Nutrition and wound healing

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Wound healing and its corresponding intimate relationship to overall nutrition has long been recognized by physicians. The importance of wound healing extends far beyond the scope of medicine, affecting numerous facets of individual and societal life. In the modern era, wound infections and delayed wound healing significantly contribute to the financial burden imposed on health care systems worldwide.

The crucial role of nutrition in cutaneous healing has been recognized since the beginning of medicine as a discipline. Some of the earliest known writings identifying this synergy date to some 2300 years ago, when Hippocrates warned of underestimating the vital role that nutrition played in health and human disease [1]. In the late 1800s, Coleman, Shaffer, and DuBois investigated the metabolic changes occurring in disease [2]. Later, Cuthbertson [3] further defined the biochemical responses to injury by studying patients and animals with long bone fractures. Responses including alterations in physiologic electrolyte levels, increased nitrogen turnover, and stimulation of the overall host metabolism were reported.

In the 1930s, Ravdin, working with other researchers at the University of Pennsylvania, showed the specific relationship between protein malnutrition and the incidence of laparotomy wound dehiscence in dogs [4–6]. Poor nutritional intake or lack of certain essential nutrients significantly alter the body’s ability to heal wounds. As interest has swung from understanding the basic physiologic mechanisms of wound healing to attempting to effect some change on the process of wound healing, investigators have explored the ability to modulate the many aspects of wound healing pharmacologically. The dynamic and complicated process of wound healing has proved to be sensitive to external manipulation of metabolic and nutritional factors. The expansion of the ability to deliver select metabolic and nutritional elements
has affected greatly the morbidity and mortality of patients sustaining serious injury or wounds.

Nutritional factors in wound repair

Malnutrition

After injury, many metabolic changes occur that collectively impair wound healing and host defenses. In fact, malnutrition often is deemed to represent only poor or inadequate nutritional intake. Malnutrition encompasses a host of factors from poor nutritional intake to overall metabolic equilibrium. Studies over the past century have shown changes in energy, carbohydrate, protein, fat, vitamin, and mineral metabolism after wounding or injury, each affecting the healing process [7]. Loss of protein from protein-calorie malnutrition leads to decreased wound tensile strength, decreased T-cell function, decreased phagocytic activity, and decreased complement and antibody levels, ultimately diminishing the body’s ability to defend the wound against infection. These immune-related compromises of malnutrition correlate clinically with increased wound complication rates and increased wound failure in lower extremity amputations and bypass procedures [8–10]. Malnutrition may preexist wounding or may be encountered secondary to the catabolic imbalance of the patient’s overall metabolic state during wound healing. Wounding leads to an increased metabolic rate, increased catecholamine levels, loss of total body water, and increased collagen and other cellular turnover [11]. The host’s catabolic response to injury has been shown to be proportional to the severity of the injury [12,13]. The body seems to prioritize healing objectives by metabolic activity. Levenson and others [14–17] showed significantly slower cutaneous wound healing in burned and traumatized animals. Conversely, liver regeneration increased in similarly burned animals. After thermal injury, these disparities in overall anabolic or catabolic state between various organs (ie, liver and skin) suggest that vital organs are preserved at the expense of other organ systems such as the skin. These differences in healing between various organ systems after injury are not well understood, but it is certain that wound healing is impaired as a result of these metabolic changes.

In a society where malnutrition is thought to have been vanquished, a significant proportion of patients do have preexisting malnutrition from decreased nutritional intake. A study of orthopedic patients, including posttrauma patients and patients undergoing total hip replacement, found that 42% of patients were malnourished [18]. A study conducted by Warnold and Lundholm [19] in 1984 evaluated 215 noncancer patients preoperatively and found that 12% showed evidence of malnutrition. Approximately 50% of all medical and surgical patients at an urban hospital in 1974 showed evidence of malnutrition [20]. Although the exact parameters used to define clinical malnutrition may vary, the potential
presence of preexisting malnutrition should not be overlooked when assessing a wound or evaluating an unwounded patient about to undergo surgery. Identification of the potential risk imposed by malnutrition is especially important in populations with underlying factors that may impede wound healing further.

An understanding of normal metabolism is helpful when planning an appropriate intervention in patients with malnutrition. One of the most basic elements required for healing is energy, which in the human host is derived from carbohydrates, protein, and fat. Dietary carbohydrates and protein provide approximately 4 kcal/g, and fats provide 9 kcal/g [21]. Reducing caloric intake by 50% in rats decreases collagen synthesis, matrix protein deposition, and granulation tissue formation [22,23]. Although in animal models, severe or prolonged protein-calorie malnutrition is necessary to impair the healing responses, in humans, modest protein-calorie malnutrition impairs fibroplasia [24]. Brief preoperative illness or decreased nutritional intake in the prewounding period has been shown to have a significant impact on collagen synthesis. This finding lends support to the concept that preoperative food intake may be more important to the wound healing process than the patient’s overall nutritional status [25]. Conversely, brief nutritional intervention by enteral or parenteral routes has been shown to overcome or prevent these impairments in the wound healing process [26,27].

Although the current literature is laden with studies attempting to delineate the exact role nutrition and supplementation play in the wound healing process, most wounds heal uneventfully. It routinely is observed in the clinical setting that wounds heal despite significant malnutrition. Patients undergoing oncologic operations often present with preoperative weight loss and malnutrition, but these patients generally heal without infection or wound dehiscence. These discrepancies between what has been shown in animal models at the basic science level and what is observed clinically can be reconciled in understanding that the body seems to give a place of preeminence to the healing wound. Albina [28] concluded that the biologic priority of the healing wound accounts for the finding that most wounds heal, even in the face of moderate preoperative and postoperative malnutrition. While establishing this biologic priority of the wound to heal, Albina [28] also noted that severe protein-calorie malnutrition and symptomatic specific nutrient deficiencies can impair wound healing to the extent that they delay the healing process. These findings should not lead clinicians to ignore the need for optimal nutrition. The goal should be to provide every patient with optimal nutrition so that this prioritization of wound healing can occur within an ideal host environment.

**Carbohydrates**

Carbohydrates, together with fats, are the primary sources of energy in the body and consequently in the wound healing process. The energy
requirements for wound healing consist mainly of the energy required to carry out collagen synthesis in the wound. Estimates of caloric requirements for a particular wound can be made knowing that protein synthesis requires 0.9 kcal/g and that a 3 cm² × 1 mm thick section of granulation tissue contains 10 mg of collagen. As such, simple wounds have little energy impact on overall metabolism, but large complicated wounds or thermal injuries can divert a disproportionate amount of energy to the healing wound [29].

Glucose is the major source of fuel used to generate cellular energy in the form of adenosine triphosphate (ATP), which in turn powers the wound healing process. The use of glucose to generate ATP is thought to be relatively inefficient, but the caloric contribution of glucose is essential in preventing the depletion of other amino acid and protein substrates. The liver, triggered by the catecholamine and cortisol surge of wounding, initiates gluconeogenesis using amino acids from degraded muscle protein. Unchecked and in the presence of inadequate carbohydrate and fat stores, this use and depletion of amino acids and protein can lead to the protein-calorie malnutrition previously described. Carbohydrates play an important role in providing the energy essential for optimal healing, but little is known about the function that different sources of carbohydrate play in this process. Gluconeogenesis is an inefficient pathway for glucose production and can result in the production of excess amounts of glucose, which may complicate wound healing, especially in diabetic patients with poor glycemic control.

Diabetic patients often experience significant impairment in the ability to heal wounds and have increased complication rates. The mechanisms at work are multifactorial and poorly understood. The microvascular and atherosclerotic changes induced in diabetics are well known to affect healing. Likewise, diabetes seems to exert an effect on the early inflammatory response and directly inhibits fibroblast and endothelial cell activity. Goodson and Hunt [30] used laboratory animals with streptozotocin-induced diabetes to show a decreased inflammatory response after wounding and diminished fibroblast and endothelial cell proliferation. Delayed epithelialization of open wounds and decreased collagen accumulation deep within the wound have been reported using this diabetic model. Barr and Joyce [31] noted decreased reendothelialization of microarterial anastomoses in streptozotocin diabetic rats and reported that this delay was not alleviated by the administration of insulin beginning at the time of surgery and extending into the postoperative period. The relationship of insulin and hyperglycemia to wound healing was clarified on the basis of experiments by Weringer and associates [32–34]. Groups of mice with dermal ear wounds were treated with antiserum to insulin (euglycemic), with 2-deoxyglucose (hyperglycemic), or with food deprivation (hypoglycemic). It was concluded that in addition to hyperglycemia, the lack of insulin itself seems to impair wound healing. Topical application of insulin to infected
skin wounds of diabetic mice or systemic administration can improve healing regardless of the route administered [35]. To achieve normal healing, however, the insulin must be given early after wounding [36]. Hyperglycemia also interferes with cellular transport of ascorbic acid into fibroblasts and leukocytes and causes decreased leukocyte chemotaxis [37].

Mann [38] suggested that a mechanism for this interruption in ascorbic acid transport might be related to the structural similarity between glucose and ascorbic acid, leading to competitive inhibition of ascorbic acid membrane transport. These effects of hyperglycemia in diabetics, specifically as they relate to leukocytes, are thought to help explain the decreased early inflammatory response and impairment of wound healing seen in diabetic patients. It also has been shown that large doses of ascorbic acid administered to streptozotocin-induced diabetic rats can reverse these effects and increase collagen production in the skin. This increase in collagen production after high-dose ascorbic acid administration in diabetic rats is accomplished through reversal of underhydroxylation and degradation and improving intracellular ribosomal collagen production [39].

The importance of controlling serum glucose levels in diabetics around the time of injury, operation, and wound healing cannot be overemphasized. The alterations in a diabetic’s metabolism after injury and after elective surgery can affect wound healing significantly by many of the mechanisms discussed earlier. In addition, diabetic patients are more susceptible to infection because of decreased host resistance. For this reason, it is crucial that physicians recognize and anticipate the needs of diabetic patients early on, before the encumbering effects of diabetes lay hold on the wound healing process.

Fats

In contrast to carbohydrates, the role of fats has not been studied widely. The earliest identification of the importance of fats was a study conducted on animals that were fed a fat-free diet, which became the first clinical description of fat or dietary lipid deficiency [40,41]. Several unsaturated fatty acids must be supplied in the diet. Linoleic acid and arachidonic acid (a product of linoleic acid) are examples of such essential fatty acids. Linolenic acid and arachidonic acid can be synthesized in humans from linoleic acid, but the rates of synthesis are inadequate for basic metabolic needs. As components or precursors of phospholipids and prostaglandins, deficiencies of these lipids cause impairment in wound healing in animals and humans [42–45]. This impairment is due to the role phospholipids play as constituents of the cellular basement membrane and the participation of prostaglandins in cellular metabolism and inflammation.

Demands for essential fatty acids increase after injury [46,47]. Deficiencies of dietary essential fatty acids were not seen frequently clinically until the introduction of prolonged parenteral feedings that did not contain fat.
Biochemical changes of essential fatty acid deficiency can manifest within 10 days of eating an entirely fat-free diet [48]. Total parenteral nutrition (TPN) is the most common cause of essential fatty acid deficiency. The administration of TPN results in a rapid onset of essential fatty acid deficiency secondary to the continuous infusion of high concentrations of glucose, which leads to elevated insulin levels, blocking lipolysis and essential fatty acid release [49].

There also has been research to define further possible benefits of specific lipid types. The ω-3 fatty acids have anti-inflammatory properties by inhibiting eicosanoid production [50–53] and other mediators, such as platelet-activating factor, interleukin-1, and tumor necrosis factor-α [54,55]. Animals consuming diets enriched with ω-3 fatty acids had weaker wounds than controls 30 days after injury. The weaker wounds did not contain less collagen; rather it is thought that the ω-3 supplementation impaired the quality, cross-linking, or spatial orientation of collagen fibrils.

**Protein**

The importance of protein in wound healing has been recognized and researched since the early 1930s. Under experimental conditions, severe protein deprivation leads to impaired healing. Clinically severe protein malnutrition, known as kwashiorkor, is recognized easily. Experimentally, rodents fed either 0% or 4% protein diets showed impaired collagen deposition, decreased skin and fascial wound breaking strength, and increased wound infection rates [56]. Acute protein deprivation in rats has been shown to impair collagen synthesis markedly with a concomitant decrease in procollagen mRNA [57].

Pure protein deficiencies are seen rarely in the clinical setting. Most patients exhibit combined protein-energy malnutrition or protein-calorie malnutrition. Protein synthesis at the wound site must be increased for collagen deposition and healing to occur. Patients with protein-calorie malnutrition have diminished hydroxyproline accumulation (an index of collagen deposition) in subcutaneously implanted polytetrafluoroethylene catheters compared with normally nourished controls [58]. The administration of individual sulfur-containing amino acids has been shown to abrogate the impaired healing in protein-deficient rats, as evidenced by increased fibroblastic proliferation and collagen accumulation. It is not possible, however, to translate these findings obtained in the context of pure protein deficiency into the setting of protein-calorie malnutrition, which is more clinically prevalent [59,60].

**Amino acids**

Although wound healing can be impaired by deficiencies in a variety of nutrients, there has been a rising interest over the last several decades in the use of individual nutrients to promote wound healing [29]. Often these
nutrients are administered in pharmacologic doses that are above the normal daily requirements. In this manner, the role of several single amino acids has been investigated. Two separate studies in the late 1940s and early 1950s showed partial resolution of healing defects in protein-deficient rats with the administration of single sulfur-containing amino acids, such as methionine and cysteine, although the clinical relevance of these findings has never been pursued [59,60].

**Branched-chain amino acids**

The branched-chain amino acids valine, leucine, and isoleucine have been used to treat liver disease and have an additional role in retaining nitrogen in sepsis, trauma, and burns [61–63]. Branched-chain amino acids support protein synthesis after injury and decrease muscle proteolysis. Serving as caloric substrates, branched-chain amino acids can be metabolized as an energy source independent of liver function [64–67]. Despite these useful properties, high supplements of branched-chain amino acids have not proved to be of any significant benefit in improving wound healing [68,69].

**Glutamine**

Glutamine is the most abundant amino acid in the body, and it accounts for approximately 20% of the total circulating free amino acid pool and 60% of the free intracellular amino acid pool [70,71]. The process of gluconeogenesis involves the shuttling of alanine and glutamine to the liver for conversion to glucose, which is used peripherally as fuel to power certain aspects of wound healing. Glutamine also is an important precursor for the synthesis of nucleotides in cells, including fibroblasts and macrophages [72,73]. Glutamine is an energy source for lymphocytes and is essential for lymphocyte proliferation [74,75]. Finally, glutamine has a crucial role in stimulating the inflammatory immune response occurring early in wound healing [76].

Given the abundant roles of glutamine in the numerous cells involved in wound healing, it is not surprising that after injury there is a rapid fall in plasma and muscle glutamine levels [77,78], which is greater than that of any other amino acid. Although efficacy of supplemental glutamine administration has been shown in some clinical situations [79], it has not proved to have any noticeable effect on wound healing [80].

**Arginine**

In the late 1940s and early 1950s, Rose [81] classified arginine as being one of the two semiessential amino acids in mammalian metabolism. Arginine is a dibasic amino acid that is synthesized endogenously from ornithine through citrulline. It is a normal constituent of numerous body proteins and is associated with a variety of essential reactions of intermediary metabolism. Arginine is absorbed from the intestine by a transport system shared with lysine, ornithine, and cysteine in an energy-dependent
and sodium-dependent fashion with substrate specificity. Arginine also shares a common uptake and transport system into fibroblasts and leukocytes with these amino acids [82].

Arginine is synthesized in adequate quantities to sustain muscle and connective tissue mass but in insufficient quantities for optimal protein biosynthesis and healing. In situations of stress or injury, in which synthesis of arginine is insufficient to meet the demands of increased protein turnover, arginine becomes an indispensable amino acid in the process of wound healing and maintenance of a positive nitrogen balance [83,84].

The role of arginine in wound healing first was shown in the 1970s, when it was hypothesized that during injury, the amino acid requirements of the adult organism would revert to those of the growing infant. Based on this hypothesis, the effect of arginine deficiency on wound healing in young adult rats was studied. Animals were fed an arginine-deficient diet for 4 to 6 weeks before wounding. When the animals were subjected to the minor trauma of a dorsal skin incision and closure, they evidenced increased postoperative weight loss, increased mortality to approximately 50%, and a notable decrease in wound breaking strength and wound collagen accumulation compared with animals fed a similarly defined diet containing arginine (Fig. 1) [84]. Subsequent experiments revealed that chow-fed rats that were not arginine deficient and were fed a diet containing an additional 1% arginine had enhanced wound healing responses as assessed by wound breaking strength and collagen synthesis compared with chow-fed controls (see Fig. 1) [84]. Similar findings were observed in parenterally fed rats given an amino acid mixture containing high doses (7.5 g/L) of arginine. These animals exhibited increased wound breaking strength, increased collagen accumulation, and enhanced immune function [85]. Likewise, mature or old rats fed diets supplemented with a combination of arginine and glycine have enhanced rates of wound collagen deposition compared with controls [86].

Several years ago, a micromodel was described that has made possible the study of the human fibroblastic response. In this model, collagen accumulation occurs in a subcutaneously placed 5-cm segment of polytetrafluoroethylene (PTFE) tubing [87]. Two studies were carried out in healthy human volunteers examining the effects of arginine supplementation using this model. In the first study, 36 young, healthy human volunteers (ages 25 to 35) were randomized into one of three groups: (1) 30 g arginine hydrochloride daily supplements (24.8 g free arginine), (2) 30 g arginine aspartate (17 g free arginine), or (3) placebo. The supplements were given for 2 weeks, after which the PTFE catheters were removed and hydroxyproline content (index of reparative collagen synthesis) was evaluated. Arginine supplementation at both doses significantly increased the amount of hydroxyproline and total protein deposition at the wound site (Fig. 2) [88]. The second study evaluated 30 elderly volunteers (age >70) who received 30 g of arginine aspartate (17 g free arginine) or placebo. This study also evaluated the fibroblastic wound response using PTFE catheters and
Fig. 1. Effect of supplemental dietary arginine added to an arginine-free (defined) diet or normal laboratory chow (1.8% arginine content) on wound healing in rats. Statistical comparison by Student t-test. FBS, fresh breaking strength of scar, g; FxBS, formalin-fixed breaking strength, g; OHP, hydroxyproline content of subcutaneously implanted polyvinyl alcohol sponges, µg/100 mg sponge dry weight.
examined epithelialization by creating a split-thickness wound on the upper thigh of each subject. The catheters in this study were analyzed for $\alpha$-amino nitrogen content (assessment of total protein accumulation), DNA accumulation (index of cellular infiltration), and hydroxyproline content [90]. There was no enhanced DNA present in the wounds of the arginine-supplemented group, suggesting that the effect of arginine is not mediated by an inflammatory mode of action (Fig. 3). Arginine supplementation had no effect on the rate of epithelialization of the skin defect, indicating that the predominant effect of arginine is on wound collagen deposition [89].

Several possible mechanisms have been postulated to explain the positive effect of arginine on wound healing. First, although arginine comprises a small amount of the collagen molecule (<5%), it is possible that

Fig. 2. Effect of 2 weeks of arginine supplementation on hydroxyproline (OHP) accumulation in subcutaneously implanted polytetrafluoroethylene catheters in young human volunteers (mean ± SEM). Groups of 12 volunteers each received a placebo (control), 30 g arginine aspartate (17 g free arginine)/d or 30 g arginine hydrochloride (24.8 g free arginine)/d for 2 weeks.

Fig. 3. Effect of arginine on wound healing parameters in healthy elderly human volunteers. Accumulation of hydroxyproline (OHP), total $\alpha$-amino N, and DNA in subcutaneously implanted polytetrafluoroethylene catheters was measured at the end of 2 weeks (mean ± SEM). Controls (n = 15) received a placebo syrup; the arginine group (n = 30) received 30 g of arginine aspartate.
supplemental arginine is providing a necessary substrate for collagen synthesis at the wound site. This could be through the direct use of arginine as substrate through the pathway arginine→ornithine→glutamic semialdehyde→proline. Arginine levels are essentially nondetectable within the wound during the later phases of wound healing when fibroplasia predominates [90]. Although ornithine levels are higher in the wound than in the plasma, further studies by Albina [91] revealed that the rate of conversion of ornithine to proline is quite low, making this mechanism of arginine use unlikely.

Second, it has been observed that the beneficial effects of supplemental arginine on wound healing are similar to the effects of growth hormone, specifically, enhanced wound breaking strength and collagen deposition [92–94]. In a study exploring this observation, hypophysectomized and normal pituitary-bearing animals were divided into two groups—one receiving growth hormone and one receiving placebo treatment—with half of the animals within each group receiving 1% dietary arginine supplementation. After wounding, the intact, arginine-supplemented animals showed increased wound breaking strength and collagen accumulation, whether growth hormone was given or not. In the hypophysectomized animals, arginine had no effect on these wound healing parameters, however, regardless of the administration of growth hormone, suggesting that the effects of arginine on wound healing in rats depend on the presence of an intact hypothalamopituitary axis [95]. In humans, arginine supplementation in doses that are able to increase wound healing also increase plasma insulin-like growth factor, the peripheral mediator of growth hormone.

Third, supplemental arginine has a unique effect on T-cell function. Arginine stimulates T-cell responses and reduces the inhibitory effect of injury and wounding on T-cell function [85,96–98]. T lymphocytes are known to be essential for normal wound healing, as evidenced by decreased wound breaking strength in animals treated with monoclonal antibodies against all T lymphocytes. T lymphocytes are found immunohistochemically throughout the various phases of wound healing in distinctive patterns. Studies have shown that each specific cell type has a modulating role on the phases of cutaneous healing. T lymphocytes interact within the dynamics of each phase of healing to accomplish a specific task, which, when considered collectively, leads to normal repair of the wound [99]. The exact mechanisms are not fully understood, but it is thought that one manner in which arginine may enhance wound healing is by stimulating these host and wound T-cell responses, which in turn would increase fibroplasia [100–102].

Finally, arginine has been identified as the unique substrate for the generation of the highly reactive radical nitric oxide (NO). Several studies suggest that NO plays a crucial role in wound healing. Inhibitors of NO have been shown to impair significantly healing of cutaneous incisional wounds and colonic anastomosis in rodents [103,104]. In vitro studies have
noted increased collagen synthesis in association with exogenous NO administration in cultured dermal fibroblasts [105]. Arginine is catabolized in wounds through two separate pathways: (1) nitric oxide synthases (NOS) and (2) arginase. The liberation of NO from arginine is catabolyzed through the NOS isoenzymes with ultimate production of citrulline. Specifically, it is the inducible isoform iNOS that is activated consistently in response to inflammatory stimuli (eg, wounding). Supranormal collagen deposition has been observed after transfection of iNOS DNA into wounds [106]. Conversely, mice lacking the iNOS gene (iNOS knockout mice) have delayed closure of excisional wounds, an impairment that is remedied by adenoviral transfer of the iNOS gene to the wound bed [107]. The functional loss of the iNOS gene abrogates the beneficial effect of arginine in wound healing, whereas wild-type mice fed arginine-supplemented diets experienced improved incisional wound healing as assessed by breaking strength and collagen deposition. This finding suggests that the iNOS pathway is at least partially responsible for the enhancement of wound healing observed with the administration of arginine [108].

**Vitamins**

The vitamins most closely associated with wound healing are vitamin C (ascorbic acid) and vitamin A. Vitamin C deficiency is well known because of its historical significance in relation to scurvy (scorbutus). The earliest accounts of this deficiency were in sailors while at sea and field armies who consumed a diet lacking fresh fruits and vegetables and subsequently developed scurvy. In the late 1800s, Osler [109] categorized and eloquently described the manifestations of this condition, noting that it had virtually disappeared as a clinical entity, owing in large part to the work of Lind. Scurvy has as its central element a failure in collagen synthesis and cross-linking [110]. The symptoms of scurvy reflect this impaired synthesis of collagen and connective tissue and include bleeding into the gingiva, skin, joints, peritoneum, pericardium, and adrenal glands. More generalized symptoms include weakness, fatigue, and depression. During the time that Osler was describing the symptoms of scurvy, the underlying defect in collagen was not understood. Crandon and colleagues [111] first revealed the significance of this “intracellular substance” (collagen) and the temporal aspects of vitamin C deficiency. In 1940, while working as a surgical resident, Crandon consumed a diet lacking vitamin C. After 3 months on this diet, a skin incision healed normally and a biopsy sample at 10 days was normal. At 6 months, a second incision healed poorly, and a 10-day biopsy sample at that time revealed a lack of “intracellular substance.” After resuming a diet supplemented with 1 g of ascorbic acid per day, healing improved, and a final biopsy sample showed increased collagen and capillary formation. These early histologic descriptions are consistent with the findings known to be associated with vitamin C deficiency today: minimal
collagen, decreased angiogenesis, and significant hemorrhage. Electron microscopy of fibroblasts from scorbutic patients reveals a dilated and disordered rough endoplasmic reticulum with diminished polysome content [112,113]. Ascorbic acid is believed to be a specific cosubstrate for the enzymes 4-hydroxylase and lysyl hydroxylase. From a biochemical standpoint, it is a reducing agent and is required for the conversion of proline and lysine to hydroxyproline and hydroxylysine [114].

Although the recommended dietary allowance for vitamin C is 60 mg/d, the clinical spectrum of its administration varies widely. In burn victims, the requirement may be 1 to 2 g/d. In human studies, 2 g was required to restore urine and tissue levels to normal after major burn injuries [115]. In animal models, the wounds of burned guinea pigs bore histologic resemblance to those of scorbutic unburned animals. When supplemental vitamin C was given, these changes were prevented. Although the dose needed in different settings may vary, there is no evidence to suggest that massive doses of ascorbic acid are of any substantial benefit to wound healing. There also is no evidence that excess vitamin C is toxic [116].

Vitamin C deficiency, in addition to impairing wound healing, has been associated with an increased susceptibility to wound infection. If wound infection does occur in the setting of vitamin C deficiency, it is apt to be more severe. These effects are thought to be attributable to the impairment of collagen synthesis interfering with walling-off of bacteria and localizing infection, impairment of neutrophil function, and impairment of complement activity [29].

McCollum and Davis initially discovered vitamin A in the early 1900s. Subsequent studies in 1941 by Brandaleone and Papper [117] showed the impairment imposed on the wound healing process by vitamin A deficiency. Ehrlich and Hunt [118] described the benefits of supplemental vitamin A on wound healing in nondeficient humans and animals in the 1960s and 1970s. They showed that vitamin A reverses the anti-inflammatory effects of corticosteroids on wound healing. The administration of vitamin A, topically or systemically, also can correct the impaired wound healing of patients on long-term steroid therapy [119,120]. Vitamin A also has been used to restore wound healing impaired by diabetes, tumor formation, cyclophosphamide, or radiation [121–124].

As alluded to earlier, vitamin A increases the inflammatory response in wounds. This increased response is thought to occur by an enhanced lysosomal membrane lability, increased macrophage influx and activation, and stimulation of collagen synthesis. In vitro studies have shown increased presence of epidermal growth factor receptors and increased collagen synthesis of fibroblast cell cultures in the presence of vitamin A [125,126]. These mechanisms still are not well understood, but it is clear vitamin A plays an important role in wound healing.

Serious injury or stress leads to increased vitamin A requirements. Large doses of corticosteroids also deplete hepatic stores of vitamin A. Decreased
serum levels of vitamin A, retinol binding protein, retinyl esters, and β-carotene have been noted after burns, fractures, and elective surgery [127–129]. In the severely injured, doses of vitamin A of 25,000 IU/d (five times the recommended daily dose) have been advocated and used without any significant side effects. Larger doses of vitamin A do not improve further wound healing, and prolonged excessive intake can be toxic [120].

The fat-soluble vitamin A and the water-soluble vitamin C are the predominant vitamins at work in the wound healing process. The other water-soluble vitamin is vitamin B complex, which seems to play little if any role in wound healing. The B vitamins play an indirect role in wound healing through their influence on host resistance. The remaining fat-soluble vitamins D, E, and K contribute little to wound healing.

Vitamin E maintains and stabilizes cellular membrane integrity, primarily by protection against destruction by oxidation [130]. Vitamin E possesses anti-inflammatory properties, similar to those of steroids, as shown by the reversal of wound healing impairment imposed by vitamin E after administration of vitamin A in the first days after wounding [131]. Vitamin E also has been shown to affect various host immune functions. As an antioxidant, it has been proposed that vitamin E could reduce injury to the wound by excessive free radicals [120]. The liberation of free radicals from inflammatory cascades in necrotic tissue, tissue colonized with microbial flora, ischemic tissue, and chronic wounds can result in depletion of free radical scavengers such as vitamin E [132,133]. This process is believed to be at work in patients with chronic lower extremity wounds. In these patients, it is not known if their relative lack of vitamin E is due to consumption of vitamin E in its antioxidant capacity or overall vitamin E deficiency, either of which could impair healing. In patients with chronic wounds of the lower extremity, some authors suggested that after healing is firmly established, vitamin E may have a role in decreasing excess scar formation, which is known to occur in chronic wounds [11].

Vitamin K is known as the antihemorrhage vitamin and is required for the carboxylation of glutamate in clotting factors II, VII, IX, and X. Vitamin K contributes little to wound healing, but its absence or deficiency leads to decreased coagulation, which consequently affects the initial phases of healing. Vitamins A and E antagonize the hemostatic properties of vitamin K. Formation of hematomas within the wound can impair healing and predispose to wound infection. This hemostatic capacity of vitamin K influences wound healing [11].

**Micronutrients**

Micronutrients are essential components of cellular function and can be divided into organic compounds, such as the vitamins already discussed, and inorganic compounds or trace elements. The term *micronutrients* refers to the extremely small quantities of these compounds found in the body.
Although these nutrients comprise only a small portion of the body’s overall nutritional needs, their importance is relied on heavily by the cellular machinery that carries out wound healing. It is difficult to associate deficits in specific minerals and trace elements to impairment in wound healing because deficiencies of micronutrients almost always are accompanied by coexisting metabolic or other nutritional disturbances. Most of these minerals and trace elements do not influence wound healing directly; rather they serve as cofactors or part of an enzyme that is essential to healing and homeostasis. Clinicians became more aware of deficiencies of these elements after the introduction of long-term parenteral nutritional solutions, which did not include supplemental minerals and trace elements. As such, it is often easier to prevent these deficiencies than to diagnose them clinically.

Magnesium is a macromineral that is essential for wound repair. Magnesium is a cofactor for many enzymes that are involved in the process of protein synthesis. The primary role of magnesium is to provide structural stability to ATP, which powers many of the processes used in collagen synthesis, making it a factor essential to wound repair. Of the numerous trace elements present in the body, copper, zinc, and iron have the closest relationship to wound healing. Copper is a required cofactor for cytochrome oxidase and the cytosolic antioxidant superoxide dismutase. Lysyl oxidase is a key copper enzyme used in the development of connective tissue, where it catalyzes the cross-linking of collagen and strengthens the collagen framework. Experimentally, impaired healing has been noted secondary to decreased copper stores in patients with Wilson’s disease and in animal models after the administration of penicillamine.

Zinc is the most well-known element in wound healing and has been used empirically in dermatologic conditions for centuries. Evidence that zinc is essential to wound healing in animals and humans first was described in the rat model in the 1930s and later in humans in the 1950s. Zinc is a cofactor for RNA and DNA polymerase and consequently is involved in DNA synthesis, protein synthesis, and cellular proliferation. Zinc deficiency impairs the crucial roles each of these processes play in wound healing. Zinc levels less than 100 μg/100 mL have been associated with impairments in wound healing. In zinc deficiency, fibroblast proliferation and collagen synthesis are decreased, leading to decreased wound strength and delayed epithelialization. These defects are readily reversed with repletion of zinc to normal levels. Immune function is impaired in zinc deficiency. Cellular and humoral elements are impaired, resulting in an increased susceptibility to wound infection and resultant increased possibility of delayed healing. Zinc levels can be depleted in settings of severe stress and in patients receiving long-term steroids. In these settings, it is recommended that patients receive vitamin A and zinc supplements to improve wound healing. The current recommended daily allowance for zinc is 15 mg. No studies have shown improvement in wound healing after the administration of zinc to patients who are not zinc deficient.
Iron is required for the hydroxylation of proline and lysine, and as a result, severe iron deficiency can result in impaired collagen production. As a part of the oxygen transport system, iron can affect wound healing, but this occurs only in settings of severe iron-deficiency anemia. In the clinical setting, iron deficiency is common and can result from blood loss, infectious causes, malnutrition, or an underlying hematopoietic disorder. In contrast to other deficiencies of trace elements, iron deficiency can be detected and treated easily [11].

Other factors affecting wound healing

Infection

The complex cascade of events discussed earlier, which comprise the body’s response to tissue injury with the purpose of restoring cutaneous integrity, occurs in the presence of various environmental factors. Any of these factors can impair the wound healing process if not effectively managed or prevented.

Sepsis, whether present as local bacterial colonization of the wound site or as a systemic inflammatory response, is one of the most formidable “environmental” obstacles to successful wound healing. Experimentally the crucial inoculum of microorganisms that significantly inhibits healing has been determined to be $10^5$ colony-forming units/cm$^2$ wound surface or gram of tissue [142,143]. In addition to appropriate antibiotic therapy, an intact, functioning immune system is vital to preventing and clearing wound infection. The immune system is tied to overall host nutrition and specific nutritional entities, such as arginine and its related metabolic pathways. In critically ill patients, it is crucial that nutritional status be optimized to provide increased substrate availability to meet the demands of tissue repair and immune function and to prevent wounds from succumbing to infection and delayed healing [144].

Evaluation of overall nutritional state

Clinicians must be aware of nutritional disturbances in wounded patients before these nutritional deficits can be corrected. The severity of the deficit must be assessed, and the caloric requirements for healing to ensue should be estimated. Kinney [145] outlined the metabolic adjustments experienced after injury as follows: (1) uncomplicated intra-abdominal surgery increases metabolic rate approximately 10%; (2) uncomplicated injuries, such as femoral fracture, increase metabolism about 20%; (3) peritonitis increases metabolism 20% to 40%; (4) third-degree burns increase metabolism 50% to 100%; and (5) fever alone increases metabolism 10% for each $1^\circ$C. Historically the sine qua non of linear nutritional status over time has been serial weight measurements. This commonly used marker for malnutrition
can be misleading, however, if the presence of abnormal amounts of body water is not taken into account. Total body water increases at approximately the same rate body protein decreases [146]. Body water also can influence the anthropometric measurements used to estimate body fat from skin-fold thickness and predetermined nomograms.

Other markers predictive of nutritional state include serum albumin and transferrin levels, total lymphocyte count, anergy-delayed hypersensitivity, urinary nitrogen, and respiratory minute volume. One of the least expensive and practical ways to estimate simple caloric requirements of seriously ill patients is respiratory minute volume. In the absence metabolic acidosis or alkalosis, with normal breathing the respiratory minute volume gives a close correlation to the patient’s metabolic rate. This information can be used to guide nutritional care. Serum albumin levels and total lymphocyte count also are useful nutritional prognosticators. In a study of nutritional status as a predictor of wound healing after amputation, normal albumin and total lymphocyte levels correlated with increased rates of healing [147]. These values also can be misinterpreted, however, if factors such as liver dysfunction, sepsis, or infection are present and not taken into account. Depressed hypersensitivity reactions to intradermally injected antigens also has been established as an indicator of nutritional status [148].

Feeding

Wound healing has been described repeatedly in this article as a complex series of cellular and biochemical events that are interdependent on the availability of energy. The substrate for the production of this wound healing energy is protein, carbohydrate, fat, amino acids, and micronutrients, which have been described previously. Specifically, it has been recommended that the calorie-to-nitrogen ratio be 120 to 150:1 during the early weeks of wound healing after severe injury, then raised to 200 to 225:1 as the body shifts to a period of positive nitrogen balance [149].

Patients who are malnourished before wounding have increased rates of wound infection and delayed wound healing. There seems to be ample evidence that nutritional repletion before planned elective operations in malnourished patients significantly reduces these complications. The exact route of administration, whether it is enteral or parenteral, may be important, but the existing data are conflicting.

TPN has been shown to reduce postoperative complications when administered to severely malnourished patients for at least 7 days preoperatively [150,151]. TPN has many associated risks, however, not the least of which is infection. Total enteral nutrition (TEN) also has associated risks, but there is growing experimental evidence that TEN is superior to TPN as a feeding modality. Studies evaluating the route of nutrition and wound healing in rats showed that TEN particularly influences the early stages of wound healing. In these studies, TEN significantly increased collagen
Fig. 4. Wound breaking strength (g, mean ± SEM) and hydroxyproline content (μg/100 mg sponge, mean ± SEM) of the sponge granulomas in enterally (total enteral nutrition [TEN]) and parenterally (total parenteral nutrition [TPN]) fed animals.
deposition and wound breaking strength compared with TPN 5 days after wounding (Fig. 4). This beneficial influence seems to disappear during the period of maximal fibroplasias, which occurs 5 to 10 days after injury. TEN seems to maintain local and systemic immune responses; preserves gut integrity, decreasing bacterial translocation; and improves protein metabolism and survival [152–155]. As already alluded to, TEN seems to exert a greater influence over the early cellular, inflammatory phase of wound healing than does TPN. This cellular phase is exquisitely sensitive to nutrient availability. The influence TEN has on systemic immune function contributes to the function and number of inflammatory cells present during early healing, ultimately affecting wound repair [156].

The exact feeding regimen should be tailored to each individual patient. In patients who are malnourished, preoperative repletion should be accomplished by the route that exposes the patient to the least risk, and if possible, elective operations should be delayed until the patient is satisfactorily supplemented. In patients who are not likely to take nutrition orally, TPN should be initiated early. The nutritional supplement should be as specific as possible to the patient’s perceived nutritional deficiency, and substrates that are turned over rapidly should be included. The amino acid arginine as previously discussed is turned over rapidly in wound healing. Of greatest importance is that nutritional deficiencies be recognized early and that repletion be initiated early because even brief periods of malnutrition can have significant negative effects on wound healing.

Summary

The relationship between host nutrition and wound healing has been the subject of study and experimentation for centuries. Despite the many years of study and a substantial knowledge base of the specific processes and factors involved, wound healing remains enigmatic. There is still much to learn about the wound-specific nutritional interventions that are available to improve wound healing. Nutrition profoundly influences the process of wound healing. Nutritional depletion exerts an inhibitory effect, and nutritional supplementation with such positive effectors as arginine can stimulate wound healing. Within this paradigm, the physician should be able to recognize patients who may be expected to have wound healing difficulties and offer early intervention to avoid wound failure.

References

[52] Prickett JD, Robinson DR, Steinberg AD. Effects of dietary enrichment with eicosapentanoic acid upon autoimmune nephritis in female NZBxNZW/F1 mice. Arthritis Rheum 1983;26:133.


