Advances and Promises of Nutritional Influences on Natural Bone Repair

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ABSTRACT: Impaired fracture healing continues to be a significant public health issue. This is more frequently observed in aging populations and patients with co-morbidities that can directly influence bone repair. Tremendous progress has been made in the development of biologics to enhance and accelerate the healing process; however, side-effects persist that can cause significant discomfort and tissue damage. This has been the impetus for the development of safe and natural strategies to hasten natural bone healing. Of the many possible approaches, nutrition represents a safe, affordable, and non-invasive strategy to positively influence each phase of fracture repair. However, our understanding of how healing can be hindered by malnutrition or enhanced with nutritional supplementation has lagged behind the advancements in both surgical management and the knowledge of molecular and cellular drivers of skeletal fracture repair. This review serves to bridge this knowledge gap as well as define the importance of nutrition during fracture healing. The extant literature clearly indicates that pre-existing nutritional deficiencies should be corrected, and nutritional status should be carefully monitored to prevent the development of malnutrition for the best possible healing outcome. It remains unclear, however, whether the provision of nutrients beyond sufficiency has any benefit on fracture repair and patient outcomes. The combined body of pre-clinical studies using a variety of animal models suggests a promising role of nutrition as an adjuvant therapy to facilitate fracture repair, but extensive research is needed, specifically at the clinical level, to clarify the utility of nutritional interventions in orthopedics. © 2019 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 38:695–707, 2020

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Bone fractures are costly and debilitating traumatic events. In the United States, over 6 million fractures occur each year, and as the global population ages and life expectancy increases, the incidence of fragility fracture will undoubtedly increase.[1] It is estimated that approximately 5–10% of individuals experience difficulty healing, which results in delayed or non-unions.[2,3] These conditions typically require intense surgical intervention and are associated with a severe decline in quality of life.[4] Thus, the costs associated with high incidence of delayed unions and malunions place considerable financial, physical, and mental burdens on individuals and highlight the need to identify affordable, effective, and safe strategies to treat bone fractures.

To date, many strategies to enhance both the quality and rate of fracture healing have been investigated. Of these, there is a handful of established clinical approaches to augment fracture repair including exogenously applied low-intensity pulsed ultrasound, orthobiologics, and bone graft surgery.[5] Though most of these strategies exhibit relatively good results, there are some notable limitations to their clinical effectiveness including costs, invasiveness, and potentially harmful side-effects that underscores the need to identify non-surgical means to improve fracture healing. Optimizing nutrition to enhance healing is an attractive approach not only due to the safe and affordable nature of nutritional factors but also due to the well-established role of nutrition in bone growth and homeostasis. This review aims to highlight the current state of the field as well as gaps in knowledge regarding the impact of nutritional status and nutritional supplements on fracture healing.

MECHANISMS OF BONE FRACTURE HEALING

Bone fractures are among the most common traumas that afflict individuals across their life-course with the lifetime risk of experiencing an osteoporotic fracture estimated to be 40–50% for women and 13–22% for men.[6,7] When a bone fractures, it is treated either operatively or non-operatively, with the management strategy being guided by the location and severity of the fracture. Non-operative treatment of fractures has several advantages including significant cost-savings for the patient,[8,9] and also fewer complications such as surgical site infections and reoperations.[10] The treatment strategy used dictates the mechanism by which the broken bone heals, which can occur either through primary (direct) fracture healing or secondary (indirect) fracture healing (Fig. 1).[11] Primary bone healing involves the direct deposition of a new bone matrix at the site of fracture that ultimately unifies the broken tissue.[11] Accurate anatomical reduction and rigid fixation are requirements for primary bone healing; thus, this mechanism of repair does not often occur in the absence of surgical intervention.[11,12] On the other hand, secondary bone healing does not require accurate anatomical reduction and is the mechanism by which

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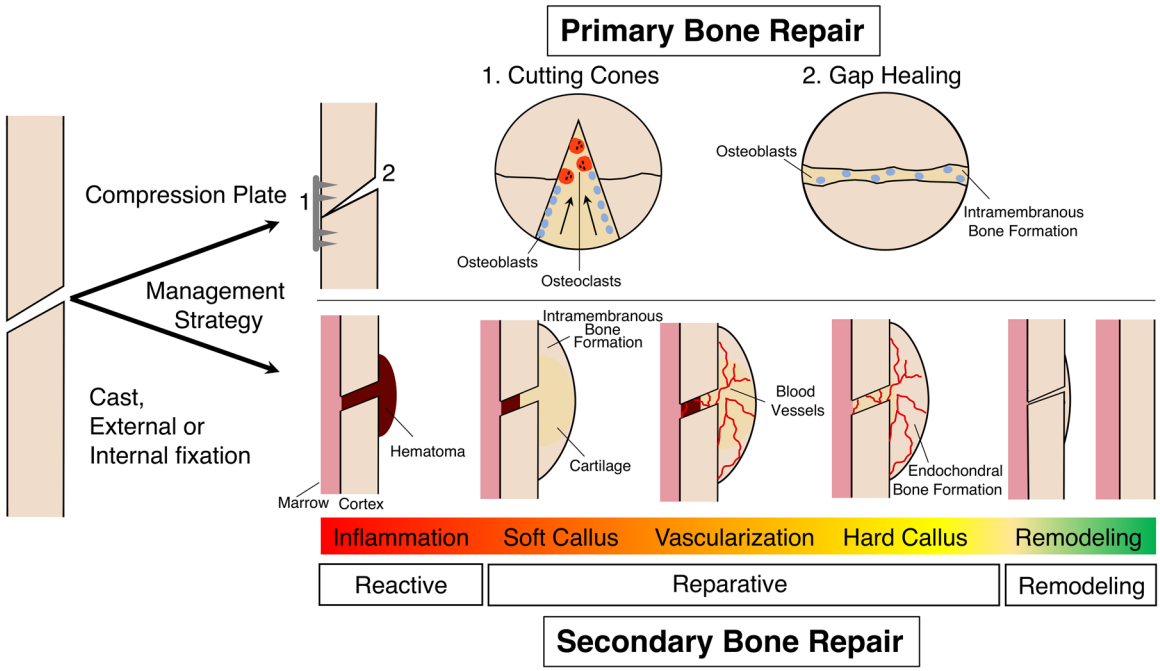

Figure 1. Mechanism of fracture repair. After a bone breaks, the treatment strategy used will dictate how it heals. Primary bone healing occurs after accurate anatomical reduction and rigid fixation and proceeds either through cutting cones or through gap healing. If the fractured fragments are close enough, osteoclasts will form cutting cones across the fracture line to re-establish osteons, that are latter filled by bone by trailing osteoblasts. Gap healing occurs when the gap between the fractured ends are further apart and proceeds through intramembranous ossification and deposition of lamellar bone between the fractured ends by osteoblasts. Fractures treated non-operatively, or through internal or external fixation heal through secondary bone healing that can be divided into three phases: an initial reactive that is followed by a longer reparative and culminates in an extended period of remodeling. Fracture ruptures the vasculature within the bone and leads to the formation of a hematoma that is infiltrated by immune cells that promote inflammation. Mesenchymal stem cells from the periosteum and bone marrow migrate to the fracture site and give rise to both bone-forming osteoblasts and cartilage forming chondrocytes that facilitate endochondral and intramembranous ossification during the reparative phase. These cells begin to form an avascular cartilaginous soft callus and as the chondrocytes hypertrophy, it signals vascular invasion of the callus. The soft callus is then resorbed via the actions of osteoclasts and chondroclasts and replaced with woven bone by osteoblasts that occur. This establishes the hard-bony callus. Finally, the coordinated actions of osteoblasts and osteoclasts remodel the hard callus into lamellar bone. Fractures are considered healed when bone stability has been restored by new bony bridges across the area of fracture. [Color figure can be viewed at wileyonlinelibrary.com]

most bones heal clinically.[11,13,14] During secondary fracture healing, the regenerative process is driven by a coordinated series of physiological events that can be grouped into an initial reactive phase, followed by a reparative phase, and culminating with a remodeling phase, all of which are described below (Fig. 1).

The reactive phase occurs immediately following trauma and is driven by the local disruption of blood vessels and surrounding soft tissue. This localized tissue injury promotes the formation of a hematoma that eventually coagulates to serve as the template for callus formation.[11,15] Simultaneously, this initiates an acute inflammatory response that recruits immune cells to the fracture site.[16,17] These cells invade the hematoma where they facilitate the removal of debris as well as secrete cytokines and chemokines that recruit immunosuppressive mesenchymal progenitor cells from the periosteum, bone marrow, and systemic circulation to resolve inflammation and initiate the reparative phase.[11,18–20]

Pluripotent mesenchymal progenitor cells that migrate to the site of injury begin to undergo chondrogenic differentiation giving rise to an avascular cartilaginous callus. As chondrocytes undergo hypertrophy, this triggers the invasion of endothelial cells that promote vascularization and mineralization of the cartilaginous matrix.[21,22] The calcified cartilage is then replaced with woven bone resulting in a hard-bony callus. Throughout this process, mesenchymal progenitor cells located at the periphery of the fracture site differentiate into osteoblasts and deposit osteoid against the existing cortex through intramembranous ossification.[11,13]

The immature woven bone of the bony callus is subsequently remodeled into lamellar bone to fully restore the bone's biomechanical properties. This occurs during the remodeling phase of fracture healing and relies on osteoclastic bone resorption and osteoblastic bone deposition.[11,23] Successful remodeling is illustrated by restoration of the cortex to the pre-fracture architecture, which occurs over months to years after the initial injury. Though bones have a remarkable capacity to naturally heal, many patient-specific factors, including nutrition can influence the progression of fracture healing.[24]

METABOLIC RESPONSE TO FRACTURE

Traumatic injuries, including fractures, depress appetite and food consumption that in turn drives weight loss.[25–29] This phenomenon has been a primary motivator for the inclusion of nutrition therapy in the holistic management

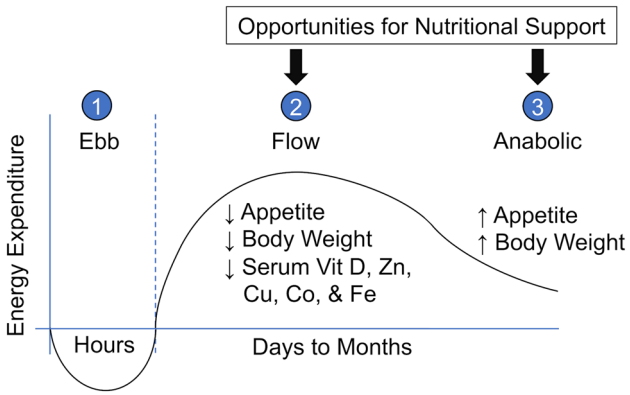

Figure 2. Metabolic response to trauma. Immediately following injury there is an initial ebb phase characterized by a decrease in energy expenditure. The body then enters into a hypermetabolic flow phase that is associated with catabolism and increased energy expenditure and decreases in serum vitamin D (Vit D), zinc (Zn), copper (Cu), cobalt (Co), and iron (Fe). The final phase is the anabolic phase in which energy expenditure returns to pre-injury levels. Nutritional interventions delivered during the flow and anabolic phases have the highest potential for best healing outcomes. [Color figure can be viewed at wileyonlinelibrary.com]

of trauma patients. Trauma induces a hypermetabolic state that is partly driven by the inflammatory reaction to injury.[30] This drives catabolic stress that is characterized by nitrogen loss and insulin resistance.[30] The metabolic response to trauma can be described by three phases: (i) a period of decreased metabolic rate; (ii) a marked increase in catabolism; and (iii) an anabolic phase that results in repair of the damaged tissue (Fig. 2). Although much attention has been placed on nutritional management of burn, head, and other non-bone trauma, little is known regarding the changes in nutritional requirements after fracture. An initial study published in 1930[31] described the development of negative nitrogen balance, tissue wasting, and weight loss in patients with long-bone fractures, which was further supported in a rat model that demonstrated an obvious hypermetabolic response to femoral fractures.[32] It is now recognized that a well-nourished patient with a fracture experiences a 20–25% increase in metabolic rate. However, those with polytrauma can experience a 30–55% increase with the magnitude of the metabolic response being dictated by the severity of the trauma.[33,34]

Beyond increases in general metabolic rate and energy expenditure, fracture induces changes in micronutrient metabolism that appear to be driven by an increased demand to facilitate callus formation and mineralization. This reactive response to fracture leads to decreased serum copper,[35,36] cobalt,[35,36] iron,[35,36] vitamin D,[37,38] and zinc,[35,36,39–41] with the latter two accumulating in fracture tissue. Calcium metabolism is also perturbed, where it is mobilized from the intact skeleton through the concerted actions of the parathyroid hormone and the systemic inflammatory response to fracture. This increase in osteoclast-mediated mobilization of calcium can result in a 2–15% reduction in bone mass of intact bones.[42–45]

These metabolic responses to fracture are of extreme clinical relevance as the incidence of clinical or more commonly sub-clinical malnutrition is high in orthopedic trauma patients.[34,46] Malnutrition when present in the context of the hypermetabolic response to trauma can impede healing. Although it is unlikely that nutrition support will be able to reverse all of the metabolic changes that occur after fracture, it does have immense clinical value as it can improve patient outcomes such as length of hospital stay and improved immune function and may facilitate natural healing.[34] In the next sections, we discuss the influence of nutritional deficiencies as well as the benefits of nutritional supplements in bone healing and patient outcomes.

IMPACT OF NUTRITIONAL DEFICIENCIES ON BONE HEALING

Nutritional insults, such as malnutrition, have been identified as important risk factors for impaired bone healing.[46] Malnutrition arises due to deficiencies, excess, or imbalances in the intake of energy and/or nutrients. Malnutrition, specifically undernutrition, is frequently observed in elderly fracture patients who are known to experience delayed healing.[46,47] This pre-existing clinical or sub-clinical malnutrition in elderly fracture patients can stem from a variety of factors including decreased appetite, decreased diet quality, and other co-morbidities that can be exacerbated by the physiological response to injury and surgical procedures that alter nutrient ingestion, absorption, and metabolic requirements.[34] Adequate intake of macronutrients (i.e., carbohydrates, lipids, and proteins) during convalescence is critically important not only to prevent depletion of endogenous glycogen, protein, and lipid stores but also to meet increased energy demands during the reparative phase of fracture healing (Fig. 3). This was highlighted in a pre-clinical model in which Wistar rats fed a restricted diet experienced impaired femoral fracture healing.[48] Beyond general caloric needs, adequate dietary protein appears to be vital for

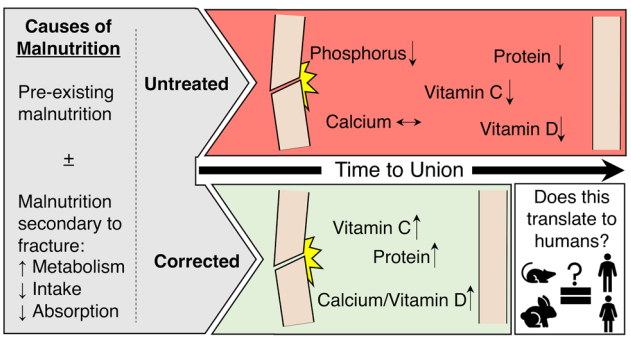

Figure 3. Effect of nutritional deficiencies on fracture healing. Orthopaedic patients often present with malnutrition, particularly nutrient deficiencies. Malnutrition can also arise from increased metabolism, decreased food consumption and nutrient absorption. When left untreated, phosphorus, protein, vitamin C, and vitamin D impair the healing process. Calcium deficiency does not have a major effect on healing. Pre-clinical studies demonstrate that correcting nutrient deficiencies using vitamin C, protein, and calcium/vitamin D supplements resumes the healing process but whether this translates to humans remains unknown. [Color figure can be viewed at wileyonlinelibrary.com]

natural fracture healing, as pre-clinical studies in rats have consistently demonstrated that protein restriction, that mimics protein malnutrition, impairs fracture healing.[28,29,49,50] Importantly, correction of protein malnutrition at time of fracture leads to more complete recovery from the effects of protein malnutrition as well as improved fracture healing.[28,50]

Micronutrient deficiencies are more likely to be observed in orthopedic trauma patients than protein malnutrition. For instance, 40–70% of elderly patients presenting with a fragility or trauma-induced fracture are vitamin D deficient,[51,52] which has been shown to increase the odds of developing a non-union (odds ratio, 1.14; 95% confidence interval, 1.05–1.22).[3] Vitamin D is important in regulating calcium homeostasis, both of which have critical roles in facilitating mineralization of the callus that requires the deposition of approximately 1.7–2.3 g of hydroxyapatite per cm^3 of bony callus.[53,54] In ovariectomized mice, only callus bone mineral density (BMD) was significantly affected by calcium and vitamin D deficient diets.[55] However, when using a genetic model of intestinal calcium malabsorption, fracture healing was unaffected compared with wildtype controls.[43] Calcium deficiency does not appear to significantly impact fracture healing, which is likely a result of stored calcium that is liberated from the intact skeleton following fracture. Along with calcium, the hydroxyapatite inorganic component of bone also requires phosphorus, and consumption of phosphate-restricted diets negatively impacts secondary fracture healing.[56–58] Severe phosphate deficiency impairs the proliferation and differentiation of mesenchymal progenitor cells, leading to an overall decrease in callus volume and an early, yet sustained increase in callus cartilage in mice with femoral fractures.[56,57] These adverse effects were attributed to decreased bone morphogenetic protein signaling-2 (BMP-2) in fracture associated mesenchymal stem cells as well as disruption of the circadian cycle that slows the progression of chondrogenic differentiation of mesenchymal stem cells. Although pre-clinical animal studies have shed light on the negative impact of severe phosphate deficiency on fracture healing, it is important to recognize that deficiency would be rarely observed clinically, as most Americans consume two to three times the recommended dietary allowances (RDA) due to the widespread availability and high bioavailability of phosphorus.[59,60]

Another important nutritional concern following fracture is vitamin C status, especially considering an estimated 40–80% of hospitalized elderly patients are deficient.[61,62] Although less frequently observed in healthy populations, vitamin C deficiency can also increase the risk of experiencing impaired fracture healing[63] that not only stems from impaired chondrogenic and osteogenic differentiation of mesenchymal stem cells but also impaired collagen maturation, an important component of bone architecture. This culminates in delayed formation of callus fibrous tissue, cartilage, and osteoid, which has been observed in a variety of pre-clinical models.[64] In the case of guinea pigs and osteogenic disorder Shionogi (ODS) rats, which cannot endogenously synthesize vitamin C, there is practically a complete inhibition of the reparative processes when given a diet lacking vitamin C or a diet that mimicked sub-clinical deficiency.[26,64,65] Comparable findings were described in a case report of a 25-year-old woman with scurvy and a proximal humerus fracture who had no radiologic evidence of healing 6 months after non-operative treatment.[66] A noticeable callus was observed shortly after correcting her vitamin C deficiency.[66] These studies collectively demonstrate that vitamin C deficiency impairs fracture healing, and importantly demonstrate that correction of deficiency can facilitate resumption of the healing process.

For the most part, these pre-clinical studies support the importance of assessing nutritional status of fractured patients in orthopedic clinics, especially the elderly, who are more likely to suffer from nutritional deficiencies. However, this comes with its own set of challenges, as the traditional proteins used to assess nutritional status (e.g., albumin, transferrin, and retinol-binding protein) are negative acute-phase proteins that decrease following traumatic injuries (Table 1). Furthermore, serum levels of many nutrients (e.g., vitamin D, zinc) decrease during the reactive response to fracture and some nutrient deficiencies such as calcium and phosphorus can only be determined using inherently flawed dietary recall assessments. These limitations suggest that traditional nutritional assessment methods may not be appropriate in the context of fracture and stress the importance of identifying reliable biomarkers of deficiency that can be used to quickly, yet accurately inform clinical decisions regarding nutritional management of fracture. In this regard, clinical studies are certainly warranted to determine if and how a patient's nutritional status can be leveraged for the best possible fracture healing outcome.

INFLUENCE OF NUTRITIONAL SUPPLEMENTS ON NATURAL BONE HEALING

During periods of rapid bone formation, like the adolescent growth spurt, nutrient requirements are higher to meet the increased demand for bone growth. Thus, it is conceivable that consumption of nutrients beyond the established RDA (Table 2) during the reparative and remodeling stages of fracture repair may be beneficial for bone healing. As fracture patients have a strong willingness to use nutritional supplements, it is important to ascertain whether nutrient supplements will have any effect on healing.[67] Several studies have sought to address this critical question, most of which were conducted using pre-clinical models (Tables 3–5). Vitamin D, calcium, and phosphorus supplements have received the most attention (Table 3) that is likely attributed to the well-established role each has in bone growth and homeostasis. Of these, vitamin D is one of the most routinely prescribed supplements to patients

Table 1. Serum Indicators of Malnutrition

Marker	Half-Life (days)	Reference Value	Advantages	Disadvantages
Albumin	14–21	3.4–5.5 g/dl	Reproducible and effected less by daily protein consumption	Long half-life makes it a poor indicator of acute malnutrition; levels decrease in trauma due to inflammation
Transferrin	10	170–370 mg/dl	Intermediate half-life, responds more rapidly to changes in protein status	Unreliable in assessment of mild malnutrition; values affected by fluid status and inflammation
Retinol-binding protein	0.5–1	3–6 mg/dl	Short half-life, responds quickly to changes in protein status and nutritional interventions	Unreliable in postoperative patients; values affected by trauma and inflammation
Prealbumin	2–3	15–36 mg/dl	Reflective of acute changes in nutritional status, unaffected by hydration status	Not a good indicator of long- term nutrition status; may vary with daily protein intake; values affected by trauma and inflammation
C-reactive protein	0.5–1	<0.8 mg/dl	Marker of general inflammation that can inform whether levels of other indicators is due to inflammatory processes or due to nutritional deficiencies	Non-specific indicator of inflammation; values affected by traumatic injuries, fractures, and surgery

Table 2. Adult Recommended Dietary Allowances (RDA) For Nutrients Related to Fracture.

Nutrient	Female RDA	Male RDA
Vitamins		
Vitamin C (mg/day)	75	90
Vitamin D (μg/day)		
19–70 years	15	15
>70 years	20	20
Vitamin E (mg/day)	15	15
Minerals		
Calcium (mg/day)		
19 to 50 years	1,000	1,000
51–70 years	1,200	1,000
>70 years	1,200	1,200
Copper (μg/day)	900	900
Phosphorus	700	700
Zinc (mg/day)	8	11
Iron (mg/day)		
19–50 years	18	8
>50 years	8	8
Macronutrients		
Protein (g/day)	46	56

with fragility fractures.[89] In support of this clinical approach to enhance healing, pre-clinical studies consistently report positive results suggesting that vitamin D may be beneficial for callus formation and healing.[27,68–70,90] Clinical data, on the other hand, are limited and difficult to interpret due to the inclusion of calcium supplements making it impossible to isolate the effect of supplemental vitamin D on bone healing. A study of 30 elderly women with a proximal humerus fracture concomitant with osteoporosis or osteopenia demonstrated that a daily supplement of 800 IU/day cholecalciferol and 1,000 mg calcium increased bone density at the fracture site after 6 weeks.[85] A separate study also found a beneficial effect of daily supplementation with 880 IU/day 25(OH)-cholecalciferol and 1,000 mg calcium on callus size in post-menopausal women with a distal radius fracture.[86] We identified a single study that examined the effects of daily vitamin D supplementation (1,200 IU) on fracture healing in deficient patients did not find any benefit of supplements on radiological healing in a middle-aged population with a fracture of the upper or lower extremity.[52] Though the evidence regarding the benefits of vitamin D supplementation in humans is limited, supplemental vitamin D does not appear to have any adverse effects on fracture healing and initiation of supplements post-fracture may promote the healing process. Unlike vitamin D, the benefit of supplemental calcium on bone healing in animal models is mixed. Ovariectomized Sprague–Dawley rats given calcium supplements had improved healing assessed radiologically, but the strength of the healed femur was not

Table 3. Summary of Pre-Clinical Studies Examining Effects of Vitamin D, Calcium, and Phosphorus Supplementation on Fracture Healing

Model	Dosage and Duration	Key Effects	Ref.
OVX C57BL/6J mice with femoral fracture	Animals fed diets deficient in calcium and vitamin D then given supplemented diets containing 2% calcium and 2,000 IU/kg vitamin D for 23 days	*Improved healing*: improved healing assessed by biomechanics and histology	[55]
Aged Wistar rats with femoral fracture	Subcutaneous injections of 250 IU vitamin D/100 g at time of fracture, and repeat injections of 125 IU/100 g body weight at 15 and 30 days post-fracture	*Improved healing*: improvements in biomechanical properties	[68]
Holman rats with femoral fracture	Animals given daily subcutaneous injections of vitamin D (25 ng) for 2 weeks then 12.5 ng vitamin D for 2 weeks	*Improved healing*: improvements in biomechanical properties	[69]
OVX Sprague–Dawley rats with femoral fracture	Daily oral supplements of vitamin D (0.1 μg/kg/day) for 6 and 16 weeks	*Improved healing*: improvements in biomechanical properties and improved callus remodeling	[70]
Guinea pigs with tibial fractures	Single intramuscular injection of vitamin D (50,000 IU/kg)	*Improved healing*: increased callus formation, vascularization, and mineralization	[71]
New Zealand White rabbits with femoral fractures	Single intramuscular injection of vitamin D (50,000 IU/kg)	*Improved healing*: increased biomechanical properties	[72]
OVX Sprague–Dawley rats with femoral fracture	Animals given drinking water containing 1% lactic-acid-hemicalcium salt for 2 months	*Improved healing*: improvements in radiological healing and smaller fracture calluses but lower biomechanical properties	[73]
129S6/SvEvTac mice with femoral osteotomy	Animals fed diets supplemented with 0.8% calcium gluconate for 10, 24, and 32 days	*Improved healing*: improvements in mechanical properties and increased callus bone mineral density	[43]
Wistar rats with fibula fracture	Animals fed diets containing 2.5% phosphorus for up to 12 weeks	*Improved healing*: faster cartilaginous-to-bony callus transition but no significant differences in tensile strength	[74]
Wistar rats with femoral fracture	Animals fed either normal (1) or phosphate deficient (2) diets then given phosphate supplements containing 125; 250; 500; or 1,000 mg/kg/day for 6 weeks	**1.** *Regular healing*: no effect of supplementation on healing in animals consuming normal diets **2.** *Improved healing*: improvement in mechanical properties of fractured bones when deficient animals were given phosphate supplements	[58]
Sprague–Dawley rats with femoral fracture	Animals fed mineral supplemented diets containing 1.5% calcium, 1.2% phosphorus, and 276.2 units/kg vitamin D for 5 weeks	*Regular healing*: no significant improvements in mechanical properties of healed bone	[49]

improved.[73] Conversely, consumption of calcium-enriched diets improved fracture healing in mice, indicated by increased callus calcium content, increased flexural rigidity, and the BMD of fractured femora.[43] Ultimately, there is a general lack of data supporting any beneficial effect of supplemental calcium on the progression of fracture healing, with the majority of available studies suggesting that calcium supplementation only marginally improves healing.

The consumption of excess phosphorus may have a positive effect during periods of rapid bone formation that occur during the reparative and remodeling phases of fracture repair. In animals, Wistar rats with fibula fracture given a high-phosphorus diet experienced more rapid healing that was apparent by the complete replacement of cartilage with woven bone at 6 weeks post-fracture.[74] In a separate study, phosphate supplements were found to have a positive effect on healing in Wistar rats that had been initially fed diets low in phosphorus, but not in rats with normal serum phosphate.[58] The evidence supporting the use of supplemental phosphate on bone repair in humans is mixed.

Table 4. Summary of Pre-Clinical Studies Examining the Effects of Macronutrient and Vitamin Supplementation on Fracture Healing

Model	Dosage and Duration	Key Effects	Ref.
Sabra rats with tibial osteotomy and laparotomy	Animals fed diets containing excess calories for 8 weeks	*Regular healing*: no significant improvements in mechanical properties of healed bone	29
Sprague–Dawley rats with femoral fracture	Animals fed diets containing 64% protein for 5 weeks	*Regular healing*: no significant improvements in mechanical properties of healed bone	49
Sprague–Dawley rats with femoral fracture	Animals fed diets containing 30% protein for 6 weeks	*Improved healing*: increased bone mineral density in callus but no improvements in mechanical properties	28
Albino rats with tibial fracture	Intravenous injection of L-glutamine/L-alanyl solution (2.0 ml/kg/day) for 7 days	*Improved healing*: faster development of cartilaginous callus at day 21	75
New Zealand White rabbits with fibula fracture	L-Glutamine/L-alanine solution (2.0 ml/kg/day) delivered via gastric catheter for 30 days	*Regular healing*: no significant differences in radiographic and histological scoring of healing	76
Guinea pigs with femoral fracture	Oral supplementation with L-arginine (100 mg/kg/day) for 2 and 4 weeks	*Improved healing*: accelerated healing and improved mechanical properties	77
Wistar rats with fractured tibias	Single intramuscular injection of vitamin C (0.5 mg/kg)	*Improved healing*: accelerated fracture repair	78
Aged Osteogenic Disorder Shionogi Rats with femoral fracture	Animals given water supplemented with vitamin C (2 mg/ml) for 5 weeks	*Improved healing*: increase in biomechanical properties	65
New Zealand White rabbits with tibial osteotomy	Daily intramuscular injections of α-tocopherol (20 mg/kg) for 30 days	*Improved healing*: increased bone formation	79
Mixed-breed dogs with tibia and fibula osteotomy	Oral supplementation with α-tocopherol acetate (100 mg/day) for 30 days	*Improved healing*: earlier bridging, mineralization, and remodeling of the fracture	80
OVX Sprague–Dawley rats with femoral fractures	Oral supplementation with α-tocopherol acetate (60 mg/day) for 14 days	*Improved healing*: improved healing	81
Sprague–Dawley rats with tibial fracture	Intraperitoneal injections of α-tocopherol (20 mg/kg/day) for 60 days	*Improved healing*: better histological and radiological indices of healing	82
New Zealand White rabbits with femoral fracture	Intramuscular injections of α-tocopherol (20 mg/kg/day) for 5 days after fracture	*Improved healing*: significantly higher histological grades of fracture healing	83
OVX Sprague–Dawley rats with femoral fractures	Animals given oral supplements of (1) α-tocopherol (60 mg/kg) or (2) tocotrienol-enriched fraction (60 mg/kg) for 6 days per week for 8 weeks	**1.** *Improved healing*: increased mechanical properties in rats given tocotrienol-enriched fraction **2.** *Regular healing*: No effect of α-tocopherol supplements on mechanical properties	84

Adult humans that consumed supplemental phosphorus (1 g/day) achieved clinical union of fractures of the femur and ankle faster than the control group.[87] Conversely, supplemental phosphate (60 mg/kg body weight) did not significantly decrease healing time in children with femoral fractures.[88]

Beyond the requirements for vitamin D, calcium, and phosphorus for mineralization of the regenerating bone, protein is also needed for cellular proliferation and collagen synthesis. Although providing excess protein to animals without pre-existing protein malnutrition did not translate to improved healing outcomes,[29,49] supplementation with specific amino acids, the building blocks of proteins, have shown some promise in promoting bone repair. Glutamine and arginine are conditionally indispensable amino acids that become essential during times of catabolic stress and are important in facilitating wound repair.[91] The preclinical studies examining the influence of glutamine on fracture healing are conflicting, with one showing

Table 5. Summary of Clinical Studies Examining Effects of Nutrient Supplementation on Fracture Healing

Patient Population	Dosage and Duration	Key Clinical Effects	Ref.
Post-menopausal women with proximal humerus fractures ($n = 30$); average age: 78 years	Oral supplementation with 800 IU vitamin D and 1 g calcium for 12 weeks	*Improved healing*: significantly increased bone content in fracture callus	[85]
Post-menopausal women with distal radius fractures ($n = 94$); average age: 74.9 y	Oral supplementation with 880 IU vitamin D and 1 g calcium for 6 weeks	*Improved healing*: significantly increased bone content in fracture callus	[86]
Adult men and women with fractures of the upper or lower extremity ($n = 167$); average age: 52.5 years	Oral supplementation with 1,200 IU vitamin D (cholecalciferol) per day for 4 months	*Regular healing:* no effect on delayed or non-union assessed radiologically	[52]
Adult men and women with femur, ankle, or wrist fractures ($n = 51$); average age: 46.9 years	Oral phosphate supplements (1 g/day) for approximately 3 months	*Improved healing*: Clinical union was significantly reduced in patients with femur (–3.8 weeks) and ankle (–6 weeks) fractures, but not wrist fractures	[87]
Male and female children with femur fractures ($n = 42$); average age: 7.4 years	Oral phosphate supplements (60 mg/kg/day) until fractured considered radiologically healed	*Regular healing*: no effect on fracture healing	[88]
Adult men and women with traumatic long-bone fracture ($n = 60$); average age: 30.6 years	Daily oral supplements of zinc (50 mg) for 60 days	*Improved healing*: increase in callus formation and accelerated healing	[39]

more rapid development of callus in rats [75] and the other showing no effect on healing in New Zealand White rabbits with fibula fractures.[76] On the other hand, arginine supplements seem to consistently improve fracture healing, as indicated by better vascularization, callus formation, mineralization, and improved mechanical properties in New Zealand White rabbits[92] and guinea pigs.[77]

Vitamin C and E are both antioxidants that have beneficial effects on bone homeostasis and fracture healing in pre-clinical studies. Vitamin C supplementation accelerated fracture repair that included prominent chondrocyte hypertrophy at day 10 and a well-developed fibrocartilaginous callus with osteoid formation on day 15,[78] and faster transition of the cartilaginous callus into the bony callus.[93] Unlike vitamin C, vitamin E supplements in the form of α-tocopherol appear to be most beneficial during the bone remodeling phase of secondary bone healing.[94] Although α-tocopherol is the major vitamin E isoform utilized by the body, ingestion of tocotrienol-enriched supplements resulted in superior biomechanical properties of healed bones compared with rats given an α-tocopherol supplement, suggesting that there may be isoform-specific effects of vitamin E on bone healing.[84]

Though our understanding of the influence of nutritional supplements on fracture healing has advanced significantly over the years, there are several gaps in our knowledge that should be addressed in future studies. Notably, there is little-to-no information on the best time and optimal duration to provide nutritional supplements to fracture patients. There is also little consensus on how much of each respective nutrient to provide. Pre-clinical studies have demonstrated that nutritional supplements can positively influence bone healing, however, whether this translates to humans remains to be established. Perhaps, future clinical studies should address this critical gap in our knowledge, and further determine whether there is a distinct patient population that would benefit most from nutritional intervention (e.g., malnourished, elderly). Finally, while the majority of the existing studies demonstrate a phenotypic effect of nutritional supplements on fracture healing, few have identified the molecular and cellular mechanisms through which nutrition influences the repair process. Research in these directions will ultimately provide the necessary information to facilitate the creation of clinically relevant nutrition-based treatment strategies for improved fracture healing.

NUTRITIONAL INFLUENCES ON PATIENT OUTCOMES

Modification of nutritional status during the early rehabilitation period is recognized as a beneficial approach to prevent functional declines and reduce complications after fracture.[95,96] Beyond the impact of nutrition on bone healing, leveraging nutritional strategies following fracture may be a unique strategy to improve patient outcomes and quality of life. Dietary protein supplementation has been the most widely studied in this regard and appears to have clinical utility with respect to recovery from fracture in humans.[28,97–100] Daily protein supplements (20 g) result in fewer systemic complications,[28,97] shorter hospital stays,[28,98–100] and lower mortality.[28] Similar to protein,

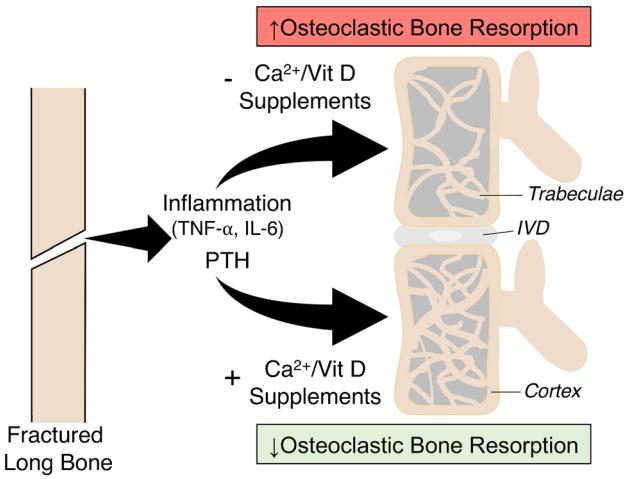

Figure 4. Mechanism by which calcium and vitamin D supplements protect against post-traumatic bone loss. After fracture, there is an increase in serum parathyroid hormone (PTH) and systemic inflammation that can liberate calcium from the intact skeleton through osteoclastic bone resorption. Consumption of calcium and vitamin D supplements prevent this bone loss from occurring. [Color figure can be viewed at wileyonlinelibrary.com]

pre-clinical studies suggest that calcium and vitamin D supplements may also impart some benefit that extends beyond healing of the fractured bone (Fig. 4). When initiated after fracture, supplemental calcium and vitamin D protected against post-traumatic bone loss in the intact skeleton.[55] Preventing bone loss in the intact skeleton may have immense clinical utility especially in geriatric patients, considering that post-traumatic bone loss is exacerbated with age.[42] Although these simple interventions can have profound impacts on post-fracture pathophysiology and recovery, poor compliance has been identified as an important factor that may limit the effectiveness of nutritional supplements.[101] Future studies should address the barriers along with strategies to overcome them to inform the best approach to deliver nutritional supplements after fracture.

EMERGING AREAS AND FUTURE PERSPECTIVES

Nontraditional Dietary Supplements

Dietary bioactive compounds (e.g., phytochemicals) are non-nutritive chemicals that are ubiquitously present in plant foods and are commonly consumed as dietary supplements. Though many of these compounds have bone-protective effects, little is known regarding their influence on bone healing.[102] Curcumin and formononetin are both bioactive compounds that have received much attention in regard to bone health, and more recently bone healing. Oral supplementation with curcumin increased the number of osteoblasts within the fracture callus through induction of autophagy[103]; whereas, formononetin increased callus vascularity and expression of VEGF and VEGF receptors within the femoral fracture callus, likely through estrogen receptor signaling.[104] Although bioactive compounds in isolation can have wide-reaching health benefits through antioxidant and anti-inflammatory actions, they are known to act additively and synergistically to elicit a more pronounced biological effect when consumed together.[105,106] A recent study demonstrated that grape seed phenolic extract that contains many phytochemicals positively influenced bone healing by increasing the mechanical strength of the healing femur.[107] Though these compounds show some promise in pre-clinical animal studies, several important questions remain (Fig. 5). The most pertinent question being whether the dose needed to impart the beneficial effect on healing can be achieved through food, or if supraphysiological levels are required that can only be achieved through dietary supplementation with isolated compounds or extracts.

Beyond metabolic changes, traumatic injuries can disrupt gut function and promote changes in the intestinal microbiome composition.[108,109] The use of probiotic supplements to influence the intestinal microbial composition after trauma is increasingly gaining attention. These dietary supplements are live microorganisms that benefit health and are commonly consumed by the elderly, with an estimated 3% of Americans over the age of 60 taking probiotics.[110] There is an increasing number of studies that suggest a protective role or probiotics on bone health.[111–116] However, to date, only one study has reported on the effects of probiotics on fracture healing, which showed that elderly patients with radius fractures who consumed the probiotic *Lactobacillus casei* Shirota displayed significant improvements in pain and functional outcomes.[117] Though healing was not assessed radiographically, this study sets an intriguing precedent that, when consumed after fracture, probiotics may be a viable strategy to accelerate the healing process thereby leading to improved quality of life. Additional studies are certainly warranted to not only assess whether probiotic supplements can accelerate or enhance bone repair but to also determine the mechanisms by which probiotic manipulation of the gut microbiome can influence the bone repair sequelae (Fig. 5).

Precision Nutrition Through Integrated Omics

The advent and recent refinement of omics-based technologies also provide a unique strategy to comprehensively define the role of nutrition in bone healing. Nutrigenomics is a promising area that marries the genomic make-up of an individual with nutritional requirements. Determining whether a patient's genotype influences metabolic requirements after fracture and whether they would respond to supplementation would be useful in designing personalized nutritional approaches to facilitate natural healing. Additionally, we are uniquely poised to address fundamental questions regarding metabolic flux during each stage of bone healing with the advancements in metabolomics to determine systemwide changes in metabolism and circulating metabolites after injury.[118] Metabolomics is

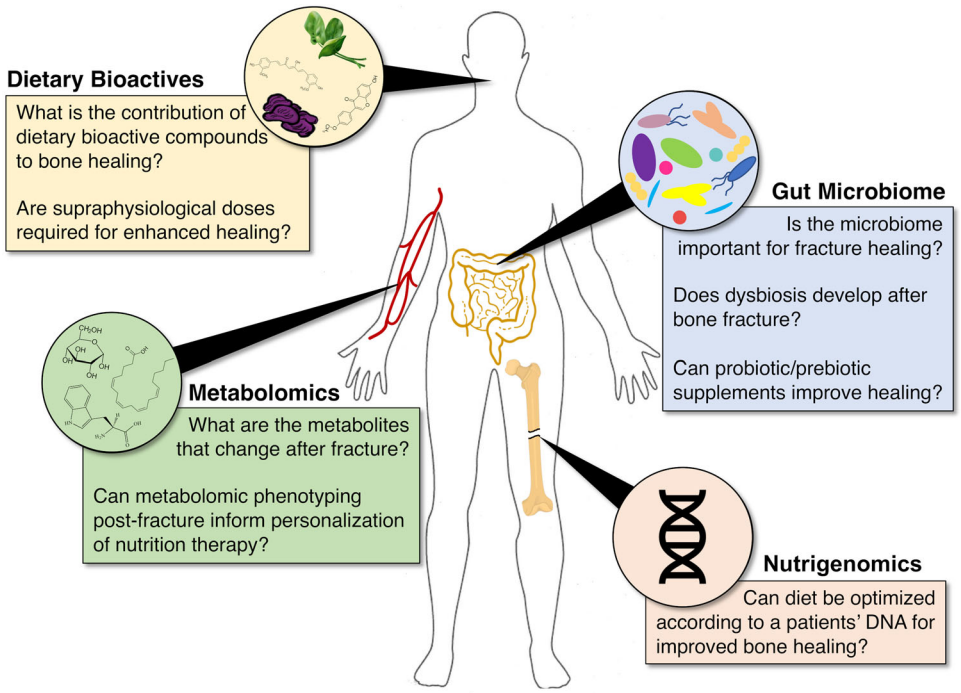

Figure 5. Emerging areas and important questions that remain. Dietary bioactive compounds, metabolomics, the microbiome, and nutrigenomics are all exciting areas that hold much promise in establishing the role of nutrition in bone healing. [Color figure can be viewed at wileyonlinelibrary.com]

the high-throughput study of metabolites (e.g., amino acids) that can simultaneously assess the substrate and product pool of metabolic pathways, providing unique insight into global shifts in metabolism in response to pathophysiological insults. A handful of studies have utilized this tool to assess metabolism after injury; however, it has yet to be applied in the context of fracture. Integrating metabolomics with nutrigenomics may prove to be a powerful tool to assess a patients' metabolic state and nutritional needs to inform the best strategy to proactively manage nutritional status for the best possible healing outcome (Fig. 5).

CONCLUSION

To date, the majority of nutrition research has focused on mechanisms to improve bone health with the goal of preventing fracture occurrence. Although prevention strategies are worthwhile and should be delineated, skeletal fracture will remain an inevitable reality. This is especially true considering the overall aging of the global population, highlighting the need to identify strategies that can enhance and accelerate the healing process. Our understanding of the molecular and cellular drivers of skeletal fracture repair has advanced significantly over the last few decades, but our knowledge of how healing can be hindered by malnutrition or enhanced with nutritional supplementation remains largely unexplored, especially in humans. Though scant, the current evidence underlines the importance of appropriate nutrition during the period of convalescence as well as the potential utility of using dietary factors to accelerate the fracture repair sequelae. However, this remains at the clinicians' discretion given the lack of high-quality clinical studies to devise a consensus nutritional intervention for fracture patients at this time. Additional well-designed pre-clinical and clinical studies are ultimately needed to fill the many gaps in our knowledge regarding the significance and usefulness of nutrition as an adjuvant therapy in orthopedics.

AUTHORS' CONTRIBUTION

J.L.R. drafted the original manuscript and contributed to revisions. H.D. critically reviewed the manuscript and contributed to revisions. Both authors have read and approved the final submitted manuscript.

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