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Nutrition, tissue oxygenation, and healing in individiuals with venous leg ulcers

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NUTRITION, TISSUE OXYGENATION, AND HEALING IN INDIVIDUALS WITH VENOUS LEG ULCERS

by

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DISSERTATION

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Dedication

This dissertation is dedicated to my late parents, Sidney William Wipke and Dorothy Elizabeth Grote Wipke, who instilled within me the importance of an education and a love for learning; and to my wonderful husband, Dan Warren Tevis, whose love and support made achieving this goal possible.

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ABSTRACT

NUTRITION, TISSUE OXYGENATION, AND HEALING IN INDIVIDUALS WITH VENOUS LEG ULCERS

Deidre D. Wipke-Tevis, RNC, PhD

University of California, San Francisco, 1996

Wound healing problems are a prevalent clinical challenge with venous ulcers. Although it is known that nutrients and oxygen are required to support repair, there are minimal data on the relationship between nutrition, tissue oxygenation, and rate of healing. The purpose of this study was to explore nutrition, tissue oxygenation, and rate of healing in individuals with venous ulcers. Nutritional risk, status, and intake, transcutaneous skin oxygen ($TcPO_2$), and wound surface area were evaluated two times, four weeks apart. The DETERMINE Public Awareness Checklist assessed nutritional risk. Nutritional status was evaluated with anthropometric measures and biochemical indices. Dietary intake was determined from two 3-day dietary records. $TcPO_2$ measures were taken in four positions at four different sites, with and without oxygen supplementation. A convenience sample of 25 subjects with a venous ulcer participated. Forty-four percent of subjects were at moderate nutritional risk; 40% were at high risk. Anthropometric measures found 20% of subjects below the 15th percentile when compared to established standards. Biochemical indicators showed that mean hemoglobin/hematocrit levels were low in males, and overall mean glucose and glycosylated hemoglobin levels were elevated. Eighty-five percent of subjects had inadequate caloric intake; 40% of subjects had an

inadequate protein intake. Mean daily zinc intake was also deficient. The initial mean wound surface area was 2.24 square centimeters (SD 2.21). The mean rate of wound closure was 0.142 cm/4 weeks (SD 0.21). Ulcers healed completely in 15% of subjects and increased in size in 25% of subjects. Females healed faster than males ($p<0.05$). When subjects received oxygen, $TcPO_2$ was significantly higher in the lying position than either the legs elevated or standing positions ($p<0.05$). A portion of venous ulcer patients were either at nutritional risk, had abnormalities in their nutritional status, and/or had inadequate intake to support healing. Venous ulcer patients should be screened for nutritional risk. Based on these data, positioning may be important for healing venous ulcers. Additional research is necessary to examine the efficacy of leg elevation to determine which position may best support healing and maximize tissue oxygenation.

Dissertation Chair: Nancy A. Stotts, RN, MN, EdD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

THE STUDY PROBLEM

Introduction

Chronic venous insufficiency (CVI) is a common medical problem in the elderly with an incidence of approximately 5.9% (Beauregard & Gilcreast, 1987) and 7 million people in the United States are affected (Coon, Willis, & Keller, 1973). Although the symptoms of CVI may vary between individuals and depend on the extent and duration of the disease, venous leg ulcers are often the ultimate sequelae of severe CVI.

Although the exact number of leg ulcers is difficult to determine (Falanga & Katz, 1991), their prevalence is approximately 1% in the Western world (Callam, Ruckley, Harper & Dale, 1985; Nelzen, Bengqvist, & Lindhagen, 1991). The most common cause of leg ulcers is venous disease (Callam et al., 1985; Nelzen et al., 1991). However, other etiologies include arterial occlusive disease, mixed arterial/venous disease, diabetes/neuropathy, vasculitis, pyoderma gangrenosum, and scleroderma (Limova & Mauro, 1995; Margolis, 1995; Sindrup, Groth, Avnstorp, Tonnesen, & Kristensen, 1987). Regardless of their etiology, leg ulcers are a chronic wound healing problem.

Wound healing requires nutrients and oxygen to support repair. Insufficient quantities of nutrients impair wound healing by prolonging inflammation, delaying fibroplasia, decreasing collagen synthesis and tensile strength, increasing capillary fragility, delaying epithelialization and increasing wound infection (Daly, Vars, & Dudrick, 1973; Goodson et al., 1987; Haydock & Hill, 1986; 1987; Jensen, Jensen, Smith, Johnston, & Dudrick, 1982; Mullen, Gertner, Buzby, Goodhart, & Rosato, 1979; Windsor, Knight, & Hill, 1988; Zaizen, Ford, Costin, & Atkinson, 1990). Recent data indicate that there is a

correlation between malnutrition and the development of pressure ulcers (Allman, et al., 1986; Olson, et al., 1996) and that poor nutrition negatively influences healing time in deep pressure ulcers (vanRijswijk & Polansky, 1994). Furthermore, data from pilot work for this study suggest that a portion of subjects with venous ulcers are at nutritional risk, have abnormalities in their nutritional status, and/or have an inadequate intake to support healing (Wipke-Tevis & Stotts, 1996).

Inadequate tissue oxygenation impairs healing by reducing fibroblast growth, collagen accumulation, angiogenesis, and epithelialization (Hunt & Pai, 1972; Knighton, Silver & Hunt, 1981; Jonsson, Jensen, Goodson, & Hunt, 1986; Pai & Hunt, 1972). In addition, ribonucleic acid production, wound strength, and capillary growth and density decrease in an hypoxic environment (Kivisaari, Vihersaari, Renvall, & Niinikoski, 1975; Knighton et al., 1981; Niinikoski, 1980, Jonsson et al., 1991). Oxygen also is important in the prevention of wound infection. Optimal bacteriocidal killing by leukocytes depends on oxygen availability for the respiratory burst (Hohn, MacKay, Halliday, & Hunt, 1976; Hohn, 1977; Jonsson, Hunt, & Mathes, 1989; Knighton, Halliday, & Hunt, 1984).

Adequate tissue oxygenation is intricately related to tissue perfusion. Perfusion influences healing in chronic wounds. Specifically, chronic wounds heal in proportion to their vascular supply (Hunt, 1991). Impaired perfusion delays granulation and epithelialization (Ahn & Mustoe, 1990), stimulates premature leukocyte activation (Moelleken, Mathes, Amerhauser, Scheunstuhl, & Hunt, 1991), impairs leukocyte diapedesis (Goldman et al., 1990), and increases wound infection and decreases collagen deposition (Jonsson et al., 1986; Jonsson et al., 1991). Oxygen delivery to the tissues is reduced as perfusion decreases and the tissues extract a greater proportion of oxygen

content from the blood (Faulkner & Hauptman, 1989). Thus, tissue partial pressure of oxygen is an indicator of wound perfusion.

Statement of the Problem

Investigators have examined the relationship between nutritional status and healing in surgical wounds and pressure ulcers. The effect of tissue hypoxia and hyperoxia on the healing of surgical wounds also has been documented. Similarly, the relationship between tissue oxygen and healing of arterial ulcers and lower extremity amputations has been investigated. However, little data are available on the nutritional or tissue oxygenation status of subjects with venous leg ulcers. Furthermore, there are minimal data on the relationship between nutrition and oxygenation and rate of healing in subjects with venous ulcers.

Purpose

The purpose of this prospective study is to explore nutrition, tissue oxygenation, and rate of healing in subjects with venous leg ulcers.

The primary aims are to:

1) describe nutritional status;

2) determine if dietary intake is adequate to meet the needs for wound healing;

3) determine if there is a relationship between the rate of wound closure and the nutritional status when the effects of perfusion are removed.

The secondary aims are to:

4) describe transcutaneous tissue oxygen values during position changes and with different levels of inspired oxygen;

5) determine if there is a relationship between glycosylated hemoglobin levels and

leukocyte vitamin C levels;

6) determine if there is a relationship between rate of wound closure and leukocyte vitamin C levels.

Significance

Wound healing problems in leg ulcers are a prevalent clinical challenge. It has been estimated that between 500,000 and 800,000 Americans have leg ulcers (Coon, Willis, & Keller, 1973). According to Falanga and Katz (1991), extrapolation of European prevalence data to the United States population would yield an estimate that between 500,000 and 1,000,000 people have leg ulcers at any one time. Research shows that chronic venous insufficiency is the most prevalent etiology of chronic leg ulceration (Burton, 1993; Callam, Harper, Dale, & Ruckley, 1987; Falanga, Moosa, Nemeth, Alstadt, & Eaglstein, 1987; Nelzen, Bergqvist, & Lindhagen, 1991; Nelzen, Bergqvist, & Lindhagen, 1993; van Rijswijk & the Multicenter Leg Ulcer Study Group, 1993). In addition, between 60% and 70% of patients have recurrent ulcerations (Falanga & Katz, 1991; Phillips, Stanton, Provan, & Lew, 1994).

Although venous leg ulcers occur in young adults (Sindrup et al., 1987; Callam et al., 1987), the prevalence of this disease is greater in women and in the elderly population (Baker, Stacey, Jopp-McKay, Hoskin, & Thompson, 1991; Callam et al., 1985; Sindrup et al., 1987; Wood & Margolis, 1992). The population of Americans 65 years of age and older is projected to increase from approximately 12% in 1988 to almost 22% by the year 2030 (White, Ham, Lipschitz, Dwyer, & Wellman, 1991). Thus, the problem of venous leg ulcers is likely to increase as the population ages. Also, according to Lipschitz, Ham, and White (1992), many older Americans are at high risk for nutritional deficits due to

chronic diseases and conditions, poor eating habits, financial hardship, social isolation, and functional limitations. Although health care professionals generally accept the importance of nutrition in both health and disease (White et al., 1991), few routinely or systematically look for signs and symptoms of poor nutritional status in patients with venous leg ulcers.

Venous leg ulcers are a classic example of chronic wounds that manifest delayed healing. Impaired healing prolongs hospital stays, increases health care costs, and may rarely cause limb loss (Bild et al., 1989; Fowler, 1991; Rudolph, Horowitz, & Jones-Putnam, 1983). One study conducted in the United States reports the average cost to heal one venous leg ulcer is $1950 with a range from $784 to $6449 (Wood & Margolis, 1992). Recent British estimates indicate that approximately $4000 per year is spent to treat each chronic leg ulcer (Greenwood, Edwards, & McCollum, 1995). On a national level, the annual cost of care for leg ulcer patients in Sweden is between $87,750 and $137 million (Lindholm, 1995). Similarly, the National Health Service in the United Kingdom indicates the annual cost to treat leg ulcers ranges from $150 to $900 million (Phillips, 1996). Based on the estimated United States prevalence by Falanga and Katz (1991) and the average cost to heal one ulcer by Wood and Margolis (1992), the average annual cost in the United States to treat venous leg ulcers would be between $975,500,000 and $1,951,000,000!

The impact of leg ulcers on an individual's life extends well beyond economic issues. Living with a leg ulcer for weeks, months, or even years influences the quality of life of the patient. The three most important aspects of life identified by elderly leg ulcer patients include freedom from pain, maintaining their independence, and the ability to remain socially active (International Committee on Wound Management {ICWM}, 1994).

Subjects with chronic leg ulcers report symptoms of fatigue, sleep disturbances, pain, fear, emotional distress, anxiety, depression, anger, and hostility (Dorman, Moffatt, & Franks, 1995; Franks, Moffatt, Connolly, Bosanquet, Oldroy, Greenhalgh, & McCollum, 1994; Lindholm, 1994; Phillips et al., 1994; Scurr, Wilson, & Coleridge-Smith, 1994; Wipke-Tevis, 1991). Subjects with chronic leg ulcers often experience periods of prolonged activity restriction, impaired mobility, and functional disability including significant reductions in activities of daily living, as well as social and leisure activities (Dorman et al., 1995; Franks et al., 1994; Franks, Bosanquet, Brown, Straub, Harper, & Ruckley, 1995; Franks, Bosanquet, Connolly, Oldroyd, Moffatt, Greenhaugh, & McCollum, 1995; ICWM, 1994; Pecoraro, Ahroni, Boyko, & Stensel, 1991; Phillips et al., 1994). The repercussions of a chronic wound such as a leg ulcer can ultimately result in personal financial problems, job loss, lifestyle and role changes, and negative self-concept and body image (Fowler, 1991; Franks et al., 1994; ICWM, 1994; Phillips et al., 1994).

Usual treatments for venous leg ulcers include the use of moist environment dressings, compression therapy, and minimizing periods of standing and sitting with the legs in a dependent position (Colerideg-Smith, Sarvin, Hasty, & Scurr, 1990; Cordts et al., 1992; Friedman & Su, 1984; Hendricks & Swallow, 1985; Kitka et al., 1988; Kolari, Pekanmaki, & Pohjola, 1988; Koone & Burton, 1989). These treatments result in healing of some ulcers; however, when ulcers become chronic, more aggressive therapy may be necessary. Additional adjunctive therapies used include electrical stimulation, split thickness skin grafts, topical application of growth factors, cultured epidermal autografts, and vascular surgical procedures (Cikrit, Nichols, & Silver, 1988; Jamieson, DeRose & Harris, 1990; Knighton et al., 1986; Limova & Grekin, 1990; Lundeburg, Eriksson &

Malm, 1992). These adjunctive therapies may be helpful; however, they also add costs.

Although it is known that nutritional status is important for healing in normally perfused tissue (Daly et al., 1972; Haydock & Hill, 1986; 1987; Windsor et al., 1988), the relationship between nutrition and perfusion in venous leg ulcers has not been adequately explored. If it were known that nutrition was a problem in this population and that a unique relationship existed between nutrition and healing of vascular ulcers, then clinicians could develop interventions to mitigate impaired healing in patients with venous leg ulcers. Such interventions could also reduce wound infections, mitigate health care costs, and minimize human suffering caused by living with a chronic wound.

CHAPTER II

PHYSIOLOGIC BACKGROUND, REVIEW OF LITERATURE, AND CONCEPTUAL FRAMEWORK

Physiologic Background

Pathophysiology of Venous Leg Ulcers

Lower extremity vascular ulcers may be due to arterial insufficiency, venous insufficiency, or a mixture of both. Although the pathogenesis of these types of vascular ulcers is different, the underlying problem preventing healing is impaired perfusion. Ultimately, the poor perfusion decreases delivery of oxygen, immune substances and nutrients to the tissue, prevents removal of metabolic end products, and impairs wound healing.

Venous insufficiency is the most prevalent etiology of chronic leg ulceration (Burton, 1994; Callam et al., 1987; Falanga et al., 1987; Nelzen, Bergqvist, & Lindhagen, 1991; 1992; van Rijswijk & the Multicenter Leg Ulcer Study Group, 1993). However, data also indicate that many patients with chronic leg ulcers demonstrate signs of coexistent arterial insufficiency and diabetes (Callam et al., 1987; Nelzen et al., 1992; Sindrup, Avnstorp, Tonnesen, Kristensen, 1987; van Rijswijk et al., 1993).

Normally, venous blood in the legs collects in the deep venous system and is returned to the heart via a combination of calf muscle contraction, one-way valves, and the negative intrathoracic pressure associated with respiration. Calf pump failure is considered the initiating event for venous insufficiency and may be caused by deep vein thrombosis, deep or communicating vein incompetence, superficial vein regurgitation and/or calf muscle failure (Browse, 1988). As the calf pump fails, intravascular

hydrostatic pressure increases, and serous fluid and erythrocytes move from the intravascular space to the interstitial space resulting in edema of the lower extremity. Eventually, the erythrocytes are broken down by enzymes releasing the pigment hemosiderin causing dark brown dermal staining. Over time, a condition termed "lipodermatosclerosis" occurs in which normal skin and subcutaneous tissue are replaced by fibrous tissue. Ultimately, this results in thick, hardened, contracted skin at the "gaiter" or ankle area.

Although it is known that the hemodynamic abnormalities present in venous insufficiency are responsible for ulceration, the exact pathogenic steps remain unknown. Previously entertained hypotheses which have been discarded include Homans' (1917) stagnant blood theory and the presence of arteriovenous connections (Haimovici, Steinman, & Caplan, 1966). Currently popular hypotheses include the fibrin cuff theory, leukocyte trapping, the "trap" hypothesis, and reperfusion injury (Browse & Burnand, 1982; Coleridge-Smith, Thomas, Scurr, & Dormandy, 1988; Falanga & Eaglstein, 1993; Greenwood et al., 1995).

Fibrin Cuff Theory. The fibrin cuff theory proposes that with increased venous hypertension, the body responds by developing an increased number of capillaries in the gaiter area of the leg. Data indicate that even in legs without ulcers, persistent venous hypertension results in increased numbers of capillary loops within the traditional ulcer bearing area (i.e., medial malleolar area) (Burnand, Whimster, Clemenson, Thomas, & Browse, 1981; Burnand, Clemenson, Whimster, Gaunt, & Browse, 1982). Furthermore, there is a significant correlation between the number of capillaries in the skin of a patient with venous disease and the fall in foot vein pressure during exercise (Burnand et al.,

1981). Fall in foot vein pressure is an indicator of the degree of valvular incompetence.

Fibrinogen leaks from the capillaries into the interstitial space due to capillary hypertension. The fibrinogen is converted into fibrin which forms circumferential cuffs around the capillaries. Histological examination of tissue in lipodermatosclerotic legs of patients with venous disease shows the presence of pericapillary fibrin (Burnand, Clemenson, Morland, Jarrett, & Browse, 1980; Burnand, Whimster, Naidoo, & Browse, 1982; Falanga et al., 1987). Interestingly, however, pericapillary fibrin deposits also have been demonstrated in patients with venous hypertension but without any clinical skin changes or ulceration (Stacey, Burnand, Bhogal, & Pattison, 1988). Similarly, patients with venous disease have been shown to have markedly elevated levels of total fibrin-related antigen and D-dimer (the total degradation product of cross-linked fibrin); thus supporting the theory that patients with venous disease have enhanced fibrin formation (Falanga, Kruskal, & Franks, 1991).

Pericapillary fibrin deposits are proposed to cause a diffusion barrier, thus, inhibiting the transfer of oxygen and nutrients from the capillaries to the tissue (Browse & Burnand, 1982). This concept is supported by findings that a sheet of commercial fibrin allows carbon dioxide exchange but impairs oxygen exchange (Burnand, Whimster, Naidoo, & Browse, 1982). Similarly, Hopkins et al. (1987) found that oxygen extraction was decreased in patients with venous leg ulcers. Furthermore, although classic work indicated that the venous blood drawn from the femoral vein of patients with venous disease had elevated oxygen levels (Blalock, 1929), transcutaneous tissue oxygen levels ($TcPO_2$) in the gaiter area of both previously ulcerated limbs and currently ulcerated limbs were significantly lower (range 12.9 to 46 mm Hg) than normals (range 59 to 62 mm Hg)

(Clyne, Ramsden, Chant, & Webster, 1985; Mani, Gorman, & White, 1986; Mani & White, 1988; Mani, White, Barrett, & Weaver, 1989; Stacey, Burnand, Pattison, Thomas, & Layer, 1987; Partsch, 1984;).

In addition to the fibrin accumulation around capillaries, data from both animal studies and humans with venous ulcers have demonstrated that interstitial tissue fibrinolysis is depressed in extremities with venous hypertension (Northeast, Gajrag, Burnand, & Browse, 1989; Gajrag & Browse, 1989). In particular, fibrin clearance and tissue plasminogen activator levels were both depressed. A preliminary report indicates that treatment with a fibrinolytic enhancer such as stanozolol decreases lipodermatosclerosis in patients with lower extremity venous disease (Browse, Jarrett, Morland, & Burnand, 1977; Burnand, et al., 1980). Specific changes observed included decreased fibrin deposition, induration, inflammation, tenderness, pigmentation, and pain.

Further support for the fibrin cuff theory arises from the tissue oximetry literature. Transcutaneous tissue oxygen ($TcPO_2$) levels are significantly lower at the ulcer site (range 5 to 46 mm Hg) in the supine position compared to the control chest site (range 46 to 70 mm Hg) in patients with venous leg ulcers (Nemeth, Falanga, Alstadt, & Eaglstein, 1989; Nemeth, Eaglstein, & Falanga, 1989; Clyne, et al., 1985; Falanga et al., 1987; Mani et al., 1986; 1989; Mani & White, 1986; Partsch, 1984; Stacey et al., 1987). Interestingly, however, Falanga and colleagues (1987) and Clyne et al. (1985) found that $TcPO_2$ levels did not change (</=1 mm Hg) in response to position changes or to the reduction of leg edema (Nemeth et al., 1989). In contrast, Dodd, Gaylarde, and Sarkay (1985) found that patients with venous ulcers (mean 17.3 mm Hg) had higher mean $TcPO_2$ levels than controls (8.8 mm Hg). These data, however, are far below those of other reports.

Furthermore, there was a fall in $TcPO_2$ upon changing to standing position. This was presumed to be a result of normal reflex vasoconstrictor responses. Dodd and colleagues (1985) attribute these differences to the use of 37^0 C electrode rather than a 45^0 C electrode. Specifically, the use of a cooler electrode prevents maximal dilatation of the capillaries and restricts the diffusion of O_2 through the skin keratin, thus allowing them to vasoactively respond to position changes.

Despite data supporting the fibrin cuff theory, there is also evidence against it. First, fibrin cuffs have been found to be discontinuous around the dermal capillary (Paredes, Tonneson, Falanga, Eaglstein, & Clark, 1990), rendering the notion of the fibrin cuffs being a barrier less likely. Furthermore, Cheatle et al. (1990) demonstrated that fibrin cuffs failed to prevent back diffusion of Xenon gas, thus casting doubts of the ability of the cuffs to actually deter oxygen diffusion. In addition, subsequent histochemical studies indicate that the cuffs around the capillaries primarily consist of macromolecules other than fibrin such as extracellular matrix proteins (Hennick et al., 1992). In addition, work by Falanga et al. (1992) found that although mean periulcer $TcPO_2$ values were lower than the mean chest reference $TcPO_2$ levels, there was no correlation between the degree of pericapillary fibrin and periulcer $TcPO_2$. Finally, researchers have shown that inhalation of oxygen produces an increase in the periulcer $TcPO_2$ in patients with leg ulcers (Falanga et al., 1987; Partsch, 1984), showing that the cuffs do not entirely block diffusion. These observations suggest that, although local tissue hypoxia is present in patients with venous ulcers, it is not specific to a fibrin diffusion barrier but related to other abnormalities in the microcirculation.

White Blood Cell Adhesion (Leukocyte Trapping). White blood cell trapping is

another hypothesis that has been proposed to explain the mechanism involved in the trophic skin changes seen in patients with venous ulcers. Observations published by Moysis and colleagues (1987) found that white blood cells accumulated in the dependent feet of subjects during prolonged venous stasis. In an effort to investigate this phenomenon further, Thomas and colleagues (1988) studied the packed cell volume of the saphenous vein in patients after sitting and lying supine. Patients with venous hypertension demonstrated a significant increase in packed cell volume after sixty minutes sitting when compared to baseline. Similarly, patients with venous hypertension had a significantly larger decrease in the ratio of WBCs to RBCs after sixty minutes of sitting when compared to baseline supine readings. These changes were not observed in normals or patients with simple varicose veins.

Thomas and colleagues (1988) suggest that the repeated accumulation of WBCs in capillaries and venules of the foot may contribute to trophic changes of the skin by causing local tissue ischemia. Local ischemia may activate hypoxic WBCs, thus stimulating the inflammatory response. Further support for this theory was demonstrated in a study by Coleridge-Smith and colleagues (1988) in which patients with venous hypertension had significantly fewer capillary loops/mm^2 after sitting with their legs dependent compared to the number of capillary loop/mm^2 when lying supine. They proposed that trapping of WBCs in the capillaries is responsible for the decrease in the number of capillaries/mm^2. Since capillaries are occluded with WBCs, blood is diverted to nonoccluded capillaries while the affected area becomes ischemic. Ischemia may result in a cycle of WBC trapping, activation, and endothelial damage (Nash, Thomas, & Dormandy, 1988). The endothelial damage caused by the oxygen metabolites and eicosonoid byproducts may

facilitate the capillary leakiness that allows fibrinogen to escape into the tissue and form the fibrin cuffs around the capillaries.

"Trap" Hypothesis. Falanga and Eaglstein (1993) have recently proposed another pathogenesis for venous ulceration. Specifically, they suggest that fibrin and other macromolecules leak into the dermis as a result of venous hypertension. These macromolecules then bind to or "trap" growth factors and other stimulating substances which then are no longer available for wound healing. The authors acknowledge that the macromolecules may not bind all the growth factors, but rather simply alter the pattern or sequence of their action. Data which support this hypothesis include: 1) the presence of fibrin within venous ulcers (Burnand et al., 1982), 2) presence of transforming growth factor Beta and tumor necrosis factors in venous ulcers (Higley et al., 1992), and 3) venous ulcer wound fluid fails to stimulate fibroblasts, endothelial cells and keratinocytes and actually inhibits some cell adhesion proteins indicating a hostile environment (Bucalo, Eaglstein, & Falanga, 1993; Grinnell, Ho, & Wysocki, 1992; Wysocki & Grinnell, 1990; Wysocki, Staiano-Coico, & Grinnell, 1993).

Ischemia-Reperfusion Injury. Other researchers suggest that mechanical WBC entrapment and capillary occlusion is not sufficient to cause skin breakdown and, in fact, leukocyte, platelet, and endothelial cell activation play an important role in venous ulcer pathogenesis (Greenwood et al., 1995). In a series of studies, Greenwood and colleagues (1995) have demonstrated that patients with venous ulcers have higher levels of neutrophil-derived oxygen free radicals than controls after 30 minutes of lower extremity dependency and after 30 minutes of re-elevation to a supine position (i.e. re-perfusion). In addition, natural plasma free radical scavengers (glutathione peroxidase in this study) are

significantly lower in patients with venous ulcers than controls. Further work by Greenwood and colleagues (1995) found that gaiter area capillary oxygen tension was significantly lower in venous insufficiency patients than age-matched normals at rest (legs supine), after 30 minutes of extremity dependency, and after 30 minutes of re-elevation of the extremity. Similarly, superoxide release increased significantly with dependency and was sustained throughout the re-elevation period. Thromboxane B_2 also rose significantly in venous ulcer patients with dependency and rose even further with re-elevation.

Collectively, these data support the hypothesis that a chronic, low grade ischemia reperfusion injury occurs in chronic venous ulcers. The ischemic phases occur when the patients sit or stand with the legs dependent. The reperfusion phases occur during exercise or when the feet are elevated. The cycle of ischemia and reperfusion is then repeated day after day and year after year.

Ischemia, Reperfusion, and Metabolites. While ischemia has been classically considered an interruption of blood flow that leads to oxygen debt, more recent attention has been focused on the buildup of metabolic end products associated with ischemia and their interaction with the tissue hypoxia that accompanies ischemia. There is increasing evidence that ischemia initiates a cascade of reactions that includes the production of inflammatory mediators, mechanical capillary plugging by leukocytes, and oxidant formation that lead to tissue injury (Engler, 1989; Ernst et al., 1987). After periods of ischemia and subsequent restoration of blood flow, tissue damage occurs and continues into the post-reperfusion period.

When blood flow to tissue slows or stops, the diminished oxygen and substrate delivery causes a reduction in oxidative phosphorylation. Anaerobic glycolysis decreases

the amount of adenosine triphosphate (ATP) that one mole of glucose produces from 36 (or 38) to 2 molecules. The lack of ATP disrupts the ion balance of the cells causing an influx of sodium, calcium (Ca^{++}), and water with subsequent cell swelling. Increased lactate production from anaerobic glycolysis lowers the cellular pH, denatures protein, and precipitates organelle and cell membrane leakiness. The increased Ca^{++} activates proteases that catalyze the conversion of hypoxanthine to xanthine and the cell releases highly reactive oxidants that further damage the microcirculation and cell membranes (West, 1986; Weiss, 1986). Eventually, lysis of myocytes and endothelial cells occurs as well as rupture of the organelles such as the mitochondria, lysosomes, and endoplasmic reticulum (West, 1986; Weiss, 1986; Carden & Korthius, 1989). The inflammatory response is subsequently initiated and leukocytes accumulate in the area.

The increased intracellular Ca^{++} activates phospholipases that attack the phospholipid structures in the cell and release arachidonic acid. Free arachidonic acid is then metabolized by two divergent pathways: the cyclooxygenase pathway and the lipoxygenase pathway (Heck, Foegh, & Ramwell, 1989; Payan & Shearn, 1989). The lipoxygenase pathway produces leukotrienes and HETE, while the cyclooxygenase pathway produces prostacycline, prostaglandins, and thromboxanes. These latter arachidonic acid byproducts further impair healing by stimulating membrane damage, edema, microthrombi, vasconstriction, and leukocytes activation.

As evidenced by the numerous theories presented, the exact mechanism of venous ulceration at the cellular level remains controversial. What does appear to be accepted is that the initiating events are valvular incompetency and venous hypertension (Burton, 1994). Furthermore, the immediate precipitating event that usually causes a venous ulcer

is superficial trauma (The Alexander House Group, 1992); and subsequent infection.

Physiology of Wound Healing

Healing is a complex dynamic process that results in restoration of anatomic continuity and function (Lazarus et al., 1994). Three major phases occur during the healing of acute and chronic wounds: the inflammatory phase, the proliferative phase, and the remodeling or maturation phase. The three major biological components of the wound healing response are the cells involved in healing, the extracellular matrix (ECM) manufactured by the cells, and the regulatory molecules which coordinate the process (Hopkinson, 1992a).

Inflammatory Phase. Upon injury to an area, local bleeding and trauma to the tissue allow von Willebrand factor (factor VIII), present in the plasma, to stimulate platelets so that they begin adhering to the exposed collagen of the vessel walls. The release of adenosine diphosphate (ADP), thromboxane A_2 (TXA_2) and platelet activating factor (PAF) activate platelets. Activated platelets extend pseudopodia and aggregate to form the platelet plug. Activated platelets also degranulate releasing ADP, calcium, and serotonin from the dense bodies. Serotonin causes immediate vasoconstriction to minimize blood loss. Additional von Willebrand factor and fibrinogen released from the alpha granules of the platelets promote further platelet aggregation and stimulate the clotting cascade. The clotting cascade proceeds in the usual fashion with the ultimate conversion of soluble fibrinogen to insoluble fibrin. In addition, the platelets also release at least several growth factors: platelet-derived growth factor (PDGF), epidermal growth factor (EGF), platelet-derived angiogenesis factor (PDAF), transforming growth factor-beta (TGF-beta), platelet factor- 4, insulin-like growth factor (IGF-1) and its binding

protein (BP-3) (Spencer, Tokunaga, & Hunt, 1993). These factors serve a variety of functions including attraction of white blood cells (WBCs), endothelial cells, smooth muscle cells, epidermal cells, and fibroblasts, as well as stimulation of new tissue formation (Knighton et al., 1989).

Tissue injury also stimulates both the complement and kinin cascades (Clark, 1988). Complement is a system of serum proteins responsible for directly lysing bacteria, opsonizing bacteria, and stimulating mast cells. Histamine, released from activated mast cells, and bradykinin promote capillary dilatation and increase vascular permeability. This results in increased localized circulation to the wound with subsequent leakage of red blood cells (RBCs), WBCs, and platelets into the area of the wounded tissue.

White blood cells provide the cellular response of the inflammatory phase of healing. The functions of the WBC in the wound include margination of the small vessels, migration to the injury site, release of chemotactic substances, phagocytosis of foreign substances, stimulation of angiogenesis, stimulation of growth factors, protein and collagen synthesis, and the reduction of oxygen to superoxide and other bacteriocidal compounds (Haslet & Hensen, 1988; Riches, 1988; Tonnesen, Worthen, & Johnston, 1988; Williams, 1988). Although all WBCs participate in wound healing, those thought to be integral are monocytes and lymphocytes (Barbul, 1990; Diegelman et al., 1987; Simpson & Ross, 1972). Neutrophils are mainly responsible to resist infection (Leibovich & Ross, 1975; 1976).

Polymorphonuclear neutrophils (PMNs) are the first to arrive at the site of injury and predominate for two to three days (Diegelmann et al., 1987; Kanzler et al., 1986). Platelet-derived 12-hydroxyeicosatetraenoic acid (12-HETE), complement 5_a ($C5_a$), and

leukotriene B_4 (LTB_4) enhance leukocyte chemotaxis (Kanzler et al., 1986; Williams, 1988). The leukocytes leave the permeable capillaries by passing between endothelial cells, enter the tissue, and, in cooperation with the aggregating platelets, release proteolytic enzymes, oxidants, and chemotactic substances (Tonneson et al., 1988; Williams, 1988). Foreign substances are marked by complement, thus enabling PMNs to recognize them. Neutrophils then form a primary line of defense against infection by initiating bacterial ingestion utilizing the processes of degranulation and microbial killing (Kanzler et al, 1986; Falcone & Caldwell, 1990; Tonneson et al., 1988).

Monocytes, attracted by complement 5_a, fibrin degradation products and TGF-beta, are the next prominent leukocytes attracted to the wounded area and they appear in the wound by approximately day five (Kanzler et al., 1986; Knighton, et al., 1989; Leibovich & Ross, 1975). Once a monocyte enters an area of inflammation, it is activated by foreign substances and hypoxia to become a macrophage and enters the injured tissue. Activated macrophages are aggressive phagocytic and bacteriocidal cells. Macrophages act to: 1) debride injured tissue, 2) process macromolecules into useful amino acids and sugars, 3) attract more macrophages, 4) secrete growth factors to stimulate fibroblast formation and activation, 5) secrete factors to stimulate angiogenesis, 6) secrete lactate which in turn stimulates collagen synthesis by fibroblasts, and secretion of vascular endothelial growth factor (VEGF) (Constant, Hunt, Zabel, Yuan, Scheuenstuhl, Suh, & Hussain, in press; Hunt & VanWinkle, 1979; Diegelmann et al., 1987; Riches, 1988: Leibovich & Ross, 1976; Thakral, Goodson, & Hunt, 1979).

Lymphocytes, the third key leukocyte to populate the wound, increase in numbers by day six (Diegelman et al., 1987; Kanzler et al., 1986; Barbul, 1990). Lymphocytes

synthesize two important lymphokines, macrophage migration inhibition factor (MIF) and macrophage activation factor (MAF) which attract and activate macrophages (Kanzler et al, 1986). Lymphs also release chemotactic factors for PMNs, basophils, eosinophils and other lymphocytes (Kanzler et al., 1986). Researchers have demonstrated that the lymphocyte releases transforming growth factor- Beta, a potent fibrogenic factor with secondary angiogenic and chemotactic properties (Kehrl et al., 1986; Sporn, Roberts, Wakefield, & Assoian, 1986). It should be noted, however, that mechanistic redundancy is a characteristic of wound healing, and many factors have similar functions.

Proliferative Phase. The formation of new tissue begins during the proliferative phase. Granulation tissue consists of macrophages, fibroblasts, and neovasculature imbedded in a loose matrix of collagen, fibronectin, and hyaluronic acid (Clark, 1988). According to Mays (1992), granulation tissue may be considered a soluble temporary extracellular matrix (ECM). Collagen synthesis and angiogenesis occur simultaneously in an interdependent manner. However, for the purposes of clarity, they are discussed separately.

The wound edges and surface are ischemic due to the interruption of the local vasculature, and healing does not progress until perfusion is restored. Angiogenesis, the growth of new vasculature, begins early after wounding and occurs from the existing intact vessels. Work by Knighton et al. (1983) indicates that hypoxia stimulates macrophages to secrete angiogenic factors. Although traditionally the lactate within the wound has been considered to be secondary to tissue hypoxia, data suggest that increasing wound oxygenation does not decrease wound lactate (Hunt, Conolly, Aronson, & Goldstein, 1978). Lactate occurs within the wound as the result of WBC metabolism,

irrespective of tissue hypoxia (Hussain, Ghani & Hunt, 1989). In response to macrophage and platelet factors such as tumor necrosis factor-alpha (TNF-alpha) and TGF-alpha, activated endothelial cells break down the basal lamina and migrate through the gap in the direction of the wound (Asmussen & Sollner, 1993). These endothelial cells form what is referred to as vascular buds. The endothelial cells multiply and form tubular structures in the direction of the hypoxic, acidotic wound. The tubular structures grow into the wound until complete loops are formed to join arterioles to venules.

The formation of new connective tissue closely follows angiogenesis. Fibroblasts arise from undifferentiated mesenchymal cells that migrate to the wound a few days after injury. Data indicate that tissue oxygen tensions of between 20 and 30 mm Hg are necessary for fibroblast proliferation (Niinikoski, Hunt, & Dunphy, 1972). The fibroblasts proceed from the wound margins into the wounded area and initially synthesize a gel-like ECM composed primarily of fibronectin and hyaluronic acid which provide a matrix for collagen fiber deposition and cell migration (Hopkinson, 1992a). Fibroblasts secrete additional components of the ECM including elastin fibers, proteoglycans, basement membrane molecules, integrins, and the collagen family of proteins. The replacement of nonsulfated glycosaminoglycans like hyaluronic acid by proteoglycans provides a more stable and resilient matrix (Daly, 1992).

Collagen is a fibrous protein whose precursors are synthesized by fibroblasts. Lactate and other factors which lower NAD^+ activate collagen synthesis (Hussain, Ghani, & Hunt, 1989). Fibroblasts synthesize three polypeptide chains which aggregate into a triple helix called procollagen. Procollagens undergo cleavage and then are called tropocollagens. Tropocollagen chains are twisted together in helical formation to form

collagen fibrils. Collagen fibrils further combine to form collagen fibers. Molecules cross-link intramolecularly and intermolecularly to provide strength. The hydroxylation of lysine and glycine is dependent upon vitamin C, iron, copper, lactate, dissolved oxygen, and alpha-ketoglutarate (Hunt & VanWinkle, 1979). Data indicate that TGF-beta and IGF-1 may be responsible for increasing collagen production and ECM components (Perry et al., 1993; Mustoe et al., 1990; Spencer et al., 1993).

The major collagen subtypes involved in cutaneous wound healing are Type I and III collagen. Type III collagen, found in embryonic connective tissue, is laid down initially in dermal wounds and forms the early matrix in healing (Hunt & Van Winkle, 1979). As the wound matures, type III collagen is replaced by type I collagen which functions to provide tensile strength and cell adhesion sites in the ECM (Hopkinson, 1992b). Normal skin also contains type IV, V, VI, and VII collagen and elastin but these are not well replaced in scar tissue.

Contraction is the inward movement of the edges of an open wound secondary to forces generated within the wound (Van Winkle, 1967). Fibroblasts contain contractile elements composed of actinomyosin microfilaments which allow them to draw together, similar to smooth muscle cells. Then, contractile fibers pull collagen fibers together thus drawing wound edges together. In open wounds healing by secondary intention, the formation of granulation tissue occurs concurrently with contraction. The degree of contraction is limited by the mobility of the surrounding tissue. For example, contraction is not effective in the foot and ankle where skin is naturally tight.

Epithelialization is the process whereby the wound is finally covered by epithelial tissue that provides a protective barrier and prevents fluid and electrolyte losses.

Epidermal basal cells flatten, lose their intracellular attachments, and form actin filaments in order to provide the ability to migrate (Daly, 1992). Epithelial cells migrate from the wound edges and move over one another until the area is covered by epidermis. When the layer is complete, the cells divide, forming additional layers of epithelium. In partial thickness wounds, new epithelial cells also migrate from hair follicles, sebaceous glands, and sweat glands. In open wounds, epithelialization is delayed until a bed of granulation tissue is established. Various epidermal growth factors released from macrophages and platelets are thought to initiate epithelialization (Alverez et al., 1983; Brown, Schultz, & Brightwell, 1984).

Remodeling or Maturation Phase. The remodeling, or maturation, phase begins approximately 21 days after injury and may continue up to 2 years. Changes in the form, bulk, and strength of the scar occur in this phase. Fibronectin is removed and the ECM loses proteoglycans and water. Remodeling involves the simultaneous lysis and removal of collagen as well as its synthesis. Increased collagenase activity is present to breakdown excess collagen fibers (Orgill & Demling, 1988). In addition, type I and III collagen are deposited in a more orderly manner to provide increased strength to the scar (Mays, 1992). However, even at optimal maturation, a scar only has 80% of the breaking strength of the original tissue.

Alterations in Healing Seen in Chronic Wounds. The normal repair process described above has been established by studying acute surgical and traumatic wounds. Healing of chronic wounds is by definition impaired, and therefore, dissimilar in several ways from acute wound healing. Although the study of healing processes in chronic wounds is currently of great interest in the literature (Baxter, 1994; Hunt, 1991; Krasner,

1990; Falanga, 1993), it is poorly understood.

Researchers have, however, revealed some of the common alterations that occur in the chronic wound. In chronic wounds the inflammatory phase of healing is prolonged as evidenced by premature leukocyte activation (Moelleken et al., 1991), impaired leukocyte diapedesis & chemotaxis (Goldman et al., 1990; Sank, Chi, Shima, Reich, & Martin, 1989) increased infection (Ahn & Mustoe, 1990; Constantine & Bolton, 1986), and prolonged inflammation (Baxter, 1994). These impairments may be related to recurrent ischemia and reperfusion (Greenwood et al., 1995).

The proliferative phase also does not progress normally in a chronic wound. Granulation and epithelialization are also impaired (Ahn & Mustoe, 1990; Sank et al., 1989). Chronic wound fluid decreases fibroblast, endothelial, and keratinocyte proliferation (Bucalo et al., 1993). Chronic wound fluid also contains activated collagenases which may break down collagen within the wound (Grinnell et al., 1992; Wysocki et al., 1993). It is also hypothesized that fibrin within the chronic wound and surrounding tissue may actually down regulate collagen synthesis (Falanga et al., 1995). Although there is evidence of increased mitotic activity and the epidermis is thickened at the edge of chronic wounds (Adair, 1977), epithelialization does not occur normally. One proposed mechanism to explain altered epithelialization is a lack of adhesion proteins within the wound (Wysocki et al., 1993).

Delayed contraction is also noted in chronic wounds. Grinnell and colleagues (1992) have proposed that the degradation of adhesion proteins such as fibronectin and vitronectin precipitate the delay in contraction. A limited amount of research suggests that calcium may play a fundamental role in the pathogenesis of chronic, ischemia wounds

(Lew, Woiheim, Waldvogen, & Pozzan, 1984; Jaconi et al., 1988; Sank et al., 1989). Additional information is needed regarding the physiologic alterations that occur within chronic wounds that deter the healing process.

Physiology of Oxygen Transport and Tissue Perfusion

Transportation of oxygen (O_2) from the external environment into the tissues is critical for most bodily functions including wound healing. Oxygen is carried in the blood either dissolved in the plasma or bound to hemoglobin (Hgb). In comparison to the amount of O_2 carried by Hgb, the amount of dissolved O_2 in the plasma is quite small. For example, assuming a partial pressure of O_2 of 100 mm Hg, only 0.3 ml of O_2 is dissolved in each 100 ml of arterial blood (Mines, 1993). In contrast, each gram of Hgb may bind with 1.34 ml of oxygen. Therefore, under normal conditions, an adult with 15 grams of Hgb per 100 ml of blood will bind and carry 20 ml of O_2 per 100 ml of arterial blood. The partial pressure of carbon dioxide (CO_2), blood pH, body temperature, presence of 2,3 diphosphoglycerate, and an abnormal O_2 structure may influence Hgb's affinity for O_2 (Mines, 1993).

Air is drawn into the lungs during inspiration, however, the barometric pressure, fraction of inspired O_2, and lung function ultimately will determine the partial pressure of the alveolar O_2. The partial pressure of arterial O_2 is then a function of the partial pressure of the alveolar O_2. The partial pressure of arterial O_2, arterial oxygen saturation, and total amount of serum Hgb are the final determinants of arterial O_2 content. The arterial O_2 content is defined as the total amount of O_2 dissolved in the plasma plus that bound to Hgb.

A variety of factors definitively determine tissue oxygenation. These include

arterial O_2 content, peripheral perfusion (flow), the arterial-cellular oxygen gradient, and cellular O_2 consumption. The movement of O_2 from the plasma to the tissue depends on three factors: 1) the partial pressure of O_2 dissolved in the plasma, 2) the partial pressure of O_2 dissolved in the cells, and 3) the cellular O_2 consumption. In order for O_2 to diffuse down a concentration gradient from the plasma to the tissue, the partial pressure of O_2 in the plasma must be greater than that in the tissues. During periods of metabolic activity, cellular needs increase and O_2 consumption rises. With increased O_2 consumption, the arterial-cellular O_2 gradient becomes steeper and more O_2 diffuses from the plasma to the tissue. The adequacy of the partial pressure of arterial O_2 is usually the limiting factor of the gradient. The partial pressure of O_2 in the plasma is supported by the reserve of O_2 carried by Hgb. This is important in tissues which consume large amounts of oxygen and less important in tissues, like wounds, which consume less (Figure 2-1).

Tissue perfusion and oxygenation are intricately connected. The major determinants of perfusion are fluid volume, cardiac output (CO), and peripheral vascular resistance (PVR). Fluid volume, CO, and PVR also have interrelationships. Cardiac output is a function of heart rate and stroke volume. Preload, afterload, and myocardial contractility are the three factors controlling stroke volume. Preload is affected by both fluid volume and afterload. Starling's law portrays the association between myocardial contractility and preload. Peripheral vascular resistance has a significant influence on afterload, and is determined by local tissue demands, autonomic nervous system stimulation, and circulating level of catecholamines.

In the majority of the body tissues, perfusion is controlled by local regulation. In skin and subcutaneous tissue (SQ), however, perfusion is primarily regulated by

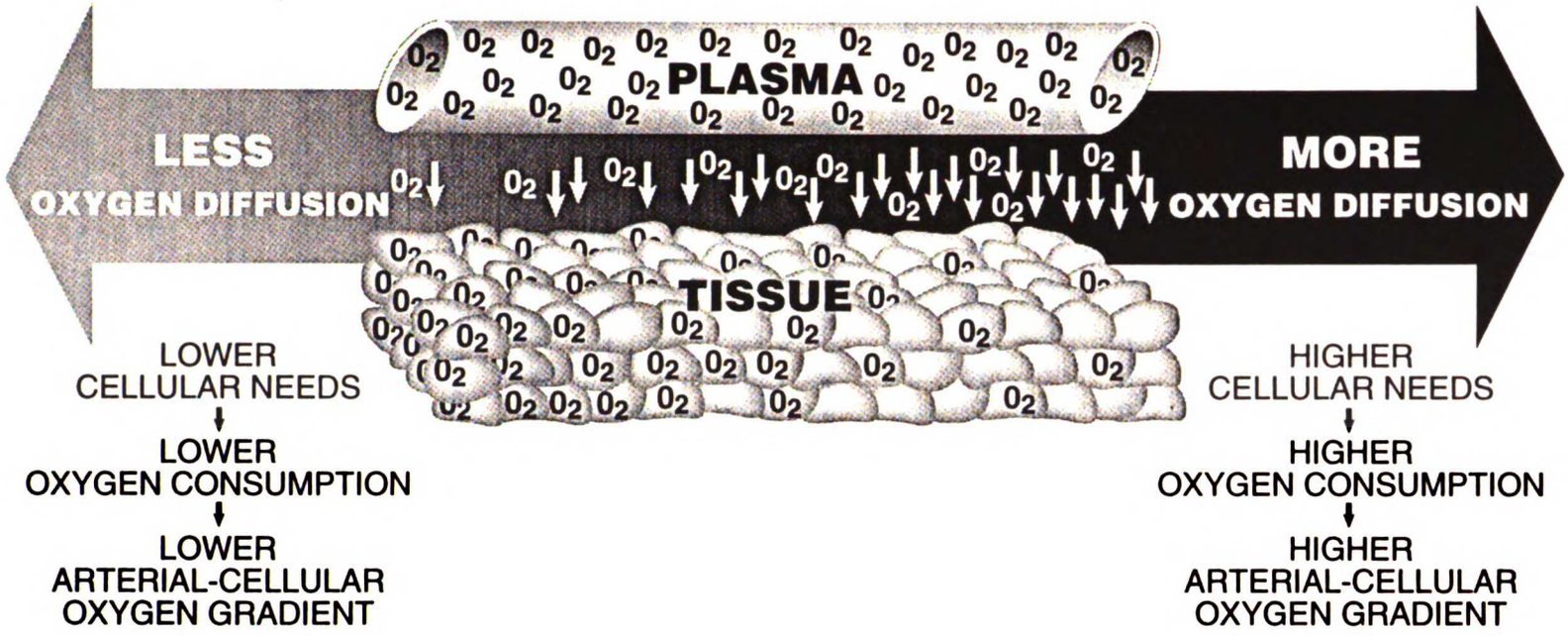


Figure 2-1. Factors contributing to the tissue partial pressure of oxygen. (From Wipke-Tevis, D.D. (1995). Subcutaneous tissue oximetry: Implications for wound healing and monitoring critically ill patients. Critical Care Clinics of North America, 7(2), 275-285; reprinted with permission).

sympathetic nervous system mechanisms (Guyton, 1986). The vasculature that supplys the skin and SQ tissue is under neural and humoral adrenergic control. The vessels are supplied directly with sympathetic vasoconstrictor fibers that secrete norepinephrine in response to neural stimulation. These vessels are also extremely sensitive to circulating catecholamines released from the adrenal medulla. The resulting vasoconstriction will decrease SQ and cutaneous perfusion and ultimately lower tissue oxygenation. This is particularly important in the extremities.

Local control mechanisms include metabolic regulation (autoregulation), myogenic control, and local changes in temperature. Local metabolic regulation plays only a small role in skin and SQ tissue blood flow control because blood flow is typically in excess of the metabolic needs of the tissue (Guyton, 1986). The skin and SQ tissue are quite sensitive to heat and cold, with resultant vasoconstriction or vasodilatation of the skin blood vessels in response to temperature changes.

Physiology of Energy Metabolism

In order to maintain physiologic functions, the human body continuously expends energy. The energy is used for three major purposes: 1) to maintain chemical and electrochemical gradients across cellular membranes for the transport of molecules and ions, 2) for the synthesis of macromolecules and biomolecules, and 3) for mechanical work accomplished by muscle contraction (Stryer, 1988). Energy also is lost as heat (Schutz & Jequier, 1994).

Adenosine triphosphate (ATP) is the immediate source of energy for cellular processes. It is energy metabolism that actually maintains the constant of supply of ATP and glucose for the body. Storage molecules such as glycogen, fat, and protein are made

when nutrients are available, retrieved from storage, and converted to ATP and glucose when nutrients are not available. Multiple metabolic pathways exist within the body that interconnect glycogen, fat, and protein reserves in order to facilitate storage and retrieval of ATP and glucose.

Ultimately, however, metabolic energy is derived from the three major macronutrients: proteins, carbohyrates, and fats. Nutrients act as sources of energy by a three stage transformation (Stryer, 1988). In the first stage, macromolecules are hydrolyzed to simpler molecules. Proteins are cleaved to make amino acids, carbohydrates are broken down to monosaccharides, and fats are degraded into fatty acids and glycerol. In the second stage, these simpler molecules are broken down into two carbon fragments and linked to Co enzyme A to form acetyl CoA. In the third stage, acetyl CoA enters the Kreb's or Citric Acid cycle and the carbons of the acetyl are completely oxidized to carbon dioxide. Hydrogens derived from this process are transferred by NAD^+/NADH to the mitochondria and ultimately joined with O_2 to form H_2O.

For each acetyl group that is oxidized, four pairs of electrons are transferred to NAD^+ and FAD. The electrons are then transferred from NADH and $FADH_2$ to oxygen in the mitochondria. The flow of electrons leads to the pumping of protons across the inner mitochondrial membrane. This sets up an electrical gradient that is then used to synthesize ATP. The free energy liberated in the hydrolysis of ATP to adenosine diphosphate (ADP) is harnessed to drive reactions that require an input of free energy. Glycolysis also synthesizes ATP but not nearly as much as the citric acid cycle coupled with oxidative phosphorylation (2 ATP versus 36 or 38 ATP).

Within the human body, energy is utilized in the form of basal energy expenditure (BEE), voluntary activity, and the thermic effect of food. The BEE constitutes the largest portion of the total energy expenditure (TEE) and represents the energy used to sustain life processes and maintain body temperature within a 24 hour period by a person who is lying quietly in a comfortable temperature and environment (Mahan & Arlin, 1992). Factors affecting BEE include body size, age, growth, pregnancy, lactation, climate, illness, endocrine activity, and body composition. The BEE can be determined a number of ways, however, it has traditionally been calculated by either direct or indirect calorimetry (Schutz & Jequier, 1994).

When direct or indirect calorimetry is not practical, BEE requirements are commonly predicted for normal humans by the Harris-Benedict equation (Harris & Benedict, 1919) as depicted in Table 2-1. To utilize the equations, the current height in centimeters, weight in kilograms, and age in years must be known. A separate equation exists to determine the BEE for men and women. This is because the equations take into account the fact that proportionately women have more adipose tissue (which is less metabolically active) than men and, therefore, have a lower metabolic rate than men of the same weight and height (Mahan & Arlin, 1992). Similarly, the decrease in the BEE associated with aging is also taken into account. The BEE does not account for the thermic effect of food or the level of activity. The inclusion of an activity factor provides an approximation of energy required for maintenance. If an individual is depleted and anabolism is required or if an individual has increased metabolic needs caused by an illness and/or injury, an injury factor should be applied to the Harris-Benedict equation. The injury factors were developed based on work done by Long and colleagues (1979). It is

important, however, to remember that the Harris-Benedict equation with adjustments for activity and injury only provides an estimate of the TEE (Zeman, 1991).

Table 2-1. Modified Harris-Benedict equation for predicting BEE and TEE.

Men: BEE (kcal) = 66.5 + 13.75W + 5.0H - 6.78A

Women: BEE (kcal) = 665.1 + 9.56W + 1.85H - 4.68A

Maintenance TEE = BEE X Activity Factor

Anabolism TEE = BEE X Activity Factor X Injury Factor

Activity Factors:	**Injury Factors**:
Bedrest 1.2	Minor Surgery 1.2
Ambulatory 1.3	Skeletal Trauma 1.33
Normal Activity 1.5-1.75	Elective Surgery 1.44
Extremely Active 2.0	Major Sepsis 1.6-1.9
	Trauma + Steroids 1.88
	Severe Burns 2.1-2.5

W = weight in kilograms, H = height in centimeters, A = age, kcal = kilocalories

Review of Literature

Critical Nutrients for Wound Healing

A well-balanced diet with an adequate amount of calories is needed for tissue growth. All nutrients are required for healing but those considered especially important to healing include protein, carbohydrates, fats, vitamin C, vitamin A, iron, and zinc. However, the exact amounts of these nutrients required for optimal healing remain unknown. Nutritional deficiencies can interfere with the wound healing process at any point.

Proteins and Amino Acids. Proteins are basic components of all living organisms and are crucial for fluid balance, antibody, hormone, enzyme, and signaling functions, as

well as providing structural building blocks for new tissue. Adequate protein is required for neovascularization, fibroplasia, wound contraction, collagen synthesis, and optimal rate of granulation tissue formation (Modolin, Bevilacqua, Margarido, & Lima-Goncalves, 1985; Haydock & Hill, 1986; Rhodes, Fliegelman, & Panzer, 1942). Protein stores are not considered an energy source. However, in starved states, skeletal muscle proteins are hydrolyzed into amino acids and converted by the liver and kidney to glucose for energy.

The influence of specific amino acids in modulating wound healing has recently been explored. For example, glutathione, a potent free radical scavenger, has been utilized to prevent reperfusion injury in skin flaps by acting synergistically with vitamin C and E to prevent free radical formation (Ehrlichman et al., 1991).

Arginine has received the most attention in the recent wound healing literature. Although arginine is an important amino acid, it is not classified as an essential amino acid except during periods of growth and conditions that result in persistent inflammation (Myrvik, 1994; Barbul, 1986). Arginine stimulates the release of prolactin, insulin, glucagon and growth hormone, and is an essential component of polyamine and nucleic acid synthesis. Several researchers have examined the immunomodulating effects of arginine in animals and humans. They have found that arginine increases thymic size and cellularity, enhances lymphocyte proliferation, augments macrophage and natural killer cell lysis of tumor targets, and increases cytokine production (Barbul et al., 1978; Barbul et al., 1981; Reynolds et al., 1988; Daly et al., 1988; Daly et al., 1992; Nirgiotis, Hennessey, & Andrassy, 1991).

Currently, the effects of arginine on wound healing are being explored in both animals and humans. Using an animal model, Nirgiotis and colleagues (1991) found that

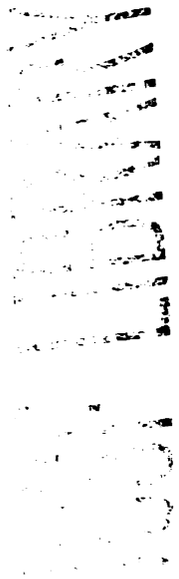
rats fed arginine demonstrated significantly higher serum albumin and protein, longer survival to bacterial challenge, improved wound bursting strength, and superior macrophage chemotaxis and killing than controls fed an arginine-free diet. Similarly, Daly and colleagues (1992) found that of 85 cancer patients undergoing upper gastrointestinal surgery randomized to receive an enteral diet with supplemental arginine, ribonucleic acid (RNA), and omega-3 fatty acids exhibited significantly fewer ($p=0.02$) infections and wound complications (11%) than those receiving standard enteral diet (37%). Furthermore, the mean length of stay in the hospital was significantly shorter ($p=0.01$) for the supplemented group (15.8 +/- 5.1 days) than the standard group (20.9 +/- 9.4 days).

Carbohydrates. Carbohydrates provide the primary fuel to meet the body's energy needs. Glucose, the building block of carbohydrates, provides the cellular fuel for nervous tissue, erythrocytes, fibroblasts, leukocytes, and promotes phagocytic activity necessary for the inflammatory phase of healing (Wolfe, 1992). Without the inflammatory process, resistance to infection is altered and collagen synthesis is impaired (Barbul, 1990; Diegelman et al., 1987; Simpson & Ross, 1972). Glycogen is the storage form for carbohydrates and in the absence of carbohydrates, liver and muscle glycogen provide the major energy source for glucose via glycogenolysis. However, the body glycogen reserves contain less than 12 hours of reserves (Anderson & Geil, 1994). Thus, after glycogen stores are utilized, the body will break down protein via gluconeogenesis or fat via lipolysis to use as fuel.

Fats and Fatty Acids. Fats also provide a source of cellular energy. Fats are important for wound healing as they are a component of cell membranes, and are used for cell processes, formation of prostaglandins, and arachidonic acid. Essential fatty acids

include linoleic and linolenic acids as well as omega-3 fatty acids. During periods of starvation, lipolysis releases free fatty acids and glycerol. Glycerol can be utilized for the formation of glucose, while free fatty acids may undergo direct oxidation and produce carbon dioxide or indirect oxidation for the formation of ketone bodies.

Current research suggests that omega-3 fatty acid supplementation may have important immunological effects for improving survival after burn injury, diminishing immunosuppression after transfusion, and reducing infection and wound complications (Daly et al., 1992). It appears that the enhanced immune function occurs when macrophages have incorporated significant amounts of omega-3 polyunsaturated fatty acids (Myrvik, 1994). They alter prostaglandin synthesis pathways to produce less dienoic and more trienoic prostaglandins. Monocyte functions such as phagocytosis, and superoxide and interleukin 1 (IL-1) production, may be modulated by means of changes in the phospholipid content and production of prostaglandin E_2 (Daly et al., 1992).



Vitamins and Minerals. In addition to macronutrients, micronutrients such as vitamin and minerals play an important role in wound healing. Vitamins are organic compounds found in nutritionally balanced diets. Vitamin A, a fat-soluble vitamin, is important for the inflammatory phase of healing, promotes epithelialization, granulation tissue formation, and collagen crosslinking (Ehrlich & Hunt, 1968; Seifter et al., 1975). Levels of vitamin A are thought to be depressed after burn or fracture injury and surgery, and bodily requirements are increased after such physiologic stressors (Williamson, 1992; Gottschlich & Warden, 1990).

For example, the skin incision breaking strength was significantly increased compared to controls when supplemental vitamin A was given to rats with femoral

fractures (Seifter et al., 1975). Similarly, Bark et al. (1984) found that supplemental vitamin A significantly increased the colon anastomotic and normal colon bursting strength ($p<0.01$) as well as the hydroxyproline accumulation ($p<0.01$) compared to normal control rats undergoing intestinal surgery. Vitamin A supplementation is particularly beneficial for wound healing in patients taking corticosteroids. Data from Ehrlich and Hunt (1968) indicate that supplemental vitamin A antagonizes some of the inhibitory effects of steroid hormones on the inflammatory process and accelerates rate of gain in tensile strength in the early stages of healing.

Vitamin C is a cofactor in collagen formation essential for the hydroxylation of proline and lysine to procollagen (Jacob, 1994). It is also critical for fibroblast replication and proper leukocyte functioning that include superoxide production, bacterial killing, and cell motility (Myrvik, 1994). Vitamin C deficiency results in underhydroxylated collagen, wound dehiscence, and increased capillary fragility (Ehrlichman et al., 1991).

Minerals are micro- and macroinorganic elements responsible for normal cellular functioning including cellular transport, signaling, and enzyme cofactors. Zinc is a cofactor for collagen formation, a component of many enzyme systems, and plays an important role in the oxidative burst of mononuclear phagocytes (Myrvik, 1994). Zinc deficiency decreases DNA and RNA polymerase activity and albumin synthesis (Fernandez-Madrid, Prasad, & Oberlas, 1973) and also impairs humoral and cellular immunity (Sandstead et al., 1982). During zinc deficiency there is a greater influx of neutrophils and monocytes which impairs wound healing via release of increased amounts of degradative enzymes, and a disruption of new tissue matrix components (Chvapil, 1980). Ultimately, zinc deficiency results in abnormal keratinization, disruption of

granulation tissue, impaired tensile strength and collagen synthesis due to altered glycine incorporation into collagen, and dehiscence (Elias & Chvapil, 1973; Powanda & Moyer, 1981).

Iron also is needed as a cofactor in the hydroxylation of proline and lysine in collagen formation. Iron enhances the bacteriocidal activity of leukocytes and is essential for O_2 transport. Many researcher have confirmed that O_2 is pivotal for fibroblast production, collagen synthesis, accumulation, and strength; angiogenesis, epithelialization, and prevention of wound infection (Hunt & Pai, 1972; Knighton et al., 1981; 1984; Niinikoski, 1980; Pai & Hunt, 1972).

Effects of Malnutrition on Healing of Surgical Wounds

Malnutrition may be characterized as either protein malnutrition (kwashiorkor), energy malnutrition (marasmus) or both. The most common presentation in the United States is protein malnutrition. Populations at risk for protein malnutrition include alcoholics and trauma victims. Energy malnutrition is more rare in our society, however, it does occur in certain high risk populations such as trauma victims, those living in poverty, and anorexics. Protein-energy malnutrition occurs mainly in patients who are chronically starved and then exposed to a stressor such as a disease process, that requires the utilization of proteins for the production of enzymes and antibodies.

As many as 50% of all hospitalized patients are malnourished (Jensen et al., 1982; Mullen et al., 1979; Reinhardt et al., 1980; Seltzer et al., 1981). Furthermore, these malnourished patients have increased morbidity and mortality. Populations at risk for malnutrition include those with difficulty obtaining or utilizing adequate nutrients, those with increased demands related to supply, and those with nutrient losses due to diarrhea,

emesis, or fistulas.

General Surgical Patients. Impairments in wound healing occur quite early in the course of protein-energy malnutrition, long before changes in anthropometrics and common biochemical indices can be detected (Ward et al., 1982; Haydock & Hill, 1986). For example, using an animal model, Ward and colleagues (1982) found that although 7 days of preoperative protein deficiency did not alter preoperative albumin and lymphocyte levels in rats, the postoperative colonic bursting strength and albumin levels were lower in the protein deficient rats than both the control rats ($p<0.01$) and the postoperatively refed rats ($p<0.005$). Windsor and colleagues (1988) observed that patients with inadequate preoperative food intake during the week before abdominal surgery produced significantly less hydroxyproline than patients with adequate preoperative intake ($p<0.005$), even though there was not a significant difference in overall body protein and fat stores between the groups. Windsor and colleagues (1988) concluded that preoperative food intake had more influence over the wound healing response than absolute losses of protein and fat for body stores. These findings are supported by work done by Goodson and colleagues (1987) which indicated a short-term decrease (<72 hours) in nutrient intake, as depicted by acute nausea and vomiting associated with appendicitis, resulted in 20% less hydroxyproline accumulation postoperatively ($p<0.02$) than cholecystectomy patients whose surgery was performed after the resolution of the acute phase of illness. The authors hypothesize that the difference in hydroxyproline accumulation may be related to the effects of acute starvation and decreased fluid volume. However, dietary intake was not actually measured in this study. Collectively, these data suggest that decreased preoperative food intake may significantly impair the ability of the body to produce

collagen for healing wounds.

In a series of studies, Haydock and colleagues examined malnutrition and wound healing in an animal model and in surgical patients using hydroxyproline deposition in Gortex tubes as a measure of wound healing response (Haydock & Hill, 1986; 1987; Haydock, Flint, Hyde, Reilly, Poole, & Hill, 1988). In a prospective, non-randomized, comparative study of surgical patients with varying degrees of malnutrition, Haydock and Hill (1986) found that the hydroxyproline deposition of normally nourished surgical patients was significantly more than both the mildly protein deficient patients (5 to 10% weight loss) ($p<0.01$) and the moderate to severely protein deficient patients (>10% weight loss) ($p<0.01$). Interestingly, however, there was no significant difference in hydroxyproline content between the mildly protein deficient and moderate to severely protein deficient groups even though the moderate to severely protein deficient patients had significantly more weight loss, and significantly lower body mass index, triceps skin fold, mid-arm circumference and serum albumin. Similarly, in another group of surgical patients, Haydock and Hill (1987) found that the hydroxyproline deposition was significantly less in malnourished patients ($p<0.01$), who were awaiting intravenous nutrition, than the normally nourished patients. In addition, in a prospective randomized study, Haydock and colleagues (1988) showed that rats receiving a protein deficient diet had decreased body weight, albumin levels, and less fibroblast and cellular invasion as well as hydroxyproline deposition in the Gortex tubing than rats fed normal chow.

Research findings from the general surgical literature suggest that insufficient nutrients impair healing by leading to prolonged inflammation, increased capillary fragility, and delayed fibroplasia and epithelialization (Daly, Vars, & Dudrick, 1972; Haydock &

Hill, 1986; 1987; Mullen et al., 1979; Windsor et al., 1988). Furthermore, patients who are malnourished or have inadequate intake prior to surgery display significantly less collagen synthesis and wound tensile strength than those who are nourished (Goodson et al., 1987; Windsor et al., 1988; Zaizen, Ford, Costin, & Atkinson, 1990; Haydock & Hill, 1986; 1987; Haydock et al., 1988).

It must be noted, however, that these studies have evaluated the effects of malnutrition or inadequate intake on healing of acute surgical wounds using either hydroxyproline deposition or wound bursting strength as the indicator of the wound healing response. These studies did not evaluate the effect of malnutrition on clinical outcomes of the patients such as delayed healing, wound dehiscence, or wound infection. Therefore, the clinical implications of mild malnutrition in general surgical patients remains unknown. Furthermore, some authors have questioned the relevance of hydroxyproline measurements as indicators of healing based on observations that tensile strength increases over time whereas collagen content usually remains stable or decreases (Albina, 1994). However, in the early phase of healing, tensile strength and collagen content are parallel.

Vascular Surgery Patients. The influence of nutritional status on the healing of acute surgical wounds has been explored in patients with peripheral arterial vascular disease undergoing revascularization and/or amputation procedures. Three prospective (Casey et al., 1983; Kay, Moreland, & Schmitter, 1987; Pedersen & Pedersen, 1992) and two retrospective (Dickhaut, Dehee, & Page, 1984; Jany & Burkus, 1988) descriptive, comparative studies examined nutritional status and healing. Results from these studies indicated the frequency of malnutrition in vascular surgery patients ranges between 22 to

72 percent. Significant predictors of impaired wound healing included serum albumin level < 3.0 g/dl (Casey et al., 1983), serum albumin < 3.5 g/dl (Dickhaut et al., 1984; Jany & Burkus, 1988; Kay et al., 1987), total lymphocyte count < 1500 cells/mm (Dickhaut et al., 1984; Jany & Burkus, 1988; Kay et al., 1987), serum transferrin level <150 mg/dl (Casey et al., 1983) and a low nutritional index score (Pedersen & Pedersen, 1992) (Table 2-2).

These data suggest that some patients with peripheral arterial vascular disease undergoing surgical procedures experience malnutrition, and those patients who are malnourished have a higher frequency of impaired wound healing as well as other complications. Several limitations should be noted within these studies. First, the construct of impaired wound healing was operationalized differently in each of these studies. Furthermore, there was no evaluation of the validity of the measure of wound healing. Consistent use of an established and valid measure of wound healing would facilitate comparisons as well as strengthen the findings of these studies.

Uncontrolled extraneous variables threaten the internal validity of these studies, especially the two retrospective studies. Potential uncontrolled extraneous variables include local wound care, perfusion status, diabetes/glucose control, surgical variables, and nutritional intake. Only two studies (Kay et al., 1987; Jany & Burkus, 1988) attempted to control some of these variables by standardizing surgical technique and postoperative wound care. Although each of the studies included a significant percentage of subjects with diabetes mellitus, no attempt was made to account for glucose levels. The literature suggests that maintenance of blood glucose levels < 200 mg/dl is necessary for optimal neutrophil activity necessary to prevent wound infection and promote normal

wound healing in diabetics (Rosenberg, 1990). Thus, without considering blood glucose levels, it is difficult to determine if impaired healing was related to nutritional status or elevated blood glucose.

Table 2-2. Summary of studies of malnutrition and healing in vascular surgery patients.

Authors	Subjects	Variables	Frequency of Malnutrition	Outcomes of Interest
Casey et al. (1983)	Peripheral Vascular Surgery Patients (n=79)	ALB, TRFN, TLC, Zinc, Anergy, Anthropometrics, Wound infection & Wound Complications	25% ALB < 3.0 g/dl, 32% TRFN < 150 mg/dl, 23% TLC < 1500/mm, 8% ZINC < 80 mg/dl	ALB >3.0 g/dl related to normal healing ($p<0.001$); TRFN< 150 mg/dl related to complicated healing ($p<0.01$)
Dickhaut et al. (1984)	Diabetic Syme's Amputation Patients (n=23)	ALB, TLC, Primary Healing of Amputation, Revision of Amputation	17% ALB < 3.5 g/dl, 4% TLC < 1500/mm, 48% Low ALB & TLC	Normal ALB & TLC related to primary healing ($p<0.0035$); Low ALB & TLC related to amputation revision ($p<0.0096$)
Kay et al. (1987)	Lower Extremity Amputation Patients (n=41)	ALB, TLC, Delayed Healing, Failed Amputation, Systemic Complications	2 % TLC < 1500/mm, 37% ALB < 3.5 g/dl, 22% Low ALB & TLC	**Malnourished group:** 16% Delayed healing, 16% Failed Amputation, 16% Systemic complications; **Nourished group:** 7% complication rate ($p<0.05$)
Jany & Burkus (1988)	Diabetic Syme's Amputation Patients (n=10)	TLC, HGB/HCT, ALB, TP, Primary Healing of Amputation, Revision of Amputation	40% ALB < 3.5 g/dl, 40% TP < 6.2 g/dl, 10% HCT < 32%, 40% TLC < 1500/mm;	50% of amputations failed & all those with amputation failures were malnourished
Pedersen & Pedersen (1992)	Lower Extremity Amputation Patients (n=47)	Nutritional Index (Weight loss, TSF, MAC, ALB, Pre-ALB), Impaired Healing, Medical Complications	72% malnourished	**Malnourished group:** More impaired healing ($p<0.05$), More medical complications ($p<0.05$), Longer Hospital Stay ($p<0.01$)

ALB=albumin, TRFN=transferrin, TLC=total lymphocyte count, HGB=hemoglobin, HCT=hematocrit, TP=total protein, TSF=triceps skin fold, MAC=mid-arm circumference, Pre-ALB=prealbumin

Malnutrition, Immune Status, and Wound Healing

Abnormalities of host defense and immunity are associated with malnutrition. Protein-calorie malnutrition has been correlated with both impaired humoral and cell-mediated immunity (Ek et al., 1990; Law, Dudrik, & Abdou, 1973; Powanda & Moyer, 1981). The resulting immunocompromised condition causes increased mortality and morbidity including impaired wound healing, development of pressure ulcers, and other medical complications (Daly, et al., 1992; Ek et al., 1990; Jensen et al., 1982; Mullen et al., 1979). For example, elderly, anergic hospitalized patients had, or developed significantly ($p<0.02$) more pressure ulcers (28%) than those elderly patients who were reactive (18.8%) (Ek et al., 1990). Immunocompromise inhibits phagocytosis, growth factors and angiogenesis, protein and collagen synthesis, and production of oxidants; thus it predisposes the individual to wound infection (Haslet & Hensen, 1988; Riches, 1988; Smith & Hartemink, 1988; Tonneson, et al., 1988; Williams, 1988).

During episodes of infection, nutrient intake is often inadequate. This may result in nutritional deficiencies (Greenhaugh & Gamelli, 1987). Mice with surgically created intra-abdominal abscesses decreased their food intake and had significantly less wound bursting strength ($p=0.0008$) than sham operation mice who resumed normal food consumption. There was not a significant difference in the wound bursting strength between the infected and nutrient-depleted sham operation mice. However, the wound bursting strength was significantly less in the infected mice compared to the sham controls ($p<0.05$) and in the nutrient-depleted sham mice compared to the sham controls ($p<0.01$). These data suggest that short-term nutritional deficits secondary to infection may be equally or more important in decreasing the host's ability to perform repair rather than

simply resistance to infection.

Malnutrition in Patients with Chronic Wounds

Malnutrition is also thought to exist in patients with chronic wounds. Some data suggest that patients either at risk for or with pressure ulcers are either malnourished or have inadequate energy and/or protein intake (Allman et al., 1986; Bergstrom & Braden, 1992; Bergstrom, Braden, & Milne, 1987; Breslow, Hallfrisch, & Goldberg, 1991; Breslow, Hallfrisch, Guy, Crawley, & Goldberg, 1993; Ek et al., 1990; Gilmore, Robinson, Posthauer, & Raymond, 1995). Similarly, limited research shows that subjects with open wounds in the home have been found to be either malnourished or have inadequate intake to support healing (Stotts & Whitney, 1990; Wipke-Tevis & Stotts, 1996).

Although not usually considered a risk factor for malnutrition, chronic wounds may precipitate malnutrition in certain patients. One study examining leg ulcer patients found that on average 2.5 to 3.0 grams of protein were lost every 24 hours while albumin accounted for 40 percent of the protein lost every 24 hours (Nylen & Wallenius, 1961). Protein losses from an open wound may seem trivial on a short term basis. It may be hypothesized, however, that protein losses may significantly contribute to hypoproteinemia in chronic ulcer patients over the long term. This hypothesis is supported by research within the pressure ulcer population which is discussed in the following section.

Pressure Ulcers. Research findings suggest that malnutrition is a problem within the population of patients with pressure ulcers. For example, early work by Allman and colleagues (1986) using a cross-sectional survey design found that 70% of hospitalized

patients with pressure ulcers were malnourished by history and examination. Subsequent work by Allman and colleagues (1995) using a prospective, inception, cohort design found that lymphopenia (<1500/mm) and decreased body weight (<58 kg) were independent and significant predictors ($p<0.05$) of pressure ulcer development in hospitalized patients with activity limitations. Similarly, Breslow and colleagues (1991) found that the nutritional status of tubefed patients with pressure ulcers was significantly worse in terms of serum albumin ($p<0.05$), hemoglobin ($p<0.05$), and cholesterol ($p<0.05$) than in tubefed patients without pressure ulcers even though the average protein intake of the pressure ulcer patients was significantly greater ($p<0.05$) than the patient controls. In addition, low serum zinc (Bergstrom et al., 1987), plasma vitamin C (ter Riet, Kessels, & Knipschild, 1995) , and leukocyte vitamin C levels (Burr & Rajan, 1972; Goode, Burns, & Walker, 1992; Taylor, Rimmer, Day, Butcher, & Dymock, 1974) have been reported in patients with pressure ulcers.

Chronic Vascular Ulcers. Fewer studies have examined nutritional status and wound healing in patients with chronic vascular ulcers. In a prospective, descriptive, comparative study, Agren and colleagues (1986) explored total serum concentrations of albumin and minerals in geriatric patients with arterial and venous leg ulcers (n=24) and controls (n=40) without ulcers. Serum albumin levels were not significantly different between the groups. However, the serum concentrations of selenium ($p<0.05$), zinc ($p<0.001$), and iron ($p<0.05$) were significantly lower; and the serum copper to zinc ratio ($p<0.001$) was elevated in the patients with vascular ulcers compared to the controls. Further analysis of the ulcer patients revealed there was not a significant difference in the serum albumin and zinc levels of patients with "poor healing" and patients with "good"

healing. There was, however, a statistically significant increase in the serum copper level ($p<0.02$) and serum copper to zinc ratio ($p<0.01$) in patients with "poor healing". It should be noted that initial ulcer sizes were fairly small. In addition, a major limitation of the study was the lack of a valid and reliable measure of healing.

In a study examining the effect of a triple layer bandage on the healing of therapy resistant venous leg ulcers, Bjellerup, Lindholm, Christensen, and Zederfeldt (1993) evaluated serum values including iron, total iron-binding capacity, hemoglobin, albumin, leukocytes, and glucose. No descriptive data on the serum values were presented within the study results. However, the authors state that "none of the serum analyses revealed pathologic values requiring intervention" (p. 57). These findings should be viewed with caution, however, since descriptive statistics of the sample's serum values were not presented.

A recent retrospective analysis of 27 patients with a variety of chronic open wounds (including venous, arterial, and pressure ulcers) who underwent reconstructive surgery found the mean preoperative serum albumin to be 3.13 +/- 0.7 g/dl with 44% of the sample having an albumin level less than 3.0 g/dl (Walter, Bartell, Paletta, & Herrmann, 1994). The hypoalbuminemic group had a 50% complication rate (e.g. flap necrosis, wound dehiscence, death) which was significantly greater ($p<0.005$) than those with an albumin level of 3.0 g/dl or greater (13% complication rate). This study is important as it suggests that hypoalbuminemia is a problem in a portion of patients with chronic leg ulcers. Furthermore, it supports previous findings which suggest there is a relationship between hypoalbuminemia and impaired wound healing.

A study in the United Kingdom has investigated vascular and nutritional

deficiencies in 50 patients with large leg ulcers (surface area > 100 $cm^{2)}$ (Balaji & Mosley, 1995). Thirty-four percent were found to have arterial insufficiency, 50% had venous insufficiency, and 16% had no vascular problems, but had serious nutritional deficiencies. Abnormally low serum nutritional indices were common in all the leg ulcer patients. Overall, nutritional deficiencies were noted in the following areas: vitamin C (60%), albumin(22%), iron (20%), zinc (18%), and folate (18%). The twenty-five venous ulcer patients in particular were found to have the following abnormalities: vitamin C deficiency (72%), hypoalbuminemia (40%), zinc deficiency (12%), folate deficiency (8%), iron deficiency (8%). Venous ulcer patients were skin grafted and had a four-layer compression bandage until the ulcer was healed. Patients with vitamin C or zinc deficiency received Ascorbic acid (500 mg three times per day) and zinc supplements (200 mg three times per day) orally. No interventions to correct protein deficiency were mentioned. Although all the venous ulcers healed prior to discharge, the authors reported a recurrence rate of 40% within 6 months. The authors conclude that nutritional deficiencies may have a role in leg ulcer etiology as well as delayed healing.

In a descriptive pilot study, Wipke-Tevis and Stotts (1996) have explored the nutritional risk, status, and intake of seven patients with venous leg ulcers. The DETERMINE Public Awareness Checklist, which assesses nutritional risk, categorized subjects as follows: 2 low risk, 2 moderate risk, and 3 at high risk. Biochemical indicators of nutritional status identified abnormalities in hemoglobin/hematocrit (n=4), albumin (n=1), transferrin (n=3), zinc (n=3), and total lymphocyte count (n=3). Dietary intake was adequate to meet caloric needs in 1 subject and inadequate in 5 subjects. Using the recent Agency for Health Care Policy and Research guidelines as an estimate of protein needs for

healing, four subjects had inadequate protein intake. Assessment of micronutrient intake showed abnormalities in vitamin C (n=2), zinc (n=4), iron (n=3), and vitamin A (n=2). Preliminary data suggest that a portion of subjects with venous ulcers are at nutritional risk, have abnormalities in their nutritional status, and/or have an inadequate nutritional intake to support wound healing.

This study has several limitations that should be noted. First, subjects were recruited during their usual clinic visit and the blood work was obtained without a period of fasting. Thus, the serum vitamin C and zinc values may be falsely elevated. Secondly, since subjects were only observed at one point in time, the long-term significance of the nutritional impairment on wound healing was not known. Finally, this was a pilot study with a very small sample size.

Limited data are available regarding the nutritional status of patients with venous ulcers and the effects on wound healing. Some researchers suggest malnutrition is a problem in the vascular ulcer population, while others do not. Additional research within the chronic ulcer population is necessary to examine nutritional status and intake as well as the long term effects of nutritional deficits on the healing of chronic vascular ulcers.

Malnutrition and Wound Healing in Diabetics

Nutritional factors also influence healing in patients with diabetes mellitus. This literature is briefly reviewed here since many patients with vascular ulcers have coexistent diabetes mellitus (Nelzen, Bergqvist, & Londhagen, 1993; Sindrup et al., 1987). Malnutrition is associated with failed wound healing in diabetics and correction of these deficiencies is thought to be necessary to promote normal wound healing (Casey et al., 1983; Dickhaut et al., 1984; Jany & Burkus, 1988). In addition, insulin deficiency is

similar to malnutrition in that they are both catabolic states with increased turnover of nutrients and increased demand for micronutrients (Reed & Mooradian, 1990). Furthermore, the polyuria frequently associated with diabetes increases urine losses of minerals, putting the patient at risk for nutritional deficiencies.

Hyperglycemia in poorly controlled diabetics is associated with impaired wound healing. The effects of hyperglycemia on wound healing have been examined in experimental animal studies (Black, Hennessey, Ford, & Andrassy, 1989; Goodson & Hunt, 1977; 1978; Seifter et al., 1981). In an early study, Streptozocin (Sz)-diabetic rats were found to have significantly higher blood and wound fluid glucose levels ($p<0.001$) and significantly less wound tensile strength and hydroxyproline accumulation ($p<0.001$) than the control rats (Goodson & Hunt, 1977). Similarly, Seifter and colleagues (1981) found that Sz-diabetic rats had significantly weaker skin incisions ($p<0.001$) and produced significantly less hydroxyproline than saline-injected control rats ($p<0.001$). Correspondingly, Black and colleagues (1989) demonstrated that Sz-diabetic rats produced less hydroxyproline ($p<0.0001$), had higher levels of glycosalated collagen ($p<0.001$), as well as increased collagenase activity ($p<0.001$) after only five days of hyperglycemia than control rats. In a later study by Goodson and Hunt (1978), however, Sz-diabetic rats receiving insulin immediately postwounding (day 1-11) produced significantly more hydroxyproline ($p<0.05$) than control Sz-diabetic rats (i.e. no insulin) or those receiving insulin on days 11 through 21 postwounding. Furthermore, when Sz-diabetic rats received insulin during days 1 through 21, the hydroxyproline deposition was quite similar to the control non-diabetic rats.

The effect of hyperglycemia on the leukocyte function has been examined in

studies of patients with poorly controlled diabetes(Bagdade, Root, & Bulger, 1974; Bagdade, Stewart, & Walters, 1980; Bagdade & Walters, 1978; Nolan, Beaty, & Bagdade, 1978). Leukocyte functions such as adherence, migration, engulfment, and intracellular killing, all of which are necessary for normal wound healing, were shown to be impaired in diabetics during episodes of hyperglycemia (Bagdade et al., 1974; 1978; Nolan et al., 1978). Decreasing blood glucose to less than 250 mg/dl was shown to improve leukocyte function (Bagdade et al., 1978; Bagdade & Walters, 1980; Nolan et al., 1978).

Protein-calorie malnutrition may also be a problem in a portion of patients with diabetes. In a study exploring the nutritional status of 79 vascular surgery patients, Casey and colleagues (1983) found that 23 diabetics were significantly more likely to develop wound healing complications than the nondiabetics ($p<0.05$). Unfortunately, the frequency of malnutrition among the subset of diabetic patients was not reported. Two other studies (Dickhaut et al., 1984; Jany & Burkus, 1988), which examined the nutritional status of diabetic patients undergoing lower extremity amputation, found malnutrition to be present in about 50% of patients. Furthermore, there was a relationship between failed healing of the amputation and the presence of malnutrition. Although the sample sizes were small, these limited findings suggest that protein malnutrition may be a problem within the population of diabetics with wounds.

The frequency of micronutrient deficiencies in patients with diabetes is thought to be more common that normal healthy adults. For example, Havivi and colleagues (1991) found the frequency of deficient plasma levels of riboflavin ($p<0.001$), zinc ($p<0.001$), carotene ($p<0.001$), hemoglobin ($p<0.001$), thiamin ($p<0.001$), retinol ($p<0.001$), and iron

($p<0.001$) were all significantly more common in 100 diabetic men and women than 112 healthy controls. Of particular interest, the percentage of diabetics with deficiencies in micronutrients important for wound healing were as follows: zinc (26%), hemoglobin (26%), leukocyte ascorbic acid (15%), and iron (6%).

Serum zinc levels have been studied quite extensively within the diabetic population. Overall, serum zinc levels have been found to be significantly lower in diabetics than controls (Car et al., 1991; Havivi et al., 1991; Walter et al., 1991). Interestingly, the changes in zinc were not observed to be related to serum glucose levels (Car et al. 1991; Havivi et al., 1990; Walter et al., 1991), urinary glucose, glycosalated hemoglobin levels, proteinuria, or diuretic use (Walter et al., 1991). Diabetics also have lower concentrations of zinc in lymphocytes, granulocytes, and platelets compared to controls (Pai & Prasad, 1988). This may be related to the hyperzincuria observed in the diabetics. Since zinc is a critical factor for wound healing, zinc supplementation in diabetics has been suggested as a means of improving healing in diabetics (Engel, Erlick, & Davis, 1981; Reed & Mooradian, 1990).

Overall, studies in diabetics suggest that vitamin A status is normal. However, impaired wound healing in diabetics is thought to be related to defective early inflammatory responses. Seifter and colleagues (1981) examined the effect of supplemental vitamin A on the wound healing of Streptozocin (Sz) induced diabetic rats with normal pre-wounding vitamin A levels. Streptozocin-diabetic rats receiving vitamin A had significantly stronger skin incision breaking strength ($p<0.01$) and doubled the hydroxyproline accumulation ($p<0.001$) over the Sz-diabetic rats not being supplemented. However, both the skin incision breaking strength ($p<0.05$) and the hydroxyproline

accumulation ($p<0.001$) of the vitamin A supplemented Sz diabetic rats remained significantly less than controls (i.e. nondiabetic rats). Additional effects of supplemental vitamin A on the Sz-diabetic rats included ameliorated immune functioning as evidenced by decreased weight loss, and improvementof adrenal enlargement, thymic involution, and lymphopenia. Seifter and colleagues (1981) propose that supplemental vitamin A improves immune response in traumatized animals and surgical patients and is likely to prove useful in preventing wound infection and promoting wound healing in surgical diabetic patients.

Research findings suggest that vitamin C metabolism is altered in diabetes mellitus. Experimental studies using an animal model have found that Sz-diabetic rats had significantly lower plasma vitamin C levels ($p<0.05$) (McLennan, Yue, Fisher, Capogreco, Heffernan, Ross, & Turtle, 1988; Yue, McLennon, Fisher, Heffernan, Capogreco, Ross, & Turtle, 1989) and significantly greater urinary excretion of vitamin C ($p<0.05$) than non-diabetic control rats (Yue et al., 1989). Similarly, other researchers have reported decreased plasma vitamin C in patients with non-insulin dependent diabetes mellitus or normals given intravenous glucose infusion (Chen, Hutchinson, Pecoraro, Lee & Labbe', 1983; Pecoraro & Chen, 1987; Som, Basu, Deb, Choudhury, Mukherjee, Chatterjee, & Chatterjee, 1981). McLennan and colleagues (1988) found that Sz-diabetic rats treated with supplemental vitamin C responded to treatment with significant increases in plasma Vitamin C ($p<0.05$) and granulation tissue prolyl hydroxylase (PRLase) activity ($p<0.05$). Prolyl hydroxylase is an Vitamin C-dependent enzyme required for collagen synthesis.

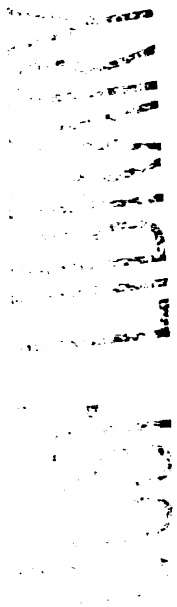
Leukocyte Vitamin C. Leukocytes are known to accumulate ascorbic acid levels well above the levels in the plasma (Evans, Currie, & Campbell, 1982) and are considered to be the best index of tissue ascorbic acid stores (Jacob, 1990). In the past, investigators

have used the total heterogeneous mixture of white cells recovered as the "buffy coat", however, this has been criticized as it is likely to provide inconsistent results since the ascorbic acid content of the different cell types vary (Evans et al., 1982; Lee, Davis, Rettmer, & Labbe', 1988; Jacob 1990). For example, mononuclear (MN) leukocytes are usually found to contain between two and three times higher concentrations of ascorbic acid than PMN cells (Evans et al., 1982; Lee et al., 1988). Unfortunately, considerable variation exists in reported levels of leukocyte vitamin C (Jacob, 1990).

The transport and storage of ascorbic acid in PMNs has been studied extensively (Moser, 1987; Lee, et al., 1988; Washko, Rotrosen, & Levine, 1991; Washko & Levine, 1992; Washko, Wang, & Levine, 1993). Ascorbic acid uptake into PMNs occurs by stereoselective active transport and is dependent on temperature, calcium, potassium, and sodium (Moser, 1987). In vitro studies have shown that glucose inhibits the uptake and accumulation of ascorbic acid in human neutrophils by influencing both the high affinity and low affinity transport activity (Lee et al., 1988; Moser, 1987; Washko et al., 1991; Washko & Levine, 1992). Interestingly, however, one study found that PMN ascorbic acid levels and glycosylated hemoglobin levels were not correlated in either control or diabetic subjects (Lee et al., 1988).

Leukocyte levels of ascorbic acid and leukocyte chemotaxis have been observed to be decreased in vivo during hyperglycemia (Chen et al., 1983; Cunningham, Ellis, McVeigh, & Calles-Escandon, 1991; Pecoraro & Chen, 1987). Mononuclear leukocyte levels of vitamin C were reduced 40% in subjects with non-insulin dependent diabetes mellitus subjected to rapid intravenous glucose infusions when compared to non-diabetics (Chen et al., 1983). Similarly, Pecoraro and Chen (1987) examined vitamin C in plasma,

MN, and PMN concentrations as well as leukocyte chemotaxis in normal male volunteers before and after an intravenous glucose infusion. The researchers found that acute, transient hyperglycemia resulted in significant, but short-term, decreases in MN (38%) and PMN (26%) Vitamin C levels, however, there was no change in leukocyte chemotactic activity. In contrast, during conditions of prolonged (up to 4 hours) hyperglycemia (blood glucose > 250 mg/dl) maintained by continuous intravenous glucose administration, plasma Vitamin C levels decreased, urinary Vitamin C excretion increased, mean MN and PMN Vitamin C levels decreased and chemotactic activity was significantly inhibited ($p<0.01$) in both MNs and PMNs (Pecoraro & Chen, 1987). In addition, MN leukocyte Vitamin C levels were also found to be significantly lower ($p<0.05$) in diabetics (33%) than age- and sex-matched, non-diabetic controls even though plasma vitamin C levels were normal and dietary vitamin C intakes were above the RDA and not different between the groups (Cunningham et al., 1991).



It has been hypothesized, then, that chronic hyperglycemia associated with diabetes results in depleted plasma and intracellular Vitamin C levels, as well as impaired leukocyte chemotactic function (Pecoraro & Chen, 1987). This mechanism may be responsible for a defective, acute inflammatory response in diabetes, increased susceptibility to infection, and the decreased tensile strength and poor collagen synthesis seen in the wounds of diabetics. These data suggest that to improve healing in diabetics it is necessary to control blood glucose with insulin and supplement vitamin C.

Wound Healing Response to Nutritional Supplementation

Impaired healing due to malnutrition can be reversed with nutritional supplementation. Nutritional supplementation improves nutritional parameters, increases

collagen synthesis, accumulation and tensile strength, and decreases wound complications such as infection and dehiscence (Bellantone et al., 1988; Delany, Demetriou, & Levenson, 1990; Daly et al., 1992; Haydock & Hill, 1987; Hoover, Ryan, & Anderson, 1980; Myers, Takiguchi, Slarsh, & Rose, 1990; Schroeder, Gillardeis, Mahr, & Hill, 1991; Smith & Hartemink, 1988; Ward et al., 1982; Zaizen et al., 1990; Zaloga, Bortenschlager, Black, & Prielipp, 1992). Nutritional supplementation also is associated with an increase in cell-mediated immunity (Ek et al., 1990; Law et al., 1973). Composition, route, and timing of nutrient intake are factors being researched in the surgical population.

Although these studies provide a foundation for understanding the effects of nutritional supplementation on wound healing, researchers have only examined the effects of nutritional supplementation on healing of primary incisions in patients undergoing surgical procedures. The dependent measures included incisional tensile strength, anastomotic bursting strength, and hydroxyproline accumulation. These dependent variables may not be appropriate for evaluation of healing of chronic wounds. In addition, much of this work is still being done using animal models and the applicability of these findings to humans remains unknown.

Nutritional Supplementation in Patients with Chronic Wounds

Limited data are available on nutritional supplementation in patients with chronic ulcers. No studies have examined the influence of caloric and/or protein supplementation in patients with vascular ulcers. Preliminary work has examined the effect of intake in patients with pressure ulcers.

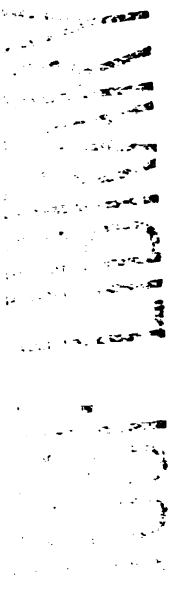
Caloric and Protein Intake. An early report of eight case studies by Mulholland and colleagues (1943) indicated that increased caloric intake alone was not sufficient to

heal pressure ulcers. However, when nitrogen balance was made positive by increasing the protein intake in the diet, pressure ulcers improved, and serum albumin, total protein, and body weight increased. Although nutrition was not the primary focus of their prospective trial of wound dressings, Gorse and Messner (1987) reported that "an adequate dietary intake" significantly improved pressure ulcer healing in patients with pressure ulcers regardless of the dressing treatment group assignment (hydrocolloid {$p<0.002$} or saline wet to dry {$p<0.02$}). Similarly, in a randomized clinical trial comparing the efficacy of air fluidized versus regular beds, Allman and colleagues (1987) indicated that the most significant baseline characteristic associated with pressure ulcer improvement was dietary protein intake with "improvers" consuming about two times more protein (51.6g/day) than "nonimprovers" (27.1g/day).

Recently, one prospective, interventional study was undertaken to examine whether nutritional intervention with a high protein diet would improve healing of pressure ulcers in malnourished nursing home patients (Breslow et al., 1993). Caloric requirements were calculated using the Harris-Benedict equation with adjustments for activity and injury, and the patients received liquid nutritional formulas containing either 24% protein or 14% protein. The authors reported that there was a significant decrease ($p<0.02$) in the total truncal pressure ulcer surface area in the 24% protein group, but not in the 14% protein group. Furthermore, in the subset of patients with stage IV pressure ulcers, the decrease in total surface area in the 24% protein group was significantly greater ($p<0.05$) than the decrease in ulcer area in the 14% protein group.

Although these results suggest that nutritional intervention with a higher protein diet improved healing of their pressure ulcers, several problems with the study limit the

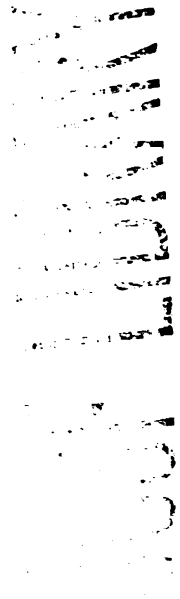
generalizability of the findings. Although initially patients were assigned in a stratified, randomized fashion based on pressure ulcer stage and type of bed, high attrition (42%) and clinical circumstances caused the authors to abandon random assignment (Breslow et al., 1993). Therefore, the final sample was not randomized to bed type, ulcer stage, or amount of protein intake. Since the route of feeding was either oral or tube feeding (again not randomized), the amount of intake within each group was not controlled. In addition, based on anecdotal data indicating three patients who received the 14% protein supplement orally disliked the taste of the supplement, it is possible that the intervention may be a confounder. Finally, the use of total truncal surface area as the dependent variable is of concern. Since pressure ulcers (particularly stage III and IV ulcers) may have significant depth as well as length and width, measurement of change in volume would have been a more accurate reflection of healing than change in wound surface area.



Vitamin C. Vitamin C is known to be important for the prevention and treatment of scurvy as well as a critical nutrient for wound healing. Nonetheless, the efficacy of supplemental vitamin C on healing of chronic wounds remains debatable. Classic work suggests that vitamin C supplementation may enhance the healing of pressure ulcers. Burr and Rajan (1972) reported that wound biopsy samples from the pressure ulcers of seven patients who received 500 mg of ascorbic acid twice a day for three days showed significantly more staining for collagen than the three patients who received a placebo for three days. Subsequently, Taylor and colleagues (1974) undertook a prospective, double blind, randomized clinical trial of 20 patients to examine the effect of supplemental ascorbic acid (500 mg twice a day) on the healing of pressure ulcers. Mean buffy coat leukocyte vitamin C levels for both groups were comparable at baseline, but were at the

lower end of the normal range for their laboratory. The mean percent reduction in area at one month was significantly greater ($p<0.005$) for the vitamin C treatment group (84%) than the placebo group (42.7%). Similarly, the mean healing rates were 2.47 square centimeters (cm)/week for the treatment group and 1.45 square cm/week for the placebo group ($p<0.005$). Limitations of the study by Taylor and colleagues (1974) include a small sample size, no measure of the overall nutritional status, and an inaccurate method of analyzing the change in wound surface area (counting graph paper squares).

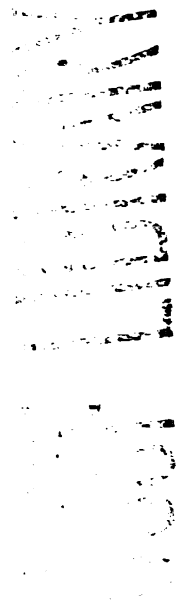
More recently, ter Riet and colleagues (1995) replicated and extended the work by Taylor and colleagues (1972) in a prospective, multicenter, blind, randomized clinical trial. The authors compared the wound closure rate and mean absolute healing rate in patients with pressure ulcers who either received 500 mg of vitamin C twice a day or 10 mg of vitamin C twice a day. Type of bed, wound cleansing and dressing regimes, and turning schedules were controlled. Over a 12 week period, neither the wound closure rate nor the healing velocity of wound surface area were different between the treatment and control groups.



Several factors make it difficult to compare the two interventional studies. In the older study by Taylor and colleagues (1974), the sample consisted of hospitalized surgical patients with large pressure ulcers (mean = $16cm^2$), low baseline buffy coat leukocyte vitamin C levels, and the control group received no supplemental vitamin C. In contrast, the contemporary study by ter Riet and colleagues (1995) had a sample of debilitated, malnourished (70%), nursing home patients with small pressure ulcers ($1.4cm^2$), relatively normal plasma vitamin C levels (over 70% had > 2 mg/L), and the "control" group received 20 mg of vitamin C every day. However, what is most striking is that the Taylor

and colleagues (1974) study was examining the effect of megadoses of vitamin C (1000 mg/day) on the healing of pressure ulcers in patients with *low levels* of vitamin C. In comparison, since 70% of patients in the ter Riet (1995) study had normal plasma levels of vitamin C at baseline, ter Riet and colleagues (1995) were comparing the effects of megadoses of vitamin C (1000 mg/day) to antiscorbutic doses of vitamin C (20 mg/day) on the healing of pressure ulcers in patients with *normal levels* of vitamin C.

Based on the limited data available, it would appear that megadoses of vitamin C do not significantly improve healing in pressure ulcer patients with normal plasma vitamin C levels. However, megadoses of Vitamin C may increase healing in subjects with pressure ulcers with low levels of tissue vitamin C. Additional well-controlled, randomized clinical trials with stratification based on baseline vitamin C status are necessary to definitively answer this question.



Zinc. Several nutritional supplementation studies have examined the use of oral zinc preparations to enhance healing of chronic wounds such as pressure and leg ulcers. Research findings suggest that subjects with chronic ulcers have significantly lower plasma zinc concentrations that control subjects (Agren, Stromberg, Rindby, & Hallmans, 1986; Greaves & Boyde, 1967; Greaves & Skillen, 1970; Hallbook & Lanner, 1972; Serjeant et al., 1970; Withers, Baker, Musa, & Dormandy, 1968). Zinc may be lost due to surgical stress, stress, sepsis, diarrhea, and/or draining wounds or fistulas (Ehrlichman et al., 1991; Williamson, 1992). However, the efficacy of oral zinc supplementation in the promotion of healing of chronic ulcers remains equivocal. Acceleration of the rate of wound healing in patients with zinc supplementation has been shown in clinical trials examining leg ulcers (Hallbook & Lanner, 1972; Husain, 1969; Serjeant, et al., 1970) and after excision of

pilonidal sinus tracts (Pories, Henzel, Rob & Strain, 1967). Similarly, however, four clinical trials also have suggested that zinc supplementation does not improve the rate of healing either in leg ulcers (Clayton, 1972; Greaves & Ive, 1972; Myers & Cherry, 1970) or pressure ulcers (Norris & Reynolds, 1971).

Careful examination of the design and methodology of these studies may explain the discrepancy of the results. The measurement of serum zinc levels and stratification of abnormal and normal subjects prior to randomization to treatment groups was not done in most of the studies. Thus, comparison of treatment versus control does not answer the question "will zinc supplementation in zinc deficient patients enhance healing?".

Perhaps the most crucial problem in these studies relates to measurement of wound healing as well as determination of the rate of wound healing versus time required to heal. In order to obtain accurate results of a clinical trial, valid and reliable measures of the dependent variable are necessary. Methods used to measure wounds in these studies included wound volume with dental impression material, and wound surface area measured with linear measurements, weighing of graph paper, and tracings with planimetry. Although there is no consensus within the field of wound healing with regards to the gold standard for measuring/evaluating wounds, tracings with planimetry are generally considered more reliable than either linear measures or weighed graph paper.

One major faulty assumption in these studies was that the rate of wound healing is constant over time. According to Haley (1979) and Gilman (1990), healing rate changes over time as a function of the wound size. Consequently, in order to evaluate the effectiveness of zinc sulfate in a clinical trial, the mean ulcer size should be similar for both the treatment and control groups. Alternatively, Gilman (1990) purposes the use of the

linear advance of the wound margin (i.e. perimeter) toward the wound center as a valid comparison of healing progress for wounds of all shapes and sizes. The linear advance of the wound margin toward the wound center may be calculated by the following equation: d = A/p; where d = linear advance, A = area, and p = perimeter. Gilman (1990) has provided a mathematical proof of his proposed parameter for measuring wound closure.

Conceptual Framework For Healing of Chronic Wounds

The conceptual framework for this study is based on research from the areas of tissue oxygenation and perfusion, immune status, and nutrition as related to healing of chronic vascular ulcers. Limited research findings also suggest that activity (Whitney, Stotts, Goodson, & Janson-Bjerklie, 1993; Whitney, Stotts, & Goodson, 1995) and psychophysiologic stress (Holden-Lund, 1988; McCarthy, Ouimet, & Daun, 1991; Wysocki, 1996) may also be important for wound healing; however, these dimensions will not be examined here.

Research findings clearly indicate that oxygen is essential for wound healing and that tissue hypoxia reduces ribonucleic acid production, capillary growth and density, fibroblast proliferation, collagen synthesis and accumulation, wound tensile strength, and epithelialization (Hunt & Pai, 1972; Kivisaari, Vihersaari, Renvall, & Niinikoski, 1975; Knighton, et al., 1981; 1984; Jonsson, Jensen, Goodson, & Hunt, 1986; Jonsson et al., 1991; Niinikoski, 1980; Pai & Hunt, 1972) and increases the risk of wound infection (Hohn, Mackay, Halliday, & Hunt, 1976; Hohn, 1977; Jonsson, Hunt & Mathes, 1989). Tissue hypoxia is intricately related to tissue perfusion. Volume status, a major determinant of perfusion, is a critical determinant of tissue oxygenation (Gottrup et al., 1989; Hunt, Goldstick, & Connolly, 1967) with even asymptomatic hypovolemia causing

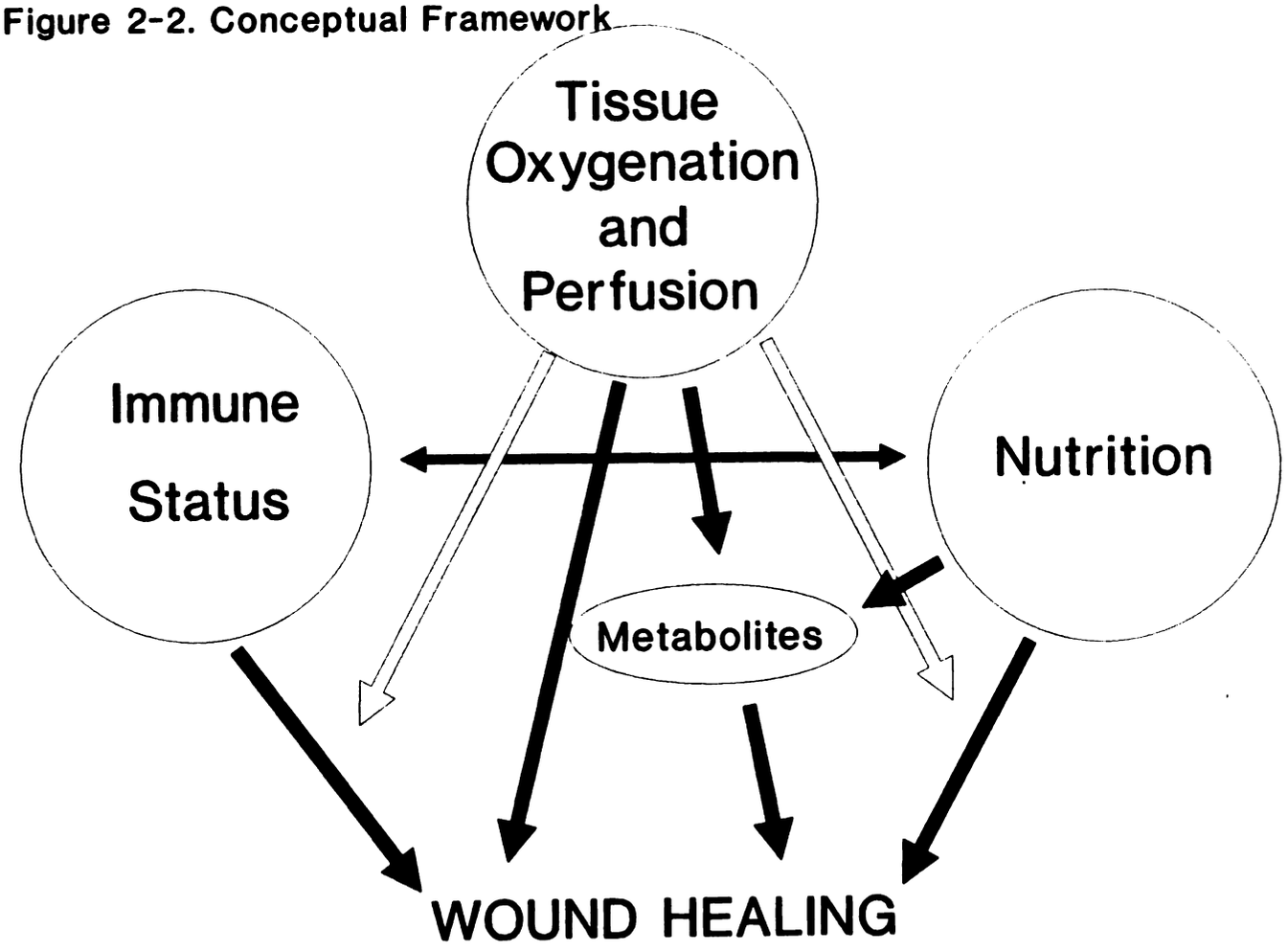
tissue hypoxia (Chang, Goodson, Gottrup, & Hunt, 1983; Gottrup et al., 1989). Perfusion is known to influence healing in chronic, ischemic wounds (Ahn & Mustoe, 1990; Constantine & Bolton, 1986; Goldman et al., 1990; Hunt, 1991; Moelleken et al., 1991). Impaired tissue oxygenation and perfusion increases the buildup of toxic metabolites. Metabolic end products such as calcium, carbon dioxide, lactate, leukotrienes, thromboxanes, and oxidants are thought to impair healing (Coleridge-Smith et al., 1988; Greenwood et al., 1995; Jaconi et al., 1988; Lew et al., 1984; Sank et al., 1989; White & Heckler, 1990; Wipke-Tevis & Stotts, 1991). Supplementation with specific nutrients is thought to prevent free radical formation (Foegh, Thomas, & Ramwell, 1990) and minimize the adverse effect of reperfusion on wound healing (Ehrlichman et al., 1991).

Wound healing requires adequate macronutrients for energy as well as specific vitamins and minerals. Insufficient quantities of these nutrients impair wound healing by causing prolonged inflammation, delayed fibroplasia, decreased collagen synthesis and tensile strength, increased capillary fragility, delayed epithelialization and increased wound infection (Daly et al., 1972; Goodson et al., 1987; Haydock & Hill, 1986; 1987; Mullen et al., 1979; Windsor et al., 1988; Zaizen et al., 1990). Abnormalities of host defense and immunity also are connected to malnutrition. Protein-calorie malnutrition is related to altered humoral and cell-mediated immunity as well as increased morbidity and mortality (Daly et al., 1992; Ek et al., 1990; Jensen et al., 1982; Law et al., 1973; Mullen et al., 1979; Powanda & Moyer, 1981). The immune system plays a crucial role in the initiation and progression of normal wound healing (reviewed in Wipke-Tevis & Stotts, 1991). Impairments in immune status, such as infection, decrease nutrient intake and also impair

wound healing (Greenhaugh & Gamelli, 1987).

Figure 2-2 depicts the hypothesized conceptual framework of critical factors affecting healing in chronic venous ulcer. Each dimension of the model has been briefly discussed in the preceding section. Critical factors affecting wound healing are immune status, nutrition, oxygenation, and perfusion. Oxygenation and perfusion are presented together as they are intricately related to one another and their unique effects are difficult to separate. Immune status, nutrition, and oxygenation/perfusion have a direct influence on wound healing as depicted by solid lines with unidirectional arrows. Oxygenation and perfusion augment the direct influence of nutrition and immune status on wound healing in that they affect the delivery of these substances as well as the function and utilization of the substances within the tissue. These augmented or indirect effects of oxygenation and perfusion are depicted by dotted lines with unidirectional arrows. Nutrition and immune status are closely interconnected with alterations in one adversely affecting the other. This interconnection is portrayed by a solid line with bidirectional arrows. Impaired oxygenation and perfusion directly increase the buildup of tissue metabolites as depicted by a solid line. Metabolic end products directly have adverse effects on wound healing, and this connection is displayed by a solid line with a unidirectional arrow. In addition, specific nutrients may prevent metabolite formation, and this relationship is depicted by a solid line with a unidirectional arrow.

Figure 2-2. Conceptual Framework



Summary

The classic approach to treatment of wounds with impaired perfusion, such as vascular ulcers, is to maximize perfusion so that sufficient oxygen, nutrients, and immune substances are available to support normal cell function and metabolites are removed. Strategies to maximize perfusion in venous ulcer patients include surgery, graduated compression stockings, and intermittent sequential pneumatic stockings (Pikanmaki, Kolari, & Kiistala, 1987; Coleridge-Smith et al., 1990; Jamieson et al., 1990; Crikrit, Nichols, & Silver, 1988; Young & Terwood, 1990). However, in many patients, even when the flow component has been optimized, ischemia and impaired healing remain. Contributing factors such nutrient availability become particularly important and a potential avenue for intervention in these patients.

Data do not exist, however, regarding the influence of nutritional interventions in patients with chronic lower extremity vascular ulcers. In fact, there currently exists a paucity of research regarding nutrition status and intake in relation to wound healing in vascular ulcer patients. Research is needed to explore this important area to enhance our understanding of nutrition's role in mitigating impaired healing in patients with chronic lower extremity vascular ulcers.

Research Questions

The primary aims were to answer the following questions:

1) What is the nutritional risk and status of subjects with venous leg ulcers? Nutritional status includes anthropometric measures (i.e. triceps skin fold, mid-arm circumference, mid-arm muscle circumference, and body mass index), serum albumin, total lymphocyte count, serum vitamin C and zinc.

2) Is the dietary intake of subjects with venous leg ulcers adequate to meet the needs for wound healing as determined by the Harris-Benedict equation with adjustments for activity and injury? Dietary intake includes total calories, protein, fats, carbohydrates, vitamin C, and zinc.

3) Is there a relationship between the rate of wound closure and the nutritional status of subjects with a venous leg ulcer when controlling for the effects of perfusion? Nutritional status includes serum albumin, total lymphocyte count, plasma vitamin C, and zinc.

The **secondary aims** were to answer the following questions:

4) How do transcutaneous tissue oxygen values at lower extremity venous ulcer sites vary with specific positions and with 21% and 40% to 60% inspired oxygen?

5) Is there a relationship between glycosylated hemoglobin levels and plasma and leukocyte vitamin C levels in subjects with a venous leg ulcer?

6) Is there a relationship between rate of wound closure and plasma and leukocyte vitamin C levels in subjects with a venous leg ulcer?

Assumptions

Measurement of nutritional status is an imperfect science at best. According to Beaton (1994), dietary intake cannot be estimated without error and probably never will be. It is difficult to assess absolute validity of dietary methods because diet is changing and the very act of observing often alters intake (Dwyer, 1994). With the use of the diet record, there is potential that adherence to dietary assessment instruction varies among subjects. If some subjects rely on memory to record food items, the accuracy of the diet record is compromised (Beerman & Dittus, 1993).

Definition of Terms and Variables

Wound healing is a complex integration of biochemical and cellular processes that result in regeneration of connective tissue, vasculature, and epithelium. A **vascular ulcer** is a type of chronic, nonhealing wound where the loss of skin or tissue integrity is caused by injury or insult; however, the major underlying problem is related to perfusion. A **venous ulcer** is a particular type of vascular ulcer typically located near the medial malleolus of the lower leg whose underlying etiology is chronic venous insufficiency. **Wound surface area** is a two-dimensional, uniplanar evaluation of wound size. **Rate of wound closure** represents the linear advance of the wound margin (i.e. perimeter) toward the wound center for the purposes of comparing the healing progress of different wounds.

Nutritional status is the physiological condition within an individual that reflects the processes involved in the assimilation and utilization of nutrients for proper body functioning and maintenance of health. Although no single measurement can classify nutritional status, a variety of anthropometric, biochemical, and immunological indicators are currently utilized to identify patients with nutritional abnormalities. Empirical indicators for nutritional status are body mass index (BMI), serum albumin, serum glucose, serum glycosylated hemoglobin, total lymphocyte count, serum zinc, plasma vitamin C, leukocyte vitamin C, triceps skin fold (TSF), mid-arm circumference (MAC), mid-arm muscle circumference (MAMC), usual Vitamin C intake, and a concurrent three day dietary intake.

Visceral protein depletion can be estimated by determining the serum albumin, transferrin, prealbumin, and retinol binding protein. Albumin was selected for study in this proposal. Albumin has a half-life of 17 to 21 days. Visceral protein depletion also is

indicated by a loss of immunocompetence as manifested by a decreased TLC (Jensen et al., 1982). The TLC is calculated as the percentage of lymphocytes multiplied by the WBC count. Dickhaut et al. (1984) reported that an albumin < 3.5 g/dl and a TLC < 1500 /ml were predictive of wound healing failure in patients undergoing amputation procedures with a sensitivity of 82% and a specificity of 75%.

Fat, skeletal muscle protein, and visceral protein are the three major body tissues that are available to meet the energy requirements of the catabolic patient (Jensen et al., 1982). Anthropometric measures are utilized to evaluate body fat and skeletal muscle protein. Body fat is assessed by measuring the TSF which includes the subcutaneous layer of fat. Body mass index is an anthropometric measure of obesity. The skeletal muscle mass is evaluated with the MAMC.

Perfusion is the blood flow to tissue that provides oxygen and nutrients and removes carbon dioxide and waste products via the circulation. The empirical indicator for perfusion status is a regional perfusion index (RPI) calculated from transcutaneous tissue oxygen ($TcPO_2$) at the wound site divided by the $TcPO_2$ level at the chest ($TcPO_2$wound/$TcPO_2$chest). A sensor is placed on the skin and the heating element warms the tissue, permitting diffusion of oxygen to the sensing electrode. When blood flow is inadequate and PaO_2 is normal or compromised, $TcPO_2$ values reflect blood perfusion since they measure the degree to which hemoglobin must be unloaded in order to meet oxygen demand (Gottrup, Gellett, Kirkegaard, Hansen, & Johannsen, 1989; Tremper, Waxman, Shoemaker, 1979). Thus, $TcPO_2$ measurements are a useful noninvasive indication of the relative perfusion of the healing wound (Rippon, Walmsley, Queen, & Lydon, 1991). Recent data indicate that measurement of $TcPO_2$ changes after

oxygen inhalation or with position changes is a more sensitive predictor of healing than $TcPO_2$ values alone or a RPI (Bacharach, Rooke, Osmundson, & Gloviczki, 1992; Conlon, Sclafani, DiResta, & Brennan, 1994).

CHAPTER 3

METHODS

Research Design

This study employed a prospective design to explore nutrition, tissue oxygenation, and healing in subjects with venous leg ulcers. Nutritional risk, status, and intake, transcutaneous skin oxygen ($TcPO_2$) values, and wound surface area were evaluated at two points in time four weeks apart.

Research Setting

The study was conducted at the General Clinical Research Center (GCRC) of the University of California, San Francisco (UCSF) Medical Center. The GCRC is a federally funded outpatient and inpatient research center located within Moffitt Hospital on the Parnassus campus of UCSF Medical Center. Application for use of the GCRC was required and the application was evaluated for scientific merit by a multidisciplinary committee. At the GCRC, the subjects were studied in a private or semi-private inpatient hospital room equipped with standard hospital equipment.

Sample

Human Subjects Assurance

Prior to the initiation of the study, the research protocol was approved by the nursing research committees at the University of California San Francisco, the San Francisco General Hospital, and the San Francisco Veterans Affairs Medical Center and the Committee on Human Research for the University of California San Francisco. Recruitment of subjects was done by contacting patients who met the study criteria, explaining the study to them, and obtaining written informed consent (Appendix 1). The

study sample reflected the multi-ethnic nature of the San Francisco Bay area and no vulnerable groups were included.

Sampling Procedures

The subjects involved a convenience sample of adults with one or more venous leg ulcers. The accessible population for the study recruitment consisted of four university-affiliated teaching hospitals and one university-affiliated home health care agency on the West coast. Each hospital had a vascular/wound clinic where subjects were recruited. The number of patients seen weekly with peripheral vascular disease and number of patients with active venous ulcers by site were as follows: vascular clinic of the university medical center 20 and 5, vascular clinic from the county hospital 25 and 6, vascular clinic of the veterans hospital 40 patients and 2, vein care clinic of the community hospital 5 and 2. The university medical center dermatology leg ulcer clinic saw 20 patients per week; of those, one had a new venous ulcer. The number of cases seen per month by the university home health care agency was approximately 300.

A recruitment flyer about the research study was posted in all of the described recruitment sites with a telephone number to call for information about the study. Recruitment flyers were provided to the home care nurses at the home care agency to distribute to venous ulcer patients under their care (Appendix 2). A public service announcement for radio (Appendix 3) and a written press release (Appendix 4) for use in the campus newspaper and local Bay area newspapers was also used for recruitment of subjects.

Subject Criteria

Subjects were patients with a vascular ulcer whose predominant etiology was venous disease, who were able to speak and understand English, had a telephone, and were willing to attend two visits four weeks apart. Potential subjects were excluded if they were receiving greater than 5 mg of prednisone (or the equivalent)/day or beta blockade therapy, had an autoimmune disease, reported a positive human immunodeficiency virus status, had a known ankle-brachial index < 0.20 or were participating in another wound healing using topical or systemic medications.

Sample Size Determination

Previous work by Hallbook and Lanner (1972) indicated that subjects with low serum zinc levels who had chronic venous leg ulcers (n = 7) had a significantly lower rate of healing of the venous ulcer than subjects with normal zinc levels (n = 7) (Mann Whitney U, p = 0.02). The low zinc group had a mean rate of healing of 1.4 mm^2/day, while the normal zinc group had a mean rate of healing of 7.2 mm^2/day. No standard deviation or error measures were reported. An effect size was determined from this data using the D-Stat Meta-Analysis software program for personal computers (Johnson, 1989). The corrected effect size for this study was determined to be 1.46. With this limited data, it appeared that zinc nutritional status had a moderate to large effect on rate of healing of venous ulcers. Data were not available, however, to calculate effect size of the other nutritional variables of interest (albumin, total lymphocyte count, vitamin C) or the covariate of perfusion in the venous ulcer population.

Because the first two aims of this study were descriptive, the sample size requirements were based upon aim 3. Aim 3 sought to determine if there was a

relationship between rate of wound closure and the nutritional status of subjects with venous leg ulcers when the effects of perfusion were statistically controlled in the analysis. Aim 3 was to be analyzed using multiple regression techniques. The covariate was perfusion, and it was estimated to have a large effect (incremental R^2=0.36). The set of four nutritional (independent) variables to be evaluated for their effect on wound healing were serum albumin, total lymphocyte count, serum zinc, and serum vitamin C. To be conservative, a moderate effect size was estimated (incremental R^2=0.18). To evaluate the interaction between nutrition and perfusion, a moderate effect was estimated (incremental R^2=0.14). Thus, a sample size of 50 subjects was needed to answer this question with an alpha of 0.01 and a power of 0.80 (S. Paul, personal communication, 8/94) (Table 3-1).

Table 3-1. Sample Size Determination for Question 3 (n = 50).

Step	Set	Number of Variables	Effect Size	Incremental R^2	Power
1	Covariate (Perfusion)	1	large	0.36	0.99
2	Nutrition	4	moderate	0.18	0.80
3	Interactions	4	moderate	0.14	0.80

Data Collection Methods

Techniques and Instruments

Digital Standing Scale. Each subject was weighed using a Scale-Tronix Stand On Scale, model 5005 (Scale-Tronix Co., White Plains, N. Y.). The company reported accuracy of +/- 0.1 kg. Height was also measured using the Scale-Tronix Stand On Scale, model 5005 (Scale-Tronix Co., White Plains, N. Y.). The company reported accuracy of +/- 2 cm. Weight in kilograms and height in square meters were used to calculate body mass index (BMI) as follows: BMI = Kg/m^2.

Lange Calipers. Using the method of Jelliffe (1966), Lange calipers were used to measure triceps skin fold thickness in millimeters (mm).

Tape Measure. A paper tape measure (Baxter Health Care, Deerfield, IL) was used to measure mid-arm circumference and wrist size in centimeters (cm).

Hematology & Chemistry Equipment. Serum nutritional parameters and complete blood cell count were performed in the laboratories of Pathlab, a division of Unilab (San Jose, CA). The method of analysis used for each test was presented in Table 3-2 (personal communication Claudette Amend, 9/29/94).

Table 3-2. Laboratory method of analysis for blood tests.

TEST	METHOD OF ANALYSIS
CBC with Differential	Flow cytometry
Serum Albumin	Olympus
Serum Zinc	Atomic Absorption
Plasma Vitamin C	Spectrophotometry
Hemoglobin A1C	Column Chromatography
Serum Glucose	Enzymatic

Wound Surface Area. The wound perimeter was measured using acetate tracings. Wound area was calculated from the acetate tracings using computerized measurement software (SigmaScan/Image, San Rafael, CA). Error can occur when the wound perimeter is traced. Thomas and Wysocki (1990) reported a measurement of error of 10% using this method. Ahroni and colleagues (1992) reported a reliability coefficient for wound tracing of 0.99 and a mean coefficient of variation of 0.043. Reproducibility of computerized planimetry was equally high with intra-rater and inter-rater reliabilities of 0.99 (Ahroni et al., 1992; Majeske, 1992) and a mean coefficient of variation of only 0.010 (Ahroni et al., 1992).

Rate of wound closure. Rate of wound closure represents the linear advance of the wound margin (i.e. perimeter) toward the wound center for the purposes of comparing the healing progress of different wounds. Gilman (1990) purposed the use of the linear advance of the wound margin (i.e. perimeter) toward the wound center as a valid comparison of healing progress for wounds of all shapes and sizes. The linear advance of the wound margin toward the wound center is calculated by the following equation: $d = A/p$; where d = linear advance, A = area, and p = perimeter.

Transcutaneous Oxygen Monitor. The perfusion status was measured using the Novametrix 840 $PtcO_2$ and $PtcCO_2$ Monitor (Novametrix-Medical Systems, Inc. Wallingford, CN). Construct validity has been established by data that indicate $PtcO_2$ measurements predict wound healing in persons with impaired perfusion (Ameli et al., 1989; Hauser, 1987; Hauser & Shoemaker, 1983; Karanfilan et al., 1986; Kram et al., 1988; Stein et al., 1989). The regional perfusion index ($PtcO_{2\,wound}$ /$PtcO_{2\,chest}$) has gained favor as it allows discernment between local and systemic causes of cutaneous ischemia. Kram et al. (1988) reported a RPI > 0.20 as predictive of healing after amputation with 100% sensitivity, 86% specificity, and 98% accuracy. Reported reliability due to electrode drift occurs at a rate of less than 1 - 2 torr per hour (Whitney, 1990) which may be alleviated by frequent calibration.

Pulse Oximeter. The arterial oxygen saturation (SaO_2) was measured by a Oximax 100 pulse oximeter (Pace Tech Medical Monitors, Inc., Clearwater, FL) that was placed on the index finger of each subject. As described by Kelleher (1989), the oximeter probe has two diodes that emit two wavelengths of light, one in the red band (660 nm) and the other in the infrared band (910 nm). Hemoglobin saturated with oxygen (i.e.

oxyhemoglobin) absorbs more infrared light and less red light; whereas the oxygen depleted blood (i.e. reduced hemoglobin) absorbs more red light. The oximeter then determines the SaO_2 by sensing differences in the absorption spectra of reduced hemoglobin and oxyhemoglobin. The Pace Tech manual reports an accuracy of +2% in the SaO_2 range of 70 to 100% and +3% in the SaO_2 range of 50 to 69%; and +2% for pulse rate in the 30 to 100 beats per minute range.

DETERMINE Public Awareness Checklist. The DETERMINE Public Awareness Checklist (PAC) was used to assess nutritional risk. The PAC was developed by the Nutrition Screening Initiative (NSI) as an initial screening tool to assess the elderly for nutritional risk. The NSI is a multidisciplinary, multi-faceted effort to promote routine nutrition screening in health and medical care settings (White et al., 1991). The critical indicators assessed by the checklist are: presence of coexistent diseases, poor eating habits, tooth/mouth pain, economic hardship, reduced social contact, taking multiple medications, involuntary weight loss/gain, self-care assistance needs, and alcohol intake. The total score was used to classify subjects into one of three descriptive categories of nutritional risk (Appendix 5).

Limited validity and reliability data are available for the DETERMINE PAC. During its development, focus groups of older Americans reviewed drafts and provided critique regarding the length, format, educational level, and presentation style (White et al., 1992). Validity and reliability testing was performed using a random sample of 449 Medicare beneficiaries aged 70 years and older (Posner et al., 1993). Based on the results of regression analyses, expert panel experiences, and existing literature, the PAC was

revised and scores were assigned to reflect each item's relative importance as an independent indicator of nutritional risk.

To evaluate the sensitivity and specificity of the PAC, scores on the PAC were compared with intake of three or more nutrients below 75% of the RDA and fair or poor perceived health status (Posner et al., 1993). Using a cutoff point of 6 as an indicator of high nutritional risk, the PAC had a sensitivity of 36.2%, a specificity of 84.9%, and a positive predictive value of 37.9% when used to identify older persons with estimated dietary intakes below 75% of the RDA for three or more nutrients. Table 3-3 provides additional sensitivity and specificity information for selected checklist score cutoff points.

Table 3-3. Diagnostic Statistics for Selected PAC Checklist Score Cut-Points. (From Posner et al. (1993). Nutrition and health risks in the elderly: The nutrition screening initiative. American Journal of Public Health, 83(7), 972-978. Copyright 1993 by the American Public Health Association. Reprinted with permission.)

	Intake of >/ = 3 Nutrients below 75% of Recommended Dietary Allowance			Perceived Health Fair or Poor		
	4 Points	6 Points	8 Points	4 Points	6 Points	8 Points
Sensitivity	60.9	36.2	23.2	72.2	45.8	25.5
Specificity	62.7	84.9	94.8	64.5	84.8	91.1
+ Predictive Value	29.2	37.9	53.3	45.9	55.6	54.5

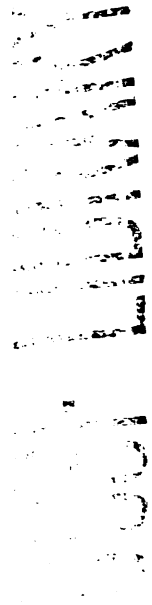
Recent work by Lowry (1994) with 106 independently living seniors aged 60 and older showed that 33% were at moderate nutritional risk (score of 3-5) and 26% were at high nutritional risk (score of 6 or more). The PAC scores were highly correlated with the number of nutritional risk factors identified on the NSI Level I screen ($r = 0.72$, $p = 0.001$). Furthermore, 75% who were classified by the PAC at nutritional risk were actually underweight (BMI < 24 kg/m^2). These data support the concurrent validity of the PAC tool.

Block Health Habits and History Questionnaire. The Health Habits and History Questionnaire (HHHQ) was developed in 1984 to assist the National Cancer Institute in identifying predictors of morbidity and mortality. The dietary component of the HHHQ consists of a quantitative food frequency questionnaire which may be used independent of the other components (Block, Coyle, Hartman & Scoppa, 1994). The food frequency questionnaire is a self-administered diet history questionnaire which asks for the frequency of consumption of 98 food items over the last year as well as usual portion size (i.e. small, medium, large) (Block et al., 1992). The questionnaire takes between 20 to 25 minutes to self-administer (Block et al., 1992). Although the HHHQ is capable of assessing nutrients as well as foods or food groups, its real advantage is that it provides knowledge of the distribution of usual nutrient intake (Block & Subar, 1992).

During instrument development, the list of foods, the nutrients associated with them, and the portion sizes associated with each food item were systematically developed by means of a data-based approach using the dietary intake data (n = 11,658) from the Second National Health and Nutrition Examination Survey (NHANES II) (Block et al., 1986). Foods were included on the questionnaire if they made an important contribution to the population's intake of energy and each of 17 nutrients in the NHANES II database. During the initial instrument development and evaluation the ability of the food list to estimate nutrient intake was compared to previously obtained one-day diet records of healthy adult men and women (n=50) using a trained coder. At the individual level, the food frequency questionnaire produced acceptable correlations when compared to the diet record: 0.73 for fat, 0.82 for energy, 0.86 for vitamin C, and 0.94 for vitamin A (Block et al., 1986). Furthermore, field administration to 1000 healthy volunteers produced mean

nutrient estimates comparable to national data and reproduced major age-and gender-specific differences.

The full-length 98 item Block HHHQ has been validated in a number of subsequent studies (Cummings et al., 1987; Sobell et al., 1989; Block et al., 1990; Coates et al., 1991; Block et al., 1992). Sobell et al (1989) examined the ability of the HHHQ to assess dietary intake 10-15 years in the past by interview and by mail (n=216). The average nutrient intake estimated by the HHHQ was within +/- 10 percent of the past diet for most nutrients. Correlations between mean 7-day diet records (obtained in 1971-1975) and retrospective estimate of diet intake by the HHHQ (in 1985) produced moderate correlations of macronutrients when subjects were interviewed: energy .50, protein, .54, fat .61 carbohydrates .45. Correlations were consistently lower for the group that was mailed the questionnaire. A detailed summary of the correlations between the HHHQ calculated nutrients and reference data (diet records/recall) nutrients for Sobell et al. (1989), Block et al. (1990), and Block et al. (1992) are available in Table 3-4.



Women aged 45-70 years who were participants in the Women's Health Trial Feasibility Study were also used to assess validity of the HHHQ (Block et al., 1990). The HHHQ was compared to the mean of three 4-day diet records collected over a one year period. The HHHQ produced group mean nutrient estimates closely approximated to the values from the diet records. Comparison between the HHHQ and diet records of women on a "normal" diet produced Pearson's correlations of moderate levels of agreement (e.g. energy r = 0.51, protein r = 0.48, carbohydrates r = 0.51, total fat r = 0.60).

Table 3-4. Summary of results from validation studies for the Health Habits and History Questionnaire. Data represent correlation between nutrients estimated from diet records and recalls and from the food frequency questionnaire. (Reprinted by permission from: Block et al. (1990). Validation of a self-administered diet history questionnaire using multiple diet records. Journal of Clinical Epidemiology, 43(12), 1327-1335, Copyright 1990 by Elsevier Science Inc.; Block et al. (1992). Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. Journal of the American Dietetic Association, 92, 686-693, Copyright The American Dietetic Association; Sobell et al. (1989). Validation of a retrospective questionnaire assessing diet 10-15 years ago. American Journal of Epidemiology, 130(1), 173-187.)

Energy or Nutrient	Sobell et al. (1989) All Responders (n=90)	Sobell et al. (1989) All Responders (n=126)	Block et al. (1990) Usual diet (N=102)	Block et al. (1990) Low-fat diet (N=158)	Block et al. (1992) HHHQ/S-M-L All Responders (n=85)
Study Design	Retrospective Interview	Retrospective Mail Survey	Concurrent	Concurrent	Concurrent
Reference Data	Mean of 2-4 7 day diet records	Mean of 2-4 7 day diet records	Mean of 3 4-day diet records	Mean of 3 4-day diet records	Mean of 4 sets of a 24-hour recall & a 3-day diet record
Sample	Black & White adult Men	Black & White Adult Men	Women Ages 45-70 Years	Women Ages 45-70 Years	Black & White Men & Women Ages 25-50 years
Energy	0.50	0.14	0.51	0.51	0.62
Protein	0.54	0.16	0.48	056	0.56
Total Fat	0.61	0.27	0.60	0.57	0.58
Carbohydrate	0.45	0.20	0.51	0.55	0.68
Saturated Fat	0.63	0.32	0.63	0.59	0.58
Oleic Acid	0.61	0.26	N/A	N/A	0.56
Linoleic Acid	0.35	0.20	N/A	N/A	0.44
Cholesterol	0.63	0.35	0.55	0.56	0.59
Crude Fiber	0.28	0.20	N/A	N/A	0.54
Calcium	0.40	0.28	0.56	0.62	0.56
Iron	0.43	0.14	0.47	0.44	0.48
Vitamin A	0.37	0.12	0.47	0.37	N/A
Thiamin	0.41	0.18	0.57	0.47	0.59
Riboflavin	0.51	0.25	0.63	0.62	0.59
Niacin	0.42	0.13	N/A	N/A	0.58
Vitamin C	0.47	0.21	0.56	0.48	0.54
Monounsaturated Fat	N/A	N/A	0.59	0.58	N/A
Polyunsaturated Fat	N/A	N/A	0.48	0.46	N/A
Sodium	N/A	N/A	0.47	0.43	0.42
Potassium	N/A	N/A	0.55	0.60	0.60

N/A = data not available.

The Block HHHQ also was compared to the University of Michigan Food Frequency Questionnaire (UMFFQ) using means from 4 day dietary record collected over a year as reference data in 85 Caucasian and African-American men and women. Although the UMFFQ tended to over-estimate energy and nutrients, the Block HHHQ means were not significantly different from food record estimates (Block et al., 1992). Furthermore, comparison between the actual food records and the HHHQ produced moderately strong correlations ranging from a low of 0.42 to a high of 0.68 for energy and specific nutrients.

The reproducibility of the HHHQ was also recently evaluated by administering the HHHQ twice at a 3- month interval to a population-based sample of middle-aged and older adults (n=211) (Mares-Perlman et al., 1993). Median age-specific correlation coefficients between the questionnaires were 0.8 in men and 0.7 in women (range 0.5-0.9).

A specialized computer software program was developed for nutrient analysis. The program multiplies the reported frequency times the reported portion size times the nutrient content and sum these over all foods (Block et al., 1992). The original program was developed for use on a mainframe computer, however adaptations were made for personal computers (Smucker et al., 1989). The software was recently revised and is now menu-driven using a turn-key system (Block et al., 1994). The computer program was subsequently re-validated using the questionnaire administered in the Women's Health Trial Feasibility Study (Block et al., 1990) using the identical reference data but using the revised software and database. Using the revised software, the mean nutrient intakes for the two versions were not dramatically different and the correlations between the FFQ and the diet records were quite similar (Block et al., 1994). According to Block et al. (1994),

the differences were trivial and consistent with random variation.

Concurrent 3-day Dietary Record. With this method, the subject records his or her actual intake each day in a diary. Three day records covering 2 weekdays and 1 weekend day have been used most frequently (Czajka-Narins, 1992). Including weekend days are important since data indicates that often intake is significantly different for women on weekend days than on weekdays (Beaton et al., 1979). Content, amount of food eaten, and food preparation techniques are recorded. The food record is most accurate when participants are trained in how to estimate quantity and record intakes using food and portion size models and measuring cups and spoons (Barrett-Connor, 1991; Czajka-Narins, 1992). Recall can be combined with the food record method when the investigator goes over the food record with the patient and asks for additional information regarding amounts and types of preparation of food (Czajka-Narins, 1992). Data suggest that foods most frequently under-reported on the food record, which may be revealed by the investigator during such structured interviews, are condiments and side-dishes (Beerman & Dittus, 1993). Telephone interviews are an accepted and valid method of obtaining the additional information regarding dietary intake from research subjects (Dwyer, 1994; Krantzler, Mullen, Schutz, Grivetti, Holden, & Meiselman, 1982).

A concurrent dietary record is preferred over dietary recall as it is consider more accurate, a better estimate of an individual's usual intake, and less affected by memory (Block, 1982; 1989). A decline in the short-term memory with age also makes the 24-hour recall method particularly problematic in assessing the diets of elderly people (van Staveren, de Groot, Blauw, & van der Wielen, 1994). Furthermore, although 24 hour recalls are a valid estimate of the mean intake for a group, a single 24 hour dietary recall is

generally considered inadequate for classifying subjects according to their usual intake of a given nutrient because the within-person variance for most nutrients is larger than the between person variance (Kushi, 1994; Barrett-Connor, 1991). Furthermore, according to Dwyer (1994), 24 hour recalls are inappropriate to use in studies involving investigations between intakes and biochemical or other health indices.

A 3-day intake was selected for this study because it is more representative of the patient's intake than a single day. A multi-day record replaces errors of memory with errors of recording and somewhat subdues the fluctuations of a single day's intake by substituting the average of several days (Block, 1982). Stuff and colleagues (1983) measured within individual agreement of daily nutrient estimates by two different methods in 40 young women and found that 3-day diet records were highly correlated with 7-day records ($r = 0.74$ to 0.90) for energy, protein, fat, carbohydrates and calcium, whereas the 1-day record was less highly correlated with the 7-day record ($r = 0.42$ to 0.63).

A 3-day record was selected over a 7-day record as patient compliance is better (Mahalko, Johnson, Gallagher, & Milne, 1985). A 7-day record requires a high degree of cooperation on the part of subjects and attrition or missing data becomes a problem. For example, Gersovitz et al. (1978) reported that 32% of their subjects failed to return the 7-day record or returned an uncodable record. Furthermore, of the 44 elderly persons who did adequately complete the 7-day record, Gersovitz et al. (1978) found that although the association between actual (weighed) and recorded values for subjects was significant in the first 2 days, the accuracy of the recording deteriorated in the last three days.

Procedure

Subject Recruitment and Informed Consent

Potential subjects who met study criteria were identified and asked to participate. Written informed consent for study participation was obtained. A copy of the consent and the Patient's Bill of Rights was given to the patient (Appendix 1). An early morning appointment was made for the subject to be admitted as an outpatient at the General Clinical Research Center (GCRC) at the university medical center for study procedures. Subjects were instructed to fast after midnight the night prior to the study.

Subject Admission History and Physical Examination

Upon arrival at the GCRC subjects removed their shoes and socks and donned a hospital gown. Height and weight were measured and recorded. Vital signs were taken and a fasting blood sample was drawn in the usual fashion. Blood samples were taken immediately to the laboratory on the GCRC unit. A regular, hospital diet with low vitamin C was provided after completion of the blood sample procedure. For subjects who identified themselves as diabetic, an 1800 calorie ADA hospital breakfast was provided.

Subjects were asked to bring a list of their medications and vitamins to the appointment so that they could be recorded during the data collection. When subjects forgot to bring a list of their medications, they were called at home to determine the names of their medications. Medical conditions reported by the subject as being diagnosed by a physician were listed on the data collection form. The subjects were asked a short series of questions regarding the duration of their wound(s), their perception of the cause of the wound, symptoms related to the wound, and their smoking history. Demographic and other relevant data were also obtained from a chart review (when a chart was available)

and included: age, gender, ethnicity, medications, and past medical and surgical history, and whether surgical debridement or infection occurred during the study period (Appendix 6).

A brief history and physical examination were performed and documented (Appendix 7). The following anthropometric measures were recorded: triceps skin fold, mid-arm circumference, wrist circumference, height, and weight. Following these measures, the subject was placed in bed to rest supine for 15 minutes.

Wound Assessment and Measurement

While the patient was reclining in the bed, the wound dressing was removed. If more than one ulcer site existed, one ulcer site was randomly selected to be studied using a random table of numbers. Using aseptic technique, a piece of acetate was placed over the wound and the wound perimeter traced three times using a fine-tipped permanent marker. The subject identification number, date, and time were noted on the tracing. The acetate tracing was cleaned with alcohol immediately after use. Gauze moistened with normal saline was applied to the wound(s).

Transcutaneous Oxygen Monitor Sensor Application

The transcutaneous oxygen monitor electrodes were heated to 44^0 C and the machine was calibrated according to the manufacturer's instructions. The skin was prepared by removing skin oil with an alcohol swab and electrodes were applied using minimal contact gel and a double sided adhesive ring. One electrode was placed at the right midclavicular line one inch below the bone. One electrode was place on the dorsal surface of the ipsilateral foot, taking care not to place the electrode over a superficial vein. Two electrodes were placed within one and one-half inches of the wound: one at the 12

o'clock position and the other at either the 3 o'clock or 9 o'clock position, taking care to avoid areas with severe lipodermatosclerosis or superficial varicosities. If the right subclavicular area was unavailable, the left subclavicular area was used. A light bath blanket was placed over the lower extremities. A pulse oximeter was attached to a finger and values were recorded. After sensor stabilization (approximately 20 minutes), measures of wound, chest, and foot $tcPO_2$ and $tcCO_2$ were recorded while the electrodes were in place. Baseline values were obtained with the subject lying supine breathing room air. Additional values were obtained with the subject breathing room air lying supine legs elevated 30°, sitting, and standing; and breathing 40% - 60 % oxygen (7 L/minute by simple face mask) lying supine, lying supine legs elevated 30°, sitting, and standing. A stabilization period of 15 minutes was allowed following changing of positions and after the addition of supplemental oxygen. For subjects unable to tolerate sitting or standing for a period of 15 minutes due to discomfort, the maximum time in each position was recorded. Room temperature and humidity were recorded.

Nutritional Assessment

During the 20 minute stabilization time, further nutritional assessment was performed. Each subject was asked to complete the 10 item "DETERMINE Your Nutritional Health" Public Awareness Checklist (Appendix 5). Subjects were also asked to complete the dietary intake section of the "Health Habits and History Questionnaire". For subjects who found the print too small to read, the principal investigator read the questions and recorded the subject's response. Prior to the conclusion of the appointment, subjects were instructed on recording a 3-day dietary intake. Common household measures including measuring spoons, measuring cups, soup/salad bowl, and

glasses were provided with accurate measurements described. Each subject was provided a 3 page diary to record their dietary intake for the next 3 days. The principal investigator called each subject at home for 3 consecutive days and recorded the dietary intake for that day. Structured probes were used by the investigator to determine exact food quantities, food preparation techniques, and condiments used. Subjects were asked to return their dietary record which was compared for accuracy of content with the telephone interview record.

Discharge Procedures

At the conclusion of the skin perfusion measurements, the wound was redressed in the same manner as the subject presented. A return appointment was made to perform repeated measures of tissue oxygenation, wound surface area, serum albumin, glucose, glycosylated hemoglobin, and 3-day dietary record 4 weeks later.

Leukocyte Cell Isolation and Sample Preparation

Beginning with subject 9, blood was drawn approximately 30 minutes prior to the completion of the oximetry studies to analyze mononuclear (MN), polymorphonuclear (PMN), and plasma (PL) vitamin C levels. Though not fasting, the hospital breakfast provided was low in vitamin C.

Vacutainer tubes (2) containing EDTA were filled with whole blood. The sample was immediately placed on ice and transported to the GCRC laboratory. Trained laboratory personnel placed three milliliters of Histopaque 1077 (Sigma Diagnostics, St. Louis, MO) into three 15 milliliter conical centrifuge tubes. Three milliliters of Histopaque 1119 (Sigma Diagnostics, St. Louis, MO) were gently underlaid beneath the Histopaque 1077 using a syringe with a long filling needle. Whole blood samples of 6

milliliters each were gently layered over the top layer of Histopaque 1077. The tubes were placed in a swinging bucket centrifuge (IEC CENTRA 7R Centrifuge) and centrifuged at room temperature (21^0 C) for 30 minutes at 700 g. Six distinct layers resulted: the upper (yellow layer) was comprised of plasma; the second, cloudy band, containing MN cells and platelets; the third, wider opaque layer comprised of Histopaque 1077; the fourth, cloudy pink band, containing granulocytes; the fifth, wider opaque layer consisting of Histopaque 1119; and finally, the bottom layer containing erythrocytes.

The plasma from each tube was pooled and placed into a labeled centrifuge tube, wrapped in foil, and placed on ice. The MN cells were carefully aspirated from the tubes, pooled, and placed into a labeled centrifuge tube and placed on ice. The PMN cells were carefully aspirated from the tubes, pooled, and placed into a labeled centrifuge tube and stored on ice. The PMN and MN cells were washed by adding 10 milliliters of phosphate buffered saline (PBS) (without Ca or Mg) to the tubes. The tubes were placed in the swinging bucket and centrifuged at room temperature (21^0 C) for 10 minutes at 700 g.

Four duplicates of 200 µl of plasma were combined with equal amounts of cold extracting solution and vortexed. Fresh extracting solution was made each day, wrapped in foil, and stored on ice. The extracting solution was comprised of 3 ml of 90% Methanol/1mM Na_2EDTA, 100 µl of 1 mM BHT, and 15 µl of 10 mM Desferal. The bullets were microcentrifuged for three minutes. The supernatant was removed and placed into labeled micro centrifuge tubes and stored in the - 80^0 C freezer.

After washing, the PMN and MN cells were removed from the centrifuge. Each cell type was carefully resuspended in 1 milliliter of PBS by gentle aspiration. The MN cells were placed on ice. In order to remove erythrocyte contamination from the PMN

cells, hypotonic lysis was performed. Three milliliters of distilled/deionized water was added to the PMN cells and gently mixed. One milliliter of 3.6% (hypertonic) sodium chloride solution was added and gently mixed. The tubes were centrifuged at room temperature for 10 minutes at 700 g.

Tryphan Blue (Sigma Chemical, St. Louis, MO) was added to the MN cells, cells were counted using a hemocytometer and microscope, and cell viability was determined. Four duplicates of 200 µl of MN cells were placed in micro centrifuge tubes and centrifuged for 3 minutes. The PBS was removed leaving a pellet of cells. 400 µl of cold extracting solution was added to each tube and thoroughly vortexed. The bullets were placed in the micro centrifuge for three minutes. The supernatant was removed and immediately placed into labeled micro centrifuge tubes and stored in the -80 degree Celsius freezer.

After lysis, the PMN cells were removed from the centrifuge. The cells were carefully resuspended in 1 milliliter of PBS by gentle aspiration. Tryphan Blue was added to the PMN cells, cells were counted using a hemocytometer and microscope, and cell viability was determined. Four duplicates of 200 µl of PMN cells were placed in micro centrifuge tubes and centrifuged for 3 minutes. The PBS was removed leaving a pellet of cells. 400 µl of cold extracting solution was added to each tube and thoroughly vortexed. The bullets were placed in the micro centrifuge for three minutes. The supernatant was removed and immediately placed into labeled micro centrifuge tubes and stored in the -80 degree Celsius freezer.

The samples were later transported on dry ice and analyzed using paired-ion, reversed-phase, high performance liquid chromatography using an electrochemical

detector at the Membrane Bioenergetics Laboratory (Lester Packer, PhD, Director), Lawrence Berkeley Laboratory, Berkeley, CA under the guidance of John MaGuire, DDS. To measure PMN and MN ascorbate, the samples were diluted with an equal volume of mobile phase immediately prior to injection of 20 to 40 µl. To measure plasma ascorbate, the samples were further extracted with an equal volume of fresh, cold extracting solution utilizing the procedure described above. The plasma supernatant was then diluted with an equal volume of mobile phase immediately prior to injection of 20 to 60 µl.

Ascorbate Standard Preparation

All standards were kept on ice during preparation. 100 µM Ascorbate stock solution was prepared using cold, degassed 90% Methanol/1 mM Desferal buffer solution. The accuracy of the Ascorbate stock solution was determined using a Perkins Elmer 5 Lambda UV/VIS Spectrophometer. The 100 µM stock solution was further diluted with the cold 90% Methanol/1 mM Desferal buffer solution to produce stock solutions of 2 µM to 40 µM of Ascorbate. These stock solutions were placed in amber vials and stored at -80^0 C. Stock solutions were stable for up to 2 months (less than 3% decay) at -80^0 C. Immediately prior to injection, the stock solutions were removed from the freezer, diluted with equal amounts of mobile phase (see below) to produce working standards of 1 µM to 20 µM of Ascorbate. Twenty µl of three working standards (1 µM to 20 µM) was injected to generate the standard curve.

Chromatography

For separation of ascorbate, a Beckman 114M solvent delivery system, an injector, equipped with a 200 microliter loop, a RP-18 5 µm (4.6 X 30 mm) precolumn, and a

Rainin Microsorb-MV 3 μm 100A C18 (4.6 X 100 mm) column were used. The mobile phase consisted of filtered 40mM sodium acetate buffer, pH 4.75; 0.54mM Na_2EDTA, 2.5% HPLC grade Methanol, and 3 ml of .5M dodecyltriethylammonium phosphate/L of mobile phase (Regis Chemical Co., Morton Grove, IL) used as the ion-pairing agent. The separations were performed at a flow rate of 1 ml/minute with a back pressure of approximately 1800 psi. The retention time for ascorbate was around 5 minutes. Both column and detector required two to three hours for equilibration. Columns were washed between usage in equal parts methanol and water.

The detection consisted of a LC-4B Amperometric detector with a glassy-carbon electrode and a Ag|AgCl reference electrode. The applied potential was set at + 0.5 V with a sensitivity setting of 20 nA. The data from HPLC analysis were digitized by PE Nelson 900 Series analytical interface (Cupertino, CA) and processed Perkin-Elmer/Nelson Analytical Turbochrome (version 2.1) data acquitions software on a 486 personal computer.

Data Analysis

Data were analyzed using Crunch 4.0 (Crunch Software Corporation, Oakland, CA) statistical package for personal computers. Descriptive statistics were determined for all variables. Body mass index was calculated using the standard formula (weight kg/height m^2). Frame size was determined based on the r value. The r value was calculated using the standard formula (height cm/wrist circumference cm). The resulting r value was then compared to a standard table (Grant, 1980). Mid-arm muscle circumference was calculated using the standard formula: MAMC (cm) = MAC (cm) -

(0.314 x TSF (mm)). The total lymphocyte count was determined by calculating the product of the WBC and the percentage of lymphocytes on the differential count.

Question 1

What is the nutritional risk and status of patients with venous leg ulcers? Nutritional status includes anthropometric measures (i.e. triceps skin fold, mid-arm circumference, mid-arm muscle circumference, and body mass index), serum albumin, total lymphocyte count, serum vitamin C and zinc. Means, standard deviations, and frequencies were used to answer question number 1 for each of the nutritional variables. Dietary intake (i.e. total calories, protein, fats, carbohydrates, vitamin A, vitamin C, iron, and zinc) were analyzed using FoodProcessor Plus 6.0 (Salem, OR) computer software for personal computers and reported descriptively.

Question 2

Is the dietary intake of patients with venous leg ulcers adequate to meet the needs for wound healing as determined by the Harris-Benedict equation with adjustments for activity and injury? Dietary intake includes total calories, protein, fats, carbohydrates, vitamin C, and zinc. To answer question number 2, the dietary intake of each individual was compared to their energy requirements as determined by the Harris-Benedict equation with adjustments for activity and injury. Protein intake was compared to the United States recommended daily allowance (RDA) for adults (0.8g protein/kg body weight/day) and the AHCPR guidelines for the treatment of pressure ulcers (1.25g protein/kg body weight/day). Micronutrient intake was compared to the RDA for adults. The frequency of subjects with dietary intake meeting or exceeding their calculated requirements was reported descriptively.

The wound surface area was calculated using an image analysis system (SigmaScan/Image, San Rafael, CA). The acetate tracing was placed on a flatbed scanner (ScanJet II, Hewlett-Packard, Palo Alto, CA) and the image was scanned using DeskScan II (Hewlet-Packard, Palo Alto, CA). The image was saved in a TIF file. The image was retrieved into the SigmaScan/Image program and the wound margins were traced using the mouse. The software calculated the wound perimeter and area. The rate of wound closure was determined using the following equation: d (average linear advance) = A (change in area) / p (average perimeter) (Gilman, 1990). A regional perfusion index (RPI) was calculated using the standard formula.

Question 3

Is there a relationship between the rate of wound closure and the nutritional status of subjects with a venous leg ulcer when controlling for the effects of perfusion? Nutritional status includes serum albumin, total lymphocyte count, plasma vitamin C, and zinc. Multiple regression analysis was planned to answer question number 3. However, due to the small sample size, that analysis could not be performed. Instead, contingency table analysis was performed using the Fisher's Exact test.

Question 4

How do transcutaneous tissue oxygen values at lower extremity venous ulcer sites vary with specific positions and with 21% and 40% to 60% inspired oxygen? Three way repeated measures analysis of variance (RMANOVA) was used to answer question number 4 at Time 1 and Time 2. If the overall Omnibus F test was significant, then post-hoc Scheffe' pairwise comparisons were performed.

Question 5

Is there a relationship between glycosylated hemoglobin levels and plasma and leukocyte vitamin C levels in subjects with a venous leg ulcer? Pearson Product Moment correlations were used to answer question number 5.

Question 6

Is there a relationship between rate of wound closure and plasma and leukocyte vitamin C levels in subjects with a venous leg ulcer? Pearson Product Moment correlations were used to answer question number 6.

CHAPTER IV

RESULTS

This study explored nutrition, tissue oxygenation, and healing in individuals with venous leg ulcers. Nutritional risk, status, and intake, transcutaneous oxygen values, and wound surface area were evaluated at two points in time four weeks apart.

Descriptive data for the sample are presented first. The results are then presented according to the questions that guided the study. Descriptive data for the variables associated with each question are presented followed by the results of the appropriate inferential statistics.

Sample Characteristics

Thirty-one patients were recruited for this study. Six patients who consented did not keep their scheduled appointments and did not respond to follow up telephone calls or letters. Twenty-five subjects attended the first visit. Two male subjects did not attend the second visit and did not respond to follow up telephone calls or letters. One female was dropped from the study when it was learned she had scleroderma. Personal medical emergencies (acute renal failure (also had scleroderma), ruptured cerebral aneurysm) prevented one male and one female patient respectively from attending their follow up visit.

Of the 25 subjects attending the first visit, 15 were men and 10 were women. Three of the dropouts were men, two were women. The subjects ranged in age from 29 to 86 years with a mean of 59.8 years (SD 15.3). The sample was ethnically diverse with 6 African-Americans, 3 Asian/Pacific Islanders, 12 Caucasians, and 4 Latinos. Two of the dropouts were Caucasian, one was Asian/Pacific Islander, two were African-American.

Each subject had at least one active ulcer site at the time of participation. Twenty-three subjects had unilateral leg ulcers, two subjects had ulcers on both legs. The average duration of the leg ulcer was 11.8 months (SD 21.1) with a range of one to 96 months. Seventeen subjects reported one or more previous venous leg ulcers. Nine subjects reported a history of leg ulcers on both legs. The locations of the study leg ulcers were as follows: 9 right medial malleolar, 7 left medial malleolar, 4 left lateral malleolar, 1 right lateral malleolar, and 4 right pretibial area.

Prior medical history revealed the presence of conditions which are associated with the development of venous insufficiency. These conditions include the following: deep vein thrombosis, varicose veins, vein surgery, hip injury/surgery, knee injury/surgery, ankle injury/surgery, and intravenous drug abuse. The frequency of these conditions within the sample are presented in Table 4-1.

Table 4-1. Summary of prior medical conditions related to venous insufficiency.

Clinical Condition	Number*	Frequency*
Varicose Veins	24	96%
Deep Vein Thrombosis or Phlebitis	9	36%
Intravenous Drug Abuse	6	24%
Knee Injury/Surgery Hip	5	20%
Vein Surgery	5	20%
Hip Injury/Surgery	4	16%
Ankle Injury/Surgery	3	12%

*not mutually exclusive categories

At the time of the initial visit, the wound care regime was assessed. Subjects reported the primary wound covering/ointment being applied, the secondary dressing being applied, and any type of compression used. These data are summarized in Tables 4-

2 and 4-3. Tables 4-2 and 4-3 are not mutually exclusive. Topical ointments and gels reported included triamcinolone acetonide cream (TAC), aloe vera gel, Carrington gel, Panafil ointment, and Polysporin with lidocaine gel. Nearly all subjects provided a moist environment for healing (22/25) and wore an external dressing (23/25). The majority (19/25) also utilized compression on the affected extremity.

Table 4-2. Primary wound coverings used by sample for venous leg ulcers.

Primary Wound Covering	Number	Frequency
Zinc Oxide + Unna's Boot	6	24%
Ointment/gel + Gauze/Telfa	4	16%
Hydrocolloid Dressing only	3	12%
Damp Saline Gauze + Kerlix	3	12%
Foam Dressing + Unna's Boot	3	12%
Ointment/gel alone	2	8%
Hydrocolloid + Unna's Boot	2	8%
Foam Dressing only	1	4%
Other (Telfa + Kerlix)	1	4%

Table 4-3. Type of compression therapy used by sample on affected leg.

Compression	Number	Frequency
Coban Wrap (over Unna's Boot)	8	32%
No Compression	6	24%
Compression Stockings	4	16%
Compression Pump	2	8%
Stockinette (over Unna's Boot)	2	8%
Compression Stockings & Circ-Aide Compression Legging	2	8%
Ace Bandage (over Unna's Boot)	1	4%

Any additional modalities being used to facilitate healing were also noted. One subject had daily home whirlpool therapy and one subject was undergoing acupuncture.

Nutritional Risk and Status

Question 1

What is the nutritional risk and status of patients with venous leg ulcers? Nutritional status includes anthropometric measures (BMI, TSF, MAC, MAMC), serum albumin, total lymphocyte count, plasma vitamin C, serum zinc, fasting glucose, and glycosylated hemoglobin (A_{1C}).

Nutritional Risk. All 25 subjects completed the Public Awareness Checklist (PAC) at the initial visit. The mean PAC score was 5.4 (SD 3.4). The PAC scores ranged from zero to 14. The PAC categorized subjects' nutritional risk as follows: four at low risk; eleven at moderate risk; and ten at high risk.

Anthropometric Measures. Baseline anthropometric data were obtained at the initial visit (Time 1). Repeat measurements of weight and body mass index were determined at Time 2. The median frame size for both males and females was large. Two subjects had a small frame, seven subjects were medium frame, and fourteen had a large frame.

The mean anthropometric measures of triceps skin fold (TSF), mid-arm circumference (MAC), and mid-arm muscle circumference (MAMC) for the sample are presented in Table 4-4. These data indicated that overall the sample was not depleted. When examined individually, some level of depletion was present. The established standards from the National Health and Nutrition Examination Study I and II were used for comparison for adult subjects up to and including 74 years of age (Frisanchco, 1984).

For subjects over 74 years of age, standards established by Falciglia and colleagues (1988) were utilized for comparison. Compared to the standards for TSF measurements, eleven subjects were above the 50th percentile, eight subjects were between the 15th and 50th percentile, four were between the 5th and 15th percentile, and 2 subjects fell below the 5th percentile. For MAC measurements, sixteen subjects were above the 50th percentile, four were between the 15th and 50th percentile, three were between the 5th and 15th percentile, and two subjects fell below the 5th percentile. For MAMC measurements, sixteen subjects were above the 50th percentile, five subjects were between the 5th and 15th percentile, four subjects were between the 15th and 50th percentile, and no subjects fell below the 5th percentile for MAMC.

Table 4-4. Mean anthropometric measures for sample with venous leg ulcers.

Anthropometric Measure	Mean	Standard Deviation	Percentile
Triceps Skin Fold (mm)			
Males (n=15)	18.3	5.9	> 75th
Females (n=10)	20.0	8.2	> 25th
Mid-arm Circumference (cm)			
Males (n=15)	35.1	5.4	> 75th
Females (n=10)	30.4	6.7	> 50th
Mid-arm Muscle Circumference (cm)			
Males (n=15)	29.3	4.4	> 50th
Females (n=10)	24.1	5.4	> 50th

The sample mean weight and body mass indices at Time 1 and 2 are displayed in Table 4-5. Utilizing accepted nutritional standards, an average body mass index of 31.3 Kg/m^2 for males and 28.4 Kg/m^2 for females indicated that overall both the men and women were obese (Bray, 1992; Bray & Gray, 1988). When evaluated individually, two individuals were underweight, seven were within their ideal body weight, six were

overweight, and the remaining nine were obese (Forse et al., 1989). Of the twenty individuals who completed both visits (four weeks apart), there was not a significant difference in body mass index at Time 1 compared to Time 2 (t=- 0.244, p=NS).

Table 4-5. Comparison of weight and body mass index at Time 1 & 2.

Anthropometric Measure	Mean	Standard Deviation
Weight at Time 1 (Kg)		
Males (n=14)*	98.8	24.7
Females (n=10)	72.7	29.8
Weight at Time 2 (Kg)		
Males (n=12)	98.2	26.6
Females (n=7)**	78.2	34.6
Body Mass Index at Time 1 (Kg/m^2)		
Males (n=14)*	31.3	7.3
Females (n=10)	28.4	9.8
Body Mass Index at Time 2 (Kg/m^2)		
Males (n=12)	31.3	8.0
Females (n=7)**	30.9	10.8

*1 Male omitted at Time 1 and 1 Female omitted at Time 2 due to missing data.

Biochemical Indicators. The sample mean biochemical indicators of nutritional status are shown in Tables 4-6 and 4-7. Missing data are the result of lost/mishandled samples during laboratory processing. Overall, the sample average values were within the reference range except for hemoglobin, hematocrit, and fasting glucose at Time 1, and glycosylated hemoglobin (A_{1C}) at both Time 1 and 2. The mean hemoglobin and hematocrit values for the males were lower than normal. The sample mean glucose and glycosylated hemoglobin (A_{1C}) levels were slightly above normal. There was not a significant difference in the overall mean serum albumin (t = -0.520, p = NS), fasting glucose (t = 1.032, p = NS), or glycosylated hemoglobin (A1C) (t = 0.788, p = NS) levels between Time 1 and 2.

Table 4-6. Mean biochemical indicators of nutritional status measured at Time 1.

Biochemical Indicators of Nutritional Status	Mean	Standard Deviation	Reference Range
Lymphocyte Count (cells/ml) (n=25)	1538.93	715.2	>1500
Hemoglobin (g/dl)			
Males (n=15)	13.15	2.16	13.5-18.0
Females (n=10)	12.38	2.05	11.0-16.0
Hematocrit (%)			
Males (n=15)	39.32	5.69	40.0-56.0
Females (n=10)	36.77	6.09	34.0-48.0
Vitamin C (mg/dl) (n=21)	0.74	0.33	0.20-1.90
Zinc (mcg/dl) (n=25)	87.52	26.30	60-130

Table 4-7. Mean biochemical indicators of nutritional status measured at Time 1 & 2.

Biochemical Indicator of Nutritional Status	Time 1 Mean (SD)	Time 2 Mean (SD)	Reference Range
Albumin (g/dl) Time 1 (n=25) Time 2 (n=20)	4.12 (0.25)	4.16 (0.26)	3.6-5.4
Glucose (mg/dl) Time 1 (n=25) Time 2 (n=20)	123.8 (57.4)	116.9 (49.5)	70-108
Glycosylated hemoglobin $_{(A1C)}$ (%) Time 1 (n=24) Time 2 (n=20)	6.35(1.86)	6.39 (1.66)	<6.2

When biochemical indicators were examined for each subject, nutritional abnormalities were identified in several areas including low hemoglobin/hematocrit, serum albumin, serum zinc, plasma vitamin C, and elevated fasting glucose and glycosylated

hemoglobin (A_{1C}) (Table 4-8). Fourteen subjects had a total lymphocyte count less than 1500 cells/ml. Of the male participants, six had low hemoglobin/hematocrit, in contrast to two females. Six subjects had a fasting glucose level > 120 mg/dl at Time 1 and four subjects had an elevated fasting glucose level at Time 2. At the initial visit, eight individuals displayed a glycosylated hemoglobin (A_{1C}) level > 6.2%, while six individuals had elevated levels at Time 2. Three individuals had low zinc levels and one had a low plasma vitamin C level. Only one individual had a slightly low serum albumin. Three subjects showed no abnormalities in any of their biochemical indices of nutritional status.

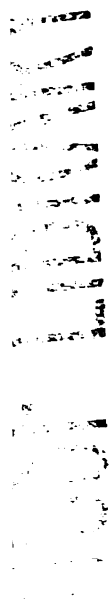


Table 4- 8. Biochemical indicators of nutritional status by subject.

ID	Subj	Alb	Tlc	Hgb	Hct	Gluc	$HgbA_{1C}$	Zinc	VitC
1	58 y/o M	4.3	2341	13.0*	38.2*	100	6.3**	81	0.60
2	64 y/o F	4.0	1329*	13.8	40.5	126**	6.4**	84	N/A
3	45 y/o M	3.9	2055	11.1*	34.3*	102	6.5**	86	1.00
4	60 y/o M	4.0	1581	14.3	41.5	292**	13.9**	75	0.50
5	86 y/o M	4.1	909*	11.5*	33.6*	108	5.7	88	0.60
6	60 y/o F	4.1	1512	12.2	38.7	112	6.9**	70	0.60
7	53 y/o F	4.2	961*	13.1	37.6	94	5.8	61	1.30
8	67 y/o F	4.4	726*	13.0	37.7	100	6.5**	120	0.83
9	48 y/o M	4.0	1258*	11.3*	35.7*	264**	9.6**	61	1.00
10	54 y/o M	4.5	1938	16.3	48.8	257**	N/A	130	N/A
11	42 y/o M	4.2	1692	14.7	42.1	100	5.8	80	0.62
12	79 y/o M	4.1	855*	15.2	43.3	95	5.3	90	0.81
13	78 y/o M	4.3	980*	13.9	42.1	106	6.0	120	1.40
14	52 y/o F	4.3	3569	15.1	46.6	93	6.6**	110	0.11*
15	68 y/o F	4.1	1276*	12.2	35.7	106	6.2	120	0.73
16	62 y/o F	3.9	728*	8.1*	24.4*	83	4.8	97	0.55
17	63 y/o F	3.5*	1116*	9.7*	29.4*	141**	5.7	30*	N/A
18	71 y/o M	4.0	1334*	13.5	40.3	140**	6.2	110	1.01
19	29 y/o M	4.3	1190*	13.6	40.7	99	5.5	58*	0.60
20	46 y/o M	4.1	3066	7.9*	25.2*	99	5.5	51*	0.44
21	78 y/o M	4.2	2300	15.0	44.1	88	4.7	76	0.22
22	82 y/o F	3.8	990*	12.9	37.2	95	5.4	79	1.01
23	76 y/o F	4.0	1081*	13.7	39.9	103	5.8	91	0.57
24	37 y/o M	4.8	1632	14.6	43.8	101	5.8	140	1.15
25	39 y/o M	4.0	2054	11.4*	36.1*	91	5.4	80	N/A

ID=Subject identification number, Subj=Subject description, Alb=Albumin, Tlc=Total lymphocyte count, Hgb=Hemoglobin, Hct=Hematocrit, Gluc=Glucose, $HgbA_{1C}$=Glycosylated hemoglobin (A_{1C}), Zinc=Zinc, VitC=Vitamin C, M=Male, F=Female, N/A=Not available.

* Value Below Normal, ** Value Above Normal.

Dietary Intake

Question 2

Is the dietary intake of patients with venous leg ulcers adequate to meet the needs for wound healing as determined by the Harris-Benedict equation with adjustments for activity and injury? Dietary intake includes total calories, protein, fats, carbohydrates, vitamin A, vitamin C, iron, and zinc.

Caloric Need. Daily caloric need was determined by using the Harris-Benedict equation with adjustment for injury and activity (Harris & Benedict, 1919; Long et al., 1979). An activity level of 1.3, reflecting an ambulatory status, and an injury factor of 1.2, indicative of minor injury, were used for all subjects. Daily caloric need, based on the 20 subjects who completed the 3 day dietary intake records at Time 1 and 2, ranged from 1543 to 3858 calories (M 2660, SD 722).

Caloric & Protein Intake. Nutritional intake data were available for 20 subjects for both visits. At Time 1, the average daily caloric intake ranged from 649 to 2894 calories (M 1779.9, SD 651.7, SE 145.7). At Time 2, the average daily caloric intake range from 755 to 3507 calories (M 1700.1, SD 666.1, SE 148.9). There was not a significant difference in the mean daily caloric intake between Time 1 and Time 2 ($t = 0.981$, $p = NS$). The overall mean daily caloric intake (based on 6 days of records) ranged from 702 to 3200 calories (M 1753.6, SD 638.9, SE 142.9).

At Time 1, the mean daily protein intake ranged from 34.5 g to 112.0 g (M 73.7, SD 21.6, SE 4.8). At Time 2, the mean daily protein intake ranged from 39.3 g to 140.0 g (M 76.4 SD 21.9, SE 4.9). The overall mean daily protein intake (based on 6 days of

Table 4-9. Caloric Need, Overall Mean Caloric Intake, Percent RDA Protein Intake, and Percent AHCPR Protein Intake for each Subject.

ID	SUBJECT	CALORIC NEED	CALORIC INTAKE	PROTEIN (% RDA)	PROTEIN (% AHCPR)
1	58 y/o M	2747	2074*	98	63*
2	64 y/o F	2351	702*	64*	41*
3	45 y/o M	2712	2168*	106	68*
4	60 y/o M	3475	N/A	N/A	N/A
5	86 y/o M	1709	1339*	145	93*
6	60 y/o F	2131	1149*	87*	56*
7	53 y/o F	2227	1067*	85*	55*
8	67 y/o F	1867	N/A	N/A	N/A
9	48 y/o M	3800	1992*	81*	52*
10	54 y/o M	3685	1633*	63*	40*
11	42 y/o M	2998	2256*	104	67*
12	79 y/o M	2760	2555*	108	69*
13	78 y/o M	2593	2055*	111	72*
14	52 y/o F	3303	1143*	48*	31*
15	68 y/o F	1768	1529*	186	119
16	62 y/o F	1856	N/A	N/A	N/A
17	63 y/o F	1820	1452*	202	129
18	71 y/o M	3689	1121*	52*	33*
19	29 y/o M	2880	3200	191	123
20	46 y/o M	N/A	N/A	N/A	N/A
21	78 y/o M	2239	2672	155	100
22	82 y/o F	1543	2107	257	164
23	76 y/o F	2383	1678*	92*	59*
24	37 y/o M	3858	1180*	102	65*
25	39 y/o M	2936	N/A	N/A	N/A

ID=Subject identification number, RDA=recommended daily allowance, AHCPR=Agency for Health Care Policy & Research, F=female, M=male, N/A=data not available.
* Below recommended intake.

records) ranged from 44.6 g to 126.0 g (M 74.5 g, SD 19.5, SE 4.4). There was not a significant difference in mean daily protein intake between Time 1 and Time 2 (t = -0.644, p = NS).

The comparison of caloric need with overall caloric intake, and protein intake with recommended daily allowance (RDA) and Agency for Health Care Policy and Research (AHCPR) guidelines is presented in Table 4-9. Intake was adequate to meet caloric needs in three subjects (15%) and inadequate in seventeen subjects (85%). The RDA guideline for protein intake for normal healthy adults is calculated as 0.8 g/kg/day. Using the RDA as a conservative estimate of protein needs for healing, twelve subjects (60%) had adequate protein intake and eight (40%) had an inadequate protein intake. The recent AHCPR guidelines for the treatment of pressure ulcers suggest that 1.25 g/kg/day of protein are necessary for healing open wounds (Bergstrom et al., 1994). When the AHCPR guidelines were used to determine protein intake adequacy, five individuals (25%) had an adequate intake and 15 (75%) had an inadequate protein intake.

Carbohydrate & Fat Intake. At Time 1, the average daily fat intake ranged from 15.3 g to 131.0 g (M 67.7, SD 34.0, SE 7.6). At Time 2, the average daily fat intake ranged from 25.6 g to 124.0 g (M 61.2, SD 27.4, SE 6.1). There was not a significant difference in the mean daily fat intake between Time 1 and 2 (t = 1.094, p = NS). The overall mean daily fat intake (based on 6 days of records) ranged from 20.5 to 119 g (M 65.2, SD 28.7, SE 6.4).

At Time 1, the mean daily carbohydrate intake ranged from 89.6 g to 461.0 g (M 228.6, SD 94.8, SE 21.2), and at Time 2, it ranged from 85.9 g to 485.0 g (M 219.5, SD 101.9, SE 22.8). There was not a significant difference in the mean daily carbohydrate

intake between Time 1 and 2 (t = 0.731, p = NS). The overall mean daily carbohydrate intake ranged from 88.1 to 473.0 g (M 226.0, SD 95.4, SE 21.3).

Micronutrient Intake. The mean daily intake at Time 1 and Time 2 as well as the overall mean daily intake (based on six days records) for vitamin A, vitamin C, iron, and zinc are presented in Table 4-10. At both Time 1 and 2, the mean daily intake of vitamin A, vitamin C, and iron exceeded the RDA for healthy adults. At both Time 1 and 2, the mean daily zinc intake was less than the RDA for adults. Similarly, the overall mean daily intake (based on 6 days) of vitamin A, vitamin C, and iron were also more than the RDA for adults, while the mean for zinc was less. Mean daily intake of vitamin A (t = 0.700, p = NS), vitamin C (t = 1.368, p = NS), iron (t = -0.207, p = NS), and zinc (t = -1.190, p = NS) were not significantly different between Time 1 and Time 2.

Table 4-10. Mean daily intake of micronutrients overall & at Time 1 & 2.

Micronutrient	Time 1 Mean (SD, SE)	Time 2 Mean (SD, SE)	Overall Mean (SD, SE)
Vitamin A (RE)	1575.2 (1869.3, 418.0)	1253.4 (1064.8, 238.1)	1226.4 (892.1, 199.5)
Vitamin A (% RDA)	166 (187, 42)	133 (106, 24)	132 (84, 19)
Vitamin C (mg)	157.7 (159.5, 35.7)	140.5 (144.1, 32.2)	152.0 (33.3, 22.2)
Vitamin C (% RDA)	263 (266, 59)	234 (240, 54)	253 (249, 56)
Iron (mg)	13.6 (4.7, 1.0)	13.8 (6.5, 1.4)	13.8 (5.1, 1.1)
Iron (% RDA)	136 (47, 10)	138 (65, 14)	138 (51, 11)
Zinc (mg)	9.0 (4.1, 0.9)	10.3 (4.8, 1.1)	9.5 (3.8, .8)
Zinc (% RDA)	65 (28, 6)	74 (31, 7)	68 (25, 6)

SD=standard deviation, SE=standard error, RE=retinol equivalents, %RDA=percent of the recommended daily allowance consumed.

Subjects reported any vitamin, mineral, and/or nutritional supplement taken on a regular basis. The number and frequency of the most frequently reported supplemental nutrients are portrayed in Table 4-11. The most commonly utilized supplements were iron, multivitamins, vitamin C, and vitamin E.

Table 4-11. Self-reported supplemental vitamin and mineral intake.*

Supplement	Number	Frequency
Iron	8	32%
Multivitamin	7	28%
Vitamin C	6	24%
Vitamin E	6	24%
Zinc	4	16%
1 or more B Vitamins	4	16%
Vitamin A	3	12%
Liquid Supplement (Ensure)	2	8%
Vitamin D	1	4%

*Some subjects took more than one supplement.

Evaluation of micronutrient intake by individual showed deficiencies in several areas (Table 4-12). Seven individuals (35%) had inadequate intake of vitamin A; six individuals (30%) had inadequate intake of vitamin C; and six (30%) had inadequate intake of iron. Only three subjects (15%) had an adequate intake of zinc, while the remaining 17 subjects (85%) zinc intake was insufficient. Seventeen subjects (85%) had inadequate dietary intake of at least one micronutrient. Four subjects (20%) had inadequate dietary intake of all four micronutrients.

Table 4-12. Mean daily micronutrient intake for each subject.

ID	SUBJ	VITAMIN A (% RDA)	VITAMIN C (%RDA)	IRON (% RDA)	ZINC (% RDA)
1	58 y/o M	128	191	157	62*
2	64 y/o F	20*	67*	70*	23*
3	45 y/o M	75*	49*	123	62*
4	60 y/o M	N/A	N/A	N/A	N/A
5	86 y/o M	67*	174	93*	52*
6	60 y/o F	57*	169	84*	47*
7	53 y/o F	38*	85*	75*	29*
8	67 y/o F	N/A	N/A	N/A	N/A
9	48 y/o M	198	334	157	85*
10	54 y/o M	158	66*	111	61*
11	42 y/o M	261	203	127	72*
12	79 y/o M	272	373	189	74*
13	78 y/o M	158	321	192	85*
14	52 y/o F	12*	43*	79*	48*
15	68 y/o F	134	292	113	64*
16	62 y/o F	N/A	N/A	N/A	N/A
17	63 y/o F	126	284	120	66*
18	71 y/o M	66*	37*	92*	38*
19	29 y/o M	213	1175	246	101
20	46 y/o M	N/A	N/A	N/A	N/A
21	78 y/o M	117	264	198	81*
22	52 y/o F	205	443	183	94*
23	76 y/o F	120	174	211	97
24	37 y/o M	296	319	135	117
25	39 y/o M	N/A	N/A	N/A	N/A

ID=Subject identification number, SUBJ=subject description, % RDA=percent of recommended daily allowance consumed, F=female, M=male, N/A=data not available.

* Below recommended intake.

Rate of Wound Closure

Question 3

Is there a relationship between the rate of wound closure and the nutritional status of individuals with venous leg ulcers when the effects of perfusion are partialed out? Nutritional status includes serum albumin, total lymphocyte count, plasma vitamin C, and serum zinc. Perfusion is measured as a regional perfusion index.

Descriptive Data. The sample mean wound size and healing data are presented in Table 4-13. The mean rate of wound closure was 0.142 cm/ four weeks (SD 0.21, SE 0.05). The mean percent of area reduction during the study was 26.0 (SD 79, SE 18). Ulcer sites completely healed in two individuals (10%). Thirteen individuals (65%) had some decrease in their total ulcer site area. In five subjects (25%), the total ulcer site area increased between Time 1 and 2. One individual developed a new ulcer site on the contralateral leg. There was not a statistically significant decrease in wound perimeter during the study (Wilcoxon = 1.792, $p = 0.07$); however, there was a significant decrease in total ulcer site area (Wilcoxon = 2.016, $p < 0.05$). A summary of the wound healing data by subject is presented in Table 4-14.

Table 4-13. Mean wound perimeter and area for sample at Time 1 and 2.

Wound Healing Parameter	Time 1 Mean (SD,SE)	Time 2 Mean (SD,SE)	Range
Perimeter (cm) (n=20)*	7.66 (4.54, 1.01)	5.62 (4.85, 1.08)	0.00 to 18.56
Area (cm^2) (n=20)**	2.24 (2.21, 0.49)	1.30 (1.65, 0.37)	0.0 to 7.55

SD=Standard deviation, SE=Standard error.
* $p > 0.05$, ** $p < 0.05$

Table 4-14. Wound healing parameters at Time 1 and Time 2 by subject.

ID	Subj	Perim1	Area1	Perim2	Area2	Closure	%Arearedc
1	58 y/o M	5.89	1.61	9.90	1.76	-0.02	-9.30
2	64 y/o F	9.2	1.88	9.55	2.39	-0.05	-27.15
3	45 y/o M	5.15	1.37	4.36	0.94	0.09	31.32
4	60 y/o M	4.11	0.83	N/A	N/A	N/A	N/A
5	86 y/o M	7.25	1.68	3.01	0.42	0.24	74.69
6	60 y/o F	15.08	2.37	2.37	0.26	0.24	89.02
7	53 y/o F	16.77	7.55	5.99	1.14	0.56	84.94
8	67 y/o F	8.09	3.30	N/A	N/A	N/A	N/A
9	48 y/o M	8.36	1.15	12.72	2.93	-0.17	-153.85
10	54 y/o M	6.86	1.17	4.44	0.64	0.09	45.60
11	42 y/o M	2.49	0.35	1.63	0.16	0.09	51.16
12	79 y/o M	3.68	0.52	5.29	1.51	-0.22	-191.42
13	78 y/o M	15.10	4.82	18.56	7.01	-0.13	-45.56
14	52 y/o F	3.01	0.56	2.84	0.48	0.03	14.40
15	68 y/o F	3.96	0.48	0.00	0.00	0.24	100.00
16	62 y/o F	10.72	1.12	N/A	N/A	N/A	N/A
17	63 y/o F	12.19	3.99	5.82	0.63	0.37	84.32
18	71 y/o M	7.81	2.67	6.26	1.16	0.21	56.49
19	29 y/o M	2.44	0.29	1.44	0.13	0.09	56.93
20	46 y/o M	14.58	1.68	N/A	N/A	N/A	N/A
21	78 y/o M	1.82	0.20	1.48	0.13	0.04	32.92
22	82 y/o F	5.91	1.07	0.00	0.00	0.36	100.00
23	76 y/o F	12.34	7.45	12.71	3.32	0.33	55.40
24	37 y/o M	7.86	3.61	4.08	1.00	0.43	72.34
25	39 y/o M	5.46	1.72	N/A	N/A	N/A	N/A

ID=Subject identification number, Subj=Subject description, PERIM1=perimeter in cm at Time1, AREA1=area in cm^2 at Time 1, PERIM2=perimeter in cm at Time2, AREA2=area in cm^2 at Time2, CLOSURE=rate of wound closure in cm during 4 week study period, %AREAREDC=percent of area reduction during 4 week study period, M=Male, F=Female, N/A=data not available.

Inferential Statistics. Multiple regression was planned to answer this question. Due to the small sample size, the large number of predictor variables, and the lack of power, the assumptions were not met and the regression could not be performed. Pearson Product Moment correlations did not reveal any significant relationships between rate of wound closure and the nutritional parameters of interest (albumin, total lymphocyte count, zinc, or vitamin C) or the regional perfusion index (Table 4-15).

Table 4-15. Pearson correlations between wound closure, nutritional indicators, and region perfusion index (RPI). (n=20)

Variable	Albumin	TLC	Vitamin C	Zinc	RPI
Wound Closure	-0.07 p=.76	-0.22 p=.36	0.12 p=.62	-0.07 p=.75	0.19 p=.43

TLC=total lymphocyte count, RPI=regional perfusion index.

In order to attempt to understand the relationships between nutrition, perfusion, and healing of venous leg ulcers, 2 X 2 continency tables were created. Variables were collapsed as follows: wound closure >/= sample mean = good healing, wound closure < sample mean = poor healing, RPI >/= 0.5 = good perfusion, RPI < 0.5 = poor perfusion, albumin >/= 3.6 & total lymphocyte count >/= 1500 = good macronutrition, albumin < 3.6 or total lymphocyte count < 1500 = poor macronutrition, vitamin C >/=0.2 and zinc >/= 60 = good micronutrition, vitamin C < 0.20 or zinc < 60 = poor micronutrition. Chi square statistics could not be used as the assumptions for the minimum number of expected frequencies were not met. Alternatively, the three 2 X 2 tables were created (Healing X Perfusion, Healing X Macronutrients, Healing X Micronutrients) and a Fisher's Exact Test was performed. None of the relationships achieved statistical significance. A summary of the results are presented in Table 4-16.

Table 4-16. Summary of results of contingency table analysis of healing, perfusion, and nutrition. (n=20).

Variables in 2 X 2 Table	Fisher's Exact Test
Healing X Macronutrients	p=0.16
Healing X Micronutrients	p=0.58
Healing X Perfusion	p=0.31

Upon further examination of the data, it appeared that the eight female subjects healed more rapidly than the 12 male subjects. Contingency table analysis was performed to explore this observation. The healing variable was collapsed as follows: wound closure >/= sample mean = good healing, wound closure < sample mean = poor healing. This observation was confirmed and the Fisher's Exact Test was significant at the $p < 0.05$ level.

TRANSCUTANEOUS TISSUE OXIMETRY

Environmental Monitoring

During the initial visit, the room temperature ranged from 21.0° C to 25.0° C with a mean of 23.0° C (SD 1.0). During the follow up visit, the room temperature ranged from 19.0° C to 25.0° C with a mean of 22.2° C (SD 1.2). The humidity ranged from < 25% to 61% with a mean of 45.1% (SD 8.8) at Time 1. At the follow up visit, the mean humidity was 47.3% (SD 12.4) with a range of <25% to 63%.

Oxygen Saturation

The overall mean oxygen saturation (SaO_2) at Time 1, regardless of position, while breathing room air was 96.5% (SD 1.3) and 99.1% (SD 0.8) with oxygen supplementation. At Time 1, the resting mean SaO_2 with breathing supplemental oxygen was significantly greater than the resting mean SaO_2 while breathing room air ($t = -9.247$, $p < 0.0001$). The

overall resting SaO_2 at Time 2, regardless of position, while breathing room air was 96.2% (SD 1.9) and 99.0% (SD 0.9) with oxygen supplementation. At Time 2, the resting mean SaO_2 with supplemental oxygen was significantly greater than the resting mean SaO_2 while breathing room air ($t = -10.722$, $P < 0.0001$).

Question 4

How do transcutaneous tissue oxygen values at venous leg ulcer sites vary with specific positions and with 21% and 40% to 60% inspired oxygen?

Four sites were used to measure transcutaneous tissue oxygen ($TcpO_2$): chest reference (C site), dorsal foot (B site), proximal periulcer at the 12 o'clock position (L site) and a lateral periulcer site at either the 3 or 9 o'clock position (A site). Twenty patients had $TcpO_2$ measured at Time 1 and Time 2. The mean resting (i.e. lying supine) $TcpO_2$ values on room air and breathing supplemental oxygen at the four sites are present in Table 4-17.

Table 4-17. Mean resting $TcpO_2$ values, while breathing oxygen and room air, for all four sites at Time 1 and Time 2.

Sensor Site Location	Time 1	Time 2
Chest Reference on RA (mm Hg)	56.2 (SD 13.6)	55.1 (SD 13.6)
Chest Reference on O_2 (mm Hg)	125.0 (SD 36.9)	124.8 (SD 46.4)
Proximal Ulcer on RA (mm Hg)	39.2 (SD 15.1)	38.9 (SD 16.0)
Proximal Ulcer on O_2 (mm Hg)	75.3 (SD 36.3)	81.6 (SD 39.1)
Lateral Ulcer on RA (mm Hg)	30.9 (SD 14.6)	35.0 (SD 17.0)
Lateral Ulcer on O_2 (mm Hg)	71.6 (SD 29.8)	81.6 (SD 39.1)
Foot Site on RA (mm Hg)	44.2 (SD 19.9)	37.6 (SD 12.5)
Foot Site on O_2 (mm Hg)	82.0 (SD 37.2)	78.7 (SD 36.5)

RA= room air, SD=standard deviation, O_2=oxygen.

Chest Reference Site (C site). At Time 1, the overall mean C site $TcpO_2$ level, averaged across all positions, while breathing room air was 57.7 mm Hg (SD 13.1) and

120.4 (SD 33.5) mm Hg with oxygen. At Time 2, the overall mean C site $TcpO_2$ level, averaged across all positions, while breathing room air was 59.3 mm Hg (SD 16.3) and 119.9 (SD 39.7) mm Hg with oxygen. At both Time 1 ($t = -8.813$, $P < 0.0001$) and Time 2 ($t = -8.206$, $P < 0.0001$), the resting mean C site $TcpO_2$ while breathing supplemental oxygen was significantly greater than the resting mean $TcpO_2$ while breathing room air.

Lower Extremity Sites. A three way univariate RMANOVA was performed for Time 1 and Time 2. The dependent variable was $TcpO_2$ and the three within factors were level of oxygen (room air versus oxygen), position (lying, legs elevated, standing, sitting), and sensor site (proximal ulcer, lateral ulcer, foot). For the within factors of position, the oxygen by position interaction, the position by site interaction, and the oxygen by position by site interaction, the Mauchly criterion achieved significance, indicating departure from sphericity. Consequently, the two RMANOVA summary tables include a column for adjusted error degrees of freedom and adjusted p-values.

At Time 1, the three way adjusted univariate RMANOVA was performed (Table 4-18) and showed a significant difference in $TcpO_2$ when subjects were breathing room air or receiving oxygen supplementation ($F = 51.110$, df 1, 19; $p < 0.0001$). The overall mean $TcpO_2$, average across position and site, while breathing room air was 37.9 mm Hg (SD 21.8) and 69.6 mm Hg (SD 32.9) with oxygen. There was no significant difference in the pattern of $TcpO_2$ with changes in position ($F = 2.804$, df 3, 21.8; $p = NS$). There was a significant difference in the pattern of $TcpO_2$ at different lower extremity sites ($F = 3.551$, 2, 38, $p < 0.04$) (Figure 4-1). Mean lateral ulcer site $TcpO_2$, regardless of position or level of oxygen, was 47.1 mm Hg (28.4), mean proximal ulcer $TcpO_2$ was 55.3 mm Hg (32.1) and mean dorsal foot site $TcpO_2$ was 58.9 mm Hg (32.2). Post-hoc Scheffe' comparisons found

that the lateral site $TcpO_2$ was significantly lower than the dorsal foot site ($p < 0.05$).

Table 4-18. Adjusted RMANOVA summary table for differences in $TcpO_2$ at the three extremity sites in response to changes in FiO_2 and position at Time 1. (n=20)

Source	df	df*	SS	MSS	F	p	p*
Between Subjects	19		164638.69				
Within Subjects	460		304423.79				
Level of Oxygen	1		120999.25	120999.25	51.110	0.000	
Error 1	19		44980.96	2367.42			
Position	3		2859.62	953.21	2.804	0.048	0.104
Error 2	57	22	19380.34	340.01			
Site	2		11719.14	5859.57	3.551	0.039	
Error 3	38		62707.03	1650.18			
Oxygen*Position	3		2928.36	976.12	6.128	0.001	0.007
Error 4	57	34	9079.27	159.28			
Oxygen*Site	2		154.03	77.01	0.300	NS	
Error 5	38		9769.64	257.10			
Position *Site	6		1183.44	197.24	2.019	0.069	0.129
Error 6	114	52	11134.72	97.67			
Oxygen*Position*Site	6		641.09	106.85	1.769	0.112	0.139
Error 7	114	82	6886.91	60.41			

FiO_2=fraction of inspired oxygen. * Indicates Huynh-Feldt adjustment for df and Alternative p-values.

There was a significant interaction between level of oxygen and position (F = 6.128, df 3, 34; p = 0.007). Figure 4-2 depicts the interaction between level of oxygen and position. Post-hoc Scheffe' comparisons found one significant pairwise comparison. The difference in $TcpO_2$ between lying and standing while breathing room air (< 1 mm) was significantly less than the difference between lying and standing while on oxygen (13.7 mm) ($p<0.05$). In this small sample of 20 subjects, the interactions of oxygen by site (F=0.300, df 2,38, p=NS), position by site (F=2.019, df 6, 51, p=NS), and oxygen by position (F=1.769, df 6, 81, p=NS) were not significant.

Figure 4-1. Pattern of $TcpO_2$, regardless of position or level of oxygen, at three lower extremity sites at Time 1. (n=20)

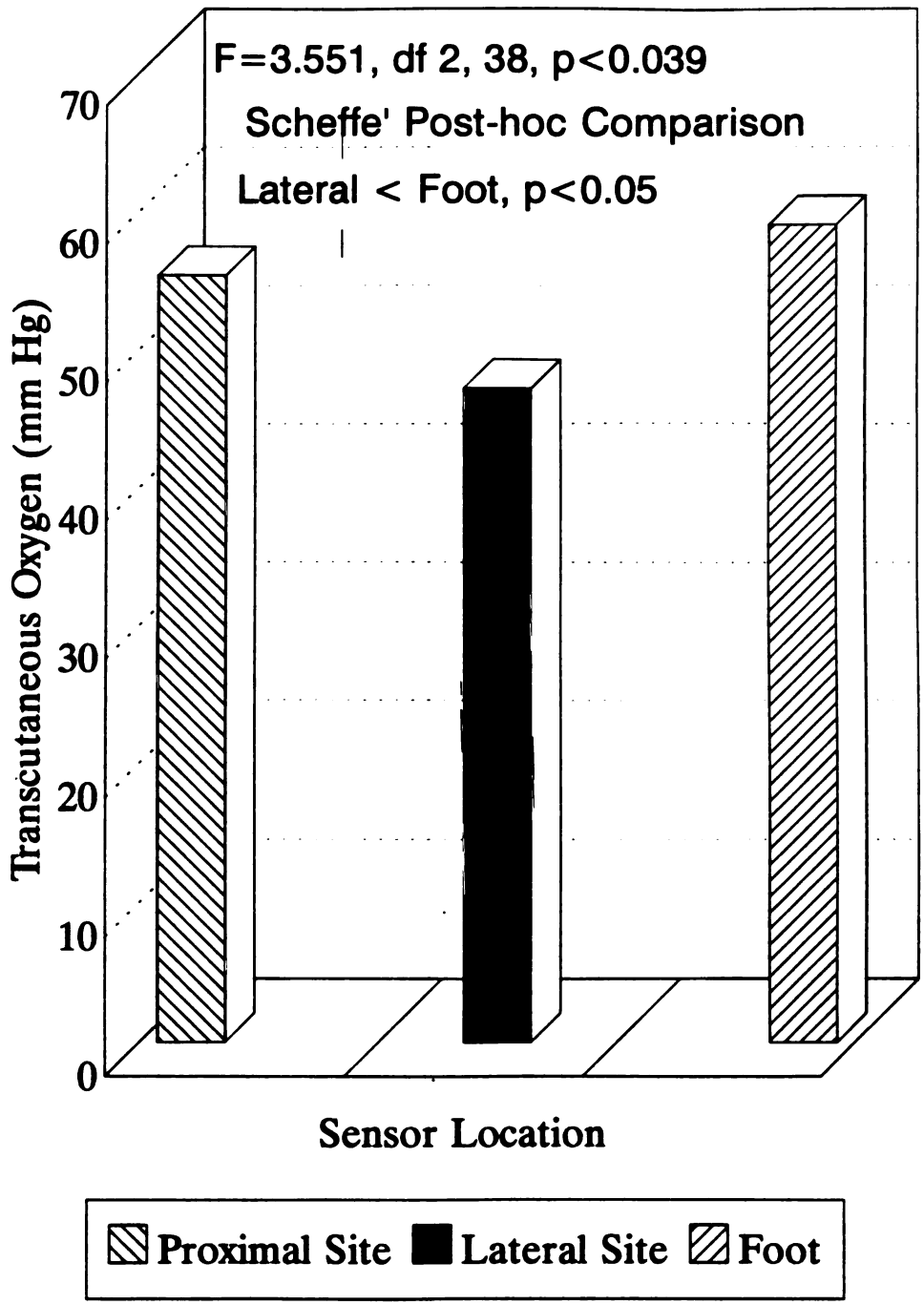
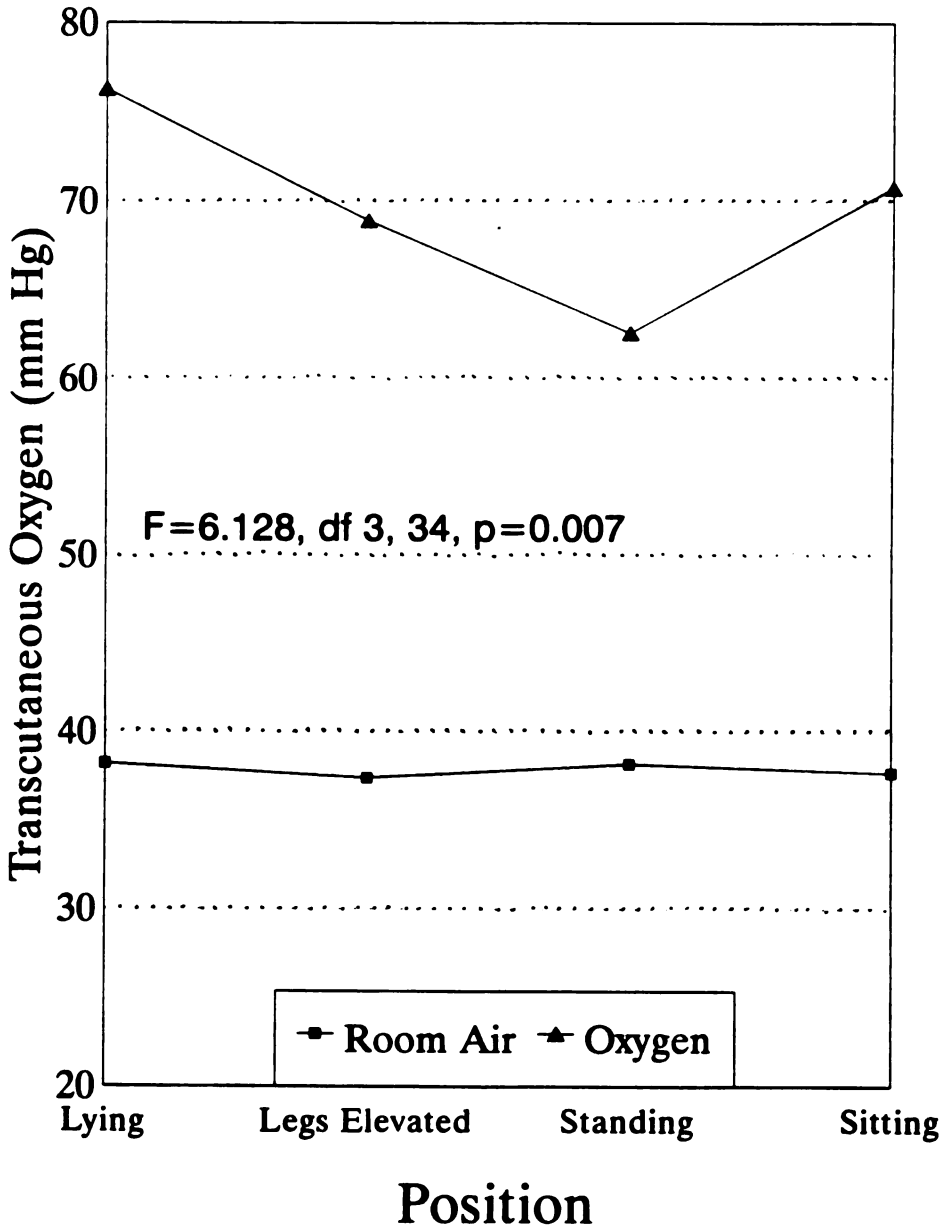


Figure 4-2. Interaction between level of oxygen and position, averaged across sites, at Time 1. (n=20)



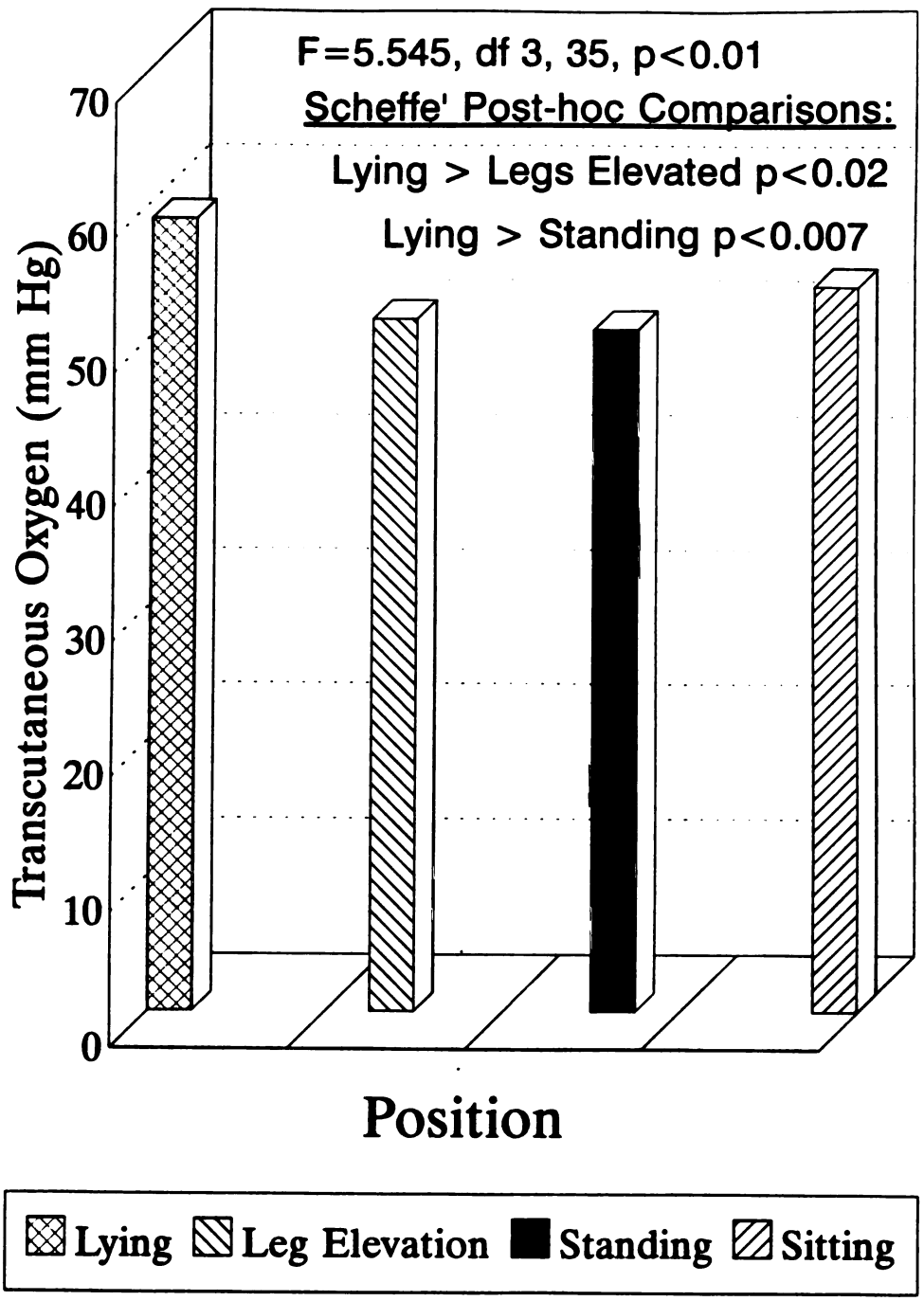
At Time 2, an adjusted univariate three way RMANOVA was performed and revealed a significant difference in $TcpO_2$ when subjects breathed room air versus supplemental oxygen ($F = 71.633$, df 1, 19; $p < 0.0001$) (Table 4-19). The overall mean $TcpO_2$, averaged across position and site, while breathing room air was 36.8 mm Hg (SD 18.0) and 70.3 mm Hg (SD 33.2) with oxygen. There was a significant difference in the pattern of $TcpO_2$ with changes in position ($F = 5.545$, df 3, 35; $p < 0.010$). Post-hoc Scheffe' pairwise comparisons found two significant comparisons. $TcpO_2$ was higher lying supine (M 58.7 mm Hg) than when the legs were elevated (M 51.3 mm Hg) ($p=0.017$), and $TcpO_2$ was higher lying (M 58.7 mm Hg) than standing (M 50.5 mm Hg) ($p=0.0062$) (Figure 4-3).

Table 4-19. Adjusted RMANOVA summary table for differences in $TcpO_2$ in response to changes in FiO_2 and position at Time 2. (n=20)

Source	df	df*	SS	MSS	F	p	p*
Between Subjects	19		159680.25				
Within Subjects	460		315432.42				
Level of Oxygen	1		134536.03	134536.03	71.633	0.000	
Error 1	19		35684.55	1878.13			
Position	3		4856.67	1618.89			
Error 2	57	35	16641.75	291.96	5.545	0.002	0.010
Site	2		2635.43	1317.71	0.684	NS	
Error 3	38		73225.24	1926.98			
Oxygen*Position	3		6012.97	2004.32	16.219	0.000	0.000
Error 4	57	43	7043.78	123.58			
Oxygen*Site	2		63.30	31.65	0.091	NS	
Error 5	38		13234.86	348.28			
Position*Site	6		2391.82	398.64	3.570	0.003	0.036
Error 6	114	39	12730.51	111.67			
Oxygen*Position*Site	6		303.94	50.66	0.951	NS	
Error 7	114	90	6071.55	53.26			

FiO_2=fraction of inspired oxygen. * Indicates Huynh-Feldt adjustment for df and Alternative p-values.

Figure 4-3. Pattern of TcpO2, regardless of level of oxygen or site, in four different positions at Time 2. (n=20)



There was not a difference in the pattern of $TcpO_2$ at different sites (F=0.684, df 2, 38, p=NS). There was a significant interaction between the level of oxygen and position (F = 16.219, df 3, 42.7; $p < 0.001$). The interaction between level of oxygen and position is presented in Figure 4-4. Post-hoc Scheffe' pairwise comparisons found five significant interactions: 1) The difference between lying and legs elevated on room air (M 3.2 mm Hg) was less than the difference between lying and legs elevated on oxygen (M 11.6 mm Hg) ($p<0.05$), 2) The difference in $TcpO_2$ between lying and standing was greater while breathing oxygen (M 19.9 mm Hg) than the difference between lying and standing while breathing room air (M -1.8 mm Hg) ($p<0.05$), 3) The difference between lying and sitting on room air (M -0.15 mm Hg) was less than the difference between lying and sitting on oxygen (M 9.8 mm Hg) ($p<0.05$), 4) The difference between legs elevated and standing on room air (M -4.9 mm Hg) was less than the difference between legs elevated and standing on oxygen (M 6.6 mm Hg) ($p<0.05$), and 5) The difference between standing and sitting on room air (M 1.7 mm Hg) was less than the difference between standing and sitting on oxygen (M -8.316 mm Hg) ($p<0.05$).

There was a significant interaction between position and site (F=3.570, df 6, 39, $p<0.04$). Figure 4-5 portrays the interaction between position and site. Post-hoc Scheffe' comparisons found one significant pairwise interaction. The difference between legs elevated and sitting (M 3.3 mm Hg) at the lateral site was less than the difference between legs elevated and sitting (M -9.2 mm Hg) at the foot site. The interactions between oxygen and site (F=0.091, 2,38, p=NS) or oxygen by position by site (F=0.951, df 6, 89.8, p=NS) were not significant.

Figure 4-4. Interaction between level of oxygen and position, regardless of site, at Time 2. (n=20)

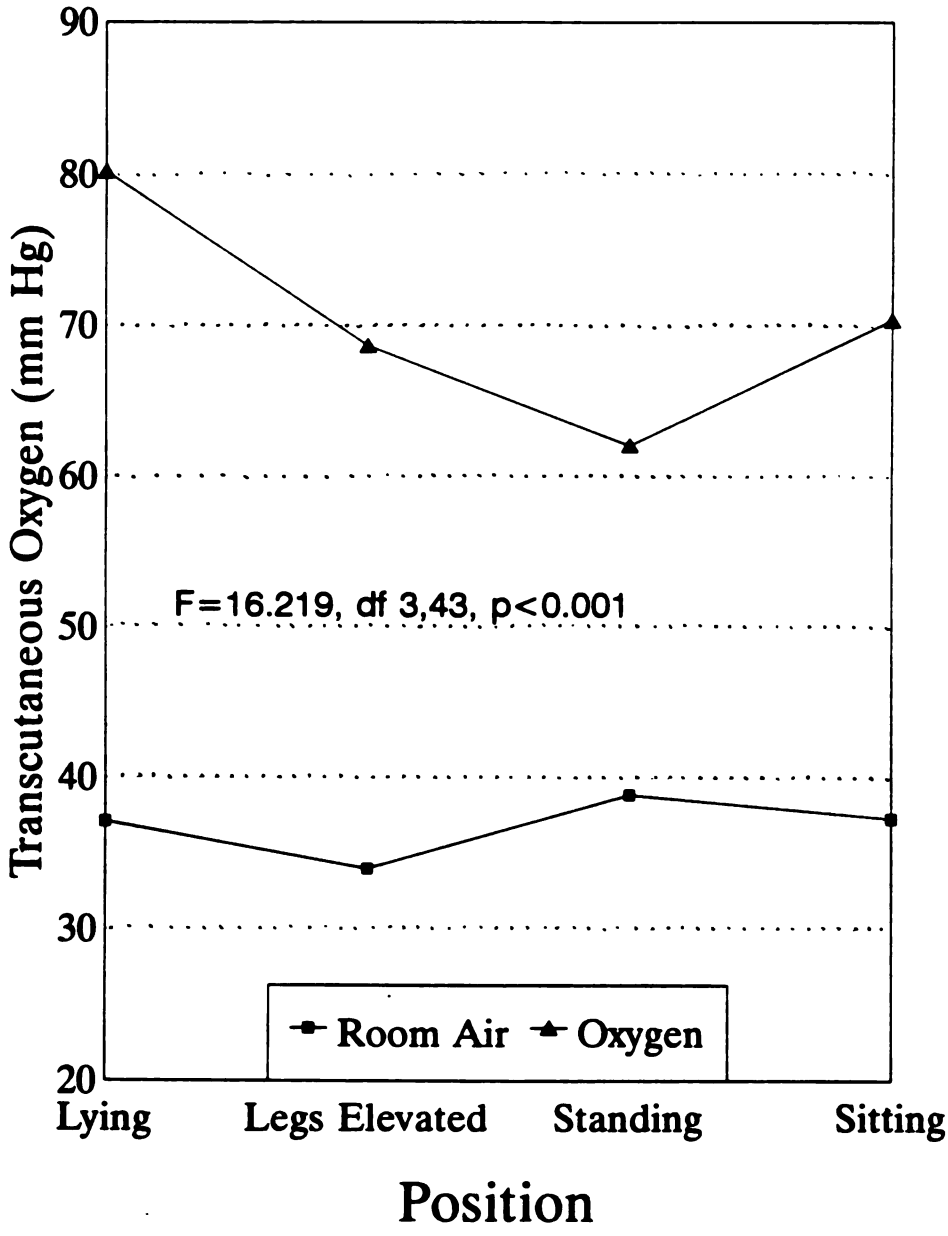
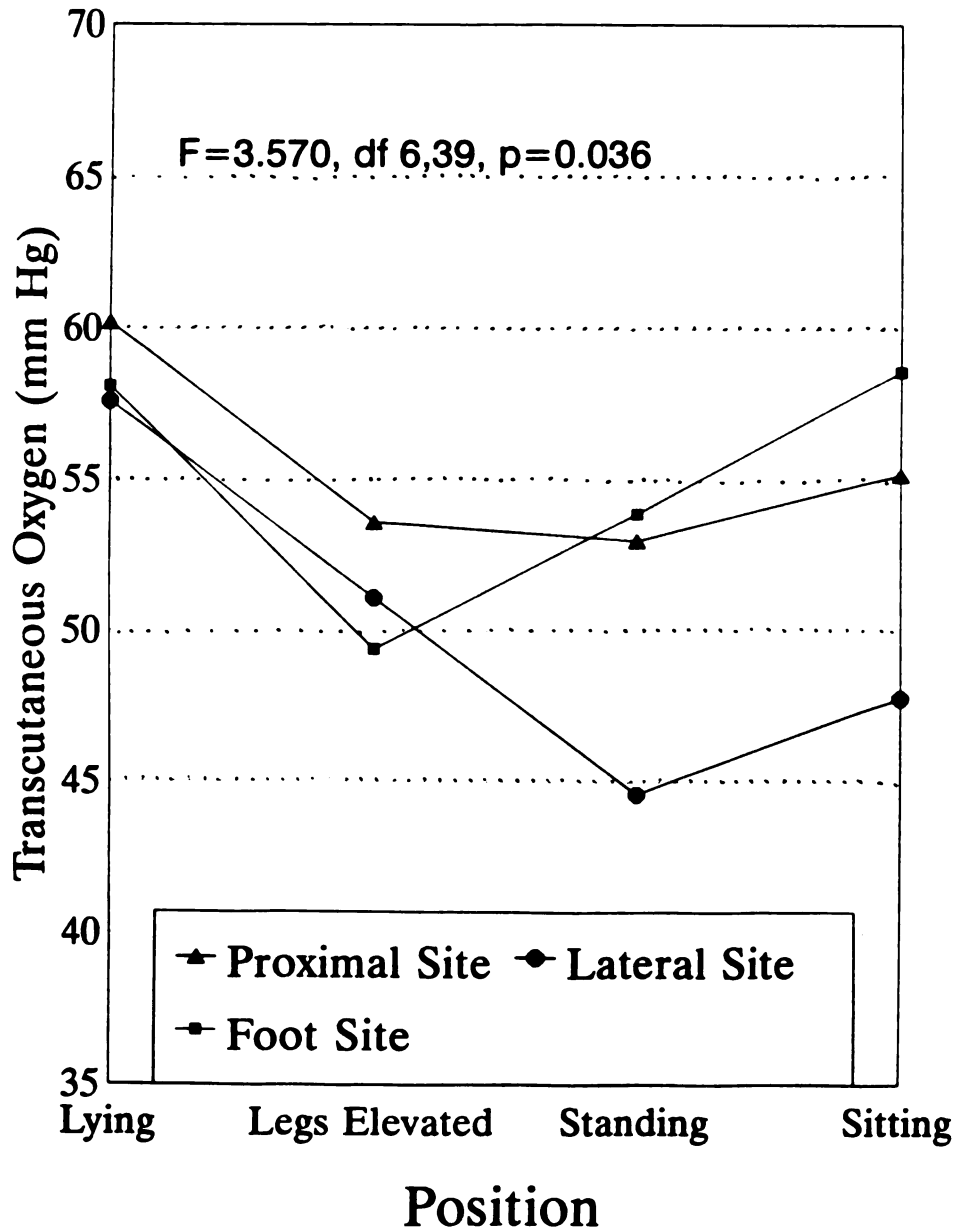


Figure 4-5. Interaction between position and site, regardless of level of oxygen, at Time 2. (n=20)



Plasma, Leukocyte Vitamin C and Glycosylated Hemoglobin (A_{1C})

Question 5

Are there any relationships between glycosylated hemoglobin (A_{1C}) and plasma, polymorphonuclear, or mononuclear vitamin C levels in subjects with venous leg ulcers?

Descriptive Data. Plasma, polymorphonuclear (PMN), and mononuclear (MN) vitamin C levels were evaluated using high performance reverse phase liquid chromatography on a subset of 15 subjects. Mean vitamin C values are provided in Table 4-20. There was no significant difference in PMN vitamin C levels between Time 1 and 2 (Wilcoxon = 0.459, p = NS), MN vitamin C levels at Time 1 and 2 (Wilcoxon = 1.070, p = NS), or plasma vitamin C levels at Time 1 and 2 (Wilcoxon = -0.533, p = NS).

Table 4-20. Mean Plasma, Polymorphonuclear (PMN), and mononuclear (MN) vitamin C levels determined using high performance liquid chromatography.

Variable	Time 1 (n=15)	Time 2 (n=10)
PMN Vitamin C (nmoles/10^8 cells)		
Mean	39.5	45.3
(SD, SE)	(SD 39.9, SE 10.3)	(SD 45.19, SE 14.3)
Range of Values	2.7 to 129.9	3.4 to 162.8
MN Vitamin C (nmoles/10^8 cells)		
Mean	223.8	206.3
(SD, SE)	(SD 118.0, SE 31.4)	(SD 182.8, SE 57.8)
Range of Values	57.9 to 456.5	31.4 to 578.5
Plasma Vitamin C (mg/dl)		
Mean	1.04	0.76
(SD, SE)	(SD 0.7, SE 0.2)	(SD 0.53, SE 0.17)
Range of Values	0.01 to 2.38	0.01 to 1.38

SD=standard deviation, SE=standard error.

Within the literature, factors identified as having an influence of vitamin C levels include, age, gender, presence of diabetes mellitus, cigarette smoking, dietary and supplemental vitamin intake (Jacob, 1990). Table 4-21 presents mean leukocyte vitamin C

levels along with the factors known to influence vitamin C levels for each subject.

Table 4- 21. Mean leukocyte vitamin C level and factors related to leukocyte vitamin C levels by subject.

SUBJ	DM	SMOKE	VITC SUPP	MEAN VITC INTAKE*	PMN1 VITC#	MN1 VITC#	PMN2 VITC#	MN2 VITC#
48 y/o M	Yes	No	No	334%	11.67	183.35	45.90	155.23
54 y/o M	Yes	Yes	No	66%	8.68	57.95	3.37	47.23
42 y/o M	No	Yes	No	203%	19.63	81.45	35.43	438.93
79 y/o M	No	No	Yes	373%	33.63	301.07	9.15	122.80
78 y/o M	No	No	Yes	321%	50.93	438.77	162.77	578.50
52 y/o F	No	Yes	No	43%	14.10	150.53	17.23	31.40
68 y/o F	No	No	Yes	292%	43.23	456.50	42.30	327.93
62 y/o F	No	No	No	323%	15.85	N/A	N/A	N/A
63 y/o F	No	No	Yes	284%	59.57	279.77	31.43	73.30
71 y/o M	Yes	No	No	37%	128.93	286.77	65.28	178.33
29 y/o M	No	No	No	1175%	129.98	203.30	39.95	109.53
46 y/o M	No	Yes	No	644%	2.68	139.13	N/A	N/A
78 y/o M	No	Yes	No	264%	32.13	205.90	N/A	N/A
82 y/o F	No	No	Yes	443%	27.5	169.62	N/A	N/A
76 y/o F	No	No	No	174%	14.0	178.88	N/A	N/A

SUBJ=subject, DM=diagnosis of diabetes mellitus, SMOKE=currently smokes, VITC SUPP=currently takes vitamin C supplement, VITC INTAKE=mean daily dietary of vitamin C, *=% of recommended daily allowance, PMN1 VITC=polymorphonuclear vitamin C level at Time1, MN1 VITC=mononuclear vitamin C level at Time1, PMN2 VITC=polymorphonuclear vitamin C level at Time2, MN2 VITC=mononuclear vitamin C level at Time 2, #=nmoles/10^8 cells, M=male, F=female, N/A=data not available.

An examination of the Pearson Product Moment correlations between glucose and glycosylated hemoglobin (A_{1C}) levels and plasma, PMN, and MN vitamin C levels, at Time 1 and 2, did not show any statistically significant relationships (Table 4-22). There appeared to be a trend of an inverse relationship between glucose and both plasma and leukocyte vitamin C levels. However, due to the small sample size and the instability of correlation coefficient in small samples, the relationships could not be determined.

Table 4-22. Correlations between glucose, glycosylated hemoglobin ($HGBA_{1C}$), and Plasma, Polymorphonuclear (PMN), and mononuclear (MN) vitamin C levels.

VARIABLE	GLUCOSE TIME 1	$HGBA_{1C}$ TIME 1
PMN Vitamin C Time 1 (n=15)	-0.13 p=.63	-0.11 p=.70
MN Vitamin C Time 1 (n=14)	-0.27 p=.34	-0.04 p=.90
Plasma Vitamin C Time 1 (n=15)	-0.33 p=.22	-0.27 p=.34
VARIABLE	**GLUCOSE TIME 2**	**$HGBA_{1C}$ TIME 2**
PMN Vitamin C Time 2 (n=10)	-0.16 p=.60	-0.34 p=.25
MN Vitamin C Time 2 (n=10)	-0.35 p=.24	-0.41 p=.17
Plasma Vitamin C Time 2 (n=10)	-0.47 p=.10	-0.53 p=.06

Leukocyte Vitamin C and Rate of Wound Closure

Question 6

Is there a relationship between rate of wound closure and leukocyte vitamin C in subjects with venous leg ulcers?

Inferential Statistics. An examination of the Pearson Product Moment Correlations between plasma, PMN, and MN vitamin C levels and rate of wound closure and percent area reduction did not show any statistically significant relationships. The correlations and their p values are presented in Table 4-23. Due to the small sample size and the instability of correlation coefficient in small samples, the relationships could not be determined.

Table 4-23. Correlations between plasma, polymorphonuclear (PMN), and mononuclear (MN) vitamin C and wound healing.

VARIABLE	RATE OF CLOSURE	% AREA REDUCTION
PMN Vitamin C Time 1 (n=13)	0.15 p=.61	0.23 p=.44
MN Vitamin C Time 1 (n=13)	-0.08 p=.79	-.10 p=.79
Plasma Vitamin C Time 1 (n=13)	-0.04 p=.89	-0.06 p=.85
PMN Vitamin C Time 2 (n=10)	-0.19 p=.59	-0.04 p=.89
MN Vitamin C Time 2 (n=10)	-0.17 p=.62	-0.02 p=.96
Plasma Vitamin C Time 2 (n=10)	0.14 p=.69	-0.04 p=.92

Summary

In an attempt to better understand the relationships between the variables in this study, a table of the major variables was created (Table 4-24). Both of the subjects that healed were female, had adequate protein intake, and used lower extremity compression. Of the five subjects whose ulcer got larger, their similarities were moderate to high nutritional risk, inadequate micronutrient intake, and abnormal biochemical indices. Four of the five subjects whose ulcer got worse were male and had an inadequate caloric intake. Moderate to high nutritional risk and inadequate micronutrient intake were commonalities of the four subjects with minimal improvement in ulcer size.

Table 4-24. Summary of nutrition, tissue oxygenation, and healing by subject.

ID	Subj	PAC	Blood	Anth	Cal	RDA Prot	Micro	TcpO2	Comp	Heal
1	58 y/o M	Mod	+	-	-	-	+	25	Yes	Worse
2	64 y/o F	Hi	+	-	+	+	+	10	No	Worse
3	45 y/o M	Mod	+	-	-	-	+	20	Yes	Min
4	60 y/o M	Mod	+	-	N/A	N/A	N/A	42	Yes	N/A
5	86 y/o M	Low	+	+	+	-	+	11	Yes	Imp
6	60 y/o F	Mod	+	-	+	+	+	32	Yes	Imp
7	53 y/o F	Mod	+	-	+	+	+	33	Yes	Imp
8	67 y/o F	Low	+	+	N/A	N/A	N/A	24	No	N/A
9	48 y/o M	Hi	+	-	+	+	+	32	Yes	Worse
10	54 y/o M	Hi	+	-	+	+	+	25	Yes	Min
11	42 y/o M	Hi	-	-	+	-	+	48	Yes	Imp
12	79 y/o M	Mod	+	-	+	-	+	39	Yes	Worse
13	78 y/o M	Mod	+	+	+	-	+	30	Yes	Worse
14	52 y/o F	Hi	+	-	+	+	+	18	No	Min
15	68 y/o F	Hi	+	+	+	-	+	33	Yes	Comp
16	62 y/o F	Hi	+	+	N/A	N/A	N/A	14	Yes	N/A
17	63 y/o F	Mod	+	+	+	-	+	34	No	Imp
18	71 y/o M	Hi	+	-	+	+	+	52	Yes	Imp
19	29 y/o M	Hi	+	-	-	-	-	33	Yes	Imp
20	46 y/o M	Mod	+	-	N/A	N/A	N/A	0	No	N/A
21	78 y/o M	Mod	-	-	-	-	+	7	Yes	Min
22	82 y/o F	Low	+	+	-	-	+	22	Yes	Comp
23	76 y/o F	Low	+	-	+	+	+	54	Yes	Imp
24	37 y/o M	Mod	-	-	+	-	-	60	Yes	Imp
25	39 y/o M	Hi	+	-	N/A	N/A	N/A	60	No	N/A

SUBJ=subject, RISK=Nutritional risk category, ANTH=anthropometrics, CAL=caloric intake, RDA PROT=recommended daily allowance of protein intake, MICRO=micronutrient intake, TcpO2= supine, room air, lateral site, transcutaneous tissue oxygen level at Time 1, HEAL=healing outcome of venous leg ulcer, M=male, F=female, Low=low nutritional risk, Mod=moderate nutritional risk, High=high nutritional risk, +=deficiency present, -=deficiency absent, N/A=data not available, WORSE=ulcer got larger, Min=minimal improvement in ulcer size, COMP=ulcer completely healed, IMP=improvement in ulcer.

This chapter presented the results of the dissertation research. In the following chapter, Chapter V; the findings will be discussed, clinical implications will be outlined, and recommendations for further research will be given.

CHAPTER V

DISCUSSION

The purpose of this prospective study was to explore nutrition, tissue oxygenation, and rate of healing in individuals with venous leg ulcers. Nutritional status and intake, transcutaneous skin oxygen values ($TcpO_2$), and wound surface area were evaluated at two points in time four weeks apart. This chapter provides a discussion for the study results in relation to: the study questions, related literature, alternative explanations, validity, conclusions, clinical implications, and recommendations for further research.

Findings in Relation to the Questions and Related Literature

Nutritional Risk and Status

Nutritional Risk. Data from the Public Awareness Checklist indicate that a portion of individuals with venous leg ulcers were at nutritional risk and/or had abnormalities in their nutritional status. Forty-four percent of the sample were at moderate risk and 40% were at high nutritional risk. These findings are congruent with pilot work (n=7) by Wipke-Tevis and Stotts (1996) that found 28% of venous leg ulcer patients were at moderate risk while 43% were at high nutritional risk. No other published research has examined the use of the PAC in individuals with open wounds.

Some data are available within other segments of the elderly population, however. Four studies of elderly, free-living Americans have reported the frequency of nutritional risk as follows: 33% to 39% moderate risk and 24% to 33% high nutritional risk (Garofalo & Hynak-Hankinson, 1995; Herndon, 1994; Lowry, 1994; Posner et al., 1993). One study which used the PAC in elderly dialysis patients found a higher frequency of risk in that 21.6% were at moderate risk and 74.3% were at high nutritional

risk (Rittgers-Simmons & Clement, 1994) . These data suggest that subjects with venous leg ulcers have a similar nutritional risk compared with other elderly subjects.

It must be noted, however, that the PAC was specifically developed for elderly subjects. Within this study, the PAC was administered to all participants regardless of their age. Specifically, there were seven male subjects under the age of 50. Although the PAC has been reported to have been used with individuals as young as 41 (Herndon, 1994), it is not known if the instrument is valid within a younger population than the one for which it was designed.

Anthropometrics. Anthropometric data were used to assess protein and fat stores within the sample. Overall, the mean arm anthropometric measures of this sample were above the 50th percentile when compared with the NHANES I and II data. Although severe depletion was rare, it was present within this sample. Eight percent of subjects had arm anthropometrics below the 5th percentile and a BMI less than 20 kg/m^2. Another 16% were mildly to moderately depleted based on arm anthropometry below the 15th percentile. Low anthropometric measures are an indicator of significantly decreased muscle and fat stores. Surgical patients with minimal stores of muscle and fat are at increased risk for morbidity and mortality (Bistrian, Blackburn, Hallowell, & Heddle, 1974).

Anthropometric measures have been used within the surgical population to assess nutritional status and predict successful wound healing. Haydock and Hill (1986, 1987) have described the anthropometrics of general surgery patients in New Zealand and found that patients with mild protein-energy malnutrition (PEM) had a mean TSF of 14.1 mm with a MAC of 28.8 cm whereas patients with moderate to severe PEM had mean TSF

between 7.2 and 11.2 mm and a MAC between 22.6 and 25.5 cm. Stotts and Whitney (1990) found that patients at home with an open surgical wound were depleted with mean TSF (mean 10.6 mm) and MAMC (mean 25.0 cm) below the median for NHANES I and II. Similarly, Casey et al. (1983) found TSF to be low in patients undergoing vascular surgery procedures (male mean < 11 mm, female mean < 18 mm).

One of the issues with arm anthropometry is the lack of established national norms for individuals above the age of 74 years. It has been suggested that arm circumference measures are preferable to skin fold measures in the elderly due to the redistribution of fat, decreased elasticity of the skin, and atrophy of subcutaneous adipocytes (Kuczmarski, 1989). For the purposes of this study, the table of norms used for subjects above the age of 74 was based on 746 elderly, white, subjects from Cincinnati between the ages of 60 and 89 years (Falciglia, O'Connor, & Gedling, 1988). The norms are likely not representative of all ethnic groups and may not be an accurate measure with this ethnically diverse sample. However, until the NHANES III data become available, it is a useful estimate.

Obesity was also a problem within these patients with venous leg ulcers. Fifty percent of both males and females were obese based on NHANES II standards. Pilot work by Wipke-Tevis and Stotts (1996) found similar results in that venous ulcer patients had a mean BMI greater than 27 kg/m^2 and arm anthropometrics above the 50th percentile. Nutrition screening of 749 elders by Posner and colleagues (1993) found that 33% were underweight (BMI less than 24 kg/m^2) and 36% were obese (BMI greater than 27 kg/m^2). Thus, it would appear that patients with venous ulcers have a higher frequency of obesity than the general population of elders.

Some controversy exists, however, with regard to the standards to be used for comparing BMI. The cutoff points for obesity established by NHANES II were 27.8 kg/m^2 for men and 27.3 kg/m^2 for women. Others have suggested that BMI norms should be adjusted according to age. The National Academy of Science's diet and health report suggests that a BMI of 22 to 27 kg/m^2 is normal for persons 45 to 54 years old, 23 to 28 kg/m^2 is normal for 55 to 65 year olds, and 24 to 29 kg/m^2 is normal for individuals over 65 years of age (Press release, Department of Health and Human Services Consensus Conference on Obesity, April 1, 1992).

Biochemical Indicators. One or more biochemical indicators of nutritional status were abnormal in a majority of the subjects (84%). Only three subjects had no abnormalities. Low levels of hemoglobin, hematocrit, and lymphocytes indicated that some subjects had inadequate reserves to synthesize cells and those cells were abnormal. The overall frequency of low hemoglobin/hematocrit was 32 % (6 males, 2 females). Similar results have been found in the pressure ulcer population (Allman et al., 1986; Breslow et al., 1993) and in patients with open surgical wounds (Stotts & Whitney, 1990). In one study of 22 patients with therapy-resistant venous leg ulcers, however, Bjellerup and colleagues (1993) reported no instance of pathologic values requiring intervention.

Causes of anemia in the elderly include gastrointestinal cancer, gastrointestinal bleeding, renal failure, vitamin B_{12} deficiency, folate deficiency, protein-calorie malnutrition, and rarely iron deficiency (Dwyer et al., 1993). Although anemia does not impair oxygen delivery in normovolemic, well perfused patients (Heughan, Grislis, & Hunt, 1974; Jensen, Goodson, Vasconez, & Hunt, 1986; Jonsson, Jensen, Goodson, Schueunstuhl, West, Hopf, & Hunt, 1991), it may be an important oxygen delivery

limiting factor in these patients where perfusion is altered and position dependent.

There was a high frequency of lymphopenia within these subjects. Fifty-six percent of venous leg ulcer subjects had a TLC < 1500 cells/ml. Pilot data (Wipke-Tevis & Stotts, 1996) and data from the vascular surgery population (Casey et al., 1983; Dickhaut et al., 1984; Jany & Burkus, 1988; Kay et al., 1987) support these findings by indicating that between 20% and 50% of patients are lymphopenic. Others have found that lymphopenia is not a problem in patients with open wounds (Bjellerup et al., 1993; Stotts & Whitney, 1990). Work by Seltzer and colleagues (1981) indicates that a low TLC is predictive of increased incidence of complications and death in critically ill patients.

Malnutrition is not the only cause of lymphopenia, however. Common causes include immunosuppression related to medications such as steroids or chemotherapy agents, cancer, autoimmune disease, and AIDS. No subjects were taking any immunosuppressive medications, had cancer, or AIDS. Two subjects with a low TLC were found to have scleroderma. Although six patients had a history of intravenous drug abuse and all had tested HIV negative in the past, they may have converted to HIV positive status. However, only one subject with a history of IVDA was lymphopenic and he had other low serum nutritional parameters.

Only one subject had a slightly low serum albumin, suggesting mild visceral protein depletion. Others have found a higher frequency of hypoalbuminemia in leg ulcer patients (44%) (Balaji & Mosely, 1995), chronic wounds requiring flaps (44%) (Walter et al., 1994), and patients undergoing vascular surgery (20 -50%) (Casey et al., 1983; Dickhaut et al., 1984; Jany & Burkus, 1988; Kay et al., 1987; Pedersen & Pedersen, 1992). The differences may be related to the small size of the leg ulcers in this study compared to the

wounds in the other studies. Specifically, with smaller ulcers nutrient losses are smaller and nutritional needs are not as great.

In pilot work, Wipke-Tevis and Stotts (1996) measured serum transferrin and found 43% had low transferrin while only 14% had low albumin. Serum transferrin is a measure of the body's ability to synthesize new cells and is considered by some to be a more sensitive indicator of marginal protein depletion because of its shorter half-life. In this study, transferrin was not measured because of the financial constraints of the study.

Micronutrients such as zinc and vitamin C have an important role in wound healing. Research suggests that decreased serum zinc levels are common in leg ulcer patients (Agren et al., 1986; Balaji & Mosley, 1995; Greaves & Boyde, 1967; Greaves & Skillen, 1970; Hallbook & Lanner, 1972; Serjeant et al., 1970) and pressure ulcer patients (Breslow et al., 1991; Breslow et al., 1993; Goode et al., 1992). Zinc is an essential micronutrient for collagen formation and epithelialization and has been demonstrated to be abnormal in malnourished individuals with impaired healing (Breslow et al., 1991; Stotts & Whitney, 1990). During this study 16 % had low zinc levels (3 subjects at Time 1 and 1 at Time 2). However, it must be noted that a large number of subjects (11/25) were on the low end of the normal range with a zinc level < to 80 mcg/dl.

Only 1 subject had low plasma vitamin C levels. Unfortunately, four individuals had missing values due to mishandled samples by the laboratory. These findings are supported by data in venous ulcer patients (Wipke-Tevis & Stotts, 1996), surgical patients with open wounds (Stotts & Whitney, 1990), and pressure ulcer patients (Bergstrom & Braden, 1992). Recently, one study of patients with large leg ulcers (> 100 cm^2) reported that 60% of subjects had low serum vitamin C. The discrepancy may be related to size of

the wounds and increased metabolic needs as well as differing amounts of nutrient losses due to variation in wound size and drainage.

The difference in results could also be related to the setting of this study. On the West coast, there is greater availability and more affordable pricing of citrus and other fruits high in vitamin C. In addition, many individuals were either taking supplemental vitamin C or multiple vitamin with vitamin C in addition to their dietary intake.

Dietary Intake

Inadequate intake of calories, protein, and/or micronutrients was present in a majority of subjects. Sufficient calories provide the energy required for cellular growth and leukocyte functioning. Based on the Harris-Benedict equation with adjustments for activity and injury, 68% had inadequate caloric intake. Similar findings are reported for venous ulcer patients (Wipke-Tevis & Stotts, 1996), surgical patients with open wounds (Stotts & Whitney, 1990), and pressure ulcer patients (Breslow et al., 1991; Breslow et al., 1993).

Since many of the subjects were obese, one of the issues is how to determine caloric need in these individuals. An episode of injury is usually not considered a good time to go on a diet and restrict calories. However, using the standard formula with adjustments for activity and injury may add sufficient calories to increase obesity rather than simply maintain stores and promote healing. It is known that adipose tissue is less metabolically active than lean body mass/muscle, therefore, in obese individuals the metabolic rate is decreased. It should be noted that caloric intake was not greater than need, however, as weight was stable from Time 1 to Time 2.

The use of the Harris-Benedict equation to determine basal energy expenditure

(BEE) has also been challenged. Some data indicate that when the Harris-Benedict equation is compared to either indirect or direct calorimetry, it systematically overestimates energy needs in healthy male and female adults an average of nine to 12 percent (Daly et al., 1985; Owen et al., 1986; Owen et al., 1987). In contrast, work by Russell and colleagues (1984) compared actual resting energy expenditure by indirect calorimetry to Harris-Benedict predicted BEE in normal adults and found no significant difference between the two methods. When Daly and colleagues (1985) reviewed the results from 15 other studies, they found that the Harris-Benedict BEE ranged from a low of 14% underestimation to a high of 19.1% over-estimation of BEE. However, in twelve of the fifteen studies, the Harris-Benedict equation was +/- 10% of the actual BEE. Although other regression equations have been developed (e.g. Mayo Foundation, Robertson-Reid, Cunningham), the Harris-Benedict equation remains the most widely used method to estimate BEE in clinical practice.

Adequate protein is needed for wound healing as it is essential for fibroblast proliferation, collagen and proteoglycan synthesis, and wound remodeling. Forty percent of the subjects in this study had protein intake less than the RDA. The RDA is really only a conservative estimate of protein needs for healing because it is based on needs for healthy adults and does not take into consideration extra needs during episodes of injury. Since albumin is not low, but arm anthropometrics and protein intake is low in a fair portion of subjects, it is possible that some subjects are wasting muscle in order to maintain their visceral protein levels. Thus, perhaps additional protein intake is warranted. The recent AHCPR guidelines for the treatment of pressure ulcers have recommended 1.25 to 1.5 g/kg/day of protein (Bergstrom et al., 1995). Using the conservative 1.25

g/kg/day as an estimate for protein needs for healing instead of the RDA, 75% of subjects did not have adequate protein intake. These guidelines have not yet been tested within patients with vascular wounds. The number of 1.25 g/kg/day is derived from work in patients with acute injury and trauma, so it is not clear whether requirements are the same in patients with chronic vascular ulcers.

In addition, it must be noted that prior to prescribing an increased protein intake at the level recommended by the AHCPR guidelines, a careful history and physical should be performed. If patients have impaired kidney and/or liver functioning, they may not be able to breakdown and utilize the protein and excrete the nitrogenous waste. This is particularly important in the population of patients with venous leg ulcers as there is naturally some decrease in kidney and liver functioning associated with aging.

The most common micronutrient with an inadequate level of intake was zinc (85%). According to the NHANES II data, the mean daily dietary intake of zinc in the United States between 1976 and 1980 was 15.5 mg (SE 0.4) in white males, 9.8 mg (SE 0.2) in white females, 12.3 mg (SE 0.8) in black males, 7.8 mg (SE 0.3) in black females (Mares-Perlman, Subar, Block, Greger, & Luby, 1995). Lower intakes were observed in women, the elderly, the less educated, and those with lower incomes. Others have reported similarly low zinc intakes in patients with open surgical wounds (Stotts & Whitney, 1990) and in patients with pressure ulcers (Bergstrom & Braden, 1992). The most likely explanation for low zinc intake is the type of foods that contain higher levels of intake. Foods high in zinc include beef, pork, dark poultry, shellfish, dairy products, and beans.

Measurement of nutritional status is not a perfect science. According to Beaton

(1994), dietary intake cannot be estimated without error and probably never will be. Furthermore, it remains difficult to assess absolute validity of dietary methods because diet is changing and the very act of observing often alters intake (Dwyer, 1994). Knowing that nutrition is being studied, subjects may consciously or unconsciously improve the quality or quantity of their intake in order to please the researcher. In contrast, under-reporting of food consumption is also problematic, especially among females and those who are overweight (Briefel et al., 1995).

The design of this study attempted to account for the potential Hawthorne effect by assessing their weight, serum albumin, glucose levels, and three day diet intake at both Time 1 and four weeks later at Time 2. Their weight, BMI, albumin, glucose level, and three day dietary intake were not significantly different between Time 1 and Time 2. These data suggest that subjects did not dramatically change their intake during the study period.

With the use of the diet record, there is potential that adherence to dietary assessment instruction varies among subjects. In this study, the use of careful instruction, models of common household measures, and a structured diet recording form assisted in minimizing this threat. One threat that may remain is the decline in short-term memory which is particularly a threat to the validity of diet recall in elderly people (van Staveren et al., 1994).

RATE OF WOUND CLOSURE

Due to the small sample size, lack of power, and the small effect sizes of nutrition and perfusion, this study was unable to answer question of whether there is a relationship between nutrition and healing in venous leg ulcers when the effects of perfusion are

removed using multiple regression. Small sample statistics were unable to uncover any relationships between nutrition, perfusion, and healing in these subjects. It must be noted, however, that one cannot conclude that there are no significant relationships between nutrition, perfusion, and healing in venous ulcer patients. Due to the small sample size, there is potential for Type II error.

Many factors may have contributed to the inability to examine this question. One potential explanation may be error in the measurement of the dependent variable (wound healing). Error can occur when the wound perimeter is traced on acetate. This is particularly a problem in patients where there is an ulcer site which contains multiple small open areas with islands of epithelium. There is greater potential for error during tracing of small ulcers. When the multiple small areas are summed for a total ulcer area, the measurement error may be increased exponentially. The study design attempted to minimize the error associated with tracing the wound perimeter by tracing each wound three times and averaging the area of the two most similar tracings.

Error can also occur during the computerized planimetry phase. For the computer program to calculate perimeter and area, the scanned wound image must be traced with a mouse on the computer screen. Again, the study design attempted to minimize the error associated with the computerized planimetry phase by tracing each scanned image three different time and averaging the results.

There may be other confounding variables that are prohibiting the effects of nutrition from being realized. For example, a large proportion of subjects had lymphopenia, and yet there was not a relationship between TLC and wound healing. Other causes of lymphopenia may have confounded the results. It is unclear why so many

subjects would have lymphopenia but not have other signs of either malnutrition or immunosuppression. However, because of the small numbers, the sample could not be stratified in order to explore the presence of confounding variables.

The design of the study and the sample itself may have also been factors in the lack of results. There were not enough subjects with moderate to severe malnutrition within the sample. The design of study, which required two four hour visits at the medical center, may have systematically eliminated those individuals most at risk. Individuals who were older, sicker, less mobile, and were unable to afford the cost of transportation to spend 8 hours of their time for the study, did not participate. Alternative study designs such as home visits may need to be explored in future studies.

The period of time between the initial assessment and follow up assessment may not be an adequate amount of time to see the differential effects of nutrition on healing. In addition, overall the wounds were quite small at the initial visit. Individuals with larger wounds may likely have greater nutritional losses from wound drainage, greater nutritional needs, and the influence of nutrition in these individuals may be more discernible.

An interesting finding was that females were more likely to heal than males. This differs from research in the cardiovascular surgery population. Data indicates that female gender is a predictor of impaired healing of saphenous vein harvest site incisions after coronary artery bypass surgery (DeLaria, Hunter, Goldin, Serry, Javid, & Najafi, 1981; Utley et al., 1989). It was hypothesized that this was related to the greater extent of adiposity on the legs of females compared to males. A closer examination of the differences between males and females with venous leg ulcers is warranted in order to understand what the predictors of healing might be.

Perhaps most interesting is the fact that the majority of subjects had both nutritional abnormalities and also had poor perfusion. One might conclude that regardless of oral intake, the real issue is whether substrates were able to get to the wound area to be utilized for healing. Future work may need to utilize a tissue marker of nutrition to further examine this question.

Transcutaneous Tissue Oximetry

Baseline readings of oxygen saturation were all greater than 95% while subjects were breathing room air. Oxygen saturations increased to 99% with supplemental oxygen administration. Oxygen saturation levels did not change with position, nor was there an interaction between oxygen and position. These data indicated that none of the subjects had severe lung disease.

Sensor Location. The mean resting room air chest reference $TcpO_2$ values were 56.2 mm Hg (SD 13.6) at Time 1 and 55.1 mm Hg (SD 13.6) four weeks later at Time 2. These values are congruent with reports in the literature on normals, and patients with peripheral arterial occlusive disease, and venous leg ulcers. Resting, room air chest reference $TcpO_2$ values in normals range from 50 to 95 mm Hg with an average of 64 to 69 mm Hg (Franzeck, Bernstein, Golbranson, & Fronek, 1982; Dowd, Linge, & Bentley, 1983a). In patients with peripheral arterial occlusive disease, resting, room air chest reference $TcpO_2$ values range from 53 to 60 mm Hg (Franzeck et al., 1982; Rhoades & King, 1986). Similarly, mean resting chest reference $TcpO_2$ values in patients with venous leg ulcers have been reported in the range of 55 to 76 mm Hg (Falanga, Moosa, Nemeth, Alstadt, & Eaglstein, 1987; Sindrup, Avnstorp, Steenfos, & Kristensen, 1987a; Nemeth, Eaglstein, & Falanga, 1989a; Nemeth, Falanga, Alstadt, & Eaglstein, 1989b).

Chest reference $TcpO_2$ values have been shown to increase significantly (range 90 to 128) when patients with arterial or venous vascular disease are given supplemental oxygen by either mask or nasal cannula (Falanga et al., 1987; Rhodes & King, 1986). This study found a similar response to oxygen supplementation with resting chest $TcpO_2$ values of 125.0 mm Hg (SD 36.9) at Time 1 and 124.8 mm Hg (SD 46.4) at Time 2. This further supports the premise that there was no severe lung disease within the sample.

Previous research indicates that $TcpO_2$ values do not change dramatically when measured along the leg in normal individuals who are lying supine and breathing room air. Mean $TcpO_2$ values along the knee range from 69 to 72 mm Hg (Franzeck et al., 1982; Dowd et al., 1983a; Stein, Provan, Prosser, Barrett, & Ameli, 1989); mean $TcpO_2$ values along the calf range from 56 to 74 mm Hg (Franzeck et al., 1982; Dowd et al., 1983a); mean $TcpO_2$ values near the medial malleolar area range from 62 to 64 mm Hg (Mani & White, 1988; Mani, White, Barrett, & Weaver, 1989); and mean foot $TcpO_2$ values range from 67 to 73 mm Hg (Dowd et al., 1983a; Stein et al., 1989). Furthermore, there are no significant changes in $TcpO_2$ values along the leg related to age or gender (Gothgen & Jacobson, 1979; Dowd, Linge & Bentley, 1983b).

In contrast, $TcpO_2$ values in the medial malleolar area in patients with venous leg ulcers are lower than in normals (Clyne, Ramsden, Chant, & Webster, 1985; Mani et al., 1989; Stacey, Burnand, Pattison, Thomas, & Layer, 1987). Transcutaneous oxygen values in the medial malleolar area in patients with venous leg ulcers are also lower than the chest or arm reference site (Mani et al., 1989; Nemeth, Eaglstein, & Falanga, 1989a; Nemeth, Falanga, Alstadt, & Eaglstein, 1989b; Falanga et al., 1987; Sindrup, Avnstorp, Steenfos, & Kristensen, 1987a; Falanga, Kirsner, Katz, Gould, Eaglstein, & McFalls,

1992). Reported mean periulcer site $TcpO_2$ values in patients with venous leg ulcers range from as low as 5 mm Hg to a high of 48 mm Hg (Clyne et al., 1985; Falanga et al., 1987; Falanga et al., 1992; Mani, Gorman, & White, 1986; Mani & White, 1988; Mani et al., 1989; Nemeth et al., 1989a; 1989b; Partsch, 1984; Sindrup, et al., 1987a).

In this study, the mean resting room air lateral periulcer site $TcpO_2$ at Time 1 was 30.9 mm Hg (SD 14.6) and 35.0 mm Hg (SD 17.0) at Time 2. The mean resting room air proximal periulcer $TcpO_2$ at Time 1 was 39.3 mm Hg (SD 15.1) and 38.9 mm Hg (SD 16.0) at Time 2. These findings are higher than those reported by some researchers (Falanga et al., 1987; 1992; Mani et al., 1986;1989; Mani & White, 1988; Nemeth et al., 1989a; 1989b; Patsch, 1984) but slightly lower than those reported by others (Clyne et al., 1985; Sindrup et al., 1987a). As expected, chest reference $TcpO_2$ values were higher than any of the lower extremity sites at both Time 1 and 2. In addition, the mean resting, foot $TcpO_2$ values of 44.2 mm Hg (SD 19.9) at Time 1 and 37.6 mm Hg (SD 12.5) at Time 2 were consistent with previously reported values (Falanga et al., 1987).

The lateral site $TcpO_2$ was lower than any other site. The lateral site (either at the 3 or 9 o'clock position) was at the exact level of ulceration and would be most reflective of the level of oxygen at the wound. The lateral ulcer site was in most cases was in the area of repeated injury and ulceration. As an area breaks down and heals repeatedly, the area becomes ischemic over time.

Oxygen Inhalation. When patients with venous leg ulcers are given supplemental oxygen, mean chest reference, periulcer site, and dorsal foot $TcpO_2$ values increase (Partsch, 1984; Falanga et al., 1987; Nemeth et al., 1989b). In this study when given supplemental oxygen, the mean resting chest reference, proximal ulcer, lateral ulcer, and

foot $TcpO_2$ values all increased to approximately twice their room air value. These findings are consistent with previous reports.

Position. Variations have been noted in $TcpO_2$ values of normal individuals with changes of position. Knee $TcpO_2$ values increased from 72.9 to 81.3 mm Hg from supine to standing (Stein et al., 1989); calf $TcpO_2$ values increased a mean of 15.1 from resting to sitting (Franzeck et al., 1982); medial malleolar $TcpO_2$ values increased a mean of 14 mm Hg from resting to standing (Clyne et al., 1985), and foot $TcpO_2$ values increased a mean 28 mm Hg from resting to sitting (Franzeck et al., 1982) and a mean of 12 mm Hg from resting to standing (Stein et al., 1989). In contrast, calf or ankle $TcpO_2$ values decrease in normal individuals an average of 8 to 10 mm Hg when the leg is elevated 10 degrees (Clyne et al., 1985; Rooke, 1992).

Reported fluctuations in $TcpO_2$ values of individuals with venous leg ulcers with changes of position have not been consistent in the literature. Dodd and colleagues (1985) found that $TcpO_2$ values were higher in subjects with venous ulcers than normals, and $TcpO_2$ decreased upon standing in both subjects and normals. In contrast, Clyne and colleagues (1985) found $TcpO_2$ values to be relatively constant in supine, foot elevated, head elevated, and standing positions. Similarly, Nemeth and colleagues (1989a) found $TcpO_2$ values to be relatively constant in supine, foot elevated and sitting positions in patients with venous leg ulcers. This difference is presumed to be a related to the difference in electrode temperature. Specifically, using a 37^0 C sensor (Dodd et al., 1985) prevents the maximal dilatation of the capillaries, restricts the diffusion of oxygen across the skin, and allows the capillaries to vasoactively respond to position changes.

On room air, there were minimal changes in $TcpO_2$ between the first measure and

the second measure one month later. These findings are consistent with the findings of Clyne and colleagues (1985) and Nemeth and colleagues (1989). However, when subjects received supplemental oxygen, differences in $TcpO_2$ were clinically evident. At Time 1, the within-factor of position approached statistical significance. At Time 2, position was significant. Specifically, $TcpO_2$ was significantly higher in the lying supine position than either the legs elevated or standing positions.

These findings have potentially clinically significant implications. A basic tenet of treatment for patients with venous ulcers is bed rest with the legs elevated above the level of the heart. The rationale for this has been to enhance venous return, decrease swelling, and maximize tissue perfusion. Based of the results from this study, periwound $TcpO_2$ decreases when the legs are elevated. As the majority of these subjects already have marginal levels of $TcpO_2$ in the periwound area, leg elevation may in fact serve to further decrease $TcpO_2$ and potentially further delay healing. Perhaps a more appropriate position would be lying supine without leg elevation. The effects of lying supine compared to leg elevation need to be evaluated to see if there were a difference in healing outcomes.

Since all peripheral sites (2 periwound and 1 foot) showed a decreased with the elevation of the legs, it may be hypothesized that all of the patients with venous leg ulcers had some degree of undetected arterial insufficiency. Research suggests that up to 50% of patients with venous disease also have some arterial disease (Callam et al., 1987). If $TcpO_2$ decreased with leg elevation, then this could mean that arterial perfusion pressure was not adequate to overcome gravity in order to maintain $TcpO_2$. Similarly, if $TcpO_2$ had increased when position changed from legs elevated to standing, this would have suggested that arterial disease predominated over venous disease because gravity

increased arterial flow and consequently maximized $TcpO_2$.

In addition, these findings reinforce the basic rule of treatment for venous ulcers which is to have patients stay off their feet as much as possible. Clearly, if $TcpO_2$ decreases while standing, this can explain the clinically seen phenomenon of increased healing with bed rest. In these subjects, $TcpO_2$ decreased dramatically when position changed to standing. This suggests that if any undetected arterial disease existed, the venous disease was dominant. In this instance gravity, increased hydrostatic pressure, and venous valvular insufficiency impeded venous return, caused venous pooling and capillary plugging with white blood cells, and slowed capillary flow which resulted in lower $TcpO_2$ during standing.

Periwound $TcpO_2$ also increased slightly when position changed from standing to sitting. This may be related to decreased effects of gravity and hydrostatic pressure. Clinicians have classically advised venous leg ulcer patients to avoid sitting. These data suggest that sitting may not be as deleterious, at least by $TcpO_2$ values, as previously thought. The comparison of the effects of sitting and standing needs to be tested to see if it makes a difference in wound healing outcomes.

One potential alternative explanation for these findings could be the confounding effects of edema on $TcpO_2$ readings. This is unlikely for several reasons. First, all measurements were taken in the morning after subjects had slept in a reclining position at home. The majority of subjects were wearing compression when arrived at research site which had to be removed prior to testing. All subjects rested in a supine position for at least 30 minutes prior to measurements being taken. In addition, observation of the equipment showed that $TcpO_2$ began dropping immediately upon change in position rather

than after the 20 minute stabilization period. In addition, some data indicate that reduction of lower extremity edema with external intermittent compression pumping does not increase $TcpO_2$ levels (Nemeth et al., 1989b).

Glucose and Leukocyte Vitamin C Levels

Of the 15 subjects who had leukocyte vitamin C levels measured, three reported a history of diabetes mellitus. The glycosylated hemoglobin level ($HgbA_{1C}$) was elevated in 3 patients and fasting glucose was elevated in 4 patients at Time 1. At Time 2, the glycosylated hemoglobin level ($HgbA_{1C}$) was elevated in 4 patients and fasting glucose was elevated in 4 patients. These data suggest that between 20 and 27% of subjects had some level of hyperglycemia during the study period. Previous reports in the literature indicated the frequency of diabetes in venous leg ulcer patients to be as high as 50 % (Nelzen, Bergqvist, & Londhagen, 1993; Sindrup, Groth, Avnstorp, Tonnesen, & Kristensen, 1987b). The frequency of diabetes in this sample was not as great as previously reported.

Reported levels of PMN vitamin C in the literature range from 7 to 32 nmoles/10^8 cells (Lee et al., 1982; Omaye et al., 1987). In this study the range was from 2.7 to 130.0 (M 39.5) nmoles/10^8 cells at Time 1 and 3.4 to 162.8 (M 45.3) nmoles/10^8 cells at Time 2. Reported levels of MN vitamin C range from 30 to 505 nmoles/10^8 cells (Lee et al., 1982; Omaye et al., 1987; VanderJaget et al., 1989). At Time 1 the MN vitamin C range was 58.0 to 456.5 (M 223.8) nmoles/10^8 cells and at Time 2 the range was 31.4 to 578.5 (M 206.3) nmoles/10^8 cells. Overall, these data are fairly consistent with previously reported results.

According to Evans et al. (1982) and Lee et al. (1988), MN vitamin C levels are

two to three times higher than PMN levels. In this study, however, when each individual's PMN level is compared to their MN level, there is a much greater concentration of vitamin C in the MN cells than the PMN cells. It is unclear why this discrepancy has occurred. However, the interpretation of leukocyte vitamin C concentrations is complicated by the different concentrations of vitamin C in various leukocyte cell fractions.

Although not statistically significant, there was a trend for glucose/HgbA_{1C} levels to be inversely related to leukocyte vitamin C values. This supports the proposed physiologic mechanism that glucose and vitamin C may have a similar transport mechanism into the cell and that during hyperglycemia neither glucose nor vitamin C can enter the cell. However, these results conflict with work by Lee and colleagues (1988) that failed to find any relationship between PMN vitamin C levels and glucose/HgbA_{1C}. Additional research is needed in this area to clarify these relationships.

One important issue is the potential for measurement error. The reduced form of vitamin C (i.e. ascorbic acid) is an unstable anti-oxidant and if exposed to light or elevated temperature converts from ascorbate to dehydroascorbate (DHAA). Methods are not available to measure dehydroascorbate directly. However, methods are available to measure total vitamin C. One can then measure ascorbate and subtract the two to determine the amount that was oxidized to DHAA. In leukocytes, a dynamic relationship exists between the oxidized and reduced forms of vitamin C and reported levels of DHAA in human leukocytes range from zero to half of the total vitamin C concentration (Schaus, Kutnink, O'Connor, & Omaye, 1986). In this study, only the reduced form (ascorbic acid) was measured. It is unknown what percentage, if any, of vitamin C within the leukocytes was DHAA. However, since the results from this study are within the

previously reported range, it is anticipated that minimal DHAA was present.

The measurement of leukocyte vitamin C levels is a very labor intensive procedure and multiple steps are required between obtaining the blood sample and ultimately determining the amount of ascorbate. Potential areas for measurement error include cell contamination, cell viability, cell counting, ascorbic acid extraction, preservation, and storage, as well as potential calibration problems with the HPLC equipment. Optimally, in future research the use of experienced technicians would minimize these potential errors.

The adverse effects of hyperglycemia on wound healing have been documented within the literature (Black et al., 1989; Goodson & Hunt, 1977;1978; Seifter et al., 1981). Hyperglycemia may cause loss of micronutrients in the urine due to polyuria. In addition, leukocyte function is known to be altered during hyperglycemia (Bagdade et al., 1974; Nolan et al., 1978) and may have effects of increased wound healing complications (Casey et al., 1983). Further research in the area of leukocyte vitamin C may assist not only in understanding the pathophysiology of impaired wound healing in diabetics, but also facilitate the development of interventions to promote healing in this high risk population.

Leukocyte Vitamin C and Rate of Wound Closure

No one has previously reported healing of venous ulcers and leukocyte vitamin C levels. A few studies have looked at buffy coat vitamin C and pressure ulcers (Burr & Rajan, 1972; Goode et al., 1992; Taylor et al., 1974). In these studies, lower levels of buffy coat vitamin C were associated with pressure ulcer formation. The use of buffy coat vitamin C has been criticized by researchers because it may give inconsistent results. For example, since the vitamin C content of the different cell types (i.e. PMN versus MN) varies widely, a shift in the differential cell count can dramatically change the results of the

total heterogeneous buffy coat vitamin C level.

Of the 20 patients who attended both visits of the study, 13 subjects had leukocyte vitamin C measures at Time 1 and 10 subjects had measures at Time 2. Mean PMN and MN vitamin C levels were not statistically different between Time 1 and Time 2. Pearson Product Moment correlations did not show any relationship between leukocyte vitamin C levels and rate of wound closure or percent area reduction. It must be recognized, however, that there was a very small number of subjects in this subanalysis. It should not be concluded that there is no relationship between these factors. There may be confounding variables such as age, gender, glucose levels, and smoking which may be preventing the relationships from being realized. In a future study with a larger sample size, the sample could be stratified by the known confounding variables. Hopefully, this stratification would assist in further exploration of this question.

Conclusions and Implications

This study was designed to explore nutrition, tissue oxygenation, and rate of healing in individuals with venous leg ulcers. Evaluation of the nutritional risk, status, and dietary intake found that a portion of patients with venous leg ulcers were at nutritional risk, had abnormal anthropometric and biochemical indicators, and inadequate dietary intake to support healing. It was proposed to determine whether there was a relationship between the rate of wound closure and nutritional status when the effects of perfusion were controlled. However, an inadequate sample size prevented the question from being answered.

Transcutaneous tissue oxygen values at venous leg ulcer sites were also examined during specific position changes and with 21% and 40 to 60% inspired oxygen. The

results indicated that $TcpO_2$ levels significantly increased when patients were given supplemental oxygen. Furthermore, during hyperoxia, differences in $TcpO_2$ as a result of position changes were more visible. Specifically, in patients with venous leg ulcers, $TcpO_2$ decreased from lying to standing position during oxygen inhalation. $TcpO_2$ values also decreased from the lying to the leg elevated position during oxygen inhalation. These changes were not as visible when subjects were breathing room air. These results challenge the common practice of prescribing leg elevation to patients with venous leg ulcers. Additional research is necessary to examine the efficacy of leg elevation versus lying supine to determine which position may best support healing and maximize tissue oxygenation.

Leukocyte vitamin C was also determined on a subset of subjects in this sample. The relationships between PMN and MN vitamin C levels, glucose/$HgbA_{1C}$ levels, and venous leg ulcer healing were explored. Unfortunately, due to the small sample size, the results were inconclusive. However, within the literature there is theoretical support for continued exploration of these questions. Additional research is necessary to establish reference values for PMN and MN vitamin C as well as explore their impact on wound healing.

Implications for Research and Practice

Many questions remain regarding nutrition, tissue oxygenation, and healing in patients with venous leg ulcers. Impaired healing of chronic leg ulcers is a tremendous financial burden on the health care system. One study reported the average cost to heal one venous leg ulcer in the United States to be $1950 with a range from $784 to $6449 (Wood & Margolis, 1992). Impaired healing of chronic leg ulcers is associated with

symptoms of fatigue, pain and discomfort, and emotional distress (Franks et al., 1994). Individuals with chronic leg ulcers often experience periods of prolonged activity restrictions and functional disability including significant reductions in activities of daily living, as well as social and leisure activity (Wipke-Tevis, D.D., unpublished manuscript, 1991). Interventions to enhance healing of leg ulcers are needed.

Based on these results, venous ulcer patients should be screened for nutritional risk. A simple tool, such as the Public Awareness Checklist utilized in this study, is one possibility. Further nutritional evaluation can be initiated as indicated. Many factors may contribute to inadequate dietary intake including financial constraints, mobility limitations, social isolation, coexistent medical problems, inadequate cooking facilities, poor eating habits, and lack of nutrition knowledge. A multidisciplinary approach may be needed to address these issues. Future studies are needed to explore both the long-term effects of malnutrition on the healing of venous ulcers and the effects of nutritional supplementation on the healing of these ulcers. The utilization of tissue markers of nutritional status may be necessary to gain a fuller understanding of nutritional alterations at the cellular level in this population. In addition, since a large portion of subjects had inadequate protein and zinc intake, these may be two important nutrients to further examine.

Based on this small study, it would appear that positioning may be a crucial aspect for healing in patients with venous leg ulcers. In fact, leg elevation may not be beneficial to tissue oxygenation. Further research is necessary to explore the relationships between positioning, transcutaneous tissue oxygen, and healing in patients with venous leg ulcers.

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UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT

A. PURPOSE AND BACKGROUND: Deidre Wipke-Tevis, RN, MS and Nancy Stotts, RN, EdD from the School of Nursing are conducting a study to explore nutrition, circulation, and healing of patients with venous ulcers (wounds on the lower legs). Because I have a venous ulcer, I am being asked to participate.

B. PROCEDURES: If I agree to be in this study, the following will happen:

1. I will not eat or drink anything except water after midnight the night before my appointments.
2. I will have the following measures completed: height, weight, and arm size measured at the first visit.
3. At the beginning of the study, and again at the end of the study, blood will be drawn to evaluate my nutritional status. Each sample will be approximately 6 teaspoons; a total of about 4 tablespoons will be drawn for the whole study. After my blood is drawn, I will be provided a meal.
4. At the beginning of the study, and again at the end of the study, my wound will be observed and the wound size will be traced by placing a piece of clear plastic over the wound.
5. At the beginning of the study, and again at the end of the study, my circulation will be checked using a sensor on my chest below my collar bone and a sensor next to the wound. The measurements will be taken while I lie on my back, sit, and stand. I will breathe oxygen from a mask during the circulation measures.
6. I will be briefly interviewed about how my wound developed and the symptoms I experience related to it at the first visit.
7. The investigator will check my medical records to gather information about my medical/surgical history and medications I take.
8. At the beginning of the study, and again at the end of the study, I will be asked to write down my food intake for three days and the researcher will call me each day at my home to obtain the information I have recorded.

Participation in the study will require two visits. Each visit will take about 3 to 3.5 hours. Recording my daily dietary intake should take about 5 minutes each day. The 3 telephone interviews will each last about 10 minutes. The total time for participation in the study will take a 7 to 8 hours over a period of 4 weeks. All study procedures will be done at the General Clinical Research Center at U.C. San Francisco.

C. RISKS/DISCOMFORTS:

1. Venipuncture: Having my blood sample drawn may cause temporary discomfort from the needle stick, bruising, and rarely infection.
2. Circulation Check: Having my circulation checked may cause the unlikely possibility of skin redness or irritation from the sensors.
3. Wound Measurement: Having my wound measured may cause me slight discomfort when the dressing is removed or the tracing is done, however, care will be taken to minimize discomfort.
4. Interviews: Answering the interviews may be an inconvenience to me but should take less than 10 minutes.
5. Recording Dietary Intake: Filling out the dietary record may be an inconvenience to me

but should take less than 5 minutes each day.

6. Confidentiality: Participation in research may involve loss of privacy. My research records will be handled as confidentially as possible within the law. No individual identities will be used in any reports or publications resulting from this study.

Treatment and Compensation for Injury: If I am injured as a result of being in this study, treatment will be available. If I am eligible for veteran's benefits, the costs of such treatment will be covered by the Department of Veterans Affairs. If not, the cost of such treatment may be covered by the Department of Veterans Affairs or the University of California depending on a number of factors. The Department of Veterans Affairs and the University do not normally provide any other form of compensation for injury. For further information about this, I may call the V. A. District counsel at (415) 750-2288 or the office of the UCSF Committee for Human Research at (415) 476-1814.

D. BENEFITS: There are no direct benefits to being in this study. The findings of this study may provide information that will help patients with wounds similar to mine.

E. ALTERNATIVES: Not to participate in this study.

F. COST: I will not be charged for the study procedures.

G. REIMBURSEMENT: In return for my time, effort, and travel expenses, I will be reimbursed $50 for my total participation in the study, paid by check approximately 6 weeks after completion of the study. If I voluntarily withdraw from the study for reasons other than medical, I will be paid $15, paid by check within approximately 4-6 weeks.

H. QUESTIONS: This study has been explained to me by Deidre Wipke-Tevis or Nancy Stotts and my questions answered. If I have further questions, I can reach Nancy Stotts at 476-4412.

I. CONSENT: I have been given copies of this consent form and the experimental Subject's Bill of Rights to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. I have the right to decline to participate or to withdraw at any point in this study without jeopardy to my medical care.

If I wish to participate, I should sign below.

Date	Subject's Signature
Date	Person Obtaining Consent

LEG ULCER PATIENTS WANTED!!!!

Be Part of a Study to Examine The Role of Nutrition and Circulation On Healing of Venous Leg Ulcers

REIMBURSEMENT $50.00

Call DEIDRE 476-4412 or Pager 719-3681

Department of Physiological Nursing, UCSF

Carol Fox, News Director
Source: Rebecca Higbee (415) 476-2557

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PUBLIC SERVICE ANNOUNCEMENT

20-seconds

THE UC SAN FRANCISCO DEPARTMENT OF PHYSIOLOGICAL NURSING IS LOOKING FOR VOLUNTEERS TO PARTICIPATE IN AN OBSERVATIONAL STUDY. IF YOU HAVE SORES OR WOUNDS ON YOUR LEGS DUE TO BAD CIRCULATION AND SPEAK ENGLISH, YOU COULD BE ELIGIBLE. STUDY PARTICIPANTS WILL BE PAID 50 DOLLARS IN COMPENSATION AT THE END OF THE STUDY. FOR MORE INFORMATION, CALL (415) 476-4412. THAT'S (415) 476-4412.

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mailed: 5/10/95 RH:venousPSA

Carol Fox, New Director
Source: Rebecca Higbee (415) 476-2557

FOR IMMEDIATE RELEASE
May 10, 1995

VOLUNTEERS NEEDED FOR UCSF STUDY ON LEG SORES

Volunteers are needed for an observational UCSF study on venous ulcers--wounds or sores that develop on the leg as a result of bad circulation.

The study, conducted by the UCSF Depart of Physiological Nursing, will look at the role of nutrition and circulation in healing venous leg ulcers.

Participants may be of any age, must have venous ulcers on their legs, speak English, have a telephone and must be able to come to two four-hour visits at UCSF four weeks apart.

Study participants will be examined and asked to fill out questionnaires. A $50 compensation fee will be available at the conclusion of the study period.

For more information, call (415) 476-4412.

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The Warning Signs of poor nutritional health are often overlooked. Use this checklist to find out if you or someone you know is at nutritional risk.

DETERMINE YOUR NUTRITIONAL HEALTH

Read the statements below. Circle the number in the yes column for those that apply to you or someone you know. For each yes answer, score the number in the box. Total your nutritional score.

	YES
I have an illness or condition that made me change the kind and/or amount of food I eat.	2
I eat fewer than 2 meals per day.	3
I eat few fruits or vegetables, or milk products.	2
I have 3 or more drinks of beer, liquor or wine almost every day.	2
I have tooth or mouth problems that make it hard for me to eat.	2
I don't always have enough money to buy the food I need.	4
I eat alone most of the time.	1
I take 3 or more different prescribed or over-the-counter drugs a day.	1
Without wanting to, I have lost or gained 10 pounds in the last 6 months.	2
I am not always physically able to shop, cook and/or feed myself.	2
TOTAL	

Total Your Nutritional Score. If it's —

0-2 **Good!** Recheck your nutritional score in 6 months.

3-5 **You are at moderate nutritional risk.** See what can be done to improve your eating habits and lifestyle. Your office on aging, senior nutrition program, senior citizens center or health department can help. Recheck your nutritional score in 3 months.

6 or more **You are at high nutritional risk.** Bring this checklist the next time you see your doctor, dietitian or other qualified health or social service professional. Talk with them about any problems you may have. Ask for help to improve your utritional health.

Remember that warning signs suggest risk, but do not represent diagnosis of any condition. Turn the page to learn more about the Warnings Signs of poor nutritional health.

These materials developed and distributed by the Nutrition Screening Initiative, a project of:

AMERICAN ACADEMY OF FAMILY PHYSICIANS
THE AMERICAN DIETETIC ASSOCIATION
NCOA NATIONAL COUNCIL ON THE AGING, INC.

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Data Collection Sheet

Subject Initials _____ Subject Number _____ Date _____

Fasting: Yes/No Blood Drawn: Yes/No Meal: Yes/No

Anthropometric Measures: Height _____cm Weight _____kg

Body Mass Index _____ Triceps Skin Fold _____mm Wrist Circumference ____cm

Mid-arm Circumference _____cm Mid-arm Muscle Circumference _____cm

Perfusion Measures Time 1: Date _____

POSITION	TIME	FIO$_2$	O$_2$ Sat	O$_2$ Wound L	CO$_2$ Wound L	O$_2$ Wound A	CO$_2$ Wound A	O$_2$ Foot B	CO$_2$ Foot B	O$_2$ REF C	CO$_2$ REF C
Lying Supine		RA									
Lying Legs Up		RA									
Lying Legs Up		O$_2$									
Lying Supine		O$_2$									
Sitting		O$_2$									
Standing		O$_2$									
Standing		RA									
Sitting		RA									

Room Temperature: Room Humidity:

LEGEND:

L - Sensor located A - Sensor located

B - Sensor located C - Sensor located

Data Collection Sheet (cont.)

Subject Initials _____ Subject Number _____ Date _____

Wound Measures Time 1: Date _____ Number Of Ulcers: _____

Location of Ulcers: _____

Wound Surface Area 1)_____ 2) _____

Interview Questions:

History of Ulcer (Onset, Duration, Previous occurrences) :

Cause of Ulcer (Underlying disease, trauma) :

Previous Types of Treatment for Ulcer (compression, dressings):

Current Type of Treatment for Ulcer (compression, dressings):

Symptoms Related to Ulcer (pain, fatigue, sleeplessness, depression):

Lifestyle Changes Caused by Ulcer (mobility, socialization):

Smoking History (Pack/years, time since quit):

Data Collection Sheet (cont.)

Subject Initials _____ Subject Number _____ Date _____

Fasting: Yes/No Blood Drawn: Yes/No Meal: Yes/No

Perfusion Measures Time 2 Date _____

POSITION	TIME	FIO_2	O_2 Sat	O_2 Wound L	CO_2 Wound L	O_2 Wound A	CO_2 Wound A	O_2 Foot B	CO_2 Foot B	O_2 REF C	CO_2 REF C
Lying Supine		RA									
Lying Legs Up		RA									
Lying Legs Up		O_2									
Lying Supine		O_2									
Sitting		O_2									
Standing		O_2									
Standing		RA									
Sitting		RA									

Room Temperature: Room Humidity:

LEGEND:

L - Sensor located **A** - Sensor located

B - Sensor located **C** - Sensor located

Wound Measures Time 2: Date _____

Number Of Ulcers: _____

Location of Ulcers: _____

Wound Surface Area 1)_____ 2) _____

Data Collection Sheet (cont.)

Subject Initials _____ Subject Number _____ Date _____

Phone Number _____ Age_____ Gender _____ Ethnicity _____

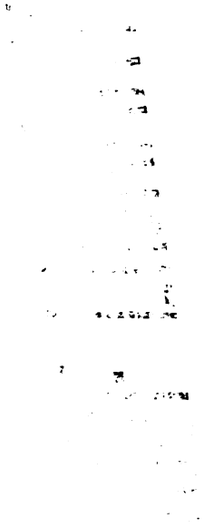
Biochemical Nutritional Measures:

TIME/ DATE	ALB	GLUC	HGB A1C	LEUK VIT C	TLC	ZINC	SERUM VIT C
Time 1 Date							
Time 2 Date							

Medications (Steroids, Vitamin/Mineral Supplements, Estrogen/Progesterone replacements, antihypertensives):

Medical/Surgical History:

Debridement &/or Treatment for Wound Infection:



GCRC Protocol #32-13:
Nutrition, Perfusion, and Healing in Individuals with Lower Extremity Venous Ulcers
SUBJECT ID NUMBER:____ DATE OF BIRTH: _____ TODAY'S DATE_____

PATIENT HISTORY

__Hispanic __Asian __African-American __Caucasian __Other_____ __Male __Female
Occupation Post-menopausal Menstruating FDLMP

CATEGORIES	NORMAL	ABNORMAL Resolved | Current	ONSET MO/YR	DESCRIBE ABNORMALITY Description/Treatment
Drug Allergies	None	|		
Other Allergies	None	|		
Cigarette Smoking	None	|		
HEENT		|		
Respiratory		|		
Cardiovascular		|		
Gastrointestinal		|		
Genitourinary		|		
Peripheral Vascular		|		
Neurologic		|		
Hematopoietic or Lymphatic		|		
Endocrine		|		
Dermatologic		|		
Musculoskeletal		|		
Surgical History		|		
Other		|		
Leg Ulcers		|		

MEDICATIONS:_____

GCRC Protocol #32-13:
Nutrition, Perfusion, and Healing in Individuals with Lower Extremity Venous Ulcers

PATIENT NAME:_____**TODAY'S DATE:**_____
SUBJECT ID NUMBER:_____ **DATE OF BIRTH:** _____

PHYSICAL EXAMINATION #1

BLOOD PRESSURE _____ **mm Hg**	**PULSE** _____ **bpm**
RESPIRATIONS _____ **per minute**	**TEMPERATURE** _____ **F or C (CIRCLE)**

SYSTEM	CHECK IF NORMAL	CHECK IF ABNORMAL	DESCRIBE ABNORMAL FINDINGS
NEUROLOGIC			
HEENT			
CARDIOVASCULAR			
PULMONARY			
ABDOMINAL			
INTEGUMENTARY			
MUSCULOSKELETAL			
PERIPHERAL VASCULAR			
OTHER			

COMMENTS_____

GCRC Protocol #32-13:
Nutrition, Perfusion, and Healing in Individuals with Lower Extremity Venous Ulcers

PATIENT NAME:_____**TODAY'S DATE** _____
SUBJECT ID NUMBER:_____ **DATE OF BIRTH:** _____

INTERIM PATIENT HISTORY

Has there been a change in the patient's status since baseline? Yes__ No__
If yes, please complete the following:

DIAGNOSIS	DATE OF ONSET	ONGOING/RESOLVED	TREATMENT
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

COMMENTS:_____

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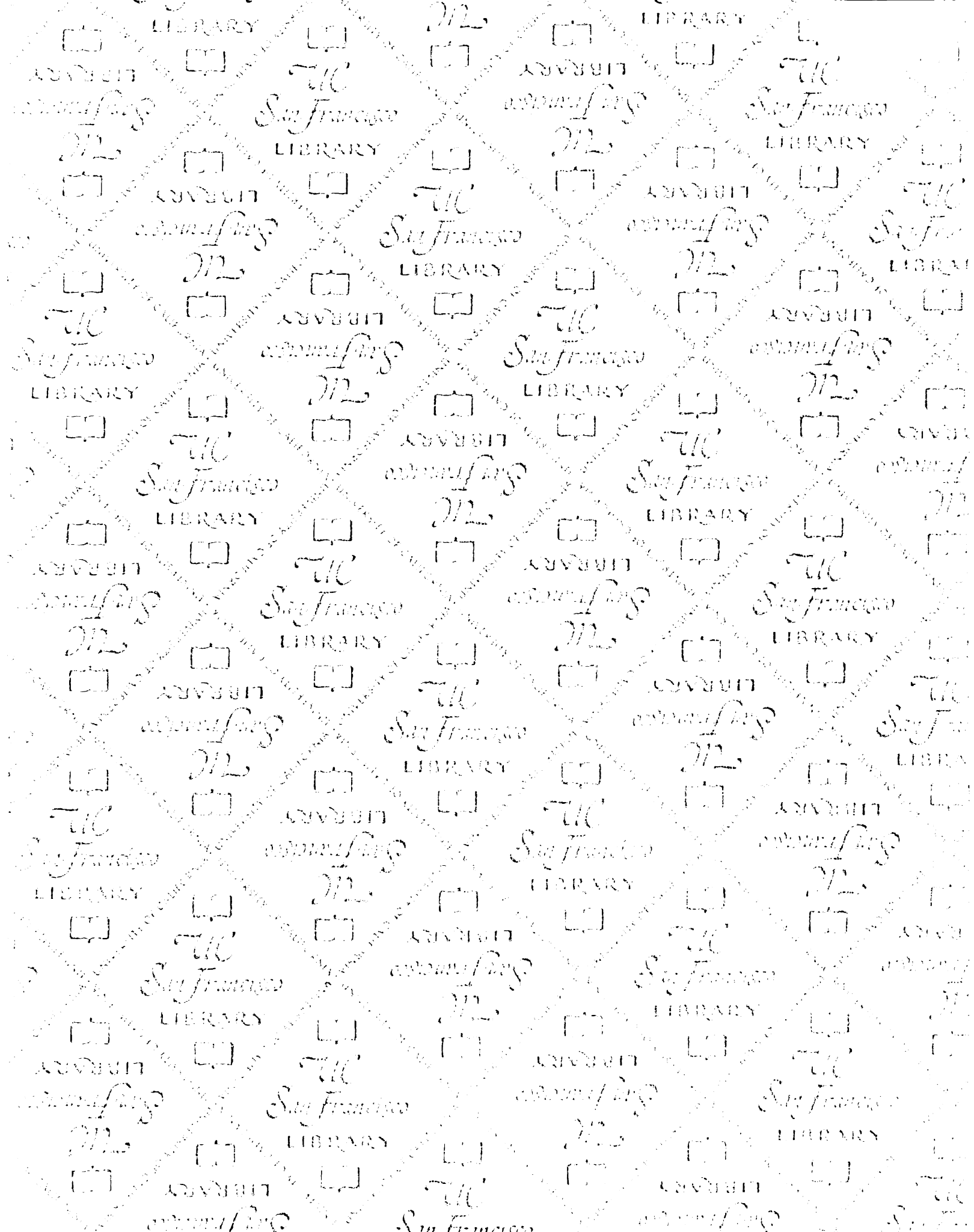
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