Nutrition and Wound Healing

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Summary: The relationship between nutrition and wound healing—after injury or surgical intervention—has been recognized for centuries. There is no doubt that adequate carbohydrate, fat, and protein intake is required for healing to take place, but research in the laboratory has suggested that other specific nutritional interventions can have significant beneficial effects on wound healing. Successful translation into the clinical arena, however, has been rare. A review of normal metabolism as it relates to wound healing in normoglycemic and diabetic individuals is presented. This is followed by an assessment of the current literature and the data that support and refute the use of specialized nutritional support in postoperative and wounded patients. The experimental evidence for the use of arginine, glutamine, vitamins, and micronutrient supplementation is described. Most of the experimental evidence in the field supporting the use of specialized nutritional support has not been borne out by clinical investigation. A summary of the clinical implications of the data is presented, with the acknowledgment that each patient's plan of care must be individualized to optimize the relationship between nutrition and wound healing. (*Plast. Reconstr. Surg.* 117 (Suppl.): 42S, 2006.)

Wound healing and nutrition have an intimate relationship that has been recognized by physicians for hundreds of years. Malnutrition or nutrient deficiencies can have a severe impact on the outcome of traumatic and surgical wounds. Wound failure, as reflected by wound infections and/or delayed healing, significantly contributes to the financial burden imposed on health care systems worldwide.

The critical role of nutrition to healing has been recognized since the beginning of medicine as a discipline. Some of the earliest known writings identifying this synergy date to roughly 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 these biochemical responses to injury in studying patients and animals with long bone fractures by demonstrating significant alterations in physiologic electrolyte levels, increased nitrogen turnover, and stimulation of the overall host metabolism.

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Received for publication November 19, 2005; revised April 5, 2006.

DOI: 10.1097/01.prs.0000225432.17501.6c

In the 1930s, Ravdin showed the specific relationship between protein malnutrition and the incidence of laparotomy wound dehiscence in dogs.[4–6] Ravdin concluded that poor nutritional intake or lack of certain essential nutrients significantly altered the body's ability to heal wounds. Interest has since swung from understanding the basic physiologic mechanisms of wound healing to attempting to modulate or enhance the process. The dynamic and complex cascade of wound healing has proved responsive to the external manipulation of metabolic and nutritional factors, but concrete changes to clinical management have been more elusive.

NUTRITIONAL FACTORS IN WOUND REPAIR

Malnutrition

Malnutrition after injury results from multiple factors, including poor nutritional intake to a host's perturbed metabolic equilibrium. Studies over the past century have shown that changes in energy, carbohydrate, protein, fat, vitamin, and mineral metabolism affect the healing process.[7] Loss of protein from protein-calorie malnutrition, the most common form of malnutrition in the world, 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 im-

mune compromises correlate clinically with increased wound complication rates and increased wound failure after clean surgical procedures.[8–10] In elderly nursing home patients, malnutrition is also associated with increased mortality, an increased risk of developing pressure ulcers, and a lower quality of life.[11–14]

Malnutrition may predate wounding or may be secondary to the catabolism resulting from the injury itself. Wounding increases metabolic rates, catecholamine levels, loss of total body water, and cellular protein turnover, resulting in an overall state of catabolism.[15] During this time of increased energy demand, muscle breakdown occurs preferentially to the use of existing fat stores, thus providing amino acids for gluconeogenesis.[16] The host's catabolic response to injury is proportional to the severity of the injury.[17,18] In fact, healing may be prioritized by metabolic activity. Levenson and others,[19–22] for example, have shown significantly slower cutaneous wound healing, but increased liver regeneration, in burned and traumatized animals. These disparities in the overall anabolic or catabolic state between various organs after thermal injury suggest that vital structures are preserved at the expense of others.

In a society where malnutrition is thought to have been eliminated, a significant proportion of medical and surgical patients have preexisting malnutrition from decreased nutritional intake. A study of orthopedic patients, including posttrauma patients and individuals undergoing total hip replacement, found that 42 percent of those studied were malnourished.[23] In a separate analysis, 12 percent of noncancer patients were found to be malnourished at preoperative evaluation.[24] Another cross-sectional study demonstrated that approximately 50 percent of medical and surgical patients in an urban hospital showed evidence of malnutrition.[25] Although the exact parameters used to define clinical malnutrition may vary, an assessment of preexisting malnutrition should be performed when evaluating a wound or a patient about to undergo operative intervention. Identification of the potential risk imposed by malnutrition is especially important in populations with other risk factors for impaired wound healing.

An understanding of normal metabolism is critical when planning a surgical procedure in malnourished patients. One of the critical 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 of energy per kilogram, and fats provide 9 kcal/kg.[26] In rats, reducing caloric intake by 50 percent results in decreased collagen synthesis, matrix protein deposition, and granulation tissue formation.[27,28] In other animal models, severe or prolonged protein-calorie malnutrition is necessary to impair the healing responses. In humans, however, only modest protein-calorie malnutrition impairs fibroplasia.[29] Finally, brief preoperative illness or decreased nutritional intake in the prewounding period has a significant effect on collagen synthesis. Preoperative food intake, then, may be more important to the wound-healing process than the patient's overall nutritional status.[30] Conversely, brief nutritional intervention by enteral or parenteral routes can overcome or prevent these impairments in the healing process.[31,32]

Although the current literature is laden with studies attempting to delineate the exact role that nutrition and nutritional supplements play in the wound-healing process, most wounds heal uneventfully, including those that occur in the setting of significant malnutrition. Patients undergoing oncologic operations, for example, often present with preoperative weight loss and malnutrition but generally heal without infection or wound dehiscence. Albina,[33] however, noted that severe protein-calorie malnutrition and symptomatic specific nutrient deficiencies can impair wound healing by delaying the healing process itself. These discrepant results underscore the importance and preeminence of healing in the posttraumatic response and should not lead clinicians to ignore the need for optimal nutrition. The primary goal, therefore, 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 source of energy in the body and, consequently, in the wound-healing process. Wounds require energy mainly for collagen synthesis. Estimates of caloric requirements for a particular wound can be determined knowing that (1) protein synthesis requires 0.9 kcal/g and (2) a 3-cm^2 × 1-mm-thick section of granulation tissue contains 10 mg of collagen. As such, simple wounds have little energy impact on overall metabolism, but thermal injuries or large complicated wounds can divert a disproportionate amount of energy to the healing milieu.[34]

Glucose is the major source of fuel used to generate cellular energy in the form of adenosine triphosphate, which in turn powers the wound-

healing process. The use of glucose to generate adenosine triphosphate is relatively inefficient, but the caloric contribution from glucose is essential in preventing the depletion of other amino acid and protein substrates. The liver, triggered by the catecholamine and cortisol surge that occurs after wounding, initiates gluconeogenesis using amino acids from degraded muscle protein. Unchecked, and in the presence of inadequate carbohydrate and fat stores, this increased gluconeogenesis can significantly deplete amino acids and protein. Although carbohydrates play an important role in providing the energy essential for optimal healing, little is known about the functions that different sources of carbohydrates play in this process. As mentioned, gluconeogenesis is a relatively inefficient pathway for glucose production that can result in the production of excess amounts of glucose. This excess may subsequently complicate wound healing, especially in diabetic patients with poor glycemic control.

Diabetic patients have a significantly impaired ability to heal wounds and, therefore, exhibit increased complication rates compared with their euglycemic counterparts. The mechanisms at work are multifactorial and yet to be clearly delineated, but they may be related to a build-up of advanced glycation endproducts in body tissues.[35] Diabetics exhibit a diminished early inflammatory response and inhibition of fibroblast and endothelial cell activity.[35–37] When inflammatory cells eventually arrive at the site of injury, they initiate a prolonged inflammatory phase that results in delayed deposition of matrix components, wound remodeling, and closure.[38] Delayed epithelialization of open wounds and decreased collagen accumulation deep within the wound have been reported in models of streptozotocin-induced diabetes.[37] Decreased reendothelialization of microarterial anastomoses has also been demonstrated, an effect that was not corrected by insulin administration at the time of surgery and that extended into the early postoperative period.[39] Work by Weringer and associates using a mouse model demonstrated that, in addition to hyperglycemia, the lack of insulin itself seems to impair wound healing.[40–42] Topical application of insulin to infected skin wounds or systemic administration in diabetic mice can improve healing,[43] but to achieve normal healing insulin must be started soon after wounding.[44]

Hyperglycemia interferes with the cellular transport of ascorbic acid into fibroblasts and leukocytes and decreases leukocyte chemotaxis.[45] Competitive inhibition of ascorbic acid membrane transport may explain this mechanism, as glucose and ascorbic acid are structurally similar.[46] This effect of hyperglycemia, specifically as it relates to leukocytes, is thought to explain the decreased early inflammatory response and impaired wound healing seen in diabetic patients. The importance of controlling serum glucose levels in diabetics around the time of injury, operation, and wound healing cannot be overemphasized. The alteration in a diabetic's metabolism after injury or elective surgery can significantly affect wound healing by any of the mechanisms discussed above. In addition, diabetic patients are more susceptible to infection because of decreased host resistance. It is crucial that physicians recognize and anticipate the needs of diabetic patients early, before the encumbering effects of diabetes lay hold to the wound-healing process. In summary, the most important factor affecting wound healing in diabetic patients involves achieving and maintaining normal glucose control.[46,47]

Fats

In contrast to carbohydrates, the role of fats has not been studied widely, although it is recognized that the demand for essential fatty acids increases after injury.[48,49] Linoleic and arachidonic acid are among the unsaturated fatty acids that must be supplied in the diet to allow for prostaglandin synthesis. Although both fatty acids can be synthesized from linoleic acid, the rate of synthesis is inadequate for basic metabolic needs. Deficiencies in these lipids can occur as early as 2 weeks after their removal from the diet, although clinical manifestations may not be apparent for 2 to 7 months.[36,50] As components or precursors of phospholipids and prostaglandins, free fatty acid deficiency impairs wound healing in animals and humans,[51–54] primarily because phospholipids are key constituents of cellular basement membranes while prostaglandins play critical roles in cellular metabolism and inflammation.

Deficiencies of dietary essential fatty acids were rarely seen clinically until the introduction of prolonged parenteral feedings that did not contain fat. Total parenteral nutrition is the most common cause of essential fatty acid deficiency; its administration results in rapid onset of essential fatty acid deficiency, which can manifest within 10 days of starting an entirely fat-free diet.[55] This, in turn, leads to elevated insulin levels, which block both lipolysis and essential fatty acid release.[56]

Additional research has sought to define benefits to wound healing of specific lipid types. The

ω-3 fatty acids, which exhibit anti-inflammatory properties by inhibiting the production of eicosanoids and other mediators, such as platelet-activating factor, interleukin-1, and tumor necrosis factor-α,[57–62] are among the most widely investigated. Animals consuming diets enriched with ω-3 fatty acids have weaker wounds because of poor quality, cross-linking, and spatial orientation of collagen fibrils.[57] The true benefit of ω-3 fatty acids, therefore, may be in their immune modulation of the host rather than in improved wound healing per se. Studies of healing burns in humans and guinea pigs, however, have demonstrated improved immune function, improved survival, and reduced infectious complications after the administration of a diet rich in ω-3 fatty acids to this specific subset of injured patients.[63,64]

Protein

The importance of protein in wound healing has been recognized and researched since the early 1930s. Experimentally, severe protein deprivation leads to impaired healing through impaired collagen synthesis and deposition, decreased skin and fascial wound-breaking strength, and increased wound infection rates.[28,65] In the clinical setting, however, pure protein deficiencies are rarely encountered; most patients exhibit combined protein-energy or protein-calorie malnutrition.

Amino Acids

Because 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.[34] Partial resolution of healing defects in protein-deficient rats was noted 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.[66,67] Arginine and glutamine, on the other hand, have been the most extensively studied amino acids in the wound-healing process.

Arginine

In the late 1940s and early 1950s, Rose[68] classified arginine as one of two semiessential amino acids in mammalian metabolism. Arginine is a dibasic amino acid synthesized endogenously from ornithine through citrulline and is a precursor for proline during collagen synthesis.[36] Arginine also has roles in maintaining positive nitrogen balance, growth factor release, and T-lymphocyte stimulation and is involved in the metabolic pathways that synthesize urea, nitric oxide, and creatine phosphate.[69,70] 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. Although arginine is synthesized in adequate quantities to sustain muscle and connective tissue mass, in situations of stress or injury, body stores of arginine decrease rapidly. It is during these times, in which its synthesis is insufficient to meet the demands of increased protein turnover, that arginine becomes an indispensable amino acid in the process of wound healing and in the maintenance of a positive nitrogen balance.[71,72]

The role of arginine in wound healing was first shown in the 1970s using an animal model, when it was hypothesized that, following 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 on wound healing in young adult rats was studied. Animals were fed an arginine-deficient diet for 4 to 6 weeks before wounding. When animals were subjected to the minor trauma of a dorsal skin incision and closure, they demonstrated increased postoperative weight loss, an increased mortality rate to approximately 50 percent, and a notable decrease in wound-breaking strength as well as wound collagen accumulation compared with animals fed a diet containing arginine (Fig. 1).[72] Subsequent experiments revealed that chow-fed rats that were not arginine-deficient and were then fed a diet containing an additional 1% arginine had enhanced wound healing, as assessed by wound-breaking strength and collagen synthesis, compared with chow-fed controls (Fig. 1).[72] Similar findings were observed in parenterally fed rats given an amino acid mixture containing high doses (7.5 g/liter) of arginine. These animals exhibited increased wound-breaking strength, increased collagen accumulation, and enhanced immune function.[73] Likewise, mature or old rats fed diets supplemented with a combination of arginine and glycine had enhanced rates of wound collagen deposition compared with controls.[74]

Twenty years ago a micromodel was described that has made it possible to study the human fibroblastic response.[75] In this model, collagen accumulation occurs in subcutaneously placed segments (5 to 7 cm long) of polytetrafluoroethylene tubing that can be removed for analysis. Two studies in healthy human volunteers examined the

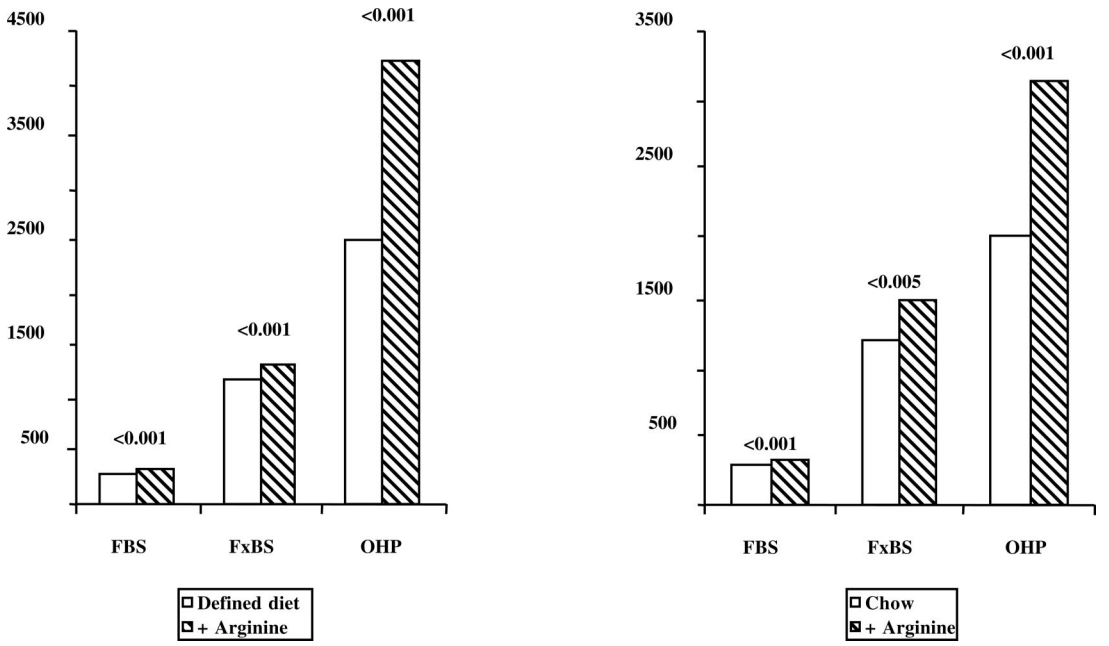

Fig. 1. Effect of supplemental dietary arginine added to an arginine-free (defined) diet or normal laboratory chow (1.8% arginine content) on wound healing in rats. Statistical comparison by Student's *t* test. *FBS*, fresh breaking strength of scar, g; *FxBS*, formalin-fixed breaking strength, g; *OHP*, hydroxyproline content of subcutaneously implanted polyvinyl alcohol sponges, μg/100 mg sponge dry weight.

effects of arginine supplementation using this model. In the first study, 36 young, healthy human volunteers (ages 25 to 35 years) were randomized to one of three groups: (1) 30-g arginine hydrochloride daily supplements (24.8 g of free arginine); (2) 30 g of arginine aspartate (17 g of free arginine); or (3) placebo. The supplements were given for 2 weeks, after which the polytetrafluoroethylene catheters were removed and the hydroxyproline content (index of reparative collagen synthesis) was determined. Arginine supplementation at both doses significantly increased the amount of hydroxyproline and total protein deposition at the wound site (Fig. 2).[76] The second study evaluated 30 elderly volunteers (age >70 years) who received 30 g of arginine aspartate or placebo. In addition to evaluating the fibroblastic wound response using the catheters, this study also examined epithelialization after creation of a split-thickness wound on the upper thigh of each subject. The catheters in this study were analyzed for α-amino nitrogen content (assessment of total protein accumulation), DNA accumulation (index of cellular infiltration), and hydroxyproline content.[77] 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 supplementa-

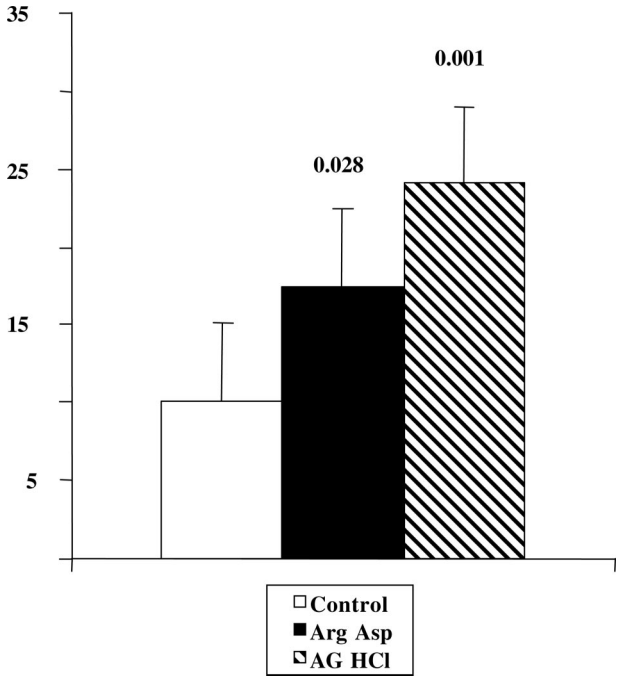

Fig. 2. Effect of 2 weeks of arginine supplementation on hydroxyproline accumulation in subcutaneously implanted polytetrafluoroethylene catheters in young human volunteers (mean ± SEM). Groups of 12 volunteers each received a placebo (control), 30 g of arginine aspartate (*Arg Asp*; 17 g of free arginine) per day, or 30 g of arginine hydrochloride (*AG HCl*; 24.8 g free arginine) per day for 2 weeks.

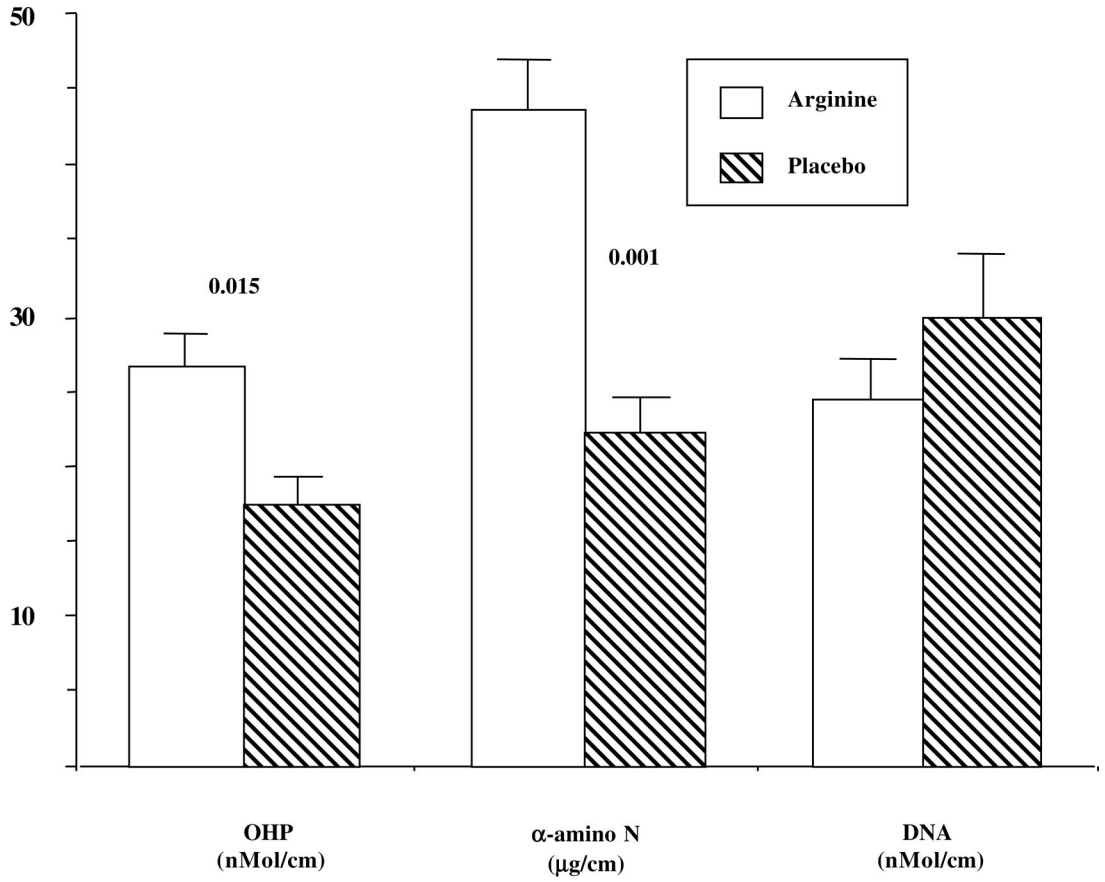

Fig. 3. Effect of arginine on wound-healing parameters in healthy elderly human volunteers. Accumulation of hydroxyproline (*OHP*), total α-amino N, and DNA in subcutaneously implanted polytetrafluoroethylene catheters was measured at the end of 2 weeks (mean ± SEM). Controls ($n = 15$) received placebo syrup; the arginine group ($n = 30$) received 30 g of arginine aspartate.

tion had no effect on the rate of epithelialization of the skin defect, indicating that the predominant effect of arginine is on wound collagen deposition.[78]

Oral arginine supplementation is well tolerated and has been the focus of recent studies of wound healing and medical outcomes. A recent randomized trial in healthy volunteers demonstrated improved collagen synthesis after dietary supplementation with arginine, glutamine, and β-hydroxy-β-methylbutyrate.[79] Despite improvements in markers of collagen biosynthesis, however, clinical evidence of improved wound healing has not been reported in most of the studies published to date. In a study by de Luis et al., patients undergoing resection for oral or laryngeal cancer were randomized to receive an enteral dietary supplement containing either fiber or fiber and arginine.[80] Postoperative infectious complications were similar in the two groups, as were plasma protein levels of albumin, transferrin, and prealbumin. Patients treated with arginine, however, had lower rates of fistula formation and, consequently, shorter hospital stays. Nursing home patients with pressure ulcers have been the most recent patient population studied with regard to arginine supplementation. One study sought to determine the tolerance of oral arginine administration and effectiveness in improving mitogen-induced lymphocyte proliferation and interleukin-2 production, two in vitro parameters of immune function.[81] Subjects tolerated the arginine supplementation well, but no improvement in immune function was observed. Prior work in volunteers and patients had shown that arginine supplementation increased mitogen-induced lymphocyte proliferation,[78,82,83] but other studies found no effect.[84–86]

Several mechanisms have been postulated to explain the positive effect of arginine on wound healing. First, the beneficial effects of supplemental arginine on wound healing are similar to the

effects seen with growth hormone.[87–89] 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—with half the animals in each group also supplemented with 1% dietary arginine. After wounding, the intact, arginine-supplemented animals demonstrated 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, again regardless of the administration of growth hormone. This result suggests that, in rats, the effects of arginine on wound healing require an intact hypothalamopituitary axis.[90] In humans, arginine supplementation in doses that have been shown to improve wound healing also increases plasma insulin-like growth factor, the peripheral mediator of growth hormone activity.[78]

Second, supplemental arginine has a unique effect on T-cell function by stimulating T-cell responses and reducing the inhibitory effect of injury and wounding on T-cell function.[73,91–93] 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. In addition, T lymphocytes can be detected immunohistochemically in distinctive patterns throughout the various phases of wound healing. Furthermore, specific T-cell types have modulating roles on different stages 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.[94] The exact mechanisms are not fully understood, but it is thought that one manner in which arginine may enhance wound healing is by stimulating the host's T-cell response, which in turn increases fibroplasia.[95–97]

Third, arginine has been identified as a unique substrate for the generation of nitric oxide, a highly reactive radical that may play a critical role in wound healing. Inhibitors of nitric oxide have been shown to significantly impair the healing of cutaneous incisional wounds and colonic anastomoses in rodents.[98,99] In vitro studies have noted increased collagen synthesis in cultured dermal fibroblasts exposed to exogenous nitric oxide.[100] Arginine is catabolized in wounds through two separate pathways, one involving nitric oxide synthases and the other by arginase.[70] Both pathways have been shown to deplete the wound environment of extracellular arginine, thus emphasizing its essential nature in wound healing.[77] The inducible isoform of nitric oxide synthase, iNOS, is most active in response to inflammatory stimuli (e.g., wounding) and generates more nitric oxide than the constitutive isoforms.[70] Supranormal collagen deposition has been observed after transfection of iNOS DNA into wounds,[101] while mice lacking the iNOS gene experience delayed closures of excisional wounds, an impairment that is remedied by adenoviral transfer of the iNOS gene into the wound bed.[102] Functional loss of the iNOS gene abrogates the beneficial effect of arginine in wound healing, whereas wild-type mice fed arginine-supplemented diets exhibit 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.[69]

Arginine supplementation may play an especially important role in the wound healing of diabetic patients. As previously described, diabetic patients exhibit an impaired inflammatory response to injury. This abnormal response is characterized by delayed neutrophil chemotaxis and impaired phagocytosis and leads to decreased concentrations of nitric oxide and growth factors as well as inadequate collagen synthesis. Animal models of diabetes, however, have demonstrated that arginine supplementation leads to greater wound-breaking strength resulting from increased levels of hydroxyproline and collagen.[103,104]

Glutamine

Glutamine is the most abundant amino acid in the body; it accounts for approximately 20 percent of the total circulating free amino acid pool and 60 percent of the free intracellular amino acid pool.[105,106] In addition to being a major respiratory fuel source, glutamine serves as a nitrogen donor for the synthesis of amino acids and amino sugars.[107,108] Glutamine is also an important precursor for the synthesis of nucleotides in cells, including fibroblasts and macrophages.[109,110] Gluconeogenesis involves the shuttling of alanine and glutamine to the liver for conversion to glucose, which is used peripherally as fuel for certain aspects of wound healing. Glutamine is also an energy source for lymphocytes and is essential for lymphocyte proliferation.[111,112] Finally, glutamine has a crucial role in stimulating the inflammatory immune response occurring early in wound healing.[105]

Given the abundant roles of glutamine in the cells involved in wound healing, it is not surprising that there is a rapid fall in plasma and muscle glutamine levels after injury.[113,114] Although efficacy of supplemental glutamine administration has been shown in some clinical situations,[115] it has not proved to have any noticeable effect on wound healing specifically.[116] Most of glutamine's benefit appears to involve improvements in gut permeability, normalization of serum levels, improved protein synthesis, and decreased hospital length of stay.[117–119]

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 and field armies who consumed a diet lacking in fresh fruits and vegetables and who subsequently developed scurvy. In the late 1800s, Osler[120] 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.[121] 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[122] 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 10 days after the injury was normal. After 6 months on this diet, however, a second incision healed poorly, and a 10-day biopsy sample 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 now known to be associated with vitamin C deficiency: minimal collagen deposition, decreased angiogenesis, and significant hemorrhage.

Although the recommended dietary allowance for vitamin C is 60 mg/d, the clinical spectrum of its administration varies widely. In major burn victims, the requirement may be as much as 2 g per day to restore urine and tissue levels to normal.[123] In animal models, the wounds of burned guinea pigs bore histologic resemblance to those of scorbutic unburned animals. These changes were prevented when supplemental vitamin C was given. Although the dose needed in different settings may vary, there is no evidence to suggest that massive doses of ascorbic acid provide any substantial benefit to wound healing. There also is no evidence that excess vitamin C is toxic.[124]

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 impaired collagen synthesis and a subsequent inability to wall off bacteria and localize infection, as well an impairment of neutrophil function and complement activity.[34]

McCollum and Davis initially discovered vitamin A in the early 1900s. Since that time it has been shown to be beneficial to the wound-healing process by stimulating epithelialization and collagen deposition by fibroblasts. Brandaleone and Papper[125] were the first to demonstrate that wound healing was impaired by vitamin A deficiency. Ehrlich and Hunt[126] subsequently described the benefits of supplemental vitamin A on wound healing in nondeficient humans and animals by showing that vitamin A can reverse 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.[127,128] Finally, vitamin A has been used to restore the impaired wound healing caused by diabetes, tumor formation, cyclophosphamide, and radiation.[129–132]

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.[36] In vitro studies have shown increased collagen synthesis of fibroblast cell cultures in the presence of vitamin A.[133,134] These mechanisms still are not well understood, but it is clear vitamin A plays in important role in wound healing.

Serious injury or stress leads to increased vitamin A requirements. Large doses of corticosteroids can 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.[135–137] 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.[138]

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 may have an indirect role in wound healing through its influence on host resistance. The remaining fat-soluble vitamins, D, E, and K, do not contribute significantly to wound healing.

Vitamin E maintains and stabilizes cellular membrane integrity, primarily by protection against destruction by oxidation.[139] 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.[140] Vitamin E also has been shown to affect various host immune functions. As an antioxidant, vitamin E may reduce injury to the wound by scavenging excess free radicals.[141] 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.[142,143] 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. Some authors have suggested that, in chronic wounds of the lower extremity, vitamin E may have a role in decreasing excess scar formation, which is known to occur in chronic wounds.[15] While two studies in animal models have suggested a beneficial effect of vitamin E supplementation on wound healing,[144,145] supplementation in humans has not been shown to have a beneficial effect on wound healing.[36,146,147]

Vitamin K is required for the carboxylation of glutamate in clotting factors II, VII, IX, and X and contributes little to direct wound healing. Its absence or deficiency, however, may lead to hematoma formation within a wound, which can impair healing and predispose to infection. It is this hemostatic capacity of vitamin K that most influences wound healing.[15]

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. Although these nutrients comprise only a small portion of the body's overall nutritional needs, they are 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 impairments in wound healing, because micronutrient deficiencies almost always are accompanied by other coexisting metabolic or 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.[34]

Magnesium is essential for wound repair and functions as a cofactor for many enzymes involved in protein and collagen synthesis.[15,139] The primary role of magnesium is to provide structural stability to adenosine triphosphate, which powers many of the processes used in collagen synthesis, thus making it a factor essential to wound repair.[127,139]

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, the cytosolic antioxidant superoxide dismutase, and for the optimal functioning of lysyl oxidase, an enzyme that catalyzes the cross-linking of collagen and strengthens the collagen framework.[138,139] Experimentally, impaired healing has been noted secondary to decreased copper stores in patients with Wilson's disease and in animals after the administration of penicillamine.[148,149]

Zinc is the most well-known element in wound healing and has been used empirically in dermatologic conditions for centuries. Zinc is a cofactor for both RNA and DNA polymerase and, therefore, is involved in DNA synthesis, protein synthe-

sis, and cellular proliferation. Zinc deficiency impairs the crucial roles each of these processes play in wound healing and leads, ultimately, to delayed wound healing.[150] 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.[34] Both cellular and humoral immune functions are impaired in zinc deficiency, resulting in an increased susceptibility to wound infection and a resultant increased probability of delayed healing. Zinc levels can be depleted in settings of severe stress[151] and in patients receiving long-term steroids.[15] In these settings, it is recommended that patients receive vitamin A and zinc supplementation to improve wound healing[152] ; 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 already zinc deficient.[153]

Iron is required for the hydroxylation of proline and lysine, and as a result, severe iron deficiency can result in impaired collagen production. Iron is also a component of the oxygen transport system and can affect wound healing in this capacity, but only in settings of severe iron-deficiency anemia. In the clinical environment, iron deficiency is common and can result from blood loss, infection, malnutrition, or an underlying hematopoietic disorder. In contrast to other deficiencies of trace elements, iron deficiency is easily detected and treated.[15,128]

OTHER FACTORS AFFECTING WOUND HEALING

Infection

The complex cascade of events that 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 they are 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 obstacles to successful wound healing. Experimentally, the crucial inoculum of microorganisms that significantly inhibits healing is 10^5 colony-forming units per square centimeter of wound surface or gram of tissue.[154,155] 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.[156]

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[157] outlined the metabolic adjustments experienced after injury as follows: (1) uncomplicated intra-abdominal surgery increases the metabolic rate approximately 10 percent; (2) uncomplicated injuries, such as femoral fracture, increase metabolism about 20 percent; (3) peritonitis increases metabolism 20 percent to 40 percent; (4) third-degree burns increase metabolism 50 percent to 100 percent; and (5) fever increases metabolism 10 percent for each 1°C above normothermia. Historically, the *sine qua non* of linear nutritional status measurements 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 that body protein decreases.[158] 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, prealbumin, retinol-binding protein 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 of metabolic acidosis or alkalosis, with normal breathing, the respiratory minute volume gives a close correlation to the patient's metabolic rate. This value can then 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 corre-

lated with increased rates of healing.[9] These values can be misinterpreted, however, if factors such as liver dysfunction, sepsis, or infection are present and not taken into account. A depressed hypersensitivity reaction to intradermally injected antigens has also been established as an indicator of nutritional status.[159]

Feeding

Wound healing has been described repeatedly in this article as a complex series of cellular and biochemical events that are dependent on energy availability. The substrates for wound-healing energy are protein, carbohydrate, fat, amino acids, and micronutrients. 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; it should then be raised to 200 to 225:1 as the body shifts to a period of positive nitrogen balance.[138]

Patients who are malnourished before injury have increased rates of wound infection and exhibit 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 enteral or parenteral, may be important, but the data are conflicting.

Total parenteral nutrition has been shown to reduce postoperative complications when administered to severely malnourished patients for at least 7 days preoperatively.[160–163] Total parenteral nutrition has many associated risks, however, not the least of which is infection. Total enteral nutrition has associated risks as well, but there is growing experimental evidence that it is superior to total parenteral nutrition as a feeding modality. Studies evaluating the route of nutrition and wound healing in rats showed that total enteral nutrition particularly influences the early stages of wound healing. In these studies, total enteral nutrition significantly increased collagen deposition and wound-breaking strength when measured within 5 days after wounding when compared with total parenteral nutrition (Fig. 4). This beneficial influence seems to disappear during the period of maximal fibroplasias, which occurs 5 to 10 days after injury. Total enteral nutrition maintains local and systemic immune responses, improves protein metabolism and survival, and preserves gut integrity, thereby decreasing bacterial translocation.[164–167] As already alluded to, total enteral nutrition seems to exert a greater influence over the early cellular, inflammatory phase of wound healing than total parenteral nutrition. This cellular phase is exquisitely sensitive to nutrient availability. The influence total enteral nutrition has on systemic immune function contributes to the function and number of inflammatory cells present during early healing, ultimately affecting wound repair.[168]

Specific feeding regimens, however, should be tailored to individual patients. In patients who are malnourished, preoperative repletion should be

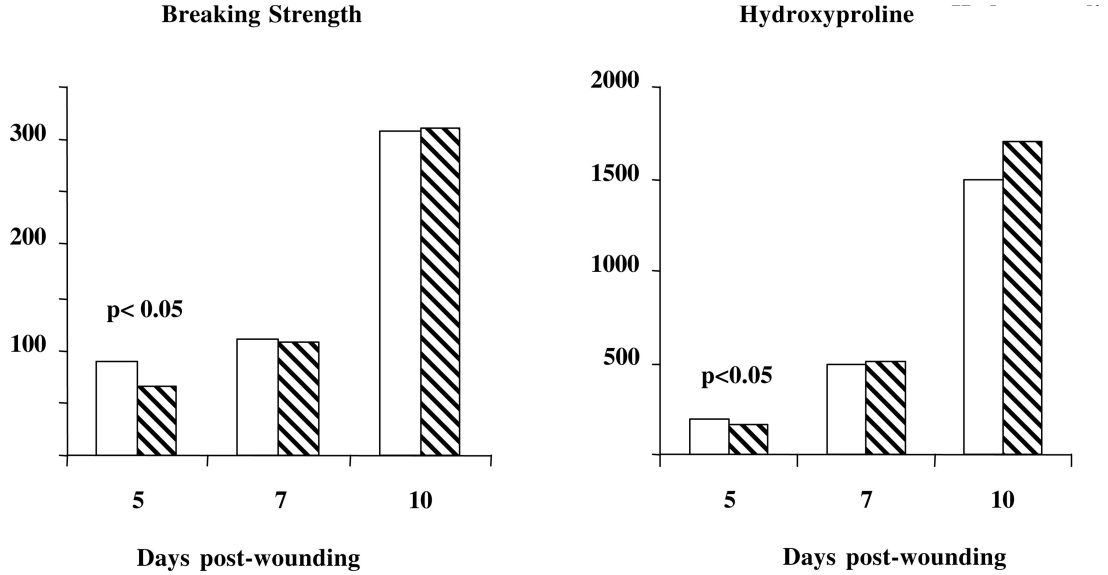

Fig. 4. Wound-breaking strength (g, mean ± SEM) and hydroxyproline content (μg/100 mg sponge, mean ± SEM) of the sponge granulomas in enterally [total enteral nutrition (open square)] and parenterally [total parenteral nutrition (striped square)] fed animals.

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, total parenteral nutrition 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 (e.g., arginine) should be included. Because even brief periods of malnutrition can have significant negative effects on wound healing, nutritional deficiencies must be recognized early and repletion initiated as soon as possible.

CLINICAL IMPLICATIONS

The clinical significance of nutrition and wound healing involves individual patients with unique needs. The goal of the physician, then, is to determine whether, when, and how nutritional supplementation is needed. There are few concrete answers. Although the benefits of perioperative nutritional support are apparent, the risk complications and increased cost need to be considered as well.

Preoperative nutritional support is generally recommended for patients with moderate (10 to 20 percent weight loss; serum albumin <3.2 g/dl to >2.5 g/dl) to severe malnutrition (>20 percent weight loss; serum albumin <2.5 g/dl)[169] and who can tolerate waiting at least 7 days for an elective operation. If intestinal function is maintained in a patient, enteral nutritional support is generally preferred, as it is associated with the maintenance of gut mucosal barrier function, the decreased activation of gut-associated lymphoid tissue, and lower costs of administration than parenteral nutrition.[170,171] While the only absolute contraindication to enteral feeding is complete intestinal obstruction, a variety of relative contraindications (e.g., high-output intestinal fistulas, acute pancreatitis, acute inflammatory bowel disease, severe diarrhea) must be considered. Enteral nutritional support may be achieved via nasogastric, nasoenteric, gastrostomy, jejunostomy, or gastrojejunal tubes, and a variety of commercial nutrition products are available for use in specialized patient populations.[172] Parenteral nutritional support is often used as the sole source of caloric intake in hospitalized patients, but there are some instances in which its use is best as a supplement to enteral feeding. Although peripheral parenteral nutrition may be easier to implement because it does not require central venous access, its nutritive value is substantially less than that of central preparations and its use is generally only indicated for less than 7 days.[173]

Serum protein markers are the best way to assess the adequacy of nutritional supplementation, as conventional methods, such as daily weight, may not be accurate in critically ill patients. While albumin is commonly used as a preoperative marker of nutritional, its half-life of 18 to 21 days precludes its use as an effective daily indicator of improvements in nutritional status. Prealbumin (half-life, 3 to 5 days) and transferrin (half-life, 7 to 10 days) should be monitored weekly in patients receiving enteral or parenteral nutritional support.

Beyond the basic understanding that general nutritional support is critical for optimal wound healing, many questions remain about the specific type of supplementation that should be used. Although glutamine and arginine have been shown to have beneficial effects on wound healing in animal models and in healthy volunteers, their clinical significance has yet to be proven. We cannot, therefore, recommend their general use in severely injured or postsurgical patients. Supplemental vitamin C appears to have beneficial effects in burn healing, whereas vitamin A is best reserved for those patients who have required long-term corticosteroid therapy. Zinc and iron supplementation, on the other hand, are best reserved for those with preexisting deficiency states.

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 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, such that depletion exerts an inhibitory effect and nutritional supplementation has a positive effect. 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.

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