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ANIMAL MODELS FOR STUDYING METAPHYSEAL BONE FRACTURE HEALING

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Abstract

An estimated 2 million osteoporotic fractures occur annually in the US, resulting in a dramatic reduction in quality of life for affected patients and a high economic burden for society. Osteoporotic fractures are frequently located in metaphyseal bone regions. They are often associated with healing complications, because of the reduced healing capacity of the diseased bone tissue, the poor primary stability of the fracture fixation in the fragile bone, and the high frequency of comorbidities in these patients. Therefore, osteoporotic fractures require optimised treatment strategies to ensure proper bone healing. Preclinical animal models can help understanding of the underlying mechanisms and development of new therapies. However, whereas diaphyseal fracture models are widely available, appropriate animal models for metaphyseal fracture healing are scarce, although essential for translational research. This review covers large and small animal models for metaphyseal fracture healing. General requirements for suitable animal models are presented, as well as advantages and disadvantages of the current models. Furthermore, differences and similarities between metaphyseal and diaphyseal bone fracture healing are discussed. Both large- and small-animal models are available for studying metaphyseal fracture healing, which mainly differ in fracture location and geometry as well as stabilisation techniques. Most common used fracture sites are distal femur and proximal tibia. Each model found in the literature has certain advantages and disadvantages; however, many lack standardisation resulting in a high variability or poor mimicking of the clinical situation. Therefore, further refinement of animal models is needed especially to study osteoporotic metaphyseal fracture healing.

Keywords: Fracture healing, animal models, metaphyseal fractures, bone regeneration.

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Introduction

Long bones are anatomically divided into the metaphyseal areas at each end and the diaphyseal region in between the metaphyseal areas. Metaphyseal bone is rich in trabecular bone surrounded by a thin cortical shell. In contrast, diaphyseal bone consist of a dense cortical bone shell enclosing the marrow cavity. Trabecular bone is more metabolically active compared to cortical bone due to its larger surface, the higher number of osteoblasts and osteoclasts as well as a good blood supply (Claes *et al.*, 2011b; Fan *et al.*, 2008). It was previously shown that osteoporosis, a disease characterised by bone loss and micro-architectural deterioration, affects trabecular bone rather than cortical bone at the beginning of the disease (Peng *et al.*, 1994). Therefore, many fractures in osteoporotic patients occur in metaphyseal bone. Most common fracture sites are the distal radius, proximal humerus, proximal femur and the spine. Each year an estimated 2 million osteoporotic fractures happen in the US, resulting in a dramatic reduction in quality of life for affected patients and a great economic burden for society (Alt and Miclau, 2016). Worldwide, one out of three women and one out of five men aged 50 years and over are at risk of osteoporotic fractures. Healing of osteoporotic fractures is often delayed due to increased osteoclast activity, decreased endochondral and intramembranous bone formation in the fracture callus, reduced angiogenesis and a disturbed inflammatory response towards fracture (Beil *et al.*, 2010; Haffner-Luntzer *et al.*, 2017; Nikolaou *et al.*, 2009). Besides this reduced healing capacity of the diseased osteoporotic bone tissue, osteoporotic fractures are challenging to treat because the weak bone quality hampers stable fracture fixation resulting in unfavourable mechanical conditions. Therefore, optimised fracture treatment strategies are required to reduce the increased complication rate in osteoporotic patients (Nikolaou *et al.*, 2009).

Preclinical animal models are essential for the development of new implants and therapeutics. So far, most animal models have recapitulated diaphyseal fracture healing. However, clinical observations and preclinical studies have indicated that diaphyseal and metaphyseal fracture healing may differ in many aspects, including the time course of healing, the extent of callus formation, the proportion of intramembranous and endochondral ossification, the source of precursor cells, the role of inflammation in the healing process, and the influence of the mechanical environment on the healing process (Sandberg and Aspenberg, 2016; Tatting *et al.*, 2018). Thus, the knowledge gained from diaphyseal healing models could not be simply transferred to metaphyseal healing. To better understand the characteristics of metaphyseal bone healing in general, and to unravel the pathomechanisms of disturbed metaphyseal healing observed in osteoporotic patients, suitable preclinical models are needed. They are a prerequisite for the development of new treatment strategies.

Here, an overview is provided of the current animal models of metaphyseal fracture healing. The advantages and limitations of the models and possible differences between metaphyseal and diaphyseal fracture healing are discussed.

Requirements for animal models to study metaphyseal fracture healing

The selection of a suitable animal model is of utmost importance to answer specific research questions and to transfer the results to the clinical situation. A preclinical model should ideally closely mimic the human situation. Differences and similarities between the model organism and the human species must be considered in general and specifically with respect to the experimentally induced fracture and the concomitant disorder (*e.g.* osteoporosis). It is essential that the metaphyseal fracture model is highly standardised and reproducible to draw valid conclusions from the experiments. Technical variation should be eliminated or substantially reduced. Further, animal welfare, as well as time and cost issues play a role in the selection of the animal model.

Large animals, such as sheep and dogs, have been used for decades in fracture healing research, and some of these studies also addressed metaphyseal bone healing (Fig. 1). Compared to small animals, large animals more closely mimic human bone physiology in terms of bone metabolism, dimensions, and mechanical loading conditions. Often, clinically available fracture fixation devices can be used due to the comparable skeletal size. Furthermore, fracture healing can be evaluated using similar tools

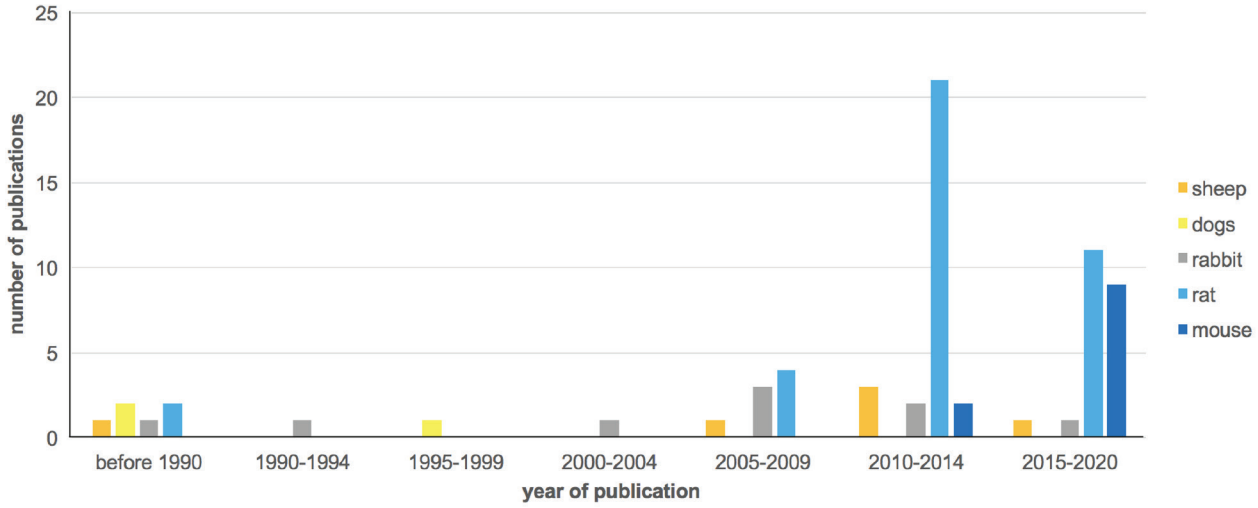

Fig. 1. Usage of different animal species for metaphyseal fracture healing research over time. Number of publications found in PMC by a systematic literature research which was performed in early June 2020. The first search was performed using the search expression *"metaphyseal" AND "fracture"*. This search resulted in 1963 publications. The second search was performed using the search expression *"cancellous" AND "fracture" NOT "metaphyseal"*. This search resulted in 3190 publications. The third search strategy was performed using the search expression *"osteotomy" AND "metaphyseal" NOT "fracture"*. This search resulted in 242 publications. In total 5395 publications were screened. 66 publications were selected for this review based on certain inclusion and exclusion criteria. Inclusion criteria were: preclinical study, *in vivo* study, fracture model, metaphyseal bone involved, full-text available in English, German or French. Exclusion criteria were: clinical study, case study from veterinarian medicine, *ex vivo* or *in vitro* study, no fracture model (*e.g.* osteolysis or bone defect model without implant fixation), no metaphyseal bone involved, published as conference abstracts or full text not available. Within the selected 66 publications, there were 5 sheep studies, 3 dog studies, 9 rabbit studies, 38 rat studies and 11 mouse studies. Simple bone drill-hole models were not included in this literature review, because they reflect defect healing rather than metaphyseal fracture healing and lack adequate mechanical stimulation.

to those used in the clinics, such as radiography and CT imaging. Additionally, as a benefit of animal models in general, *ex vivo* analysis such as histological evaluation is possible. On the other hand, large animal experiments are space and cost intensive and possible molecular analyses are limited, because transgenic animals are hardly available and molecular analytical tools (*e.g.* antibodies) often missing (Oheim *et al.*, 2016). Furthermore, the usage of drugs in the experimental setting can be expensive due to the large body weight. Importantly, the induction of osteoporosis to study osteoporotic fracture healing is challenging in sheep (Oheim *et al.*, 2016). Ovariectomy (OVX), a commonly used model to mimic postmenopausal osteoporosis, is not sufficient to induce significant bone loss in sheep. OVX needs to be combined with other treatments such as glucocorticoid application, calcium deficient diet or surgical procedures, *e.g.* disconnection of the hypothalamus-pituitary axis (Oheim *et al.*, 2013; Oheim *et al.*, 2016; Oheim *et al.*, 2017), making these models much more complex and challenging their translational relevance.

In recent years small animals, including rabbits, rats and mice, have become more and more popular for metaphyseal fracture healing research (Fig. 1). These species are relatively easy to handle, as well as being cost and space effective. Further, the bone healing process is much faster in small animals compared to larger species, resulting in shorter experimental time periods. A major advantage is the availability of genetically modified animals, which allows the unravelling of molecular mechanisms of the fracture healing process. This is especially true for mice, but there are also some transgenic rabbit and rat lines available. Furthermore, the small size of the animals allows cost-effective treatments with drugs, antibodies, or other biological substances of interest. Additionally, significant bone loss can be induced in rodents by OVX and aging, if a study of metaphyseal fracture healing in a postmenopausal model or age-related osteoporosis is desired. The disadvantage of small animal models is that bone structure, metabolism and size are very different from humans. Furthermore, the loading situation is not comparable. Due to the small skeletal size, technical problems can arise in creating standardised fractures. Custom fracture fixation devices need to be developed as well as standardised surgical techniques. Since the metaphyseal area in the long bones of adult mice is very small, it can be challenging to establish a fracture protocol especially for those animals.

Basically, standardised mechanical conditions at the fracture site are essential to reduce interindividual variation and gain reproducible data, because bone healing is highly dependent on the tissue strains acting at the fracture site (Claes, 2017). Small interfragmentary strains may result in intramembranous ossification, the direct conversion of mesenchymal tissue into bone, whereas moderate strains induce endochondral ossification, in which a cartilaginous matrix intermediate is converted to bone (also known as callus healing). High interfragmentary strains impair bone healing. This seems to be true for both diaphyseal and metaphyseal fracture healing (Claes *et al.*, 2011a; Hinsenkamp *et al.*, 1984; Sandberg and Aspenberg, 2016; Uhthoff and Rahn, 1981). The mechanical conditions in the healing zone are determined by the location and geometry of the fracture, the stability of fracture fixation and the mechanical loading of the fractured limb. Therefore, the model must be highly standardised with respect to these features. Furthermore, from a translational perspective a suitable animal model should closely mimic fracture types observed in the clinics.

Methodology for systematic literature research

A systematic literature research was performed using PubMed Central® (PMC) in early June 2020.

The first search was performed using the search expression *"metaphyseal" AND "fracture"*. This search resulted in 1963 publications. The second search was performed using the search expression *"cancellous" AND "fracture" NOT "metaphyseal"*. This search resulted in 3190 publications**.** The third search strategy was performed using the expression *"osteotomy" AND "metaphyseal" NOT "fracture"*. This search resulted in 242 publications.

In total, 5395 publications were screened. 66 publications were selected for this review, based on certain inclusion and exclusion criteria. Inclusion criteria were: preclinical study, *in vivo* study, fracture model, metaphyseal bone involved, full-text available in English, German or French. Exclusion criteria were: clinical study, case study from veterinarian medicine, *ex vivo* or *in vitro* study, no fracture model (*e.g.* osteolysis or bone defect model without implant fixation), no metaphyseal bone involved, published as conference abstracts or full text not available.

Within the selected 66 publications, there were 5 sheep studies, 3 dog studies, 9 rabbit studies, 38 rat studies and 11 mouse studies. Simple bone drill-hole models were not included in this literature review, because they reflect defect healing rather than metaphyseal fracture healing and lack adequate mechanical stimulation.

Large animal models for metaphyseal fracture healing

Sheep

Studies using sheep as a model organism for metaphyseal fracture healing research are scarce. Only 5 papers were found in PMC. The studies differ in the localisation of the fracture and the possibility of defining and controlling the mechanical conditions at the fracture site (Table 1).

Heiss *et al*. developed a metaphyseal fracture model at the proximal ulna with the primary aim

of testing a new bone glue (Heiss *et al.*, 2010). A metaphyseal segment of 20 mm length was removed from the proximal ulna, reinserted, and then fixed with the bone adhesive or not (control animals). The authors reported that the sheep loaded their leg normally from the first postoperative day onwards, indicating that the procedure was well tolerated. Most fractures were bridged with newly formed bone after 3 months. External callus formation was only observed in the control group, suggesting that the fracture was unstable, if the fragment was not fixed with the glue. The advantages of this metaphyseal fracture model were early weight-bearing, even if the bone segment was not fixed, and – with respect to animal welfare – the absence of adverse events in the postoperative period. However, the results showed a relatively high variance within the groups, suggesting difficulties in technical standardisation and/or varying mechanical conditions. Furthermore, the location of the fracture at the proximal ulna does not reflect a common clinical situation.

The other available studies used metaphyseal fracture models at the distal femur (Alagboso *et al.*, 2019; Bindl *et al.*, 2013; Claes *et al.*, 2011a; Claes *et al.*, 2009). In one study, a full wedge-shaped osteotomy was created at the lateral femoral condyle, which was rigidly stabilised by a custom-made locking plate (Alagboso *et al.*, 2019). All animals tolerated the surgery well and returned to full weight-bearing within the first 3 postoperative days. After 3 months, intramembranous bone formation was observed in the fracture gap with some variation in bony bridging scores (healing scores ranged from 4 to 9) within the groups. Biomechanical indentation testing revealed no significant differences in stiffness between healed and intact bone indicating successful healing. No external callus was found at the lateral aspect of the osteotomy, only a small periosteal callus was observed at its medial site. In comparison to the above-mentioned model, this sheep model might provide a higher degree of standardisation. Furthermore, the authors state that the full discontinuity of the osteotomy gap may better reflect the clinical situation than a partial osteotomy at the distal femur as used in other studies (Claes *et al.*, 2011a; Claes *et al.*, 2009).

In order to study the influence of mechanical loading on metaphyseal fracture healing, Claes *et al.* also established a sheep model at the distal femur (Claes *et al.*, 2009). A partial osteotomy (3 mm) was created at the femoral condyle close to the trochlea. The deflection of the osteotomy gap during limb loading was controlled by distance plates inserted at the proximal end of the gap. All animals tolerated the surgery well and returned to normal activity after 3 d. Bone healing was analysed after 8 weeks in different regions of interest in the osteotomy gap that displayed different magnitudes of interfragmentary strains. In areas with strains below 5 %, significantly less bone was formed compared to areas with higher strains (6 to 20 %). Strains larger than 20 % induced fibrocartilage formation. From these results, it was suggested that metaphyseal bone healing follows similar biomechanical principles to diaphyseal healing, although no external callus was formed. This metaphyseal fracture model was also used in an osteoporotic sheep model (Bindl *et al.*, 2013). The advantages of this model are the high degree of standardisation and the exactly controllable mechanical conditions.

In summary, to date only few models have been established for studying metaphyseal fracture healing in sheep. Most studies have been conducted

Table 1. Sheep models for metaphyseal fracture healing.

Strain	Age	Sex	Fracture site	Fracture model		Osteoporosis model	Healing outcome	References
mountain sheep, black head sheep, merino sheep	3-6 years	female	distal femur	open partial osteotomy (3 mm gap), plate implanted to limit interfragmentary movement		ovariectomy, hypothalamic-pituitary disconnection	interfragmentary strains at the fracture site determine type of tissue formation: endochondral and intramembranous bone formation	Claes *et al.*, 2009 Claes *et al.*, 2010 Bindl *et al.*, 2013
merino sheep	5-8 years	female	proximal ulna	open osteotomy (20 mm gap), no fixation or bone glue fixation		N/A	healing by intramembranous bone formation	Heiss *et al.*, 2010
merino sheep	6-7 years	female	distal femur	open wedge-shaped osteotomy, plate fixation		N/A	healing by intramembranous bone formation	Alagboso *et al.*, 2019

at the distal femur. Depending on the localisation and stabilisation of the fractures, healing occurs in 2 to 3 months without pronounced external callus formation. To our best knowledge, only one study used osteoporotic sheep to mimic metaphyseal fracture healing in osteoporotic patients.

Dogs

Only 3 studies were found which used dogs to study metaphyseal fracture healing, all of them at the distal femur (Table 2). Uhthoff *et al*. created a standardised wedge-shaped fracture gap in the distal femur (Uhthoff and Rahn, 1981). Since the osteotomy was incomplete, no fixation device was used. After 4 weeks, the fractures displayed the first signs of intramembranous ossification, suggesting a high mechanical stability. Later time points were not investigated. Furthermore, the same group established an intercondylar femoral osteotomy model (Uhthoff *et al*., 1987). A lateral osteotomy was sawed, the bone fragments reduced and fixed by two cortical screws. The authors reported that the operation was well tolerated and that all fractures healed uneventfully by direct bone formation within 14 weeks. This was confirmed by another group that used the same model to test bioabsorbable screws (An *et al*., 1998). This model is highly standardised due to the standardised osteotomy and the complete reduction of the fracture gap.

In conclusion, the described models resulted in direct bone formation at the fracture site. Complete healing took up to 14 weeks. Ethical considerations might be the reason for the low number of studies in dogs. No osteoporotic dog models were used.

Small animal models for metaphyseal fracture healing

Rabbits

Rabbits have been used for a long time as a model organism for metaphyseal fracture healing research (Table 3). Fracture locations vary from proximal fibula, proximal and distal tibia and distal femur. In fact, rabbits are the model organism with the widest spectrum of described models at different fracture sites.

Matos *et al*. established an osteotomy model in the proximal rabbit fibula, which was stabilised by the intact tibia (Matos *et al*., 2007). The very stable and standardised fracture geometry led to predominantly intramembranous bone formation. Furthermore, the authors were able to demonstrate a robust osteoanabolic effect of zoledronic acid in this model. However, the proximal fibula is not a common location for metaphyseal fractures in the clinics.

Tsiridis *et al*. (Tsiridis *et al*., 2007) created a unicortical, wedge-shaped osteotomy at the distal tibia. The lateral gap size was 4 mm. The fracture was fixed by a custom-made external fixator. All fractures were healed after 4 weeks and did not display external callus formation. Advantages of this model are the clinically relevant fracture location and the good reproducibility. This model was successfully

Table 2. Dog models for metaphyseal fracture healing.

Strain	Age	Sex	Fracture site	Fracture model		Osteoporosis model	Healing outcome	References
Beagles	adult	N/A	distal femur	incomplete osteotomy, no fixation		N/A	appearance of external callus depends on fracture stability	Uhthoff *et al*., 1981
N/A	3-8 years	N/A	distal femur	open intercondylar 25 mm long osteotomy, fixation with cortical screws		N/A	healing by direct bone formation	Uhthoff *et al*., 1987
N/A	adult	male	distal femur	open lateral femoral condyle osteotomy, fixation with two screws		N/A	healing by direct bone formation	An *et al*., 1998

used to analyse the effects of parathyroid hormone (PTH) and bone morphogenetic protein (BMP)-7 on metaphyseal fracture healing (Morgan *et al.*, 2008; Tsiridis *et al.*, 2007).

Other models were created at the proximal tibia. Han *et al.* introduced an oblique proximal tibia fracture model (Han *et al.*, 2012). They created a bilateral splitting fracture through the tibial plateau. The fracture was fixed by a lateral compression screw. The authors reported that only one animal suffered from fracture displacement, indicating a high degree of standardisation in this model. No external callus was formed, and histological analysis revealed osteoblast proliferation along the bone trabeculae as early as 1 week after fracture and intramembranous bone formation after 2 weeks. Bony bridging occurred 4 weeks after surgery. Chen *et al.* published a vertical osteotomy model at the proximal tibia in rabbits (Chen *et al.*, 2015). After anatomical reduction, the fracture was fixed with a compression screw. The authors reported intramembranous bone formation at the pre-existing bone trabeculae and already in the bone marrow 5 d after surgery. After 2 weeks, the fracture gap was filled with new bone trabeculae. The original bone structure was restored between week 4 and 8 after fracture. Also in this study, no signs of external callus formation and endochondral bone formation were

Table 3. Rabbit models for metaphyseal fracture healing.

Strain	Age	Sex	Fracture site	Fracture model		Osteoporosis model	Healing outcome	Reference
N/A	adult	N/A	distal femur	incomplete osteotomy, no fixation		N/A	appearance of external callus depends on fracture stability	Uhthoff *et al.*, 1981
New Zealand white	16-48 weeks	either	distal femur	open transverse osteotomy (0.15 mm gap), fixation by intramedullary PLLA rod and PGA membrane		N/A	healing by periosteal bone formation	Ashammakhi *et al.*, 1994
Japanese white	6 months	N/A	proximal tibia	bilateral, open osteotomy, fixation by a K-wire staple		N/A	healing by intramembranous bone formation, small external callus	Chen *et al.*, 2004
New Zealand white	1.5 months	male	proximal fibula	open osteotomy (0.5 mm gap)		N/A	healing by intramembranous bone formation	Matos *et al.*, 2007
New Zealand white	16-18 weeks	female	distal tibia	open, unicortical, wedge-shaped osteotomy (lateral gap size 4 mm), fixation by external fixator		N/A	healing by external callus formation	Tsiridis *et al.*, 2007 Morgan *et al.*, 2008
New Zealand white	6 months	N/A	proximal tibia	bilateral, oblique fracture through the tibial plateau, fixation by compression screw		N/A	healing by intramembranous bone formation	Han *et al.*, 2012
New Zealand white	8 months	female	distal femur	open, half-round fracture 10 mm proximal to the distal femur end, fixation by different tubes and bone cements		osteoporosis by ovariectomy and glucocorticoid treatment	healing by intramembranous bone formation	Nishizuka *et al.*, 2014
New Zealand white	6 months	N/A	proximal tibia	open vertical osteotomy at the medial tibial plateau, fixation by compression screw		N/A	healing by intramembranous bone formation	Chen *et al.*, 2015

found throughout the whole fracture healing process. Another study established a proximal tibia osteotomy model which was stabilised by K-wires (Chen *et al*., 2004). Also in this model, all fractures healed without pronounced external callus formation. Histological analysis revealed both intramembranous and endochondral bone formation in the fracture area, indicating higher interfragmentary tissue strains. In this study, fractures healed between 4 and 8 weeks after surgery. In summary, several models were described at the proximal tibia in rabbits using different fracture fixation devices, resulting in different degrees of mechanical stability and therefore different fracture healing modes.

Many studies using rabbit for metaphyseal fracture healing research are conducted on the distal femur. To investigate the influence of fracture geometry and gap size, Uhthoff *et al*. performed an incomplete osteotomy on the rabbit distal femur (Uhthoff and Rahn, 1981). No fracture fixation was used. Gap sizes varied greatly and, in some animals, spontaneous fractures occurred during the experimental period. Histological analysis revealed that the healing course was highly dependent on the stability of the fractures. Rabbits with spontaneous fractures displayed external callus formation and endochondral ossification at the fracture site. More stable fractures healed by intramembranous ossification, without external callus formation, within 3 weeks. This model lacks standardisation and cannot be recommended for ethical reasons. Ashammakhi *et al*. published a complete metaphyseal osteotomy model (Ashammakhi *et al*., 1994). They sawed a 0.15 mm osteotomy gap in the distal femur metaphysis and stabilised the fracture by using an intramedullary rod. However, the authors reported a high rate of adverse events, both during the surgery and the post-operative time course, due to implant failure – indicating an insufficient fracture stabilisation in this model. Nishizuka *et al*. tested a different fixation material for an incomplete, half-round fracture of the rabbit distal femur (Nishizuka *et al*., 2014). They used OVX rabbits treated with glucocorticoids to induce severe osteoporosis. Fractures were stabilised with different configurations. Dependent on the used fracture fixation device, there was a high rate of adverse events after surgery, indicating the need for a well-controlled model to study metaphyseal fracture healing at the rabbit distal femur.

In conclusion, several rabbit models were developed to study metaphyseal fracture healing at different fracture locations, with different fracture geometries and fixations. Healing occurred within 4 to 8 weeks. Both intramembranous ossification and endochondral ossification were found, dependent on the stability of the used fracture configuration. Some studies showed a high rate of adverse events due to implant failure, indicating the need to further improve metaphyseal fracture models in rabbits.

Rats

For metaphyseal fracture healing research, rats have been most commonly used. The models are located either at the proximal tibia or the distal femur, but vary widely regarding fracture geometry and stabilisation. Closed and open model were used with and without fixation devices (Table 4).

Aspenberg *et al*. proposed a very simple model to study metaphyseal bone healing (Meunier and Aspenberg, 2006). The group created a unicortical drill hole into the trabecular bone at the medial site of the proximal tibia and inserted a screw. Bone formation was analysed histologically, and the interfacial shear strength was measured using a pull-out test. The pull-out force increased during the experimental time period of 14 d, indicating bony integration of the implant. This model may be very simple and highly standardised; however, it reflects osseointegration of an implant rather than metaphyseal healing normally occurring between two bony fragments. Nevertheless, the authors used this model in many further studies to investigate the effects of unloading and various treatments, such as cyclooxygenase 2 (Cox2) inhibition, PTH, bisphosphonates, Dickkopf-1 inhibition, tissue necrosis factor α inhibition, Sclerostin inhibition and receptor activator of the NF-κB ligand (RANKL) inhibition on trabecular bone healing (Agholme *et al*., 2011; Agholme *et al*., 2014; Aspenberg *et al*., 2008; Bernhardsson *et al*., 2015a; Bernhardsson *et al*., 2015b; Meunier and Aspenberg, 2006; Sandberg *et al*., 2012; Sandberg *et al*., 2014). The model was also used to compare metaphyseal and diaphyseal fracture healing in rats (Bernhardsson *et al*., 2015b; Sandberg and Aspenberg, 2015a). These latter studies demonstrated that the inflammatory response differs between the two type of fractures, whereas both follow similar biological principles regarding osteoanabolic pathways.

Jarry *et al*. (Jarry and Uhthoff, 1971) used different "real" fracture models at the distal femur and the proximal tibia. To study the influence of the fracture type and of biomechanical stimuli, the authors created closed fractures or performed osteotomies in an open approach. The fracture was left unfixed or stabilised by an intramedullary K-wire. Some experimental groups were immobilised. The main outcome of this study was that external callus formation and endochondral ossification was found under mechanically unstable conditions, whereas intramembranous bone healing without periosteal callus formation was observed in more stable situations. This indicates that, also in the rat, metaphyseal fracture healing is highly dependent on the mechanical environment. However, the mechanical conditions can hardly be controlled in the models used and may vary highly within the different groups, even after fixation of the fracture with an intramedullary pin – which does not provide rotational and shear stability (Histing *et al*., 2011).

Table 4. Rat models for metaphyseal fracture healing.

Strain	Age	Sex	Fracture site	Fracture model		Osteoporosis model	Healing outcome	References
Sprague-Dawley	N/A	N/A	distal femur or proximal tibia	open and closed fractures/ osteotomies, fixation by K-wire or no fixation		N/A	appearance of external callus depends on fracture stability	Jarry *et al.*, 1971
N/A	adult	N/A	distal femur	open, oblique osteotomy, no fixation		N/A	appearance of external callus depends on fracture stability	Uhthoff *et al.*, 1981
Sprague-Dawley	8-20 weeks	either	proximal tibia	open, unicortical drill hole defect, inserted screw (1.7 mm diameter, 3 mm length)		unloading by muscle paralysis	healing by intramembranous bone formation	Meunier *et al.*, 2006 Aspenberg *et al.*, 2008 Agholme *et al.*, 2011 Sandberg *et al.*, 2012 Agholme *et al.*, 2013 Sandberg *et al.*, 2014 Bernhardsson *et al.*, 2015
Wistar	20 weeks	male	distal femur	open, transverse transcondylar osteotomy, fixation with PLLA-rod		N/A	healing by periosteal callus formation	Handolin *et al.*, 2007
Wistar	7-9 months	female	proximal tibia	open, complete mid-sagittal osteotomy, fixation with cerclage		ovariectomy (OVX), ageing, glucocorticoid-induced osteoporosis	healing by intramembranous bone formation	Nozaka *et al.*, 2008 Aonuma *et al.*, 2011 Aonuma *et al.*, 2014 Kawano *et al.*, 2017
Sprague-Dawley	12 weeks - 11 months	either	proximal tibia	open osteotomy (gap 0.5 mm), fixation by five-hole T-shaped miniplate		OVX, orchidectomy (ORX)	healing by intramembranous bone formation with small periosteal callus in the absence of fibula fractures; large periosteal callus formation in the presence of fibula fractures	Stuermer *et al.*, 2010 Kolios *et al.*, 2010 Komrakova *et al.*, 2010 Komrakova *et al.*, 2011 Komrakova *et al.*, 2013 Stuermer *et al.*, 2014 Komrakova *et al.*, 2015 Saul *et al.*, 2018
Sprague-Dawley	26 weeks, 7-8 months	female	distal femur	open wedge-shaped osteotomy (lateral height of 3, 4 or 5 mm, medial height 1 mm), fixation by 7-hole T-shaped miniplate		OVX and multi-deficient diet	osteotomy with 5 mm lateral height displayed non-union formation, healing of smaller defects by intramembranous bone formation with small periosteal callus formation	Alt *et al.*, 2013 Thormann *et al.*, 2013 Lips *et al.*, 2013 Lips *et al.*, 2014 Thormann *et al.*, 2014 Kampschulte *et al.*, 2016 Ray *et al.*, 2016 Ray *et al.*, 2018
Sprague-Dawley	12 weeks	female	proximal tibia	open osteotomy (gap 1 mm), fixation by five-hole Y-shaped miniplate		OVX	healing by periosteal callus formation	Mackert *et al.*, 2017

Further metaphyseal fracture models were established in the distal rat femur. After creating an oblique osteotomy, Uhthoff *et al*. observed exclusively intramembranous bone formation, indicating a high degree of mechanical stability, although no fracture fixation device was used (Uhthoff and Rahn, 1981). In this model, the bones were completely healed within a period of 42 d. Despite being rather simple from a technical aspect, this model may provide a relatively high degree of standardisation. Handolin *et al*. performed a transverse transcondylar osteotomy, which was stabilised with an intramedullary rod (Handolin *et al*., 2007). The authors described periosteal callus formation and intramembranous ossification at the fracture site. All osteotomies were bridged with newly formed bone after 12 weeks. However, 10 % of the animals displayed implant failure and were excluded from the study. This model was further used to analyse the influence of low-intensity pulsed ultrasound on metaphyseal fracture healing. Its disadvantages are the high rate of adverse events and the relatively long healing time. Alt *et al*. established a critical size osteotomy model with the primary aim of testing bone grafts to support metaphyseal bone healing (Alt *et al*., 2013). They created a complete wedge-shaped osteotomy at the distal femur with a lateral gap size of 3 to 5 mm and stabilised the fragments with a commercially available T-shaped miniplate. Whereas the rats with the 3 mm gap healed completely, the 5 mm osteotomies did not heal after 6 weeks, indicating a critical size for this defect. The authors also used this model to demonstrate that metaphyseal bone healing is impaired in ovariectomised, osteoporotic rats (Thormann *et al*., 2014). This model is well standardised, allows variation of the gap size and can further be used to study the effect of inserting biomaterials into the gap (Kampschulte *et al*., 2016; Lips *et al*., 2013; Lips *et al*., 2014; Ray *et al*., 2018; Ray *et al*., 2016; Thormann *et al*., 2013).

Besides the distal femur, many studies selected the proximal tibia as a fracture location. Nozaka *et al*. created a vertical osteotomy model at the proximal tibia in ovariectomised, osteoporotic rats (Nozaka *et al*., 2008). A mid-sagittal osteotomy was sawed from the joint surface to the diaphysis of the tibia. The osteotomy was fixed with a circumferential cerclage. The osteoporotic rats displayed reduced bone formation compared to non-OVX animals. 40 % and 27 % of bony union was observed in the control and osteoporotic rats, respectively, after 5 weeks. This model was further used to investigate the effects of low intensity pulsed ultrasound (LIPUS), alendronate and PTH on metaphyseal fracture healing in OVX or aged rats or in rats with glucocorticoid-induced osteoporosis (Aonuma *et al*., 2011; Aonuma *et al*., 2014; Kawano *et al*., 2017). A disadvantage of this model is the relatively long healing period, as most of the fractures were not healed after 5 weeks.

Stuermer *et al*. introduced a transverse osteotomy model at the proximal tibia (Stuermer *et al*., 2010b). An osteotomy with a gap size of 0.5 mm was created 7 mm distal to the tibial plateau. The fracture was stabilised by a commercially available T-shaped miniplate. Some animals, which accidentally suffered from additional fibula fractures, developed a large external fracture callus at the tibia due to the unstable mechanical situation and had to be excluded from the study. The rats with isolated tibial fractures displayed mainly endosteal healing with moderate periosteal callus formation. The fractures were bridged with bone after 35 d. This model is well standardised, but the drop-out rate is relatively high due to additional fibula fractures, indicating that the operating procedure is probably challenging. The model was used in further studies to investigate the effects of oestrogen, oestrogen-receptor modulators, PTH, alendronate, strontium ranelate, zileuton and whole-body vibration on osteoporotic metaphyseal fracture healing (Kolios *et al*., 2010a; Kolios *et al*., 2010b; Kolios *et al*., 2009; Komrakova *et al*., 2011a; Komrakova *et al*., 2013; Komrakova *et al*., 2011b; Komrakova *et al*., 2010; Komrakova *et al*., 2015; Saul *et al*., 2018; Stuermer *et al*., 2014; Stuermer *et al*., 2010a; Stuermer *et al*., 2010b). In later studies, the authors even used bilateral tibial osteotomies. A very similar fracture model was recently used to evaluate the effects of an extracorporeal shockwave therapy on metaphyseal fracture healing in ovariectomised rats. These authors created a 1 mm osteotomy gap and used a Y-shaped miniplate for fracture fixation (Mackert *et al*., 2017). Others used a similar model, but with a smaller gap size (0.35 mm) to investigate the effects of a vibration treatment on metaphyseal fracture healing in osteoporotic rats (Choy *et al*., 2020; Wong *et al*., 2019).

Concluding, various rat models with different fracture geometries and fixation techniques were established for use at the distal femur or the proximal tibia. The fracture healing time varied between 35 d and 12 weeks, dependent on the model. Many rat studies used osteoporosis models to study osteoporotic metaphyseal fracture healing; however, variability was rather high especially in osteoporotic animals.

Mice

Despite their small skeleton, which makes operative procedures challenging, mice are increasingly used for metaphyseal fracture healing research (Fig. 1). 11 studies were found in PMC, all published within the last 8 years (Table 5). The fracture models vary widely, but similar to the rat studies, the most common fracture locations are the distal femur and the proximal tibia. Only one study used the proximal femur as a more clinically relevant fracture location.

The first study to use mice for metaphyseal fracture healing research was published by Histing *et al*. (2012). The authors conducted an open transverse osteotomy with a 0.22 mm gap size at the distal femur, stabilised by a commercially available 3-hole locking plate designed specifically for mice. The authors

observed no adverse events during the experimental time period. In all animals, the plates and osteotomies were located correctly, indicating technical feasibility. After 5 weeks, all fractures were almost completely healed. Biomechanical testing revealed a significantly higher rotational stiffness 5 weeks after fracture (90 % compared to unfractured bone) compared to 2 weeks (30 %). Histological analysis demonstrated that the fractures healed without external callus formation by intramembranous ossification, indicating high mechanical stability. This model seems to provide a high degree of standardisation. A disadvantage might be that it is technically difficult to fix a plate on the small mouse bone. This requires experienced surgeons. A very similar fracture model but with a large gap size of 1.2 mm was used for a further study, in which a bone graft was tested in osteoporotic mice (Kauschke *et al.*, 2018).

Another fracture model at the distal femur was published by Han *et al.* (Han *et al.*, 2015). The authors created an intercondylar fracture by splitting the bone with a scalpel. The fractures were stable without any fixation, but the authors reported that the fracture geometry varied between the animals. Most fractures already displayed bony bridging after 7 d. The authors reported that fractures healed by intramembranous bone formation. Existing bone trabeculae were activated, and then redundant bone was resorbed by osteoclasts. Disadvantages of this model are the lack of standardisation and the clinically rather rare fracture type.

The simplest model for studying metaphyseal bone healing at the proximal tibia of mice was proposed by the Aspenberg *et al.* group, according to their above-mentioned rat studies (Meunier and Aspenberg, 2006). A unicortical drill hole of 0.6 mm diameter was created in the metaphyseal bone of the proximal tibia and a 0.7 mm-diameter and 1 mm-length screw was inserted. The screws were firmly integrated into the bone within 2 weeks. The new bone in the interface was formed by intramembranous ossification. The disadvantage of this model was already mentioned above. It does not really reflect metaphyseal fracture healing but rather osseointegration of an artificial implant surface. The group used this mouse model to recapitulate some of their rat studies and investigated the effects of glucocorticoids, alendronate and PTH on trabecular bone healing (Bernhardsson and Aspenberg, 2018; Sandberg and Aspenberg, 2015a; Sandberg *et al.*, 2017). Furthermore, the immune reaction towards the bone injury was analysed in this model as well as the effects of this fracture type on the

Table 5. Mouse models for metaphyseal fracture healing.

Strain	Age	Sex	Fracture site	Fracture model		Osteoporosis model	Healing outcome	References
CD-1, B6.129S6-CF1-Chrm-3^{tm1Jwe}	12-16 weeks	female	distal femur	open transverse osteotomy (0.22 mm or 1.2 mm gap), fixation by 3-hole locking plate		osteoporosis induced by M3 mAChR KO	healing by intramembranous bone formation	Histing *et al.*, 2012 Kauschke *et al.*, 2018
C57BL/6	24 weeks	N/A	proximal tibia	open transverse osteotomy, fixation by 27G needle		N/A	healing by external callus and endochondral bone formation	Tu *et al.*, 2014
Balb/c	8-10 weeks	male	distal femur	open, intercondylar fracture, no fixation		N/A	healing by intramembranous bone formation	Han *et al.*, 2015
C57BL/6	10 weeks	either	proximal tibia	open, unicortical drill hole defect, inserted screw (1 mm length, 0.7 mm diameter)		N/A	healing by intramembranous bone formation	Sandberg *et al.*, 2015 Sandberg *et al.*, 2016 Tätting *et al.*, 2017 Bernhardsson *et al.*, 2018 Tätting *et al.*, 2018
C57BL/6J	12 weeks	female	proximal femur	open, transverse osteotomy between the third and lesser trochanter (0.4 mm gap size), fixation by 24G needle		N/A	healing by external callus and endochondral bone formation	Haffner-Luntzer *et al.*, 2020

intact skeleton (Tatting *et al.*, 2017; Tatting *et al.*, 2018). This model was also used to compare metaphyseal and diaphyseal fracture healing (Tatting *et al.*, 2018).

Tu *et al*. (Tu *et al.*, 2014) introduced a transverse osteotomy model at the proximal mouse tibia. Tibiae were stabilised by inserting a 27G needle into the intramedullary cavity. Displacement of the tibial fractures and additional fibula fractures were frequently observed. 4 weeks after surgery, most fractures displayed bony union. Fractures healed by external callus formation and endochondral ossification, indicating relatively high interfragmentary tissue strains. The disadvantages of this model are the low degree of standardisation and uncontrolled mechanical conditions due to the poor facture fixation.

The Institute of Orthopaedic Research and Biomechanics group at Ulm established a novel fracture model at the mouse proximal femur, because many osteoporotic fractures occur in this area in humans (Haffner-Luntzer *et al.*, 2020). A transverse osteotomy between the third and the lesser trochanter was created using a 0.44 mm Gigli wire saw and fixed with an intramedullary a 24G needle. The mice tolerated the operation well and loaded the limb normally immediately after the operation. No adverse events were observed during the experimental time period. All animals displayed successful bony union at day 21 after surgery. Histological analysis revealed periosteal callus formation and endochondral ossification. Advantages of this model are the clinically relevant fracture location and the easy surgical protocol. Disadvantages are that the intramedullary needle does not provide rotational stability and that only few bone trabeculae are found in the metaphyseal region of the proximal mouse femur.

In summary, several metaphyseal fracture healing models have been described in mice. Most studies were conducted on the distal femur and the proximal tibia, one study investigated healing at the proximal femur. Fracture healing occurred between 1 and 5 weeks after surgery, dependent on the model. So far, there are no studies available comparing metaphyseal fracture healing in osteoporotic and non-osteoporotic mice in a translationally relevant model. Because mice are the best model to study molecular mechanisms, metaphyseal fracture healing models should be further refined.

Differences between metaphyseal and diaphyseal fracture healing

It is widely believed that metaphyseal bone heals without external callus formation by direct bone formation. In contrast, diaphyseal fracture healing is more likely to show periosteal callus formation and endochondral ossification. However, concluding from the different models reviewed here, preclinical data suggest that both intramembranous and endochondral bone formation can also occur in metaphyseal bone healing, dependent on interfragmentary strains acting at the fracture site, as it was shown for diaphyseal healing. Less stable fracture fixations (intramedullary needles/wires, cerclages) were more likely to heal by periosteal callus formation and endochondral ossification, whereas stable fracture fixations (plates, compression screws) or anatomically reduced fracture gaps mostly heal by direct bone formation without external callus formation. Indeed, Claes *et al*. demonstrated that metaphyseal fracture healing follows similar biomechanical rules to diaphyseal healing in sheep (Claes *et al.*, 2011a). Also clinically, both types of bone formation can be found in metaphyseal bone depending on the fracture location, the fracture geometry and the fixation technique (Hinsenkamp *et al.*, 1984). However, healing without pronounced external callus is more likely in metaphyseal than in diaphyseal fractures and metaphyseal fractures are considered to heal faster (McKibbin, 1978).

Several studies were conducted to compare metaphyseal and diaphyseal fracture healing with respect to biological factors. Significant differences were found between the two types of fractures. Diaphyseal fracture healing seems to be highly dependent on the activation of periosteal progenitor cells, even if other stem cell sources also contribute to bone formation (Blokhuis, 2017). In contrast, metaphyseal healing is less dependent on periosteal cells, since a higher number of active osteogenic cells are available at the existing trabeculae and in the bone marrow in between (Sandberg and Aspenberg, 2016). Furthermore, osteoblasts isolated from different locations display a different proliferation and differentiation capacity, which could contribute to the different healing capacities of different bone locations (Martinez *et al.*, 1999). This was not only true for metaphyseal *vs*. diaphyseal bone, but also for different bones. Furthermore, blood flow and vascularisation were shown to be different in the diaphyseal and metaphyseal regions of the bone, with higher vascularisation in the metaphyseal bone (Blokhuis, 2017; Sandberg and Aspenberg, 2016). This indicates the fracture location might significantly impact the healing capacity and might account for the faster healing of metaphyseal fractures (McKibbin, 1978).

It was also suggested that the inflammatory response plays a crucial role in diaphyseal healing, but may be less important in metaphyseal bone healing (Tatting *et al.*, 2018). Furthermore, metaphyseal fractures led to a rather acute inflammatory response, whereas diaphyseal fractures led to a more chronic inflammation. In line with that, anti-inflammatory drugs such as NSAIDs or glucocorticoids exert greater effects on diaphyseal than metaphyseal healing (Sandberg and Aspenberg, 2015a; Sandberg and Aspenberg, 2015b). However, attention has to be paid regarding these studies since a diaphyseal complete fracture model with higher interfragmentary

movements and a higher degree of trauma was compared to a metaphyseal drill-hole model with almost no biomechanical stimulation and less tissue trauma. Further studies are necessary to compare similar boundary conditions in metaphyseal and diaphyseal bone to investigate potential differences in the inflammatory response.

Some studies stated that an antiresorptive treatment with bisphosphonates supports fracture healing, both in metaphyseal and diaphyseal bone (Kolios *et al.*, 2010b; Morse *et al.*, 2020; Sandberg *et al.*, 2017). Several studies demonstrated that treatment with various anti-resorptive drugs lead to a larger callus with increased mechanical stiffness and strength, because of delayed external callus remodelling. Thus, it could be speculated that diaphyseal fractures, which mostly heal by secondary healing by means of external callus formation, might benefit to a greater extend from antiresorptive treatment that metaphyseal fractures.

Not surprisingly, osteoanabolic treatments support healing of both metaphyseal and diaphyseal fractures. This was shown for PTH, which increases osteoblast differentiation and bone formation independent of the fracture location (Bernhardsson and Aspenberg, 2018; Li *et al.*, 2017; Nozaka *et al.*, 2008). Also, the activation of the crucial osteoanabolic Wnt/β-catenin signalling pathway, by antagonising the Wnt inhibitor Dkk1, accelerates both metaphyseal and diaphyseal fracture healing (Agholme *et al.*, 2011; Jin *et al.*, 2015).

To conclude, antiresorptive and osteoanabolic treatment strategies support both diaphyseal and metaphyseal healing. However, because direct and indirect bone formation are differentially regulated and contribute in different proportions to metaphyseal or diaphyseal healing, the effect size of the different interventions might be different in both conditions. However, comparative studies are widely missing.

Summary

Both large (sheep and dogs) and small (rabbits, rats and mice) animal models are available to study metaphyseal fracture healing. Mainly used fracture locations are the proximal tibia and distal femur; however, some studies also used proximal ulna (sheep), proximal fibula (rabbit), distal tibia (rabbit) and proximal femur (mouse). Various fracture fixation techniques have been developed for the different animal species and fracture locations. Healing times varies greatly between the species and the fracture models. Mice display the fastest metaphyseal fracture healing with bony union between 1 and 5 weeks, depending on fracture localisation and type, whereas dog and sheep fracture healing take the longest with 2 to 3 months healing time. Each model found in the literature has certain advantages and disadvantages; however, many lack standardisation resulting in a high variability of the healing outcome or poorly mimic the clinical situation. Furthermore, in some manuscripts, description of the surgical technique is not detailed enough to allow repetition of the experiments.

The animal studies reviewed here indicate that metaphyseal and diaphyseal fracture healing follow similar basic biological and biomechanical principles, even if they may display specific differences regarding the progenitor cell source, the involvement of inflammatory processes, or the contribution of different biological pathways. However, so far, comparative studies are rare. Most studies directly comparing diaphyseal to metaphyseal fracture healing used animal models with different trauma severities for both type of injuries. This might influence the type and course of fracture healing even more than the fracture location. Therefore, further investigations are needed to unravel mechanistic molecular and cellular differences.

The multiple preclinical studies reviewed here showed various healing patterns depending on the biomechanical conditions acting at the fracture site. Stable fracture situations induced intramembranous ossification, whereas endochondral bone formation and external callus formation was observed under unstable conditions. A standardised fracture fixation is important to avoid high intra-experimental variability in metaphyseal fracture healing studies.

Since metaphyseal fractures are very common in osteoporotic individuals, specific animal models to mimic osteoporotic metaphyseal fracture healing are desirable (Marmor *et al.*, 2015). Several large and small animal models have been developed to compare non-osteoporotic and osteoporotic metaphyseal fracture healing. However, many studies demonstrated high variability, especially in osteoporotic animals, and lack detection of significant differences between non-osteoporotic and osteoporotic fracture healing. Therefore, there is the great need for further refinement of animal models for osteoporotic metaphyseal fracture healing because suitable preclinical models are essential for the development of new treatment strategies.

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Discussion with Reviewers

Stephan Zeiter: One of the important outcomes of this literature review was the finding that many metaphyseal studies heal by endochondral repair rather than only intramembranous ossification. Please discuss the clinical significance of this finding and suggest some key preclinical questions that follow this finding.
Authors: Because osteoporotic fractures frequently occur in metaphyseal bone, it would be highly interesting and clinically relevant to also investigate differences between non-osteoporotic and osteoporotic metaphyseal fracture healing in terms of mechanobiology. One key question would be whether the threshold of mechanical stimuli leading to either endochondral or intramembranous fracture healing might differ dependent on the osteoporosis status of the patients. It has been postulated previously that mechanotransduction in the aged and/or osteoporotic organism differs from young patients. However, this question, to date, is clearly not answered and would be an important subject for further studies.

Stephan Zeiter: The finding that the inflammatory state is different between metaphyseal and diaphyseal fractures is very interesting. Can the authors postulate what the fundamental difference may be and some future studies that could explore this?
Authors: There are very few studies investigating the difference in the inflammatory response occurring after metaphyseal and diaphyseal fractures. These studies postulated that metaphyseal fracture healing displays a rather acute inflammatory response profile, whereas diaphyseal fracture healing displays are more chronic inflammatory profile. However, because the models used in these studies vary greatly in trauma severity, it would be highly pertinent to further analyse this important question in a more standardised setting. Furthermore, it would be highly relevant to shed light on the time course of inflammation between the different types of fractures also regarding different inflammatory cells and molecules.

Chelsea Bahney: This is a great paper and excellent overview. I would challenge the overall concept of it. Why do we need all these imperfect animal models when we have millions of humans suffering these fractures and we are operating on them? We need to be smarter to understand this problem clinically. We have advanced non-radiation involving imaging, we can standardise treatment we can take biopsies at the surgery and during healing, we can take blood tests etc...
Authors: The reviewer is completely correct in that we need more translational and clinical research on the topic of metaphyseal fracture healing. Using advanced clinical imaging techniques would help to understand in which clinical settings metaphyseal fractures heal by endochondral or intramembranous ossification. Taking biopsies, analysing the fracture haematoma, which is frequently removed anyway, and taking blood samples will help to obtain greater insight into biological and molecular processes involved in metaphyseal fracture healing. However, preclinical studies using animal models are absolutely necessary to investigate fracture healing under standardised experimental conditions. In the clinical setting, patient-specific factors like age, sex, drug intake, BMI and many others not currently known might influence the healing in a way we cannot yet predict. Furthermore, there are specific

research questions which cannot be answered by clinical research, for example, which role does a specific molecule play during bone regeneration and can we utilise this molecule as a target for biological therapy? In conclusion, in an ideal world, preclinical, translational and clinical researchers would work together on important orthopaedic topics with the goal of benefitting the patient.

Zsolt Balogh: A very important aspect of preclnical studies is their reproducibility. The insufficient/inappropriate reporting of animal studies has been discussed extensively (*e.g.* ARRIVE guidelines) as a possible reason for difficulties in reproducing published work. Based on the papers included in this review, do the authors think that these models have been described in sufficient detail to be able reproduce them? I would especially be interested in the description of the surgical technique. This could be an interesting aspect to be included in the manuscript.

Authors: The reviewer raised a very important point. Indeed, many of the published studies on metaphyseal fracture healing reported about adverse effects, animal welfare aspects and inclusion/exclusion criteria for animals as it is recommended by the ARRIVE guidelines (although many papers were published before these guidelines were established). However, not all studies included these data, and the description of the surgical technique is frequently not detailed enough to allow repetition of the experiment. We added this information to the summary statement of the manuscript.

Editor's note: The Scientific Editor responsible for this paper was Chris Evans.