice analysis of the tissue distribution (soft callus, hard callus and cortical bone) for the number of voxels per transverse CT slice along the 3.75-mm-long ROI in the longitudinal direction, centred around the fracture and encompassing the callus formed after fracture. Slice data are averaged for all fractures in all study groups (N=40). Voxels were cubes with a side length of 50.7 µm.

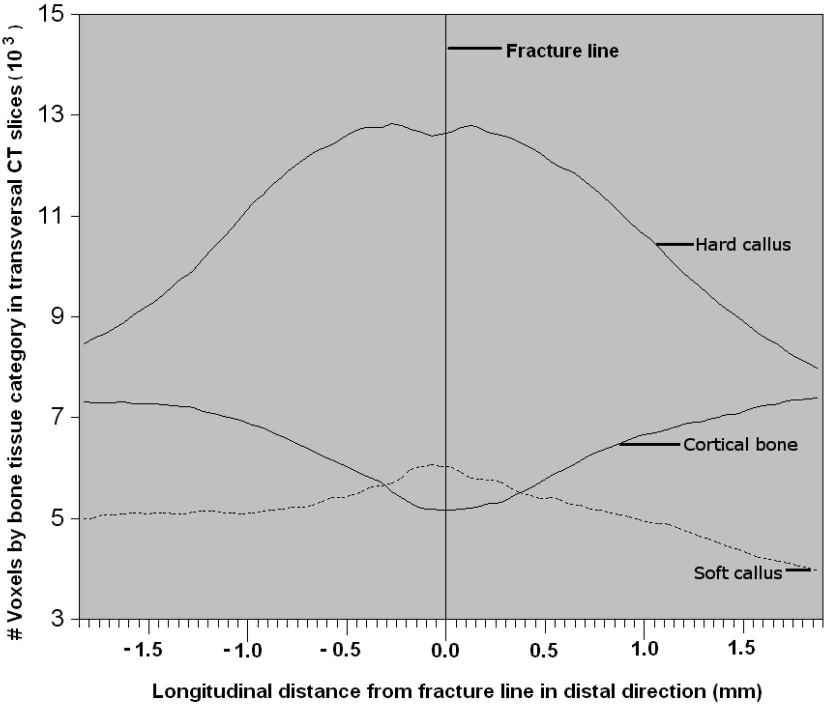
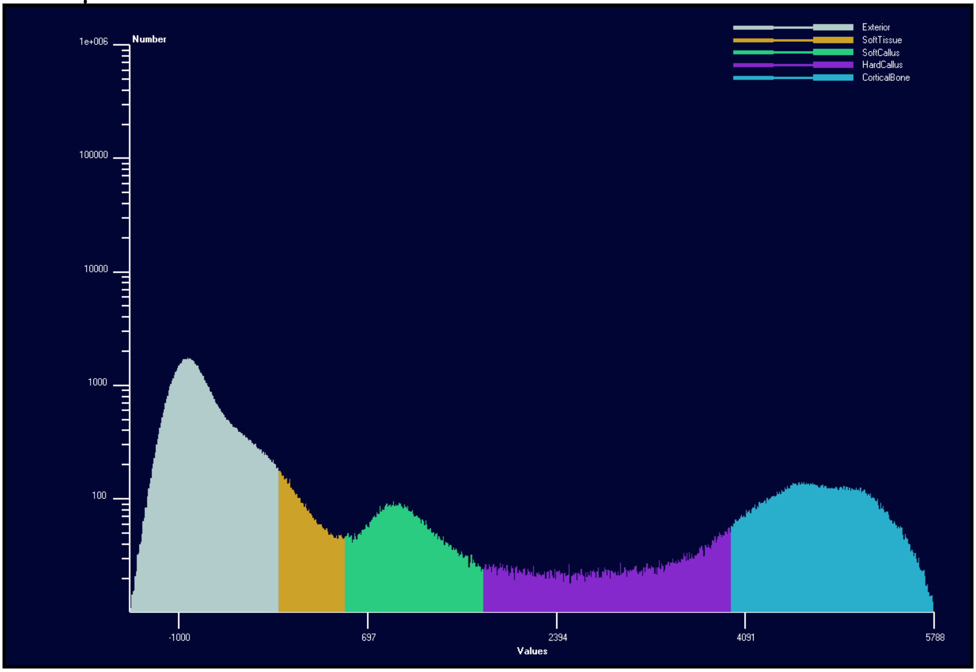


Figure 10. Histogram of the voxel distribution of micro-CT scans of the intact rat tibia. The ROI included a 3.75-mm-long segment (75 slices) of the tibia in the longitudinal direction. The voxels are segmented based on CT values into the exterior (<–7 mg/cm3), soft tissues and fat (–7–171 mg/cm3), soft callus (171–540 mg/cm3), hard callus (540–1200 mg/cm3) and cortical bone (>1200 mg/ cm3). Voxels were cubes with a side length of 50.7 µm.



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9 Original publications