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CALCIUM AND VITAMIN D IN BONE FRACTURE HEALING AND POST-TRAUMATIC BONE TURNOVER

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Abstract

Calcium and vitamin D are essential for maintaining bone health. Therefore, deficiencies in calcium and vitamin D are major risk factors for osteoporosis development. Because sufficient amounts of calcium are also required for fracture-callus mineralisation, compromised bone repair that is frequently observed in osteoporotic patients might be attributed to calcium and vitamin D deficiencies. Consequently, calcium and vitamin D supplementation represents a potential strategy for treating compromised fracture healing in osteoporotic patients. Growing clinical evidence suggests that a fracture event may induce post-traumatic bone loss in the non-fractured skeleton, particularly in osteoporotic patients, which might further exacerbate osteoporosis and increase the risk of secondary fractures. Because the skeleton represents the main source of calcium, which is increasingly required during fracture-callus mineralisation, post-traumatic calcium mobilisation might occur under conditions of insufficient calcium and vitamin D status. However, to date, investigations of the roles of calcium and vitamin D in bone repair and post-traumatic bone turnover are very limited.

The current review summarises the state of the literature, focusing on the role of calcium and vitamin D in fracture healing and post-traumatic bone turnover, and critically discusses the therapeutic potential of calcium and vitamin D supplementation in this context.

Keywords: Calcium, vitamin D, fracture healing, post-traumatic bone loss, osteoporosis.

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List of abbreviations

1,25-VitD$_3$	1,25-dihydroxy-vitamin-D$_3$
25-VitD$_3$	25-hydroxy-vitamin-D$_3$
Alpl	alkaline phosphatase
ASBMR	American Society of Bone and Mineral Research
BGLAP	bone gamma-carboxyglutamate protein
BMD	bone mineral density
Cckbr	cholecystokinin gastrin B receptor
CYP24A1	cytochrome P450 family 24 subfamily A member 1
FGF23	fibroblast growth factor 23
IL	interleukin
IOF	International Osteoporosis Foundation
OPG	osteoprotegerin
OVX	ovariectomised
PTH	parathyroid hormone
RANK	receptor activator of NF-κB
RANKL	RANK ligand
SPP1	secreted phosphoprotein 1
TNFSF11	tumour necrosis factor superfamily member 11
VDR	vitamin D receptor

Introduction

Osteoporosis is globally the most common age-related skeletal disease, characterised by a progressive decline in bone mass and disruption of the bone micro-architecture, resulting in an increased risk of fragility fractures. Numerous risk factors influence osteoporosis development, including oestrogen decline during menopause, immobilisation, old age and nutrition (Rachner *et al.*, 2011). In respect to nutrition, major osteoporosis risk factors are an insufficient calcium supply and a reduced vitamin D status. Calcium is the main mineral present in bones, where it provides skeletal strength and serves as a reservoir for maintaining blood calcium levels in a physiological range. Vitamin D is the key controller of calcium homeostasis by regulating intestinal calcium absorption, renal calcium reabsorption and bone remodelling (Amling *et al.*, 1999; Li *et al.*, 1997). Both calcium and vitamin D deficiencies promote bone loss through increased bone resorption in order to maintain the blood calcium concentration (Lips and van Schoor, 2011; Peacock, 2010). Vitamin D deficiency is recognised as a global health problem and is expected to increase further because of demographic changes reflecting an aging population (Hosseinnezhad and Holick, 2013). Because of the important roles of calcium and vitamin D in bone health, basic osteoporosis therapy includes their supplementation for individuals at high risk of osteoporosis, including aged postmenopausal females, when dietary calcium and vitamin D intake are insufficient (Cosman *et al.*, 2014). However, osteoporosis treatment is rarely applied in clinics because osteoporosis is a silent disease, which is primarily diagnosed after patients have experienced the first fragility fracture. Even after such a fracture, under-treatment is common, because only 10-20 % of patients receive adequate treatment (Bellantonio *et al.*, 2001; Follin *et al.*, 2003).

Both calcium and vitamin D play key roles in bone mineralisation, which is also part of the fracture-healing process (Claes *et al.*, 2012; Einhorn and Gerstenfeld, 2015). Therefore, it is likely that calcium and vitamin D deficiencies contribute to fracture-healing complications, which are frequently observed in osteoporotic, postmenopausal patients (Nikolaou *et al.*, 2009). However, only limited experimental and even fewer clinical studies investigate the role of calcium and vitamin D in fracture healing, as reviewed here. In addition, only a few investigate post-traumatic changes in the non-fractured skeleton, particularly under calcium- and vitamin-D-deficient conditions. There is growing clinical evidence of systemic bone loss following a fracture, as indicated by a reduction in the total bone mass of 2-15 %, compared to values immediately post-fracture or age-matched controls without fracture (Fox *et al.*, 2000; Karlsson *et al.*, 2000). Systemic bone loss may explain the clinical observation of an increased risk of secondary fractures (Ahmed *et al.*, 2013; Lyles *et al.*, 2008). Experimental data suggest that calcium and vitamin D deficiencies might exacerbate post-traumatic bone loss (Fischer *et al.*, 2017; Haffner-Luntzer *et al.*, 2016). Hereby, calcium, which is required for fracture-callus mineralisation, is increasingly mobilised from the remote skeleton to guarantee sufficient bone repair since the dietary calcium supply does not meet calcium requirements for callus mineralisation. These findings imply a clinical therapeutic requirement for calcium and vitamin D supplementation after fracture.

The scope of the current review was to summarise and analyse the known experimental and clinical data on the role of calcium and vitamin D in regular and osteoporotic fracture healing, as well as in post-traumatic bone turnover. Moreover, the therapeutic potential of calcium and vitamin D supplementation in the clinic, in particular in the prevention of fracture-healing complications and post-traumatic bone loss, is discussed based on the reviewed literature.

Bone remodelling

Bone is dynamically remodelled throughout the entire lifespan to replace damaged bone and adapt to mechanical load by the balanced and coupled actions of bone-forming osteoblasts and bone-resorbing osteoclasts. Balanced bone remodelling is essential for the maintenance of bone mass and skeletal integrity. The complex process of bone remodelling is regulated by a variety of endogenous and exogenous factors. Primarily, it is regulated through the RANK/RANKL/OPG system acting directly on osteoblast/stroma cells and osteoclast precursors. RANKL, expressed by osteoblasts/stroma cells, binds to the RANK receptor on osteoclast precursors inducing osteoclastogenesis (Suda *et al.*, 1999). Osteoblast-produced OPG functions as a decoy receptor, blocking the effects of RANKL (Hsu *et al.*, 1999). Many local and systemic factors regulating bone remodelling – including transforming growth factor-β, bone morphogenic proteins, cytokines-like IL-1β, IL-6 and tumour necrosis factor-α, hormones such as PTH, 1,25-VitD_3 and oestrogen – mainly signal by influencing the RANK/RANKL/OPG system on osteoblasts/stroma cells, thus keeping the system in balance (Crockett *et al.*, 2011). In addition to these endogenous determinants, exogenous factors, including mechanical loading and nutrition, are involved. Osteocytes sense and respond to mechanical stimuli by the induction of osteo-anabolic signals, including nitric oxide and prostaglandins (Robling *et al.*, 2006). Calcium and vitamin D are the main nutrients exerting important functions in bone remodelling (Gennari, 2001).

Imbalances in bone remodelling can drive bone metabolism towards increased bone formation, favouring abnormal bone gain, as seen in osteosclerosis, or towards increased bone resorption, resulting in bone loss, as observed in osteoporosis.

Calcium and vitamin D involvement in bone remodelling and homeostasis

Dietary composition, in particular, the amounts of calcium and vitamin D, play an important role in bone remodelling and skeletal integrity (Gennari, 2001). Approximately 99 % of the body's calcium is present in bones and teeth, stored as hydroxyapatite responsible for tissue mineralisation. In bone, it provides skeletal strength and serves as a calcium reservoir to maintain constant blood calcium levels. Therefore, calcium is required for skeletal growth, development and maintenance. Calcium requirements differ during the lifetime, depending on the varying needs due to growth in childhood and youth, or during pregnancy and lactation (Nordin, 1997). Calcium as an essential element is only available through the diet. However, dietary calcium recommendations vary widely among countries. For example, the recommended daily calcium intake for the elderly above 65 years in Germany is 1000 mg, in the USA 1200 mg and in the UK 700 mg (German Nutrition Society, 2013; Institute of Medicine, 2011; Prentice, 2013). Differences might arise from variations in data acquisition and interpretation and cultural aspects, including geographic locations, lifestyle and genetics. Under physiological conditions, approximately 30-40 % of the ingested calcium is absorbed by the gut. Several factors influence calcium absorption rate, including total calcium amount, nature of the calcium complex, acidic conditions in the stomach and small intestine, age and vitamin D status (Fleet, 2017; Krause *et al.*, 2015; Schinke *et al.*, 2009). In the case of high dietary calcium intake, calcium is absorbed by passive diffusion. By contrast, normal to low dietary calcium intake requires active calcium absorption, which is regulated by the active 1,25-VitD_3 acting on the VDR in the intestine (Christakos *et al.*, 2014). Therefore, vitamin D plays a key role in calcium and bone homeostasis.

The fat-soluble vitamin D (cholecalciferol) is ingested as part of the diet or is synthesised in the skin upon ultraviolet-B irradiation (Holick *et al.*, 1980). To acquire physiologic activity, vitamin D is firstly hydroxylated in the liver on carbon 25 by 25-hydoxylase, producing 25-VitD_3 (calcifediol). In the kidney, 25-VitD_3 is secondly hydroxylated on carbon 1 by a 1-α-hydroxylase, producing the biologically active 1,25-VitD_3 (calcitriol). In the target tissues, 1,25-VitD_3 binds to its nuclear VDR, a family member of the steroid/thyroid hormone receptors, and functions as a transcription factor. As a heterodimer mainly bound to the retinoid-X-receptor, the VDR binds to vitamin D response elements on the DNA, thus modulating the transcription of numerous vitamin D target genes, including the bone-related genes osteocalcin (BGLAP), osteopontin (SPP1) and RANKL (TNFSF11). The VDR is expressed by most mammalian organs and their respective cell types, including the immune system, gastro-intestinal tract, reproductive organs, kidney, parathyroid gland, skin, heart, brain, muscles and bones, thus suggesting a broad variety of biological functions (Christakos *et al.*, 2016). However, the key function of 1,25-VitD_3/VDR is the regulation of mineral and bone homeostasis (Li *et al.*, 1998).

1,25-VitD_3, PTH and FGF23 (a key regulator of phosphate and vitamin D metabolism) are the main factors maintaining constant blood calcium and phosphate levels by regulating: i) calcium and phosphate absorption in the intestine and reabsorption in the kidney, ii) bone resorption. The main inducer of this complex regulatory network is a change in serum calcium levels, since constant calcium concentrations are essential for many biological functions. The inactivation of the calcium-sensing receptor in the parathyroid glands, resulting from a fall in serum calcium levels, stimulates PTH release (Fig. 1). Circulating PTH binds to its receptor in the kidney, where it enhances calcium reabsorption, phosphate excretion and 1,25-VitD_3 production. Both circulating PTH and 1,25-VitD_3 bind their respective receptors on osteoblasts, thus increasing RANKL expression, which stimulates osteoclastic bone resorption and the release of calcium and phosphate into the circulation. Consequently, calcium levels are restored and negative feedback mechanisms are induced, including the release of calcitonin from the thyroid gland, which reduces calcium reabsorption in the kidney and absorption in the gut and inhibits osteoclastic bone resorption, thus maintaining calcium levels in a physiological range (Fig. 1).

The parallel increase of phosphate levels during the calcium-mediated PTH and 1,25-VitD_3 actions requires the phosphate-lowering actions of FGF23, independent of PTH (Fig. 1). The 32 kDa FGF23, which belongs to the FGF19 subfamily, is increasingly released by osteocytes in bones upon high circulating phosphate and 1,25-VitD_3 concentrations (Liu *et al.*, 2006). In the kidney, FGF23, associated with its cofactor Klotho, binds the FGF1 receptor, thus decreasing renal phosphate reabsorption and increasing its excretion (Urakawa *et al.*, 2006). In addition, FGF23 further reduces phosphate levels by inhibiting 1,25-VitD_3 production in the kidney and probably PTH production in the parathyroid gland (Ben-Dov *et al.*, 2007; Shimada *et al.*, 2004). Several inherited syndromes characterised by abnormalities in FGF23 levels are associated with osteomalacia, thus strengthening the role of FGF23 in bone homeostasis (Shimada *et al.*, 2001; Weber *et al.*, 2003; Yamazaki *et al.*, 2002). In addition, as shown in experimental and clinical studies, genetic Klotho deficiency is associated with reduced bone mass (Kuro-o *et al.*, 1997; Yamada *et al.*, 2005; Zarrabeitia *et al.*, 2007). In contrast, studies investigating associations of serum Klotho and bone mass report controversial results (Baldan *et al.*, 2015; Chalhoub *et al.*, 2016). In osteoporotic elderly, no associations between serum Klotho and bone loss and fracture risk are detected (Chalhoub *et al.*, 2016). Since the membrane-bound Klotho protein, which is required for FGF23 function in bone, is not measurable in the blood, the

relevance of the secreted Klotho protein as a marker for osteoporosis is uncertain and needs to be further examined.

In conclusion, the process of bone remodelling is essential for adapting to the changes in calcium levels. However, a low dietary calcium supply or long-lasting vitamin D deficiency, which considerably reduces intestinal calcium absorption and increases PTH concentrations, stimulates bone turnover and excessive bone resorption to restore systemic calcium levels. These mechanisms favour bone loss and osteoporosis development.

Calcium and vitamin D in osteoporosis

Osteoporosis is globally the most common skeletal disease, affecting alone in Europe, USA and Japan an estimated 75 million people, as stated by the IOF in 1997 (Consensus Development Conference, 1997). Due to demographic changes of an aging population, the number has increased in the last decades and will further increase in the future. In 2010, an estimated number of 53.6 and 27.5 million people suffered of osteoporosis in the USA and Europe, respectively (Hernlund *et al.*, 2013; Wright *et al.*, 2014). The IOF estimates that more than 200 million women

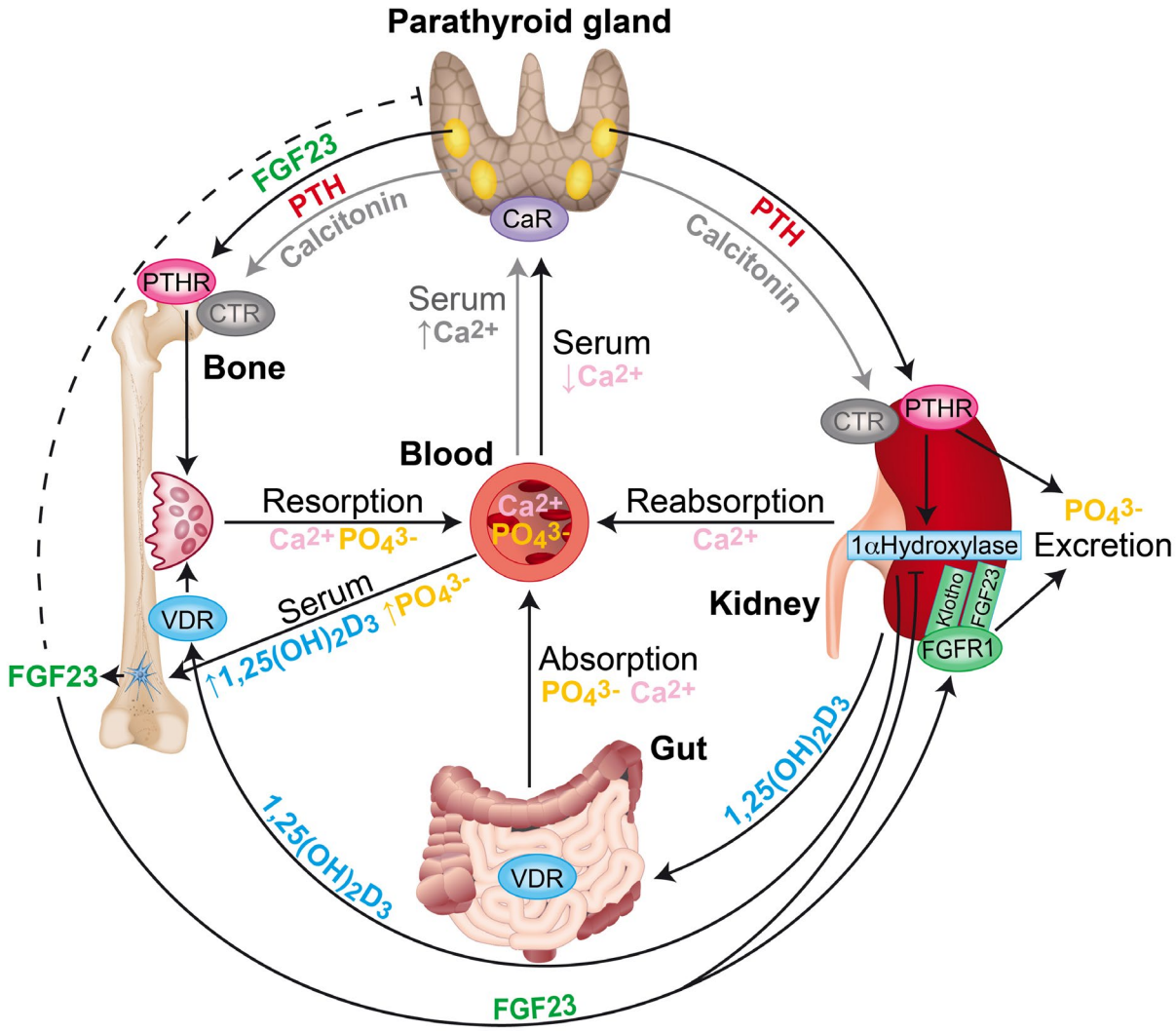

Fig. 1. Regulation of calcium and bone homeostasis. Upon a fall in serum calcium, PTH is secreted from the parathyroid glands. Circulating PTH increases calcium mobilisation from bones and stimulates both calcium reabsorption and 1,25-VitD_3 synthesis in the kidneys. 1,25-VitD_3 increases calcium absorption in the gut. In response to PTH and 1,25-VitD_3 actions, phosphate levels increase, stimulating FGF23 secretion from bone osteocytes. In the kidney, FGF23 promotes phosphate excretion and inhibits 1,25-VitD_3 production. FGF23 might further inhibit PTH secretion. Restored/high serum calcium levels trigger negative feedback loops, including calcitonin acting on its own receptor, thus reducing renal calcium reabsorption and inhibiting osteoclastic bone resorption (illustrated in bright grey). Ca = calcium; PO = phosphate; PTH = parathyroid hormone; 1,25-$(OH)_2D_3$ = 1,25-dihydroxy-vitamin D_3; FGF23 = fibroblast growth factor 23; VDR = vitamin D receptor; PTHR = parathyroid hormone receptor; FGFR1 = fibroblast growth factor receptor 1; CaR = calcium sensing receptor; CAR = calcitonin receptor.

worldwide suffer from osteoporosis (Kanis, 2007). The disease is characterised by a progressive decline in bone mass and disruption of bone microarchitecture because of the unbalanced activities of osteoclasts and osteoblasts, where bone resorption exceeds bone formation. According to the World Health Organization, osteoporosis is defined by a reduction in BMD of 2.5 or more standard deviations below the mean BMD of young adults (Cosman *et al.*, 2014). Osteoporosis is associated with an increased risk of fragility fractures, mainly occurring at the spine, hip and wrist. The lifetime risk of an osteoporotic fracture is 40 % in females *versus* 27 % in males (Cooley and Jones, 2001; Kanis *et al.*, 2000). The most common form is postmenopausal osteoporosis, which results from a decline in oestrogen levels because of menopause, and globally affects an estimated 200 million females (Kanis, 2007). Overall, a panel of risk factors, including genetics, gender, age, comorbidities and their therapies, life style and nutrition influence osteoporosis development.

Calcium and vitamin D deficiencies represent the main risk factors influencing osteoporosis development. Changes in serum calcium entail adaptations in bone remodelling, as in the case of increased bone resorption induced by low serum calcium. With a mean daily calcium intake of approximately 700-800 mg in adults in Germany (European Food Safety Authority, 2006), the calcium supply remains slightly below the recommendation of 1000 mg/d (German Nutrition Society, 2013). However, both intestinal calcium absorption and dietary calcium intake decrease with increasing age (Ireland and Fordtran, 1973; Wakimoto and Block, 2001). Such low-to-normal dietary calcium intake requires active intestinal transport by 1,25-$VitD_3$ (McCormick, 2002). However, vitamin D deficiency is common (Holick and Chen, 2008). One main reason is the low dietary vitamin D intake, because only a few foods have a high vitamin D content, including oily fish, eggs, milk and some dairy products. Because eating habits change with age, the lowest vitamin D supply from the diet is in those above 65 years (Wakimoto and Block, 2001). However, endogenous vitamin D synthesis in the skin upon sunlight exposure supplies 80-90 % of the required vitamin D. Nevertheless, numerous factors, including geographic location, latitude, season, time spent outdoors and clothing, influence dermal vitamin D synthesis. For example, solar irradiation during winter is insufficient to produce vitamin D in people resident in northern countries (Webb *et al.*, 1988). To guarantee optimal bone metabolism and bone health, systemic 25-$VitD_3$ levels of 75 nmol/L or more (> 30 ng/mL) are required, because lower 25-$VitD_3$ concentrations are associated with bone mineralisation defects (Priemel *et al.*, 2010; von Domarus *et al.*, 2011). Globally, it is estimated that vitamin D deficiency affects approximately 3 billion people (Hossein-nezhad and Holick, 2013). In Europe, roughly 80 % of the population displays insufficient 25-$VitD_3$ serum levels below 75 nmol/L (< 30 ng/mL) (Gonzalez-Gross *et al.*, 2012; Holick *et al.*, 2011). Particularly in Germany, widespread vitamin D deficiency is common and occurs through all ages (Hintzpeter *et al.*, 2008a; Hintzpeter *et al.*, 2008b). However, the effects are even more pronounced in the elderly (Schilling, 2012), due to reduced dermal vitamin D-synthesis capacity with age (MacLaughlin and Holick, 1985) and reduced sunlight exposure because of immobility and less time spent outdoor (Bruyere *et al.*, 2014; Holick *et al.*, 1980). Indeed, in Germany, the mean 25-$VitD_3$ levels are 46.2 nmol/L in adults and 39.1 nmol/L over 65 years (German Nutrition Society, 2012). Particularly in risk groups, including elderly and postmenopausal females, severe vitamin D deficiency might interfere with bone health, because of reduced intestinal calcium absorption capacity. Indeed, several epidemiological studies demonstrate associations between low 25-$VitD_3$ levels and reduced bone mass on one side and increased risk of falls and fragility fractures on the other (Bischoff-Ferrari *et al.*, 2014; Kuchuk *et al.*, 2009). Therefore, preventive and therapeutic actions, including nutrient supplementation and food fortification, ensuring minimum circulating 25-$VitD_3$ levels (75 nmol/L or 30 ng/mL) are required to preserve and restore bone health (Brown *et al.*, 2013a; Brown *et al.*, 2013b).

For osteoporosis and fracture prevention, practical guidelines of the National Osteoporosis Foundation recommend calcium and vitamin D supplementation for high-risk groups, including postmenopausal females (> 50 years old), when dietary calcium and vitamin D intake is insufficient (Cosman *et al.*, 2014). In addition, calcium and vitamin D supplementation is, as basal therapy, always part of other osteoporosis treatments, including bisphosphonates, intermittent PTH application or novel drugs targeting sclerostin, RANKL or cathepsin K (Rachner *et al.*, 2011; Tabatabaei-Malazy *et al.*, 2017). However, the efficiency and safety of calcium, vitamin D or combined calcium and vitamin D supplementations are critically discussed. On the one hand, several clinical trials observe a reduction in hip and non-vertebral fractures because of calcium and vitamin D supplementation (Bischoff-Ferrari *et al.*, 2008; Bischoff-Ferrari *et al.*, 2005; Chapuy *et al.*, 1992; Warensjo *et al.*, 2011). In addition, a meta-analysis by Tang *et al.* (2007) report that calcium and vitamin D supplementation reduce osteoporotic-fracture occurrence, thus corroborating the beneficial effects of calcium and vitamin D supplementation on fracture risk reduction. On the other hand, some meta-analyses do not observe associations between calcium and vitamin D supplementation and fracture-risk reduction (Bolland *et al.*, 2015; Peacock *et al.*, 2000; Zhao *et al.*, 2017). However, most of these studies are conducted in healthy community-dwelling adults, who clearly do not profit from calcium or vitamin D supplements. By contrast, risk groups of elderly, particularly postmenopausal

females, displaying reduced calcium intake and insufficient vitamin D status benefit from calcium and vitamin D supplements (Harvey *et al.*, 2017; Tang *et al.*, 2007). Confirming this, a very recently published statement of the ASBMR warns of reports highlighting no beneficial effects of calcium and vitamin D supplementation in community-dwelling adults, because such meta-analyses frequently focus only on healthy adults. The ASBMR clearly state that these findings do not apply to osteoporotic patients or patients taking bone protective drugs, including bisphosphonates and PTH, because calcium and vitamin D supplementation, as a part of a basal therapy, influences drug efficiency and treatment success (Web ref. 1). However, a general population-based treatment with calcium and vitamin D is not recommended, because beneficial health effects are, to date, not demonstrated (Harvey *et al.*, 2017). Sufficient calcium and vitamin D should be obtained through the diet or skin synthesis. However, when this is not achievable, supplements are recommended for risk groups (Cosman *et al.*, 2014). To exclude critically discussed and still scientifically unproven side effects of increased calcium levels due to supplementation – which might favour the development of kidney stones, cardiovascular diseases or gastrointestinal symptoms – patients with a history of these diseases should be monitored for serum parameters of calcium metabolism. However, the tolerable upper calcium limit posing no risk of adverse health effects for the general population is 2500 mg/d, which is not reached by the dietary supply or calcium supplements, commonly containing 500-1200 mg of calcium (European Food Safety Authority, 2006).

Osteoporosis is frequently under-treated and under-diagnosed until patients have experienced the first osteoporotic fracture. Even after such a fracture, only 10-20 % of patients receive adequate osteoporosis treatment, including calcium and vitamin D supplementations (Bellantonio *et al.*, 2001; Follin *et al.*, 2003; Weaver *et al.*, 2017). The main reasons are a lack of diagnosis and therapy initiation, possibly because of side effects, uncertain efficiencies, high costs and patient's health status, compliance and motivation. In addition, the primary goal of trauma surgeons is fracture stabilisation and reduction, which might be one reason for the lack of diagnosis and treatment initiation (Weaver *et al.*, 2017). Interdisciplinary approaches strengthening the collaboration of trauma surgeons, osteologists and nutritionists might reduce the existing therapy deficit, which may further reduce the risk of fragility fractures.

Fracture healing

Fractures caused by osteoporosis occur in one third of females and one fifth of males over 50 years. Globally, approximately 9 million people with osteoporosis suffer a fracture annually (Johnell and Kanis, 2006). Thus, because of ongoing demographic changes with an aging population, the incidence of fractures will further increase. Bone fractures normally heal without complications and any scar formation. However, under specific conditions, including old age, impaired health status, comorbidities and severe injuries/traumas, the repair process may fail (Claes *et al.*, 2012). Therefore, a total of 5-10 % of all fractures display disturbed bone healing (Zura *et al.*, 2016). Bone fracture healing is a highly dynamic, complex and tightly regulated process that involves the interplay of many cells and molecular mediators, including growth factors and cytokines, and is further influenced by the biomechanical environment of the fracture-healing zone. The repair process proceeds in three overlapping phases: inflammation, repair and remodelling. The inflammatory phase is characterised by tissue and cell damage, rupture of blood vessels and recruitment of immune cells to the fracture haematoma. During the repair phase, intramembranous and endochondral ossification drive fracture-callus formation and growth towards the fracture gap until bony bridging. During remodelling, the newly formed woven bone is replaced by lamellar bone, thus restoring the original bone structure and stability (Claes *et al.*, 2012). The changes that occur with the onset of osteoporosis might interfere with this complex and tightly regulated repair process. Indeed, compromised bone repair is frequently observed in osteoporotic patients (Cornell *et al.*, 2003; Nikolaou *et al.*, 2009; Zura *et al.*, 2016). However, pathomechanisms are still poorly understood and further research is needed.

Osteoporotic fracture healing

Osteoporotic fractures are more frequently associated with complications, including infections and implant failure, resulting in expensive aftercare with prolonged hospitalisation periods and increased morbidity and mortality rates. Implant failure occurs in approximately 10-25 % of osteoporotic fracture cases (von Ruden and Augat, 2016). The total complication rate, not only including bone-related complications but also infections, pneumonia and anaemia, is approximately 60 % after hip and 50 % after vertebral fractures and increases with age (Lehmann *et al.*, 2016). Nikolaou *et al.* (2009) demonstrate that fracture-healing time is significantly prolonged in older osteoporotic patients, thus indicating a delay of the repair process. Furthermore, half of the osteoporotic patients do not fully recover after hip injury (Cornell *et al.*, 2003). Epidemiological analysis show that the risk of fracture non-union is significantly increased in osteoporotic patients (Zura *et al.*, 2016). Therefore, osteoporosis affects the bone-regenerative capacity, resulting in fracture-healing complications. However, it is still strongly debated whether osteoporotic bones heal worse because of poor fixation stability in fragile bone or whether the

Table 1. Summary of experimental and clinical studies on the effects of calcium and vitamin D on fracture healing. s.c.: subcutaneous; i.m.: intramuscular.

Study and fracture type	Treatment	Fracture-healing outcome	Authors
Experimental studies			
Male rats (age: 2 weeks) hind leg fracture	Calcium- and/or phosphorus-deficient diet	Impaired healing: ↓ callus mineralisation	Doepfner, 1970
Male rats femur drill hole defect	Injection (s.c.) of 1,25-$VitD_3$	Improved healing:↑ biomechanical properties	Lindgren *et al.*, 1981
Male rats (age: mature) femur fracture, intramedullary fixation	Calcium-/vitamin D-deficient diet Calcium-/vitamin D-supplemented diet	Regular healing Regular healing	Einhorn *et al.*, 1986
Male guinea pigs tibia fracture	Single high-dose injection (i.m.) of vitamin D	Improved healing: ↑ blood supply, callus formation and mineralisation	Omeroglu *et al.*, 1997b
Male rabbits (age: 3 months) femur fracture, intramedullary fixation	Single high-dose injection (i.m.) of vitamin D	Improved healing: ↑ biomechanical competence	Omeroglu *et al.*, 1997a
Male rats (age: 18 months) femur fracture, intramedullary fixation	Injection (s.c.) of 25-$VitD_3$	Improved healing: ↑ biomechanical competence	Delgado-Marinez *et al.*, 1998
Female rats (age: 2 months) ovariectomy, femur fracture, intramedullary fixation	Calcium-deficient diet	Impaired healing: ↓ bone content, biomechanical properties	Namkung-Matthai *et al.*, 2001
Male rats Tibia fracture, intramedullary fixation	Injections (i.m.) of calcium/vitamin D	Improved healing: ↑ biomechanical properties	Aslan *et al.*, 2006
Female rats (age: 10 weeks) ovariectomy, tibia fracture, intramedullary fixation	Vitamin D-deficient diet	Regular healing	Melhus *et al.*, 2007
Female rats (age 6: months) ovariectomy, femur fracture, intramedullary fixation	1,25-$VitD_3$ supplementation (oral gavage)	Improved healing: ↑ biomechanical properties, bone content	Fu *et al.*, 2009
Female mice (age: 26 weeks) femur fracture, external fixation	Calcium deficiency due to malabsorption Calcium-supplemented diet	Regular healing Improved healing: ↑ biomechanical properties,	Haffner-Luntzer *et al.*, 2016
Female mice (age: 26 weeks) ovariectomy, femur fracture, external fixation	Calcium-/vitamin D-deficient diet Calcium-/vitamin D-supplemented diet	Regular healing Improved healing: ↑ biomechanical properties	Fischer *et al.*, 2017
Clinical studies			
Postmenopausal women (n = 30; mean age: 78 years), randomised placebo-controlled study, proximal humerus fracture	Calcium/vitamin D supplementation	Improved healing: ↑ bone content in fracture callus	Doetsch *et al.*, 2004

biological healing potential is reduced (Nikolaou *et al.*, 2009). Clinical trials frequently highlight the surgical complications of osteoporotic fracture treatment, whereas experimental studies have the advantage of investigating the biological regeneration capacity and the fracture-healing outcome more reliably. During recent years, several experimental studies, mostly performed in OVX rodents, mimicking the clinical picture of oestrogen depletion after menopause, reveal a reduced regeneration potential during all stages of bone repair. This is indicated by a reduced amount of newly-formed bone and diminished biomechanical competence of the fracture callus (Meyer *et al.*, 2001; Namkung-Matthai *et al.*, 2001; Wang *et al.*, 2005), as well as changes in angiogenesis (Sun *et al.*, 2012), cartilage formation (Islam *et al.*, 2005) and osteoblast and osteoclast functions in the fracture callus (Islam *et al.*, 2005; Xu *et al.*, 2004). Because osteoporosis is a multifactorial disease, the biological reasons for impaired bone repair are manifold, including a disturbed immune response after fracture (Haffner-Luntzer *et al.*, 2017), disturbances in osteo-anabolic signalling pathways (Heilmann *et al.*, 2013; Ke *et al.*, 2012; Xu *et al.*, 2014) and changes in oestrogen levels and signalling (Beil *et al.*, 2010; Haffner-Luntzer *et al.*, 2018; Wehrle *et al.*, 2015).

Many experimental studies report delayed and disturbed fracture healing in osteoporosis. However, the pathomechanisms of osteoporotic fracture healing are multifactorial and still poorly understood, thus needing further investigation. Regarding the increasing number of elderly, osteoporosis and fragility fractures, the identification of the mechanisms disturbing osteoporotic bone healing is of considerable clinical relevance for the development of new therapeutic strategies. Notably, the role of calcium and vitamin D in fracture healing in this context is, to date, poorly investigated.

Calcium and vitamin D involvement in bone repair

Because calcium and vitamin D play pivotal roles in bone remodelling and mineralisation and while mineralisation is an essential part of fracture-callus formation, both nutrients may influence the fracture-healing process. Calcium is essential for callus mineralisation: approximately 1.7-2.3 g of hydroxyapatite deposition is needed per cm^3 of bony callus, as shown by determining calcium kinetics with radioactive Ca^{45} during callus development in rats (Bauer, 1954; Herman and Richelle, 1961; Lemaire, 1966). Therefore, it is likely that deficiencies in calcium and vitamin D negatively influence fracture healing. Notably, there is only a limited number of conflicting experimental studies, some published many decades ago, investigating the effect of calcium and/or vitamin D deficiency on fracture healing (Table 1). Some of these animal studies are already reviewed by Eschle and Aeschlimann (2011). Rats deficient in dietary calcium display a reduced fracture callus mineralisation, as shown by a lower callus weight (Doepfner, 1970). Similarly, osteoporotic rats with calcium deficiency exhibit diminished callus bone content, quality and biomechanical competence (Namkung-Matthai *et al.*, 2001). By contrast, regular fracture healing is observed in calcium- and vitamin-D-deficient rats (Einhorn *et al.*, 1986), osteoporotic rats receiving a vitamin-D-deficient diet (Melhus *et al.*, 2007) and mice displaying intestinal calcium malabsorption (Haffner-Luntzer *et al.*, 2016). Differences in results may arise from the widely varying experimental conditions. In addition, only a few studies consider the aspect of osteoporosis/oestrogen-deficiency. However, elderly patients with healing complications and non-unions are more frequently vitamin-D-deficient when compared to patients with uneventful bone regeneration (Di Monaco *et al.*, 2006; Pourfeizi *et al.*, 2013; Warner *et al.*, 2016). Summarising these experimental and clinical data, the influence of calcium and vitamin D deficiency on bone repair is greatly debated and poorly investigated. More preclinical and clinical studies are needed.

Many more studies investigate the effects of calcium and vitamin D supplementation on fracture-healing (Table 1). Most of these studies are performed in rats that are injected with vitamin D (cholecalciferol), 25-$VitD_3$ or 1,25-$VitD_3$ after fracture. Improved healing is observed, as indicated by improved biomechanical properties and increased bone content of the fractured bones (Aslan *et al.*, 2006; Delgado-Martinez *et al.*, 1998; Lindgren *et al.*, 1981; Omeroglu *et al.*, 1997a; Omeroglu *et al.*, 1997b) as well as an accelerated fracture-callus remodelling after oral 1,25-$VitD_3$ administration (Fu *et al.*, 2009). By contrast, rats receiving a mineral-enriched diet containing calcium and vitamin D display a regular fracture healing (Einhorn *et al.*, 1986). Haffner-Luntzer *et al.* (2016) observe improved fracture healing as demonstrated by increased flexural rigidity of the fractured femora and enhanced callus bone formation in mice fed with a calcium-supplemented diet. In agreement with these findings, fracture healing is also improved in OVX-mice that display chronic calcium and vitamin D deficiency before fracture when supplemented with calcium and vitamin D immediately after fracture occurrence (Fischer *et al.*, 2017). These mice exhibit improved biomechanical fracture-callus properties and the highest percentage of bony-bridged fracture gaps, indicating successful fracture healing. Cellular parameters are also changed, because osteoclasts are reduced and osteoblasts increased in the calli of supplemented mice (Fischer *et al.*, 2017). Corroborating these findings, a prospective, randomised placebo-controlled clinical study, with postmenopausal females receiving calcium and vitamin D after fracture, describe increased bone content in the fractured area 6 weeks after proximal humerus fracture because of supplementation, compared to placebo-receiving controls (Doetsch *et al.*, 2004). However, this is currently the only clinical study investigating the fracture-healing outcome. In

several clinical studies calcium and vitamin D are supplemented after fracture, but these studies focus on the effects on total BMD, fracture prevention or changes in serum bone parameters and not specifically on bone healing itself (Harwood *et al.*, 2004; Kolb *et al.*, 2013).

Most experimental and clinical studies support beneficial effects of calcium and vitamin D supplementation during bone repair. However, as previously reviewed by others, clinical studies addressing the effects of calcium and vitamin D on fracture healing are rare and data on beneficial effects remain inconclusive (Gorter *et al.*, 2014; Sprague *et al.*, 2016). Within the reviewed human studies, only a few investigate the fracture-healing outcome, whereas most only measured the effects of supplementation on changes in 25-VitD_3 levels. Therefore, more clinical placebo-controlled randomised trials are needed, in which patients, particularly with chronic calcium and vitamin D deficiency, are supplemented with both nutrients during the healing process and the fracture-healing outcome is evaluated.

Both indirect and direct effects of calcium and vitamin D in bone repair are discussed. Fischer *et al.* (2017) observe improved calcium balance as shown by reduced serum PTH levels in calcium- and vitamin-D-supplemented mice. These findings corroborate the overall accepted assumption that the positive effects of vitamin D are mainly indirect through its endocrine actions on calcium homeostasis, thus increasing systemic calcium availability. The authors listed in Table 1 share this opinion. However, direct effects of vitamin D locally in the fracture callus could also possibly influence the healing process. Increased VDR expression is found in the fracture callus of supplemented mice, thus supporting this hypothesis (Fischer *et al.*, 2017). VDR expression is described for a broad variety of body's cells, including cells of the immune and skeletal systems (Christakos, 2002; van Driel *et al.*, 2006; van Leeuwen *et al.*, 1991; Wang *et al.*, 2014; Zarei *et al.*, 2016). *In vitro* experiments demonstrate that 1,25-VitD_3 binding to the VDR on osteoblasts enhances osteoblast differentiation and mineralisation, as indicated by an increased expression of the osteoblast differentiation marker *Alpl* (Matsumoto *et al.*, 1991; van Driel *et al.*, 2006; Woeckel *et al.*, 2010). Interestingly, *Alpl* expression in the fracture callus of mice supplemented with calcium and vitamin D is also increased. Therefore, enhanced VDR signalling in the fracture callus might contribute to the improved fracture healing observed after calcium and vitamin D supplementation. However, the exact mechanisms need to be determined in the future. For this purpose, also the local expression of 1,25-VitD_3 target genes and signalling pathways, including genes of vitamin D metabolism itself (*e.g.* CYP24A1 enzyme for vitamin D degradation) and of bone metabolism (*e.g. Rankl, Opg*, Wingless signalling), as well as systemic 1,25-VitD_3 levels should be addressed. In addition, other regulatory factors controlling calcium and vitamin D metabolism should be included in any future analysis. Fischer *et al.* (2017) observe that vitamin D and phosphate-regulating FGF23 are increased in mice supplemented with calcium and vitamin D after fracture. In agreement with this, increased FGF23 levels are observed in clinics after successful and uneventful hip-replacement therapy (Goebel *et al.*, 2009). Using a sheep model of regular and delayed fracture healing, Goebel *et al.* (2009) further observe an up-regulation of the FGF23 mRNA in the fracture callus during regular fracture healing, but not under conditions of delayed healing. By contrast, Fischer *et al.* (2017) do not detect any differences in the local gene and protein expression of FGF23 and its main receptor FGFR1 in the fracture callus of supplemented mice as compared to control and calcium- and vitamin-D-deficient mice. Differences in FGF23 expression patterns might be due to the different animal models used. Therefore, further studies are needed to clarify the observations of systemically increased FGF23 turnover after fracture due to supplementation.

In conclusion, most experimental and clinical studies observe a positive influence of calcium and vitamin D supplementation on the fracture-healing outcome. These findings support the therapeutic potential of calcium and vitamin D supplementation after fracture in clinics for osteoporotic patients with insufficient calcium/vitamin D status. These patients frequently suffer from fracture-healing complications, which might be ameliorated in the case of a sufficient calcium and vitamin D supply.

Post-traumatic bone turnover

In addition to fracture-healing complications, osteoporotic patients who have experienced their first fragility fracture further display a 2 to 4-fold increased risk of a future fracture (Ahmed *et al.*, 2013; Center *et al.*, 2007; Kanis *et al.*, 2004; Klotzbuecher *et al.*, 2000; Lyles *et al.*, 2008; Omsland *et al.*, 2013). The fracture risk is higher in the first years after fracture and declines thereafter; however, it always remains higher when compared to patients without a previous fracture (Johnell *et al.*, 2001; Lindsay *et al.*, 2001; Ryg *et al.*, 2009). Subsequent fractures are further related to a reduced mobility and quality of life and an increased mortality in comparison to the first fragility fracture (Bliuc *et al.*, 2009; Sawalha and Parker, 2012). Therefore, therapeutic strategies reducing the secondary fracture risk are needed. However, the reasons for the increased risk of secondary fractures are currently unknown but might be the consequence of an accelerated decline in systemic bone mass in these individuals.

Systemic bone loss following fracture

Clinical data suggest systemic bone loss following fracture (Table 2**a**,**b**). Bone loss occurring at the broken extremity, particularly in close proximity to the fracture area, is well described and mainly

Table 2a. Summary of clinical studies examining systemic changes in bone mineral density after fracture. BMD: bone mineral density; DEXA: dual energy X-ray absorptiometry; qCT: quantitative computed tomography.

Study population	**BMD measurement** (site, time points, method)	Reference values/group	Outcome- BMD change (%)	Conclusion	Authors
Elderly patients (n = 83, mean age: 77 years), hip fracture	Lumbar spine, non-fractured hip 12 months DEXA	Baseline values	Hip: − 5.4 % (12 months) Spine: − 2.4 % (12 months)	Systemic bone loss after fracture	Dirschl *et al.*, 1997
Elderly patients (n = 85, mean age: 73 years), hip fracture	Lumbar spine, non-fractured hip 12 months, 6 years DEXA	Baseline values	Hip: − 4.3 % (12 months) Lumbar spine: − 1.8 % (12 months) Hip: + 7.7% (1 to 6 years) Lumbar spine: + 4.5 % (1 to 6 years)	Initial rapid systemic bone loss; after 6 years bone loss almost recovered	Dirschl *et al.*, 2000
Elderly females with osteoporosis (n = 108; mean age: 67 years), vertebral fracture and/or Colle's fracture	Lumbar spine, non-fractured radius Fracture occurred 1-15 years before DEXA	Age-matched healthy females (n = 42, mean age: 67 years)	Spine: − 19 % Radius: − 16 %	Systemic bone loss after fracture	Eastell *et al.*, 1989
Adult patients (n = 26; mean age: 35 years), tibia fracture	Femur, calcaneus at fractured and non-fractured leg 3, 6 and 12 months DEXA	Baseline values	Fractured leg: − 4 % (3 months), − 2.5-4 % (12 months) Non-fractured leg: no BMD changes	Bone loss at the fractured leg	Emami *et al.*, 2001
Adult patients (n = 21, mean age: 43 years), tibia fracture	Fractured leg, non-fractured leg 1, 2, 3, 4 ,5 and 6 months 5-11 years DEXA	Contralateral leg plus age-matched healthy controls (n = 10, mean age: 45 years)	Fractured leg: − 53 % (6 months) − 47 % (5-11 years) No differences in BMD between non-fractured leg and control group	Bone loss at the fractured leg, persistent after years	Eyres and Kanis, 1995
Elderly females (n = 205, mean age: 81 years), hip fracture	Non-fractured hip, total body 3 and 10 d, 2, 6 and 12 months DEXA	Baseline values	Hip: − 4.6 % (12 months) Total body: − 2.3 % (12 months)	Systemic bone loss after fracture	Fox *et al.*, 2000
Elderly females (n = 23, mean age: 62 years), Colle's fracture	Lumbar spine, radius within 5 weeks after fracture qCT	Age-matched healthy females	Spine: − 20 % Radius: − 5.2 %	Systemic bone loss after fracture	Härmä and Karjalainen, 1986
Elderly females (n = 20, mean age: 63 years), forearm fracture	Fractured forearm and hand, non-fractured forearm and hand 6, 12, 26 and 52 weeks DEXA	Baseline values	Hand: − 9 % (6, 52 weeks) Forearm: − 18 % (6 weeks), − 11 % (52 weeks) Non-fractured hand: no BMD changes	Bone loss at the fractured arm	Ingle *et al.*, 1999a
Elderly patients (n = 14, mean age: 63 years), ankle fracture	Fractured and non-fractured leg 6, 12, 26 and 52 weeks DEXA	Baseline values	Distal ankle: − 13 % (6 weeks) Hip: − 3 % (26 weeks) Non-fractured leg: no BMD changes	Bone loss at the fractured foot; no recovery of hip bone loss after 52 weeks	Ingle *et al.*, 1999b

Table 2b. Summary of clinical studies examining systemic changes in bone mineral density after fracture. BMD: bone mineral density; DEXA: dual energy X-ray absorptiometry; qCT: quantitative computed tomography.

Study population	**BMD measurement** (site, time points, method)	Reference values/group	Outcome-BMD change (%)	Conclusion	Authors
Elderly females (n = 40, mean age: 63 years), forearm fracture	Fractured hand, non-fractured hand, lumbar spine DEXA	Age-matched healthy females (n = 95, mean age: 67 years)	Fractured hand: − 6.2 % Non-fractured hand: − 3.2 % Spine: − 6.4 %	Systemic bone loss after fracture	Ingle and Eastell, 2001
Elderly patients (n = 102, mean age: 77 years), hip fracture	Fractured hip, non-fractured hip 10 d, 4 and 12 months DEXA	Baseline values	Fractured hip: − 7 % (12 months) Non-fractured hip: − 5 % (12 months)	Loss of bone mass after fracture, however, greater in the fractured hip	Karlsson *et al.*, 1996
Adult patients (n = 26, mean age: 57 years), tibial osteotomy	Fractured leg, non-fractured leg, total body, lumbar spine 3, 14, 42, 120, 270 and 450 d DEXA	Baseline values	Fractured leg: − 35 % (9 months) Total body: − 5 % (9 months) Spine: − 15 % (9 months) Non-fractured leg: − 10 % (9 months)	Systemic bone loss after fracture; reversal of bone loss in non-fractured bones incomplete: − 20% relative to baseline	Karlsson *et al.*, 2000
Adult females (n = 36, 50-73 years), Colle's fracture	Lumbar spine within 9-21 months after fracture Dual photon absorptiometry	Age-matched healthy females	Spine: − 9 %	Systemic bone loss after fracture	Krolner *et al.*, 1982
Adult patients (n = 12, mean age: 38 years), tibia fracture	Fractured leg, non-fractured leg, spine 1 week, 3 and 6 months, DEXA	Baseline values	Fractured leg: − 7-14 % (6 months) Non-fractured leg: no BMD change Spine: no BMD changes	Bone loss at the fractured leg	Petersen *et al.*, 1997
Adult patients (n = 7, mean age: 20 years), tibia fracture	Fractured leg, non-fractured leg 10, 20, 30, 60 and 120 d Dual photon absorptiometry	Baseline values	Fractured leg: − 50 % (120 d) Non-fractured leg: no BMD changes	Bone loss at the fractured leg	Ulivieri *et al.*, 1990
Elderly patients (n = 11, mean age: 63 years), tibia fracture	Fractured hip, non-fractured hip, lumbar spine 5 years, DEXA	Baseline values	Fractured hip: − 2.9-4.7 % (5 years) (compared to − 12.5-5.1 % after 1 year) Non-fractured hip: no BMD changes	Bone loss at the fractured leg; after 5 years some recovery, but not to baseline	Van der Poest Clement *et al.*, 1999
Elderly patients (n = 16, mean age: 60 years), tibia fracture	Fractured hip, non-fractured hip, lumbar spine 3, 6 and 12 months, DEXA	Baseline values	Fractured hip: − 6-15 % (12 months) Non-fractured hip: − 2 % (12 months) Spine: no changes in BMD	Loss of bone mass after fracture, however, greater in the fractured hip	Van der Wiel *et al.*, 1994
Adult patients (n = 18, mean age: 34 years), tibia fracture	Fractured leg, non-fractured leg 2, 8, 12, 24 and 52 weeks DEXA	Baseline values	Fractured leg: − 10-28 % (24 weeks) − 9-20 % (52 weeks) Non-fractured leg: no BMD changes	Bone loss at the fractured leg	Veitch *et al.*, 2006
Elderly females (n = 19, mean age: 64 years), Colle's fracture	Fractured forearm, non-fractured forearm (wrist) Gamma absorptiometry	Baseline values	Fractured forearm: − 18 % (4 months) Non-fractured wrist: − 10 % (6 months)	Systemic bone loss after fracture	Westlin, 1974
Elderly females (n = 205, mean age: 65 years), hip fracture	Non-fractured hip, total body 3, 10, 60, 180 and 365 d DEXA	Baseline values	Non-fractured hip: − 2.1-4.6 % (1 year) Total body: − 2.3 % (1 year)	Systemic bone loss after fracture	Yu-Yahiro *et al.*, 2001

results from disuse because of immobilisation (Karlsson *et al.*, 1996; Van der Wiel *et al.*, 1994). This locally restricted bone mass loss normally recovers after successful bone repair. However, in old and osteoporotic individuals, the deficit in bone mass persists for years, rarely ever achieving pre-fracture mineral levels (Eyres and Kanis, 1995; Ingle *et al.*, 1999a; Karlsson *et al.*, 2000). Furthermore, changes in bone mineral levels are also observed in distal skeletal sites, including the unaffected extremity, spine and whole body of the elderly (Eastell *et al.*, 1989; Härmä and Karjalainen, 1986; Ingle and Eastell, 2001; Krolner *et al.*, 1982; Westlin, 1974). Clinical studies that examine systemic bone loss, including in the non-fractured extremity, spine and whole body, are summarised in Table 2**a**,**b**. Highlighting some of these studies, Fox *et al.* (2000) report that in 205 community-dwelling postmenopausal females displaying a proximal femur fracture, bone loss at the contralateral hip is 4.6 % and in the whole body 2.3 % after 12 months as compared to baseline values. Karlsson *et al.* (2000) observe a reduction in lumbar spine BMD of up to 15 % after 9 months. Dirschl *et al.* (1997) detect a mean decline in bone mass of 2.4 % in the lumbar spine and 5.4 % in the contralateral hip 1 year after an osteoporosis-related hip fracture. By contrast, some clinical data exist where no decline in bone mass of the uninjured extremity or the whole body after fracture is reported (Emami *et al.*, 2001; Ingle *et al.*, 1999b; Petersen *et al.*, 1997; Ulivieri *et al.*, 1990; Veitch *et al.*, 2006). However, the studies that do not observe systemic BMD changes after fracture frequently examine a younger fracture cohort, suggesting that post-traumatic bone loss particularly affects the aged population. Moreover, some report total or at least partial recovery of bone mass after 5 or 6 years (Dirschl *et al.*, 2000; van der Poest Clement *et al.*, 1999). Differences may result from variations in the investigated cohorts, including in age, gender, fracture type and treatment. Furthermore, there are considerable differences in the location of the measured bone area and to which values the data were referring to (*e.g.* to baseline or to an additional control cohort without fracture). Nevertheless, based on most of the clinical data, it is hypothesised that a fracture might induce systemic bone loss, which is particularly enhanced in elderly and osteoporotic patients, thus further reducing bone properties in this risk group.

In 205 postmenopausal females, Yu-Yahiro *et al.* (2001) observe an association between the reduction in bone mass after hip fracture and an increase in serum PTH levels. PTH levels in these females remain elevated even 1 year after fracture. A rise in serum PTH levels after fracture is also found in other clinical and one experimental study (Haffner-Luntzer *et al.*, 2016; Hitz *et al.*, 2007; Meller *et al.*, 1984). In conclusion, it is hypothesised that a fracture-induced increase in serum PTH levels induces systemic bone loss and may explain the increased risk of subsequent fractures. Possible reasons for post-traumatic bone loss include a reduced calcium supply, immobilisation and an increased inflammatory status after fracture. However, experimental studies that provide the opportunity to study the basic underlying mechanism of a fracture-induced bone loss independent of other influencing factors, including immobilisation, are mostly lacking. The first indications for post-traumatic bone loss are reported by Lane *et al.* (1984), who show that the calcium-to-phosphorus ratio increases in the skeleton of rats and rabbits after fracture, thus suggesting for the first time a decrease in the amount of calcium in the whole skeleton following a fracture. Using a sheep model, Augat and Claes (2008) confirm increased post-traumatic osteopenia in proximity to the fracture site. Regarding systemic changes in bone mass, Christiansen *et al.* (2015) observe a loss of trabecular bone mass in the lumbar spine of mice 10 d after knee injury, which is associated with an avulsion fracture of the distal femur. 2 and 4 weeks after femur fracture, Emami *et al.* (Age-dependent systemic bone loss and recovery following femoral fracture in mice. In: ORS 2017 Annual Meeting San Diego. Poster Nr. 0714) detect bone loss at distinct sites in middle-aged mice that do not recover bone mass after 6 weeks as compared to young mice. The authors observe increased IL-6 levels at day 3 after fracture in middle-aged mice and hypothesise that the increased inflammatory response after injury may be responsible for systemic bone loss. However, mechanisms inducing increased systemic bone loss need to be further determined.

Role of calcium and vitamin D in post-traumatic bone turnover

Based on the important function of both calcium and vitamin D in bone homeostasis, these nutrients may also play a critical role in post-traumatic bone turnover. Supporting this assumption, a significant correlation between the amount of post-traumatic bone loss on one side and insufficient dietary calcium intake and reduced serum vitamin D levels on the other is observed in the clinic (Dirschl *et al.*, 1997). Indeed, Haffner-Luntzer *et al.* (2016) confirm the involvement of calcium and vitamin D in post-traumatic bone turnover. They demonstrate that mice with intestinal calcium malabsorption, Cckbr-deficient mice (lacking Cckbr, necessary for gastric acidification), display increased serum PTH levels after fracture, which is accompanied by elevated osteoclastic bone resorption in the non-fractured skeleton. Notably, fracture healing is unaffected in Cckbr-deficient mice (Haffner-Luntzer *et al.*, 2016). These results indicate for the first time that under conditions of insufficient dietary calcium supply, the calcium needed for callus mineralisation is increasingly mobilised from distal skeletal sites in favour of successful bone repair.

Confirming the previous results obtained in mice with intestinal calcium malabsorption, Fischer *et al.* (2017) demonstrate that calcium- and vitamin-D-

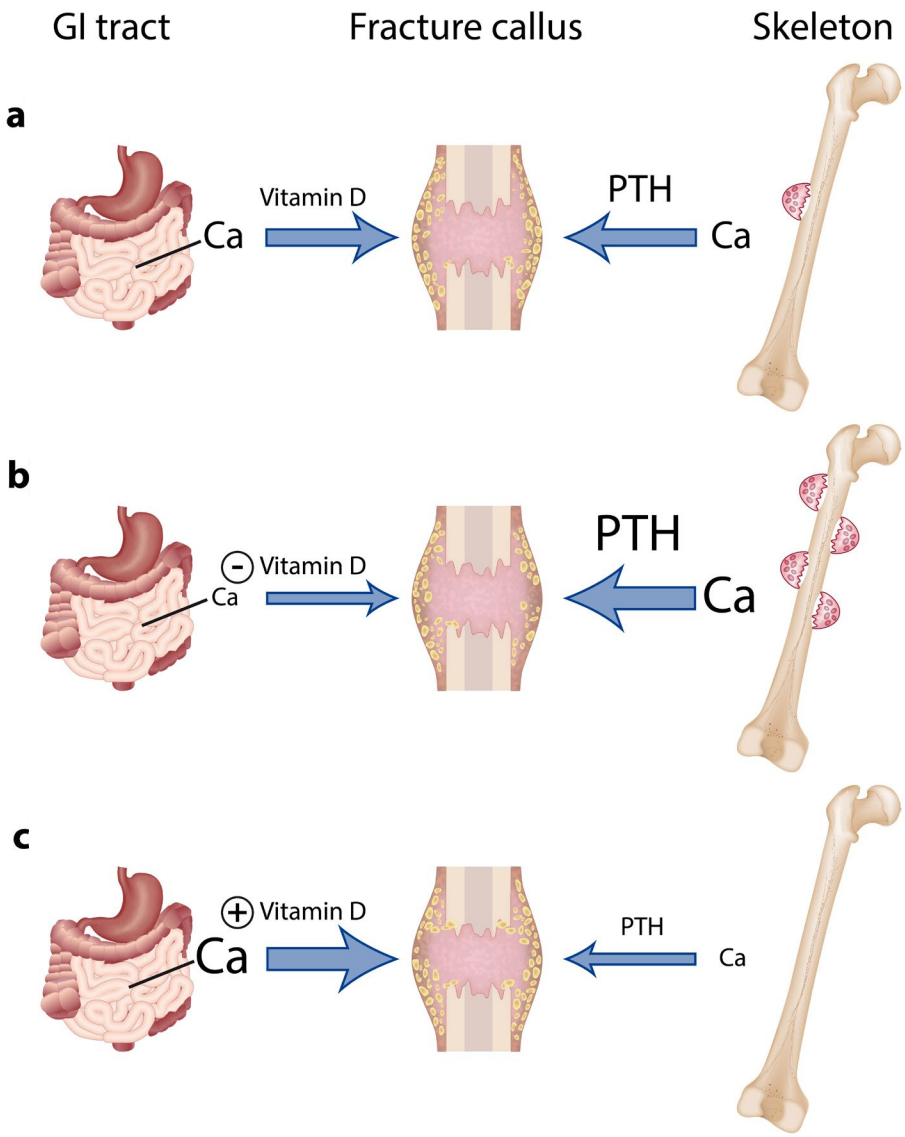

Fig. 2. Model of the impact of calcium and vitamin D on post-traumatic bone turnover. (**a**) Healthy state of normal calcium supply and vitamin D status satisfactory for successful fracture healing and skeletal health. (**b**) Insufficient calcium supply and reduced vitamin D stimulate PTH-induced osteoclastic bone resorption in the non-fractured skeleton to guarantee calcium needs for fracture-callus mineralisation, however, further worsening skeletal health. (**c**) Increased calcium supply and sufficient vitamin D provide calcium needs for fracture healing without increasing calcium mobilisation from non-fractured skeletal sites. Ca = calcium.

deficient mice similarly display a rise in PTH serum levels after fracture and that fracture healing is only marginally disturbed in these mice. In addition, fractured calcium- and vitamin D-deficient mice exhibit significantly more osteoclasts in their lumbar vertebrae relative to non-fractured deficient mice, indicating enhanced osteoclastic bone resorption in the remote skeleton after fracture. In addition, increased osteoclastic bone resorption in fractured deficient mice result in bone loss (Fischer *et al.*, 2017). These results indicate that when a fracture occurs under calcium- and vitamin-D-deficient conditions, the increasing need for calcium stimulates an increase in serum PTH levels to induce osteoclastic bone resorption at distal skeletal sites. Because of the increased calcium mobilisation, sufficient amounts of calcium can be provided for fracture callus mineralisation. The increased post-traumatic calcium mobilisation might be the reason why bone repair is not or only marginally disturbed under calcium and vitamin D deficiency, however, at the clear expense of bone properties (Fig. 2). It might also explain the results of most of the other experimental studies, which do not observe a delay in fracture healing under calcium- and vitamin-D-deficient conditions. The enhanced post-traumatic calcium mobilisation might explain the dramatically increased risk of secondary fractures.

Importantly, Fischer *et al.* (2017) observe no increase in PTH serum levels and osteoclastic bone resorption when deficient mice are supplemented with calcium and vitamin D directly after fracture. Furthermore, they do not observe a decline in bone properties at distal skeletal sites because of calcium and vitamin D treatment. Additionally, in mice displaying intestinal calcium malabsorption, calcium supplementation reduce secondary hyperparathyroidism and bone resorption in the non-fractured skeleton (Haffner-Luntzer *et al.*, 2016). Confirming these results, the only experimental study that investigate the effect of vitamin D on post-traumatic bone turnover observe that 1,25-VitD_3 injections in rats reduce post-fracture osteopenia (Lindgren *et al.*, 1981). Supporting the experimental findings, calcium and vitamin D supplementation after fracture can increase lumbar BMD in elderly patients with hip fracture when compared to patients of the placebo group, where lumbar BMD decrease and PTH levels increase (Hitz *et al.*, 2007).

Concluding, these findings clearly indicate the need for calcium and vitamin D supplementation during fracture healing in patients with calcium and vitamin D deficiency or osteoporosis to prevent systemic bone loss following fracture and to reduce the risk of secondary fractures. In addition, multiple-injured patients, who display many fractures and thus require larger amounts of calcium for the mineralisation of numerous fracture calli, might benefit from calcium and vitamin D supplementation during fracture healing.

Summary and future directions

This review summarises the current literature on how calcium and vitamin D influence fracture healing and post-traumatic bone turnover. Data reviewed here demonstrate that the role of calcium and vitamin D in fracture healing is still poorly investigated and existing results are inconsistent. However, there is increasing evidence that calcium and vitamin D deficiency enhances systemic bone loss after fracture, because the calcium necessary for fracture callus mineralisation is increasingly mobilised from the remote skeleton in favour of fracture healing when the calcium and vitamin D status is insufficient. This increased post-traumatic bone loss might exacerbate osteoporosis and explain the 3-fold increased risk of secondary fractures in the clinic. Importantly, experimental data show that calcium and vitamin D supplementation, initiated after fracture and continued during the entire healing process, prevents post-traumatic bone loss. In addition, several experimental studies observe an improvement in bone regeneration because of calcium and vitamin D supplementation. These findings imply a high clinical potential of calcium and vitamin D supplementation on fracture-healing outcome and post-traumatic bone turnover. However, there is a paucity of clinical research that has investigated the effects of calcium and vitamin D on fracture healing or post-traumatic bone turnover. More clinical studies should be performed in which calcium and vitamin D are supplemented during the fracture-healing process of patients displaying osteoporosis, calcium and vitamin D deficiency or multiple fractures.

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Discussion with Reviewer

Richard Stange: It would be of interest to know about fracture healing during pregnancy. Probably a rare event, but a challenging situation.

Authors: Due to increasing calcium needs required for the foetus skeletal development and to meet their own calcium needs, pregnant females must provide a sufficient amount of 1000 mg of calcium per day (Kovacs, 2014, additional reference). This calcium demand is nearly met by an increase in calcium absorption efficiency of around 50 % starting from the third month (Kovacs and Kronenberg, 1997; Kovacs, 2011, additional references). However, the maternal skeleton also serves as a calcium reservoir. Clinical studies report increased levels of bone resorption markers and marginally reduced bone mass in pregnant as compared to non-pregnant females. However, osteoporosis and fractures rarely occur during pregnancy (Kovacs, 2011; Purdie *et al.*, 1988; Moller *et al.*, 2012, additional references). Herath *et al.* (2017) report in a retro-prospective study that out of 114,673 pregnant females 33 sustain a fracture, with an ankle fracture as the most common one. Nevertheless, during conditions of additional calcium demand, as in the case of a fracture event plus insufficient calcium and vitamin D supply, bone resorption and bone loss might be increased. Based on previous findings (Fischer *et al.*, 2017), it can be assumed that during fracture healing of pregnant females with additional calcium and/or vitamin D deficiency, the calcium needed for foetal development and fracture callus mineralisation might be increasingly mobilised from the intact skeleton through an

increase in parathyroid hormone levels. Thus, fracture healing will be undisturbed, but increased bone resorption will reduce intact bone mass. Based on our knowledge, there are no experimental and clinical studies available investigating fracture healing outcome during pregnancy. There are only a few case reports outlining fracture healing in healthy pregnant females, with one showing even accelerated fracture union (Hadji *et al.*, 2017; Herath *et al.*, 2017; Tv *et al.*, 2015, additional references). However, none of these reports investigate if there are changes also in the remaining skeleton. Long-term consequences of fractures during pregnancy are not known so far and should be considered in the future. Based on a search of the literature as well as previous findings, it is recommended that pregnant females, especially calcium- and vitamin-D-deficient ones, who suffer a fracture, should consume calcium and vitamin D, at least during the fracture healing time course, to prevent skeletal bone loss. This aspect was not included in the discussion of the subject because published evidence is scarce.

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Editor's note: The Scientific Editor responsible for this paper was Juerg Gasser.