Title
The effect of electrical stimulation on foot skin perfusion in persons with or at risk for diabetic foot ulcers

Permalink
https://escholarship.org/uc/item/3sp2q8s1

Author
Gilcreast, Darlene Mary

Publication Date
1995

Peer reviewed|Thesis/dissertation
The Effect of Electrical Stimulation on Foot Skin Perfusion in Persons with or at Risk for Diabetic Foot Ulcers

by
Darlene M. Gilcreast, Major, Army Nurse Corps

DISSERTATION
Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in
Nursing

in the
GRADUATE DIVISION

of the
UNIVERSITY OF CALIFORNIA

San Francisco
copyright (1995)
by
Darlene M. Gilcreast
Major, U.S. Army Nurse Corps
DEDICATION

This dissertation is dedicated to

John "Jay" Gilcreast,

My husband who had the vision to send me to college,
the dedication to lovingly support me in that endeavor,
and the charity to overlook my shortcomings
while the task was accomplished.
ACKNOWLEDGEMENTS

There are many people who made this research possible. I thank the U.S. Army Nurse Corps for providing financial support and duty time to complete the doctoral program. This research was funded by Tri-Service Military Nursing Research Grant N94-010. Deep gratitude is expressed for the generous support provided of equipment and supplies loaned by Mr. Robert Wingrove, Sr., Medical Devices, Inc. (Minneapolis, MN) and Parks Medical Instruments, Inc. (Aloha, OR). I thank Colonel (Retired) Louise Ryan, AN; Lieutenant Colonel (Retired) Linda Bryant, AN; and Lieutenant Colonel Wayne Voelmeck, AN, who helped me to be selected for the doctoral program. I also thank Colonel Carol Reineck for her role in the Tri-Service Military Nursing Research Workshops.

I acknowledge the wonderful support provided by the members of my dissertation committee. Dr. Nancy A. Stotts served as chairperson of the committee, my academic advisor, and mentor. Dr. Stotts’ is very dedicated to students and provides hours of guidance being available, no matter the request, day, nor hour. In addition, Dr. Stotts taught me wound care, fostered relationships to promote quality research, and clarified CRUNCH for me. Dr. Erika S. Froelicher demonstrates finesse and knowledge as a researcher I find awesome. Dr. Froelicher’s critiques always fostered positive self-esteem in her students in
addition to improving the quality of research. Dr. Kathryn A. Lee possesses exemplary research skills and clarified statistics for me. Dr. Lee’s insights added immensely to the design and analysis of this project. Dr. Lucinda L. Baker demystified the electrical stimulation literature for me and her ten years of experience in electrical stimulation for clinical treatment provided an invaluable service to this work. Dr. Baker selflessly gave of her time and efforts, spending whole days traveling to participate in committee work. I found Dr. Baker’s generosity amazing, since she is faculty at another university and served without compensation. The work of this committee was without a doubt the finest example of collegial synergism I have witnessed.

Gratitude is expressed to Kathryn Moss, DPM, who provided podiatric consultation on the conduct and completion of the study. Dr. Moss taught me foot neurological assessment techniques and provided assistance with clinical decision making. I thank T. K. Hunt, MD, who allowed me to observe in his wound healing clinic; Morton Altman, DPM, Jeffrey Page, DPM, and John Sorenson, DPM, who opened collaboration with the California College of Podiatric Medicine; and Linda Hughes, RN, RVT, and Janet Wyleczyk, RVT, for teaching me vascular assessment. I also thank Kathryn O’Brien, FNP, for her assistance in recruitment.
Gratitude is expressed especially to the subjects who participated in this study. They gave unselfishly of their time and shared personal information to foster research and improve the lives of people with diabetes.

I thank my fellow doctoral students (Major Kathryn Dolter, AN; Commander Janice Stinson, USNR, NC and LCDR (Retired) Ralf H. Stinson, USN; Major Stacey Young-McCaughan, AN and Patrick McCaughan; Sister Chris Wood, SNJM; Deidre Wipke-Tevis, Marcia Ryder, Laura Thompson, Kathy Wood, Gayle Shiba, Jeanne Kempiennan, Joan Fair, Jeanette Koshar, Keeta Lewis, Daphne Stannard, Martha Moon, and JoAnn Daugherty) for their critique and interest in my project. The support of these people was extremely valuable to me. Finally, I thank my family, who has patiently understood my dedication to nursing, my clients, education, and the U.S. Army Nurse Corps.
ABSTRACT

The Effect of Electrical Stimulation on Foot Skin Perfusion in Persons with or at risk for Diabetic Foot Ulcers

Darlene M. Gilcreast, PhD, Major, U.S. Army Nurse Corps

The primary purpose of this study was to investigate whether electrical stimulation (HVMPC, 100V, 100pps, negative polarity) would increase skin perfusion in persons with diabetes who have or at risk for foot ulcers. Secondary purposes were to examine the effects of glucose control, peripheral neuropathy, peripheral vascular disease, Wagner Class, gender, ethnicity, age, and medications on skin perfusion in response to electrical stimulation (ES).

The inclusion criteria were persons with diabetes who were at least 40 years of age, did not use tobacco, were at risk of foot ulceration, had one great toe, and were English speaking. The sample was composed of 132 subjects; 55 were Black and 77 were Non-Black; 72 were male and 60 were female. Mean age of subjects was 66 years (9.6), mean duration of diabetes was 15 years (11). There were 33 ulcers in 24 subjects.

After obtaining consent, baseline TcpO₂ level was obtained, ES was applied, and TcpO₂ measurements were recorded at 30 and 60 minutes. Levels of neuropathy, peripheral vascular disease, ulcer severity, gender, ethnicity, age and vasoactive medications were measured.
Data showed a mean decrease in foot $\text{TcPO}_2$ levels ($M = 5\text{mmHg}, \text{SD 17}$) in response to ES. ANOVA with post-hoc Scheffe was statistically significant ($p < 0.05$). Moderator variables did not significantly influence skin perfusion ($p > 0.05$). Data demonstrated two response levels; 35 (26%) subjects increased $\text{TcPO}_2$ ($M = 14\text{mmHg}, \text{SD 19}$) and 97 (72%) subjects decreased $\text{TcPO}_2$ ($M 12\text{mmHg}, \text{SD 9}$). Logistic regression did not identify any statistically significant predictors of increased $\text{TcPO}_2$ ($p > 0.05$).

This treatment offers the possibility of helping a significant number of persons with diabetes to increase skin blood flow and more rapidly heal foot ulcers. Future research is needed to determine whether will ES will significantly augment standard treatment in persons with or at risk for diabetic foot ulcers.
# Table of Contents

DEDICTION............................................................................................................. ii  
ACKNOWLEDGEMENTS........................................................................................... iii  
ABSTRACT................................................................................................................ vi  
TABLE OF CONTENTS............................................................................................. viii  
LIST OF TABLES...................................................................................................... xi  
LIST OF FIGURES.................................................................................................... xiii  
CHAPTER 1................................................................................................................ 1  
THE STUDY PROBLEM............................................................................................. 1  
   The Purpose of the Study....................................................................................... 2  
   Significance of the Study..................................................................................... 3  
CHAPTER 2................................................................................................................ 5  
BACKGROUND OF THE STUDY AND LITERATURE REVIEW.............................. 5  
   Prevalence of Diabetes......................................................................................... 5  
   Physiology of Skin Perfusion and Oxygen Transport......................................... 6  
   Microvascular Defect in Diabetic Subjects........................................................... 8  
   Peripheral Neuropathy......................................................................................... 12  
   Diabetic Foot Ulcers............................................................................................ 13  
   Physiology of Wound Healing............................................................................ 14  
   Hemostasis and Inflammation............................................................................. 15  
   Epithelialization................................................................................................ 16  
   Angiogenesis and Granulation Tissue Formation................................................. 17  
   Diabetic Wound Healing...................................................................................... 18  
   Electrical Stimulation: An Adjunct to Wound Healing......................................... 20  
      Background of ES............................................................................................ 20  
      Types of ES.................................................................................................... 22  
      Studies Exploring ES for Soft Tissue Wound Healing.................................... 25  
      Studies of ES Effects on Cells and Tissues....................................................... 26  
      Animal Studies of ES and Wound Healing..................................................... 27  
      Use of ES in Human Tissue Repair.................................................................. 50  
      Electrical Stimulation and Perfusion in Humans............................................ 68  
      Summary of the Literature Review.................................................................. 82  
CHAPTER 3................................................................................................................ 85  
METHOD.................................................................................................................... 85  
   Research Hypotheses......................................................................................... 85  
   Primary Null Hypothesis..................................................................................... 85  
   Secondary Null Hypotheses................................................................................. 85
APPENDIX A ................................................................. 174

University of California, San Francisco,
Experimental Subjects Bill of Rights ................. 175
University of California, San Francisco,
Consent to Be a Research Subject:
"Electrical Stimulation and Diabetic Foot
Skin Perfusion" .................................................. 177
Subject Data Collection Form ............................. 180

APPENDIX B ................................................................. 181

Figure 1. Foot Diagram ................................. 182

APPENDIX C ................................................................. 183

Pilot Study Data ................................. 184
LIST OF TABLES

Chapter 2

Table 2-1 Electrical Stimulation in Cellular (In Vitro) Models.................................29
Table 2-2 Studies of the Effect of Electrical Stimulation on Edema in Animal Models.....................30
Table 2-3 Studies of Electrical Stimulation and Wound Healing in Animals before 1990...............36
Table 2-4 Studies of Electrical Stimulation in Animal Models after 1990.................................41
Table 2-5 Early Human Wound Healing Studies (Before 1990)........................................51
Table 2-6 Recent Human Wound Healing Studies (1990 to Present)........................................56
Table 2-7 Early Human Studies by Kaada and Colleagues of the Effect of ES on Skin Perfusion........71
Table 2-8 Recent Human Studies of the Effect of ES on Skin Perfusion.................................72

Chapter 3

Table 3-1 Wagner Class Scale.................................................................92
Table 3-2 Data Collection Procedure.................................

Chapter 4

Table 4-1 Sample Characteristics (n = 132).............................115
Table 4-2 Type of Diabetes Management, HemoglobinA1c, and Age..............................................115
Table 4-3 Site of Electrical Stimulation (n = 133).............116
Table 4-4 Mean Vascular Characteristics, All Subjects (n = 132)............................................117
Table 4-5 Mean TcpO2 Readings (mmHg), All Subjects (n = 132).............................................118
Table 4-6 Repeated Measures Analysis of Variance for Difference in TcpO2 in Response to ES Treatment, Within All Subjects (n = 132).................................120
Table 4-7 Repeated Measures Analysis of Variance of 
TcpO2 in Response to ES Treatment, Within 
All Subjects (n=132)..........................120

Table 4-8 Repeated Measures Analysis of Variance of 
TcpO2 in Response to ES Treatment, Within 
All Subjects by Glucose Level with Age, 
Ethnicity, and Gender as Covariates (n=132)...122

Table 4-9 Repeated Measures Analysis of Variance of 
TcpO2 in Response to ES Treatment, Within 
All Subjects by Peripheral Neuropathy Level 
with Age, Ethnicity, and Gender as Covariates 
(n=132)........................................123

Table 4-10 Repeated Measures Analysis of Variance of 
TcpO2 in Response to ES Treatment, Within 
All Subjects by Peripheral Vascular Disease 
Level with Age, Ethnicity, and Gender as 
Covariates (n=132)............................123

Table 4-11 Repeated Measures Analysis of Variance of 
TcpO2 in Response to ES Treatment, Within 
All Subjects by Wagner Class Level with Age, 
Ethnicity, and Gender as Covariates (n=132)...124

Table 4-12 Responders vs Nonresponders: Mean TcpO2 
and TcpCO2 Readings (mmHg) in Response to 
ES.............................................125

Table 4-13 Ulcer Number, VPT, HgbA1C, and Vascular Levels: 
Responders vs. Nonresponders (n=132).........126

Table 4-14 Nitrate-Containing Medications...............127

Table 4-15 One-Way ANOVA, Change in TcpO2, Baseline 
to End of Treatment, by Subjects Taking 
Nitrate-Containing Medications vs. Subjects 
Who Did Not (n=132)..........................128

Table 4-16 One-Way ANOVA, Change in TcpO2, Baseline 
to End of Recovery, by Subjects Taking 
Nitrate-Containing Medications vs. Subjects 
Who Did Not (n=132)..........................128

Table 4-17 Calcium-Channel Blocking Drugs.............129

Table 4-18 Logistic Regression Odds Ratios and 95% 
Confidence Intervals for 8 Moderator 
Variables to Predict Nonresponse to ES........13
LIST OF FIGURES

Appendix C.................................................................184

Figure 1. Foot Diagram.................................................184
CHAPTER 1
THE STUDY PROBLEM

Statement of the Problem

Approximately 26,000 persons with diabetes undergo lower extremity amputation annually, most due to failure of foot wounds to heal (Pecoraro, Reiber, & Burgess, 1990). The problem is particularly common in minority populations (American Diabetes Association, 1993). Diabetic foot wounds result in disability, suffering, disruption of activities, and account for 24% of hospital days related to diabetes each year. Foot care costs billions of dollars annually (Hunter, Cathcart-Silberberg, Langemo, Olson, Hanson, Burd, & Sauvage, 1992; Huse, Oster, Killen, & Lacey, 1986).

A treatment is needed to speed wound healing in persons with diabetes to reduce the possibility of extremity amputation. One of the adjunctive therapies available is electrical stimulation. Electrical stimulation (ES) has been shown to increase blood flow to the skin (Kaada & Eielsen, 1982), attract cells that promote tissue repair (Orida & Feldman, 1982), and increase the number of receptors on cells for transforming growth factor-beta (Falanga, Bourguignon, & Bourguignon, 1987). Research has shown ES promotes wound healing of ulcers of mixed etiologies, especially pressure ulcers in spinal-cord-injured subjects (Feedar, Kloth, & Gentzkow, 1991), and venous stasis ulcers in diabetic subjects (Lundeberg,
Eriksson, & Malm, 1992). Increased blood flow to the area has been seen with ES (Dodgen, Johnson, Baker, & Chambers, 1987). Since wound nutrition is an essential element in wound healing and oxygen is a vital substrate for metabolism, it is possible that ES could promote wound healing of foot ulcers in persons with diabetes. To date, only a ten-year-old abstract of a pilot study involving solely subjects with diabetic foot ulcers is reported in the literature (Alon, Azaria, & Stein, 1986).

Although persons with diabetes have been included in ES studies, large studies limited to diabetic subjects with foot ulcers have not been reported. Study of this area requires consideration of glucose control, neuropathy, peripheral vascular disease, severity of ulceration, gender, ethnicity, medications, and concomitant disease, as these factors may alter the effects of ES on perfusion in subjects with diabetic foot ulcers.

The Purpose of the Study

The purpose of this study is to examine the effect of ES on skin perfusion in the feet of persons with diabetes who have, or are at risk for, foot ulcers. The independent variable is ES provided as high-voltage, monophasic pulsed current (HVMPC) and the dependent variable is skin perfusion measured by transcutaneous oximetry in millimeters of mercury (mmHg). Secondary purposes are to examine the effects of glucose control, level of neuropathy, level of
peripheral vascular disease, Wagner Scale classification, gender, ethnicity, medications, and age on skin perfusion in response to ES.

Significance of the Study

Diabetic foot ulcers are due to multiple factors, including neuropathy, changes in foot architecture, and arteriovenous shunting (Grunfeld, 1991). They result in considerable healthcare costs, disability, and often result in amputation (Pecoraro, Ahroni, Boyko, & Stensel, 1991).

Standard wound care for subjects with foot ulcers includes providing a physiologic environment conducive to healing, such as, removing weight bearing from the injured tissue and control of diabetes. This treatment results in healing of some ulcers; however, when ulcers become chronic, adjunctive therapy may be needed to promote healing.

Electrical stimulation is an adjunctive therapy that has been shown to increase healing in subjects with pressure ulcers, foot ulcers, and leg ulcers (Stefanovska, Vodovnik, Benko, & Turk, 1993; Alon, Azaria, & Stein, 1986; and Lundeberg, Eriksson, & Malm, 1992). The mechanism of action is thought to be temporary sympatholytic interference with vasoconstriction, thus increasing blood flow (as with a surgical lumbar sympathectomy) (Rutherford & Shannon, 1995). Although foot ulcers in diabetes are an important type of chronic wound, ES has not been widely studied in this population.
If it were shown that ES enhances blood flow in persons with, or at risk for, foot ulcers; then it is possible that ES could be used to enhance perfusion in persons with foot ulcers, improve healing, and shorten wound healing time. Such a therapy could reduce risk of infection, result in healthcare cost savings, and decrease human suffering caused by disability.
Prevalence of Diabetes

Counting both diagnosed and undiagnosed cases of diabetes, approximately 13 million people (or 5.2 percent of the population) in the United States have diabetes mellitus. According to 1990 Centers for Disease Control statistics, 27.6 persons per 1,000 United States residents have diabetes ("Morbidity and Mortality Weekly Report," 1990). Among persons 20 to 74 years of age, this number includes 6.2% of Whites, 10.2% of Blacks, 13.0% of Hispanics, and 28% of Pima Indians. The prevalence of diabetes increases with age, resulting in half of the persons with diabetes being over 55 years of age (American Diabetes Association, 1993). At age 65 years, 103.9 persons per 1,000 have diabetes (17% of Whites, 25% of Blacks, 33% of Hispanics, and 50% of Pima Indians). The prevalence of diabetes among native Alaskans is lower than the general population in the United States; however, it varies by Alaskan subgroup with the Aleuts being the highest at 2.7% (American Diabetes Association, 1993).

Blacks have twice as many lower-extremity amputations as Whites (Fain, 1993). The rate is highest for Black males. Males have a higher rate (5.3 per 1,000) than females (3.6 per 1,000) (Connell, 1991).
Ninety percent of people with diabetes have non-insulin-dependent diabetes (NIDDM); 10% have insulin-dependent diabetes (IDDM) (Fain, 1993). Twenty-five years after diagnosis, 50 percent of individuals have neuropathy (Bransome, 1992). Among diabetic subjects in a Veterans' Affairs outpatient clinic, 9% had a history of ulceration, 1% had active ulceration, 4% had toe amputation, and 2% had leg amputation (Holewski, Stess, Graf, & Grunfeld, 1989).

Physiology of Skin Perfusion and Oxygen Transport

The skin blood flow normally serves to regulate body temperature, causing wide fluctuations in perfused capillaries. Nutritional requirements of the skin are usually small. Perfusion is normally adjusted according to the metabolic demand of the tissue by cytokines (vasoactive substances) with the overall regulation determined by the sympathetic nervous system, which controls the precapillary sphincter and regulates vascular smooth muscle tone in arterioles. The capillaries maximally dilate whenever the nerve supply is interrupted. A local regulatory effect is also seen in response to epinephrine, norepinephrine, and other vasoactive substances (cytokines) (Berne, & Levy, 1986). The relative influence of the sympathetic nervous system and local mediators varies among the tissues it innervates.
Control by the sympathetic nervous system is through the sodium-potassium pump and calcium flux (Berne & Levy, 1986). The sodium-potassium pump is the first messenger, with calcium channels (calmodulin), cyclic adenosine monophosphate (cAMP), and inositol phosphate acting as second messengers (Lee, Canaday, & Doong, 1993). Smooth muscle contraction occurs as a result of increased calcium levels causing phosphorylation activating myosin light chains. The phosphorylation of the myosin forms bridges between actin and myosin fibers resulting in vasoconstriction. Reduction in calcium levels causes inactivation of the myosin light chains, resulting in vasodilation (Taubman, 1990).

Some of the 18 cytokines known to be vasoactive substances are adenosine triphosphate (ATP), epinephrine, norepinephrine, nitric oxide, acetylcholine, substance P, serotonin, histamine, vasopressin, angiotensin II, and dopamine (Edvinsson & Uddman, 1993). Neuromodulators, substances that influence the effect of neurotransmitters, result in effects specific to the tissue being signalled, for example alpha and beta receptors in cardiac tissue. Substance P has a direct role in triggering the inflammatory response in wounded tissue, controlling blood flow to the area (Low & Reed, 1993).
Microvascular Defects in Diabetic Subjects

The exact mechanism that contributes to microvascular defects in persons with diabetes is not yet known. It involves a complex interplay between hyperglycemia, peripheral neuropathy, and peripheral vascular disease (Winegrad & Simmons, 1992).

Peripheral vascular disease in diabetic subjects shows thickened capillary basement membranes and accelerated atherosclerosis. The thickened basement membrane does not prevent diffusion of oxygen, but blood flow is reduced due to increased blood viscosity and glycation of serum proteins. Diabetes decreases oxygen availability because glycated hemoglobin is more resistant to giving up oxygen to the tissues (Cianci & Hunt, 1993). The glycation of the red-blood cell membrane interferes with its deformability, thus limiting its ability to pass through capillaries. This creates greater arteriolar and capillary resistance, necessitating a higher pressure to maintain capillary perfusion (Cohen & Tesfamariam, 1992). The increased pressure causes extrusion of serum proteins into the tissues, leading to tissue edema.

Hyperglycemia causes alterations in vascular signaling of second messengers by decreasing cellular inositol through aberrant glucose metabolism known as the "polyol pathway" and through loss of electrolytes by excessive urination (Greene, Lattimer, & Sima, 1988). Excess glucose is
converted to its polyol derivative (sorbitol) by aldose reductase in non-insulin requiring tissues leading to excessive production of diacylglycerol and activation of protein kinase C.

Myoinositol depletion is linked to the development of peripheral vascular disease in diabetes. Vascular dysfunction includes hemodynamic alterations and increased permeability of the microcirculation to plasma proteins. Excessive protein kinase C leads to vasoconstriction of the microvasculature (Ruderman, Gupta, & Sussman, 1992). Protein kinase C affects a variety of cellular processes, including ion transport via the sodium-potassium pump. Relaxation of capillaries to receive an increase in blood flow is caused by the endothelin-derived relaxing factor, nitric oxide, which is derived from intracellular arginine. Nitric oxide is secreted in response to acetylcholine from the sympathetic nervous system. Acetylcholine increases intracellular calcium by the action of phospholipase C, causing capillary dilation (Cohen & Tesfamariam, 1992).

Due to the production of "polyols" in persons with elevated blood sugar, nitric oxide is not released, or is "quenched" upon release, despite increased tissue demand for oxygen. Instead, prostaglandins, which cause constriction, are released. The resulting effect is vasospasm and ischemia (Ruderman, Gupta, & Sussman, 1992).
Hyperglycemia changes flow characteristics of blood in other ways (McMillan, 1993). Red blood cells (RBCs) normally comprise 40% of blood. Blood flow involves the physical properties of viscosity, drag, and shear, which are influenced by vascular turbulence. Increased glomerular filtration and oncotic draw of water into the urine causes hemoconcentration. Hemoconcentration raises viscosity, places RBCs closer together, and increases drag and shear.

Red blood cells normally repel each other and endothelial cells, due to a net positive charge on the cellular membranes, which keeps the RBCs suspended in the solution and away from vascular walls. With glycation, electrical properties of the RBCs change and raise the tendency to coagulation. Flow is further impeded due to decreased flexibility of RBCs.

Edmonds, Nicolaides, and Watkins (1986) have found the incidence of microvascular defects, particularly arteriovenous shunting, increases with diabetic foot ulceration. Subjects with ulceration showed consistently higher diastolic flow than diabetic subjects without ulceration (p < 0.002). Their study showed that foot ulceration does not usually develop in the absence of peripheral neuropathy and subjects with ulcers had sympathetic denervation. This denervation also increases vessel permeability to albumin leading to peripheral edema, increased intracutaneous pressure, and decreased capillary perfusion.
Moenckeberg's sclerosis, common in persons with diabetes, is a type of calcification of the medial layer of blood vessels, usually between the knee and ankle, which results in abnormal hardness of the vessels. It is closely associated with diabetic neuropathy (Ritz, Friedman, & Osbourne, 1992). In small and large arteries, damage is done to the endothelium causing cellular leaks into the tunica media. Protein molecules, platelets, macrophages, and cholesterol deposit in the smooth muscle causing vessel walls to thicken. Cianci and Hunt (1993) call this process hyalinization of capillary walls and state it may lead to capillary obstruction.

High blood flow to the lower legs in diabetic persons due to the force of gravity and peripheral neuropathy causes an unusual stretch on blood vessels, increasing plaque formation and calcification. Hypertrophy of the vessel walls results in deposition of more cholesterol, calcium, and collagen, which cause plaque and vessel narrowing. Because of the increased calcification, vessels lose their ability to distend and recoil, and become noncompressible, which results in falsely elevated blood pressure measures (Allen, Anderson, Walker, & Sicard, 1993).

During wound healing, skin nutritional requirements greatly increase. Oxygen is used for oxidative glycolysis to maximize energy availability in the body and decrease lactic acidosis. The oxidative burst is used by phagocytes
for killing bacteria. Fibroblasts use oxygen to cross-link, hydroxylate, and secrete collagen. The majority of oxygen is carried in the blood bound to hemoglobin, but must be dissolved in the plasma in order to diffuse to the tissues and be used in wound healing.

Peripheral Neuropathy

Diabetic peripheral neuropathy is defined as abnormal function or structure of peripheral nerves caused by diabetes (Dyck, Karnes, O’Brien, 1987). Studies by Greene and associates (1988) identified the polyol pathway, that converts excessive blood glucose to sorbitol and related compounds, which causes injury to vasculature and nerves.

Chronic blood glucose levels above 200 mg/dl are linked to an increased incidence of neuropathy. This relationship is supported by data that show nerve conduction improvement in subjects who achieve good glucose control (<180mg/dl) (Weber & Cardile, 1990). Autopsies on persons with diabetes suggest damage is due to nerve ischemia related to microvascular changes. Peripheral nerves have a high affinity for sodium-dependent transport systems, which are abnormal with high glucose levels (Greene, Lattimer, & Sima, 1988). Phosphoinositide, a source of inositol, is stored in metabolically inactive myelin, which is seen histologically to deteriorate in the presence of hyperglycemia and polyols. Demyelination decreases nerve conduction.
A circular pathway is entered in which neuropathy promotes vascular dysfunction which promotes neuropathy. Electrical stimulation may prove to be an intervention to help diabetic subjects interrupt this circular process and restore nerve function before irreversible damage occurs (Dodgen, Johnson, Baker, & Chambers, 1987).

**Diabetic Foot Ulcers**

Diabetic foot wounds consist of a variety of injuries to the foot, including trauma. The most common foot wounds due to diabetes are neurotrophic ulcers and vascular ulcers (Grunfeld, 1991). It is important to distinguish between neurotrophic diabetic foot ulcers and vascular diabetic foot ulcers, as pathophysiology and treatment are different (Grunfeld, 1991; Harrelson, 1989).

Neuropathic ulcers occur predominantly on the plantar (weight-bearing) surface of the foot, but may occur on the lateral and medial aspects. Peripheral neuropathy results in an imbalance between flexors and extensors causing a "clawing deformity" of the foot (Harrelson, 1989). The clawing deformity pulls cushioning fat pads forward, thinning them, and increasing pressure on the plantar surface of the foot. Increased pressure with decreased sensation results in tissue ischemia, necrosis, and foot ulceration in persons with diabetes (Grunfeld, 1991).

Diabetic neurotrophic ulcers may present as a necrotic area that is covered by callus. In this case, the ulcer
exists underneath the callus. Ulcers beneath calluses bleed into the tissue evidenced by hemosiderin in the center of the callus. Ulcers are normally shallow, but may extend to the deeper tissues of the foot, even to bone (Harrelson, 1989).

Vascular ulcers in diabetic subjects usually result from arterial causes, such as atherosclerotic lesions producing emboli and decreasing flow, cardiac pump failure, and peripheral edema. These ulcers often begin as distal gangrenous lesions ("blue-toe syndrome") (Grunfeld, 1991). Necrotic tissue may slough or become a nidus for infection, resulting in an ulcer. According to Litner & Tombloom (1984), 64% of diabetic vascular ulcers occur on the toes, 16% on the heel, 10% on the dorsa of the feet, and 10% on the metatarsal heads.

Venous ulcers, which occur on the leg and ankle, may occur in diabetic persons, but the etiology is due to venous disease which is not specific to diabetes. Therefore, venous ulcers are not diabetic ulcers per se.

The Physiology of Wound Healing

Soft tissue wound healing is repair resulting in continuity of the skin and underlying structures being reestablished (Stotts, 1993). In humans, the majority of tissue is not regenerated, but patched with scar tissue to close the break (Clark, 1993).
Hemostasis and Inflammation

Injury causes a break in local blood vessels with extravasation of blood components. Platelet aggregation initiates clotting and the wound healing process (Kirsner, Eaglstein, 1993). The clot stops bleeding and provides a matrix for cellular migration. The coagulation cascade, complement pathways, and injured cells secrete vasoactive and chemotactic factors that attract inflammatory cells (Clark, 1993). Platelets in the clot and other cells in the area of injury secrete growth factors, which activate cells to engage in repair.

Neutrophils, the first to arrive at the injury, clear foreign debris, including bacteria. Integrins found on the surface of neutrophils, along with elastase and collagenase, enhance cell-matrix interactions attracting monocytes (Kirsner & Eaglstein, 1993). Monocytes are drawn to the area by extracellular matrix chemoattractants (collagen, elastin, and fibronectin) which are surrounded by vasoactive thrombin. In the tissue, colony stimulating factor transforms monocytes to macrophages capable of engulfing cellular debris (Clark, 1993).

Macrophages, fibroblasts, and blood vessel endothelial cells move into the wound approximately 48 hours after wounding, showing an interdependence among these cells in tissue repair (Clark, 1993). Macrophages provide a source of cytokines, fibroblasts construct new extracellular
matrix, and blood vessels provide a source of nutrients. Fibroblasts also produce cytokines, which stimulate the repair process at the tissue level (Clark, 1993).

Macrophages secrete collagenases to assist in debridement and factors which recruit other inflammatory cells. Under proper circumstances macrophages secrete peptide growth factors, interleukin-1, platelet-derived growth factor, transforming growth factor-beta, tumor necrosing factor-alpha, fibroblast growth factor, and insulin-like growth factor-1 (Clark, 1993). The activities of the macrophages induce angiogenesis (Kirsner & Eaglstein, 1993).

Epithelialization

Approximately 12 hours after injury, epidermal cells begin to migrate into the wound. Epidermal cells at the margins of the wound flatten and develop pseudopodia. While migrating, epidermal cells do not divide. Fibronectin produced by plasma allows for the migration of the cells across the surface. The growth factors, transforming growth factor-beta and epithelial growth factor, promote cellular migration, possibly through increased fibronectin production. Hyaluronic acid provides a matrix that is easily penetrated by ingrowing cells. Extracellular proteins, laminin and Type IV collagen, responsible for dermal adhesion, disappear at this time. Epithelial cells at the margin of the wound proliferate to provide a source
of cells to migrate across the wound (Kirsner & Eaglstein, 1993).

**Angiogenesis and Granulation Tissue Formation**

Granulation tissue is formed next. Wound healing requires an adequate blood supply to deliver oxygen, nutrients, and growth factors for tissue reconstruction. New blood vessels begin to grow into the wound only three days after injury. Vascular buds form from existing blood vessels stimulated by substances released into the wound bed by inflammatory cells. These buds grow toward the center of the wound stimulated by an increased carbon dioxide concentration and lower pH (Cooper, 1990).

New tissue includes macrophages, fibroblasts, loose connective tissue, and blood vessels. The main cell responsible for tissue regeneration at this time is the fibroblast (Clark, 1993). Collagen formation requires adequate amounts of iron, zinc, ascorbic acid, alpha-ketoglutarate, and oxygen (Cooper, 1990).

**Contraction**

As granulation tissue matures, the wound space is filled with extracellular matrix (fibronectrin, hyaluronic acid, proteoglycans, and collagen) (Clark, 1993). Fibroblasts transform becoming myofibroblasts containing actin filaments, enabling them to contract. Myofibroblasts form lines and contract the cell-to-cell matrix.
Contraction accounts for up to 40% of the decrease in wound size. Partial-thickness wounds contract less than full-thickness wounds, and superficial wounds contract hardly at all (Kirsner & Eaglstein, 1993).

**Diabetic Wound Healing**

According to Pecoraro (1991a), defective wound healing in persons with diabetes is a major cause of limb loss. The mechanism underlying the defect is not entirely understood. Problems experienced during diabetic wound healing include retarded closure, delayed contraction, abnormal granulocyte activity, altered platelet function, interference with collagen synthesis, and hyperglycemic effects on red blood cells (Pearl & Kanat, 1988).

Many proteins involved in wound healing are glycated and do not respond in the usual way. Granulocytes are delayed in moving into the wound bed, show decreased ability to engulf and kill bacteria, and have altered chemotaxis. Normally, a high lactate content in the wound stimulates release of growth factors by macrophages resulting in angiogenesis (Cianci & Hunt, 1993). However, diabetic macrophages are slow in responding.

Platelets display hyperaggregation and excessive secretion of thromboxane in the presence of high blood sugar, promoting vasoconstriction. Elevated serum glucose results in secretion of endothelin-1 (a constricting agent)
by endothelial cells, reducing perfusion (Yamauchi, Keizo, Takayanagi, Umeda, & Nawata, 1990).

Viscosity of the blood is affected by the glycation of proteins, particularly red blood cells, which make up 40% of the blood (McMillan, 1993). Poiseuille's law states the quantity of fluid flowing through a tube is directly proportional to the pressure gradient, the viscosity coefficient, and the fourth power of the radius of the tube (Morain & Colen, 1990). Thus, all of these factors combine to significantly reduce blood flow.

The skin in persons with diabetes often does not receive appropriate quantities of oxygen and nutrients for healing due to sluggish circulation brought about by increased blood viscosity and autonomic neuropathy (Edmonds, 1986). Autonomic neuropathy causes persistently high lower extremity blood flow on standing, but lowers skin nutritive flow by microvessel shunting (McMillan, 1993). Products of cellular metabolism are also not removed adequately.

With the inflammatory response hampered, diabetic subjects are unable to mount an adequate response to mediate normal healing and a chronic inflammatory process often is set up (Pearl & Kanat, 1988). Emara, Hou, Faria, Fivenson, and Ladin (1995) have demonstrated that chronic wound fluid suppresses T-cell mediated mitogenesis, and retards wound healing. The work of Stacey, Lainez, Skender-Kalnenas, and Morrison (1995) supported these findings.
Cellular replication, collagen synthesis to rebuild tissues, combatting infection, and wound contraction all require oxygen. The lack of perfusion to wounds in diabetes plays a direct role in delaying healing (Cianci & Hunt, 1993). A treatment which increases perfusion, and thus skin oxygen, could prove helpful in speeding wound healing.

Electrical Stimulation: An Adjunct to Wound Healing

Lee, Canaday, and Doong (1993) suggest that ES mimics the natural process of electrochemical signaling when applied to the skin. Various forms of ES have been reported to increase local circulation. Carr, Delaney, Westerman, and Roberts (1993) explain the effect of ES on circulation as "noxious" stimulation of the "axon reflex" resulting in the release of neuropeptides from sympathetic nerve terminals producing vasodilation or by release of vasoactive substances from mast cells.

Electrical stimulation of wounds has been shown to promote epithelialization (Alvarez, Mertz, Smerbeck, & Eaglstein, 1983). It attracts inflammatory cells (Orida & Feldman, 1982), reduces edema (Bettany, Fish, & Mendel, 1990), and increases DNA production in fibroblasts (Bourguignon, & Bourguignon, 1987).

Background of Electrical Stimulation

One basis for ES as a therapy is the naturally occurring electrical field of the human body. The body has
an electrical field that retains a polar orientation throughout life, being more positive over the head and central nervous system and negative over the extremities (Becker, 1985). Barker, Jaffe, and Vanable (1982) termed this natural phenomenon the "skin battery" and showed electrical flow occurs by sodium-ion channels (Jaffe & Vanable, 1985).

Following preliminary work, Foulds and Barker (1983) extensively mapped the adult human-skin battery. There were no differences in the map due to age or gender (Foulds & Barker, 1983). In general, moist areas have higher voltage potentials than dryer areas and hairy regions have lower potentials than areas free from hair (Foulds & Barker, 1983). The skin battery is capable of generating electrical potentials up to 80 millivolts (mV), or 1 microampere per millimeter (uA/mm) of wound length. A full-thickness wound extends entirely through the skin and has a potential of zero, as the skin battery is short circuited. However, two or three millimeters from the wound, the skin potential is normal. Lateral voltage gradients in the area of wounds are $140 \pm 20 \text{ mV/mm}^2$. Normal skin potentials are 40 to 80 mV (Jaffe & Vanable, 1984).

Borgens, Vanable, and Jaffe (1979) showed that frogs partially regenerated amputated limbs in response to external electrical forces. Natural direct currents have been recorded coming from children’s accidentally amputated
fingertips, "the current of injury" (Illingworth & Barker, 1980). It was shown that the currents did not come from nervous tissue, as the current was also present in denervated tissue. Electrical stimulation was subsequently used to stimulate regeneration in children with partial finger amputations (Borgens, 1989). These data are empirical evidence that an externally applied electrical force, similar to the naturally occurring skin battery, may influence soft tissue regeneration in humans.

Presumed mechanisms by which ES promotes wound healing are redistribution of blood flow to the skin improving skin nutrition, simulation of the current of injury by sodium-ion channels which stimulate the repair process, reduction of edema in the area, and limiting the area of injury by restoring blood flow (Chu, McManus, Matylevich, & Mason, 1995).

Types of Electrical Stimulation

Many names for types of ES can be found in the literature. The most widely accepted source on ES is written by Kloth and Feedar (1990), and will be used as the reference for identifying electrical types (Sussman, 1993).

Four types of ES have been widely used for soft tissue wound healing applications: low voltage, constant microamperage direct current (LVCMDC); low voltage, pulsed microamperage current (LVPMC); high-voltage, monophasic pulsed current (HVMPC); and low-voltage, pulsed
milliamperage current, also known as transcutaneous electrical nerve stimulation (TENS) (Kloth & Feedar, 1990). Baker and colleagues (1988) and Stefanovska and colleagues (1993) have used a fifth-type, symmetrical biphasic waveforms (SBP), which is not reported in Kloth & Feedar (1990), but is described in Low and Reed (1993).

Low-voltage, constant microamperage direct current (LVCMDC) is the continuous unidirectional flow of current, less than 100 volts (V), that does not stop nor reverse during treatment. Current is delivered to tissues via an electrode of either positive or negative polarity. A second electrode, opposite in polarity to the first electrode, is placed at least 15 centimeters from the first electrode over a large muscle group. Whenever possible, the positive electrode is placed closer to the head (Kloth & Feedar, 1990). Protocols for LVCMDC call for 200 microamperes (uA) to 1,000 uA to be passed through a wound for two to four hours per day.

Low-voltage, pulsed microamperage current (LVPMC) is used with either constant polarity monophasic pulses or reversing (biphasic) pulses through paired probes or carbonized rubber electrodes. This form of therapy has not been widely tested (Kloth & Feedar, 1990).

High-voltage, monophasic pulsed current (HVMPC), between 1 and 128 pulses per second (pps), is an interrupted flow of current, which usually does not reverse polarity
during treatment. It may be used in a biphasic form in which the polarity reverses with alternating pulses. Total current delivered to tissues is around 1.0 mA/cm². Higher voltages are required to accomplish this with a short, pulsed current. Since the stimulus is stronger than LVCMDC or LVPMC, treatment time is reduced (Kloth & Feedar, 1990). The main feature of this type of current is its short pulse duration.

Treatments using HVMPC are applied over moistened saline gauze packing the wound or by self-adhering electrodes lateral to the wound. Voltages are typically between 75 and 200V, and frequency is generally set around 100 pps. Voltage is normally set at a barely perceptible paresthesia or a twitch of underlying muscle, and pulse duration is frequently set at less than 100 microseconds (us). Treatment periods between 20 minutes and one hour are reported, but the most common is 30 minutes, two times per day (Kloth & Feedar, 1990). The most frequently used method starts with negative polarity and alternates polarity every three days or when a healing plateau is observed (Gentzkow, Alon, Taler, Eltorai, & Montroy, 1993).

Low-voltage, pulsed milliamperage current (TENS) typically uses symmetric biphasic pulses. Pulsed currents of this type have been applied to hands at the acupuncture Hoku point (the web space between the thumb and index finger) and, through an unknown mechanism, observed to
produce vasodilation in the ipsilateral lower extremity (Kaada, 1982; Ernst & Lee, 1985). The protocol recommended by Kaada and colleagues (1982, 1983) uses between 1 and 125pps with 20 to 250ms pulse duration. This is applied two to three times daily for 30 to 45 minutes (Kloth & Feedar, 1990).

Studies Exploring ES for Soft Tissue Wound Healing

Research on ES relevant to wound healing can be classified by type of model used for the study. Early investigations of the effect of ES on isolated cells and tissues involved in wound healing actually followed clinical application. Animal models were explored with quasi-experimental and experimental studies. Finally, promising approaches were studied in humans.

A problem encountered in examining this literature is lack of standardization. Investigators use a variety of wave forms and intensities, often calling the ES by new names. They also use old names in new ways, creating confusion about the waveform actually used. Studies frequently did not list all of the parameters of the ES forms used, making it difficult to know what was used as the treatment. Various types of electrodes, positioning of electrodes, and size of electrodes are used. Altering these parameters changes the density of the current delivered to the tissues, making comparison among studies difficult.

In an attempt to clarify the confusion, studies have
been classified as closely as possible to the kinds of ES defined by Kloth and Feedar (1990). Where it is known that the waveform is different, these differences are indicated.

**Studies of ES Effects on Cells and Tissues**

Various types of cells which are involved in wound healing have been studied. Orida and Feldman (1982) found macrophages move toward the positive pole in electrophoresis gel in response to 0.35-3.25 mA current. Cooper and Schliwa (1985) found single epidermal cells and cell clusters migrate toward the negative pole in DC electric fields of 0.5 to 15 volts/centimeter (V/cm). Keratinocytes extended lamellipodial extensions with actomyosin networks which were reversibly inhibited by calcium-channel antagonists; demonstrating that calcium-ion channels are involved in the effect of ES (Cooper & Schliwa, 1985).

Erickson and Nuccitelli (1984) observed ES influenced galvanotaxis when quail fibroblasts migrated toward a negative pole of 1 to 10 mV/mm, well below the endogenous electrical field known to exist in mammals. Patel, Xie, Young, and Poo (1985) observed the effects of electrical polarity on the growth of Xenopus (tadpole) neurons in culture. The magnitude of the current required to induce a growth cone was found to be in the order of picoamperes per square micrometer, close to that generated at the muscle
cell surface by acetylcholine molecules during the early phase of nerve-muscle contact.

Recently, Goldman and Pollack (1995) examined the activity of fibroblasts in a collagen "dermal equivalent" matrix in vitro. Tritiated thymidine and DNA were measured in response to an electrical stimulus felt to be equivalent to the endogenous "skin battery," between 30 and 1,000 mV/m at frequencies between 3 and 1,000Hz. Findings showed that fibroblasts had the greatest synthetic activity (+70%) between 37 and 50mV at 10 cycles per second (p < .01). Increases in DNA occurred at 40 and 50mV, but DNA did not increase at 100 cycles per second (p < .05).

Direct electrical currents from 1 microampere to 30,000uA stimulated production of ATP in rat skin (Cheng, VanHoof, Bockx, Hoogmartens, Mulier, DeDucker, Sansen, & DeLoecker, 1982). A HVMPC of less than 300 volts increased protein and DNA synthesis in human fibroblasts; however, voltages greater than 300 volts hindered synthesis (Bourguignon & Bourguignon, 1987). Falanga, Bourguignon, and Bourguignon (1987) increased fibroblast receptors for transforming growth factor-beta with HVMPC. These studies suggest mechanisms of galvanotaxis and chemotaxis caused by the influence of ES could favorably influence wound healing.

**Animal Studies of ES and Wound Healing**

Studies of animal models are listed in Tables 2-2, 2-3, 2-4, and 2-5. Table 2-2 summarizes the major
components of the effect of ES on edema. Table 2-3 summarizes ES animal studies before 1990 with wound healing as the dependent variable. Table 2-4 summarizes animal studies after 1990 with wound healing as the dependent variable.

Early studies began with Young in 1966. Some early studies are unrefined due to electrical technology at the time. Interpretation is questionable in some studies due to small numbers and lack of controls.

Using two groups of dogs (n = 4/group), Young (1966) found HVMPC of 150V, (polarity is not reported) five minute duration for 14 days promoted healing of ischemic tissue induced by 12 hours of tourniquet occlusion. Dogs in the treatment group showed evidence of sensation, less edema, less weight loss, and had less necrosis than control dogs. At the conclusion of the study, treated dogs walked without limping and untreated dogs developed severe gangrene. Results show HVMPC reduced edema and promoted circulation.

Reed (1988) studied the effect of HVMPC (10, 30, or 50V, polarity not reported) on the formation of edema in hamster cheeks with histamine infusion. Intravenous fluorescein-labeled dextran was used for the marker. Histamine produced edema in all groups; however, it was less in the hamsters treated with 30 or 50V HVMPC. Reed concluded that, beyond a certain threshold (10V), HVMPC
Table 2–1. Electrical Stimulation in Cellular (In Vitro) Models

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Cell Type</th>
<th>Amplitude, Current, Frequency, Pulse Duration</th>
<th>Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng et al., (1982)</td>
<td>Rat skin cells</td>
<td>1μA–30,000 μA</td>
<td>Radio-immunoassay</td>
<td>ATP increased 3 times 50-1000μA</td>
</tr>
<tr>
<td>Orida &amp; Feldman (1982)</td>
<td>Murine Macrophage</td>
<td>&lt;12V/cm</td>
<td>Movement of cells, cellular receptors</td>
<td>Moved towards cathode, receptors moved to cathode side</td>
</tr>
<tr>
<td>Erickson &amp; Muccitelli (1984)</td>
<td>Quail somite fibroblast</td>
<td>50-100mV per mm</td>
<td>Movement of cells</td>
<td>Migrated towards cathode</td>
</tr>
<tr>
<td>Patel, Xie, Young, &amp; Poo (1985)</td>
<td>Xenopus embryonic neurons</td>
<td>0.1V/cm 50 nA, 50 Hz, 5 ms (equal to signal induced by acetylcholine at neuromuscular junction)</td>
<td>Videorecording change in position</td>
<td>Negative currents increased growth, positive currents decreased growth</td>
</tr>
<tr>
<td>Cooper &amp; Schliwa (1985)</td>
<td>Fish epidermal cells</td>
<td>0.5-15V/cm</td>
<td>Photomicroscopy; cytoskeletal stain</td>
<td>Cells moved to cathode, Verapamil inhibited</td>
</tr>
<tr>
<td>Bourguignon &amp; Bourguignon (1987)</td>
<td>Human fibroblasts</td>
<td>50-300V</td>
<td>Tritiated proline &amp; tritiated thymidine</td>
<td>Protein &amp; DNA synthesis increased</td>
</tr>
<tr>
<td>Falanga, Bourguignon &amp; Bourguignon (1987)</td>
<td>Human dermal fibroblasts</td>
<td>100 pps 100 V</td>
<td>TGF-B receptors RIA</td>
<td>Elect. stim. increased TGF-B receptors</td>
</tr>
<tr>
<td>Goldman &amp; Pollack (1995)</td>
<td>Human dermal fibroblasts</td>
<td>30mV to 1,000mV, 3 Hz to 1,000Hz</td>
<td>Total DNA ³H-thymidine</td>
<td>Incr. @ 40mV, 10Hz &amp; 50mV, 10Hz but not 100Hz</td>
</tr>
</tbody>
</table>

Abbreviations used in the table:
3H-thymidine=tritiated thymidine
RIA =radioimmunoassay
Hz =Hertz (cycles/sec.)
TGF-B=transforming growth factor-beta
Table 2-2. Studies of the Effect of ES on Edema in Animal Models

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Animal/Number</th>
<th>Type of ES &amp; Polarity</th>
<th>Measurement</th>
<th>Results (Tx = Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (1966)</td>
<td>8 Dogs</td>
<td>HVMPC, 150V Polarity not reported (NR)</td>
<td>Description</td>
<td>Tx dogs walked without limp, had less necrosis than controls</td>
</tr>
<tr>
<td>Reed (1988)</td>
<td>14 Hamsters</td>
<td>HVMPC, 10V, 30V, 50V, &lt;100us</td>
<td>Capillary leakage by fluorescein microscopy</td>
<td>Leakage less in 30V &amp; 50V group (p &lt; .01)</td>
</tr>
<tr>
<td>Bettany, Fish, Mendel (1990)</td>
<td>20 Frogs</td>
<td>HVMPC, - pol, 75 us, Vc contraction</td>
<td>Edema, volume plethysmography</td>
<td>Tx limbs less edema than control limbs</td>
</tr>
<tr>
<td>Karnes, Mendel, &amp; Fish (1991)</td>
<td>20 Frogs</td>
<td>PLIDC, - pol</td>
<td>Edema, volume plethysmography</td>
<td>No significant difference, but trend for Tx limbs &lt; controls</td>
</tr>
<tr>
<td>Fish, Mendel, Schultz, &amp; Gottstein-Yerke (1991)</td>
<td>14 Frogs</td>
<td>HVMPC, + pol</td>
<td>Edema, volume plethysmography</td>
<td>No significant diff. by ANOVA. Recommend - pol to reduce edema</td>
</tr>
<tr>
<td>Taylor, Fish, Mendel, &amp; Burton (1991)</td>
<td>24 Frogs</td>
<td>HVMPC, - Pol, single Tx</td>
<td>Edema, volume plethysmography</td>
<td>1 Tx - pol significantly reduced (p&lt;.003) edema, Tx effect lasted 4h</td>
</tr>
<tr>
<td>Taylor, Fish, Mendel, &amp; Burton (1991)</td>
<td>12 Frogs</td>
<td>HVMPC, - pol of intensity to produce muscle contraction</td>
<td>Edema, volume plethysmography</td>
<td>4 Tx over 24h did not significantly reduce edema (p = 0.85).</td>
</tr>
<tr>
<td>Cosgrove, Bell, Fischer, et al. (1991)</td>
<td>44 Rats</td>
<td>HVMPC, sham tx, &amp; variable muscle stimulation pol NR</td>
<td>Edema, hindpaw volume</td>
<td>No significant difference between Tx and control groups</td>
</tr>
<tr>
<td>Chu, McManus, Matylevich, &amp; Mason (1995)</td>
<td>Sprague Dawley rats (? number)</td>
<td>LVCMDC, + pol, 40 uA</td>
<td>Quantity of Evans blue (EB) in scald wounds</td>
<td>DC Tx wounds 9 times less EB than controls or sham wounds (p &lt; .01)</td>
</tr>
</tbody>
</table>

Abbreviations in Table:
- pol = polarity
- Tx = treatment
- uA = microamperes
reduced microvessel leakiness, and thus retarded edema formation.

Bettany, Fish, and Mendel (1990) explored the effect of HVMPC, negative polarity, 75us duration, 120Hz, 10% below minimal muscle contraction, on edema formation caused by impact injury in 20 anesthetized frogs. Volumes of frog hindlegs were measured by water displacement when immersed in a small cylinder. Treated limbs had less edema than control limbs (p < .01); however, the amount of current required to produce minimal muscle contraction steadily increased throughout the trial.

Karnes, Mendel, and Fish (1991) measured effectiveness of LVMPC, negative polarity in reducing edema formation in the hindlimbs of 20 frogs following induced trauma. The quantity of edema was measured by volume displacement in a small fluid chamber. One ml of fluid displaced was equal to one ml of edema. The intensity of the treatment was 90% of motor threshold using 100Hz and 620 us pulse durations. Treatment was applied four times for 30-minutes followed by 30-minute rest periods. Contralateral limbs served as controls.

Karnes et al. (1991) found volumes of treated limbs were less than untreated limbs up to 17 hours post trauma, but the one-way analysis of variance did not show a significant change in volume caused by LVMPC. Findings are
similar to other findings produced by these investigators using this same model (Bettany, Fish & Mendel, 1990).

Fish, Mendel, Schultz, and Gottstein-Yerke (1991) studied the effect of 30-minute HVMPC treatment, applied four times, on edema formation following trauma in 14 anesthetized frogs. The stimulus was at an intensity approximately 90% of muscle contraction threshold, 120Hz, with positive polarity and was followed by a 30-minute rest period. Hindlimbs were measured prior to trauma by volume displacement, trauma was uniformly induced on both limbs, one limb was treated with ES, and the other untreated limb served as a control.

Again using water displacement, measurements were taken after trauma, after treatments, and after rest periods. Data were presented as changes from pretrauma volumes in ml/kg of body weight. Statistical analysis was by two-way ANOVA with repeated measures. Despite aggressive treatment, virtually equal bilateral edema occurred. These findings are contrasted with the reduction of edema this group of investigators found using negative polarity.

Taylor, Fish, Mendel, and Burton (1991) investigated the effect of a single HVMPC, negative polarity treatment, on edema following trauma (as already described) at various times postinjury (1.5, 3.0, 4.5, 8.0, 17.0, 20.0, and 24.0 hours post trauma). One randomly-selected limb received 120Hz of HVMPC at 90% of motor threshold. The dependent
variable was volume change expressed in ml/Kg body weight, analyzed by two-way RMANOVA (time and treatment group).

Findings were that a single, negative HVMPC treatment curbed edema in treated limbs ($p = 0.003$). Volumes were significantly less up to four hours after treatment. Therefore, the investigators suggest treatments should be given every four hours. An abstract is all that is available on this study and detailed breakdowns of volume changes versus treatment time post-injury are not provided.

Taylor, Fish, Mendel, and Burton (1991) studied the effect of HVMPC induced muscle contractions on the formation of edema in 12 frogs with induced trauma to their hindlimbs. The model was the same as that previously described. Four treatments of 30 minutes each were given followed by 30-minute rest periods. Intensity of the treatment was adjusted to just produce muscle contractions that resulted in minimal hip, knee, and ankle movements. Findings were that this intensity of this treatment did not reduce edema. Implications of these findings compared with the other findings of these investigators suggest that treatment stimuli may have been too intense to be beneficial in preventing edema.

Cosgrove, Bell, Fischer, Fowler, Jones, Myaing, Riddlespurger, Seaman, Tepper, and Alon (1991) compared the effects of two types of ES commonly used, HVMPC (100 pps of 5us duration) and variable muscle stimulation (VMS, 250 pps,
200us duration), on edema in rats paws at four times following induced trauma (24, 48, 72, and 96 hours). Rats were randomized to one of the two treatment groups or control. One hour of ES was administered to the two treatment groups, and the control group received a sham treatment. Significant differences between groups were not found. This is probably due to the fact that edema had already formed prior to treatment.

Chu, McManus, Matylevich, and Mason (1995) report using silver nylon with DC current to reduce edema, measured by extravasation of Evans blue (EB) following full-thickness thermal injury in Sprague-Dawley rats (number not reported). Burns were induced by uniform scalds over 20% of the body surface area on the dorsum of the rats. The intensity of current was 40uA of positive polarity compared with a sham treatment group and a no treatment control group. Findings were that both immediate and delayed application of the DC current resulted in less extravasation of the EB dye (p = 0.001). Only an abstract is available to assess this study, details of the experiment are not known.

Studies of the effect of ES on edema formation in animals offer a body of literature supporting a basis for ES to positively affect wound healing. The studies suggest negative polarity is more effective than positive polarity in suppressing edema, treatments may be too intense or too weak to provide an effect, and a treatment time of around 30
minutes may produce a positive effect, if all other parameters of treatment are appropriate. This body of literature demonstrates efforts to clarify the components of ES that may promote wound healing.

Table 2-3 presents studies of the effects of ES on wound healing in animal models. Alvarez, Mertz, Smerbeck, and Eaglstein (1983) used LVCMDC to increase epidermal wound healing in 11 pigs with surgically induced back wounds (n = 150 wounds on each animal divided into three treatment groups: active, sham, and control). Actively treated wounds were stimulated continuously for seven days and compared with sham treated and control wounds. Wound healing was measured by epidermal growth over the wound, tissue analysis (protein, collagen, and DNA content), and assessment of the microbial flora. Epithelialization was measured by chemically separating the excised healed epidermis from the dermis and macroscopically examining it for pinholes. Completely healed wounds, those without defects in epithelialization on macroscopic examination, were divided by the number of total wounds examined to obtain a percentage of healed wounds.

Epithelialization was greatest in actively stimulated wounds when compared with sham treated and control wounds (p < .05). Sham treated wounds had greater healing than control
Table 2-3. Studies of ES and Wound Healing in Animals before 1990

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Animal/Number</th>
<th>Type of Current</th>
<th>Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alvarez, Mertz, Smerbeck, &amp; Eaglstein (1983)</td>
<td>11 Pigs (150 wounds on each animal)</td>
<td>LVCMDC, + pol</td>
<td>Epi* &amp; col*</td>
<td>DC treated wounds &gt;* col &amp; epi &gt; cont</td>
</tr>
<tr>
<td>Smith, Romansky, Vomero &amp; Davis (1984)</td>
<td>65 Mice diabetic &amp; cont Mice</td>
<td>LVCMDC, 1V &amp; 20V pol NR</td>
<td>TS* &amp; H*</td>
<td>TS exp &gt; cont, Level of healing &gt; in exp than cont group</td>
</tr>
<tr>
<td>Stromberg (1988)</td>
<td>7 Pigs w/13 wounds</td>
<td>HVMPC +/- pol alt</td>
<td>wound closure rate</td>
<td>- Pol closure &lt; cont group; alt Pol closure &gt; cont group</td>
</tr>
<tr>
<td>Davis &amp; Mertz (1988)</td>
<td>7 Pigs w/10 wounds</td>
<td>HVMPC - pol X 1d + pol X 7d</td>
<td>Epithelialization (epi)</td>
<td>Tx group &gt; cont group</td>
</tr>
<tr>
<td>Cruz, Bayron, &amp; Suarez (1989)</td>
<td>20 Pigs</td>
<td>HVMPC - pol</td>
<td>Healing rate &amp; H</td>
<td>Tx group &gt; healing &amp; &gt; number fibroblasts</td>
</tr>
</tbody>
</table>

Abbreviations and Symbols used in table:

> = greater than
< = less than
alt = alternating
pol = polarity
H = histology
NR = not reported
TX = treatment
V = volts
exp = experimental
TS = tensile
strength
wounds, which were left open to air (p < .05). Actively stimulated wounds had more collagen and DNA than control or sham treated wounds (p< .05). Bacterial growth was lower in ES treated wounds on day 2; but on days 4 and 6, there were no significant differences in bacterial growth. This study showed LVCMDC promoted greater epithelialization and DNA synthesis than sham treatment or the control treatment. The sham treatment promoted more epithelization than the control treatment, perhaps due to the occlusive dressing the treatment provided for the wound (p < .05).

Smith, Romansky, Vamero, and Davis (1984) investigated the effect of LVCMDC at three levels (control, no charge; 1V, 10ma; 20V, 20 ma; polarity not reported) on surgical wound healing in 60 normal and diabetic adult mice divided into six groups. Wounds were surgically created on the dorsum of each mouse. Wound tensile strength was assessed at ten days and tissue examined microscopically. Tensile strength was classified as poor, moderate, and good.

The number of diabetic mice with poor wound healing decreased as amperage increased. Eight diabetic mice, four in each ES group, achieved good wound healing, while no diabetic control mice achieved good healing. Histologic examination showed diabetic stimulated wounds resembled normal control wounds in number of blood vessels and thickness of the epidermis (Smith et al., 1984).
Brown and colleagues generated a series of three studies, Brown and Gogia (1986); Brown, McDonnell, and Menton (1988); and Brown, McDonnell, and Menton (1989) to explore the effect of polarity of HVMPC on surgical wounds in rabbits. The first study used negative polarity, the second used positive polarity, and the third used alternating polarity.

In the first two studies, 40 rabbits were randomized to four groups. Wounds were stimulated for two hours twice daily with 80pps, 100us, between 30 and 60V (voltage was decreased or increased to achieve a barely perceptible contraction). Treated wounds were compared to control and sham treated wounds after three and seven days (Brown & Gogia, 1986; Brown, McDonnell, & Menton, 1988). Wound size, tensile strength, and histology were dependent variables. The investigators concluded negative polarity decreased wound size and increased tensile strength for the first three days after injury, but did not facilitate wound healing after that time. Brown and colleagues state histological analyses were questionable because wounds had been "pulled apart" by tensile strength testing prior to histological examination (Brown, McDonnell, & Menton, 1988).

In the third study, Brown, McDonnell, and Menton (1989) used the same treatment as the previous two studies, but polarity was negative for the first three days, then switched to positive polarity the last four days. Thirty-
six rabbits were randomized to treatment and control groups and healing was evaluated only at seven days. Wound closure was measured to assess the extent of healing after animals were sacrificed. However, the method used for measurement was not included in the study report. Wound closure was 100% for HVMPC treated wounds and 87% for control wounds (p<.02). Tensile strength and epithelialization were not significantly different. Brown and colleagues (1989) concluded changing polarity was preferable to maintaining polarity, either positive or negative, throughout treatment.

Cruz, Bayron, and Suarez (1989) used 20 pigs, with two electrode-induced full-thickness burn wounds of the same size, to study the effect of HVMPC (175V, less than 100us, 60pps) on wound contraction and fibroblast formation. One wound was used for tissue analysis, the other used for area measurement. Treatment used negative polarity for ten minutes daily. All wounds were left without dressings between treatments. Control animals were fitted with inactive electrodes during the stimulation period. Stimulated wounds healed faster than controls (p<.001), with 40% of them totally healed at the end of the four week study. No control wounds healed by the end of the study. On histologic examination, more fibroblasts were present in stimulated wounds compared with controls (p<.001).

Stromberg (1988) studied the effect of HVMPC (35mA, less than 100us, 128pps) and polarity on wound contraction
in seven pigs with 13 wounds, each full-thickness and 8cm in diameter. Wounds were treated for 30 minutes twice per day. The dependent variable was residual open wound area. Negative polarity treated wounds were larger (93% original size) than control wounds (58%) after two weeks. The alternating polarity treated wounds were smaller (18% original size) than either of the other two groups at two weeks (Stromberg, 1988). After four weeks, wound sizes were: negative polarity 36%, control 9%, and alternating polarity 0% of their original size. This study, although using a small number of subjects, supports the findings of Brown, McDonnell, and Menton (1989).

Davis and Mertz (1988) studied the effect of HVMPC (30 mA, 140us, 128pps), with alternating polarity, applied 30 minutes per day for seven days, on epithelization of surgically induced partial thickness wounds in seven pigs with ten wounds each. Polarity of treatment electrodes was negative on Day 0 and positive Days 1-6. Measurement was made by percentage of harvested wounds in a category with total epithelization (no flaws) when examined macroscopically. Stimulated wounds were 100% epithelialized compared to 80% in control wounds at the end of the seven-day study (p<.025) (Davis & Mertz, 1988).

Davis, Cazzaniga, Reich, and Mertz (1990) further explored the effect of electrode polarity on healing using the pig model. Both treatment groups, negative polarity on
### Table 2-4. Studies of ES in Animal Models 1990 to Present

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Animal/Number</th>
<th>Type of Current &amp; Polarity</th>
<th>Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis, Cazzaniga, Reich &amp; Mertz (1990)</td>
<td>7 Pigs w/10 wounds</td>
<td>HVMP, pol -1d/+6d + 7d vs. Cont</td>
<td>% of completely epi wounds</td>
<td>Both Tx group &gt; epi than cont group - pol 1d best.</td>
</tr>
<tr>
<td>Chu, McManus, Mason, Okerberg, &amp; Pruitt (1990)</td>
<td>220 Male guinea pigs</td>
<td>LVCMDC, 40uA X 2d, 20uA x 3d + pol</td>
<td>Epi time, revascularization time (revasc)</td>
<td>ES group 100% healed on 12d; cont group 50% healed by 16d.</td>
</tr>
<tr>
<td>Chu, McManus, Okerberg, Mason, &amp; Pruitt (1991)</td>
<td>120 Male guinea pigs</td>
<td>LVCMDC, 40uA X 2d, 20uA X 3d. + pol silver nylon</td>
<td>India-ink measure of revasc.</td>
<td>ES wounds &gt; adherence; revasc of ES wounds by 2d &gt; cont</td>
</tr>
<tr>
<td>Reich, Cazzaniga, Mertz, et al. (1991)</td>
<td>4 Pigs w/80 wounds</td>
<td>HVMP, + pol</td>
<td>Mast cell count, microscopic exam</td>
<td>No. of mast cells in Tx wounds &lt; cont wounds</td>
</tr>
<tr>
<td>Mertz, Davis, Cazzaniga, et al. (1993)</td>
<td>24 Pigs w/72 wounds</td>
<td>HVMP, 4 pol groups: -1d/+ 2d to 7d + all 7d - all 7d - even days/+ odd days</td>
<td>% of completely epithelialized wounds</td>
<td>Wounds with - pol 1d/+ pol, 16% healed; only - pol, 5% healed; other groups, 0% epi</td>
</tr>
<tr>
<td>Kambic, Reyes, Manning, Waters, &amp; Reger (1993)</td>
<td>20 Pigs w/20 wounds</td>
<td>LVCMDC &amp; HVMP 200uA/cm2 X 30 days, - pol</td>
<td>Tensile strength</td>
<td>AC and DC stim. wounds = in strength</td>
</tr>
<tr>
<td>Agren, Engel, &amp; Mertz (1994)</td>
<td>4 Pigs w/72 wounds</td>
<td>HVMP + hydrogel dressing, + and - pol</td>
<td>Collagenase extraction</td>
<td>Control wounds &lt; collagenase than hydrogel tx wounds &lt; collagenase than wounds tx w/both hydrogel &amp; HVMP</td>
</tr>
</tbody>
</table>

**Abbreviations and Symbols used in table:**

> = greater than  
epi = epidermalization  
< = less than  
exp = experimental  
alt = alternating  
H = histology  
col = collagen  
NR = not reported  
d = day  
pol = polarity  
V = volts  
TS = tensile strength  
Tx = treatment
Day 0 followed by positive polarity Days 1-6 and positive polarity Days 0-6, had increased epithelization compared to sham treated wounds (p<.025). Davis and colleagues (1990) recommend at least one day of negative polarity. How they reached this conclusion is unclear, as all that was available is an abstract of their research.

Reich, Cazzaniga, Mertz, Kerdel, and Eaglstein (1991) investigated the effect of HVMPC on the number of mast cells in healing wounds, since these may be related to skin healing problems such as hypertrophic scars and atopic dermatitis. In hypertrophic scars, the number of mast cells does not decrease with the healing of the wound (Rothe, Nowalk, & Kerdel, 1990). Daily punch biopsies were used to obtain wound specimens, mast cells were stained and counted. The number of mast cells in treated wounds was significantly less than controls on the first three days. The greatest difference occurred on Day 2, with control wounds having three times more mast cells than treated wounds (p<.001). No evidence of mast cell degranulation (ghost cells) was seen on electron microscopy. Reich and colleagues suggest this treatment could be effective in reducing mast cell-mediated abnormalities of wound healing.

Mertz, Davis, Cazzaniga, Cheng, Reich, and Eaglstein (1993) explored the effects of four HVMPC treatment regimens of various polarities on wound healing in 24 pigs over eight days. Polarity types were: Treatment 1--negative on Day 0
followed by positive for Days 1 through 7, Treatment 2--positive throughout the trial, Treatment 3--negative throughout the trial, Treatment 4--negative on even days and positive on odd days. These were compared to a control group of animals that were treated with a sham treatment. Measurement of wound healing was by percent of wounds in a treatment category that were completely epithelialized (no defects in harvested wounds upon macroscopic examination). Control group wounds (five pigs) were 100% healed on Day 7.

Treatment Group 1 wounds, negative on Day 0 followed by positive polarity (six pigs), were 100% healed by Day 6. Treatment Group 2 wounds, positive polarity only (four pigs), were 100% healed on Day 6. Treatment Group 3 wounds, negative only (five pigs), were only 86% healed by Day 7. Treatment Group 4 wounds, alternating polarity daily (four pigs), were only 90% healed by Day 7. In order to compare treatments, Mertz and colleagues calculated a healing-time-50, the time it takes for 50% of the wounds to completely epithelialize. They concluded that positive polarity alone improved healing by 9% (decreased healing time by one day), negative polarity alone decreased healing by 9%, negative on Day 0 followed by positive polarity improved healing by 20%, and alternating polarity every day decreased healing by 45%.

This study builds on previous work by Brown and colleagues and provides a much needed evaluation of the effect of polarity on ES of wound healing. This is the only study
found that presents a healing-time-50 as a way of comparing treatments. This measure provides a uniform way of comparing different wound healing treatments.

Agren, Engel, and Mertz (1994) assessed the effect of HVMPC (4µA/cm², 128 pps, positive polarity) and hydrogel dressing on healing of partial-thickness burns using a pig model. The authors state the hydrogel dressing used has been shown to conduct electrical currents efficiently. Assessment was done daily by measuring collagenase levels by bioassay taken from punch biopsies in the center of the wounds. Collagenase was chosen since it is an important enzyme in tissue repair and returns to baseline once epithelization is complete. Collagenase assists in the debridement of necrotic tissue from the wound (Kloth & Miller, 1990), participates in migration of epidermal cells across the granulating wound (Feedar & Kloth, 1990), and is found in any tissue which is undergoing rapid remodeling (Stryer, 1988).

Collagenase was 1.5 times higher than controls in the wounds treated with hydrogel alone and was 3.0 times higher than controls in wounds treated with hydrogel and HVMPC. This study proposes that one mechanism by which HVMPC may increase wound healing is by increasing collagenase.

Chu, McManus, Mason, Okerberg, and Pruitt (1990) investigated the influence of LVCMDA 40µA (1V, positive polarity) for two days followed by 20µA for three days on
healing quality of split-thickness grafts in 200 guinea pigs (treatment group n = 180, control group n = 40). The purpose of this study was to find a way to reduce the time required to produce skin suitable for autoskin graft in burned subjects using a guinea pig model (Chu et al., 1990). A standardized scald method was used to induce burns in the guinea pigs. Silver nylon was used both as a dressing and as a treatment conductive electrode. After five days, dressings and stimulation were no longer applied. Gross and microscopic comparisons of wound healing in ten treatment and two control animals were made on days 2, 3, 4, 7, 14, and 90 days postburn. Dependent variables were healing time, wound contraction, and histological quality (evidenced by hair follicle preservation, vascularization, and suitability for skin graft). India ink was perfused via an artery to mark vessels for microscopic examination prior to sacrifice.

After wounds reepithelialized, 120 animals from the treatment group and 20 animals from the control group had split-thickness grafts harvested. Each graft was divided in half and the posterior portion grafted onto the anterior wound portion of the wound bed. The anterior portion of the graft was discarded. Each animal then had an anterior grafted wound segment with an open posterior donor site of equal size. Animals were again dressed with the silver nylon and treated as before. This model was used for
multiple harvesting in 60 guinea pigs. Second graft and donor sites were treated as before. Tissue was examined from ten animals 14 days post-treatment in each group to determine hair follicle survival (Chu, et al., 1990).

By 12 days postburn, all animals in the treatment group completely reepithelialized their wounds. In contrast, only 50% of the animals in the control group reepithelialized. The remaining control wounds were partially covered with a thick eschar at 16 days postburn. Microscopic examination showed approximately 30% of the original dermis was replaced by a layer of granulation tissue and surviving hair follicles were rare in this group. Control wounds not healed by 16 days required several additional weeks for healing and could not be used for subsequent skin grafting. Autografts taken from reepithelialized treated wounds were firmly adherent four days after grafting. Microscopic examination showed the presence of india ink in the grafted tissue, indicating that a union between graft and wound microcirculation had been established. In addition, there was an unusual hyperplasia of hair follicle epithelium at the graft-wound interface, which was most obvious four days after grafting. By seven days postgrafting, hyperplasia of hair follicle epithelium at the graft-wound interface was less prominent and nearly normal hair follicles had returned. After 12 days postgrafting, normal guinea pig skin predominated (Chu, et al., 1990).
Autografts from reepithelialized controls first showed evidence of revascularization and weak adherence seven days after grafting (five days later than treated animals). Further, degeneration of hair follicles occurred by one week postgrafting (Chu et al., 1990).

By 48 hours after skin graft harvest, treated donor sites showed evidence of epithelial cell migration and hair follicle formation. No significant inflammatory reaction was seen. Samples taken at three days showed donor wounds covered with new epidermis 3 to 8 cells thick. All treated animals had healed grafted wounds and donor sites by 14 days post harvest. No gross contraction was obvious 2 weeks or 3 months after harvesting, and both donor and graft sites expanded with growth of the animals. Hair density was only slightly reduced.

The second harvesting required several days longer to heal than primary donor sites. After 16 days following the second harvesting, minimal subepidermal fibroblast proliferation was present in the donor site. Donor sites of control animals showed neutrophil accumulation at the wound surface 48 hours after initial graft harvest. Donor wounds after harvesting in control animals required more than three weeks to reepithelialize (Chu, et al., 1990). The investigators concluded that DC current markedly reduces the time required for healing and improves the quality of the healed wounds, making them suitable for multiple skin
grafts. The same data were reported in another publication the following year (Chu, McManus, Okerberg, Mason, & Pruitt, 1991).

Chu and associates (1990) speculated that the improvement seen in the healing was due to prevention of deleterious events occurring in the tissue following injury causing cell damage and death; thus, LVCMDC limited tissue destruction. All treated wounds showed less inflammation, granulation tissue, and fibrosis than controls. The observed shortening of healing time could be due to the increased number of surviving hair follicles producing more epithelial cells, enabling cells to migrate from islands within the wound. The investigators believe ES prevented circulatory stasis following injury, thus preventing ischemia. Chu, et al. (1990) stated that reduction in tissue loss reduced inflammation. This was supported by the microscopic findings.

The findings of Chu and associates (1990) show that very low current applied constantly is very effective with a silver nylon dressing/electrode. As pointed out by Hunt in his comments regarding this research (Chu, et al., 1990), the electrode used in this study is more physiologic, as it provides a diffusion of current into the wound while most other studies use electrodes inducing current lateral to the wound.
Kambic, Reyes, Manning, Waters, and Reger (1993) compared the effects of alternating current (AC) and direct current (DC) on the healing of Grade IV pressure ulcers in pigs. A percutaneous cancellous screw was used to make uniform wounds in denervated pig hind legs in three groups: denervated control wounds, denervated AC stimulated wounds, and denervated DC stimulated wounds. Healing was measured by tensile strength and histological comparison of samples to control wounds. The scar tissue produced in the two treatment groups were similar with increased vascularity and reduction of wound volume, when compared to controls. The work of Kambic and colleagues (1993) demonstrates ES promotes wound healing, regardless of wave form.

These studies demonstrate a positive effect of ES on healing wounds of various etiologies in various animal models. Although results obtained in animal models are not directly applicable to humans, the studies provide guidelines for selecting safe and effective parameters for use in human subjects.

Up to 175 volts have been used with high voltage pulsed current with pulse durations up to 145us duration (Cruz, Bayron, & Suarez, 1989). Evidence shows wave forms that achieve 30 milliamperes (mA) per centimeter squared provide good results (Stromberg, 1988); however, Chu and associates (1990) suggest this is much too strong. Variable results have been found among experienced researchers using highly
refined designs within a consistent program of research (Mertz et al., 1983-1994).

The findings of Reich et al. (1991) are not consistent with the findings and proposed mechanisms of Chu et al. (1990); however, different ES types were used. Thus, evidence suggests that Goldman and Pollack (1995) are correct in suggesting that the type of ES that benefits wound healing may be within a vary narrow dosage range and type. Therefore, more studies are needed to define the optimum ES type and range. No adverse effects have been reported with this therapy regardless of ES type and range, indicating it is potentially safe for humans.

Use of ES in Human Tissue Repair


Carley and Wainapel (1985) conducted a matched group study of the effect of LVCMDC on vascular ulcers in 30 hospital inpatients, paired according to age, diagnosis, wound etiology, and ulcer size. One member of each pair was randomly assigned to treatment of two hours, twice daily. The dependent variable was ulcer volume measured to the nearest cubic millimeter. Negative polarity was used for
<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Subjects</th>
<th>Type of Current &amp; Polarity</th>
<th>Wound Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gault &amp; Gatens (1976)</td>
<td>100 DU 66 pts.</td>
<td>LVCMDC 200-1,000uA pol NR</td>
<td>HR/week</td>
<td>Treatment group healed 2 times faster</td>
</tr>
<tr>
<td>Newton &amp; Karselis (1983)</td>
<td>40 healthy subjects, intact skin</td>
<td>HVMPC - pol</td>
<td>Skin pH under electrode</td>
<td>No sig. pH change</td>
</tr>
<tr>
<td>Barron, Jacobson, &amp; Tidd (1985)</td>
<td>6 DU</td>
<td>LVCMDC biphasic &amp; - pol</td>
<td>% healing</td>
<td>2, 100%; 3, 99%; 1, 55%</td>
</tr>
<tr>
<td>Carley &amp; Wainapel (1985)</td>
<td>30 DU</td>
<td>LVCMDC - 3d/+ pol until healed</td>
<td>HR</td>
<td>Treatment group healed twice as fast as control group*</td>
</tr>
<tr>
<td>Alon, Azaria, &amp; Stein (1986)</td>
<td>15 diabetic foot ulcer</td>
<td>HVMPC, + Pol</td>
<td>Corr. Coeff HR w/size &amp; duration</td>
<td>12/15 healed</td>
</tr>
<tr>
<td>Fakhri &amp; Amin (1987)</td>
<td>20 burned males, LE</td>
<td>LVCMDC pol NR</td>
<td>Days to heal</td>
<td>19/20 healed</td>
</tr>
<tr>
<td>Kloth &amp; Feedar (1988)</td>
<td>16 DU</td>
<td>HVMPC alt pol</td>
<td>HR/week</td>
<td>Treatment group: 100% (45%/wk) Control group: wounds increased</td>
</tr>
</tbody>
</table>

* statistically significant, p < .05

**Abbreviations and Symbols used in table:**
- > = greater than
- < = less than
- exp = experimental
- alt = alternating
- d = day
- DU = decubitus ulcers
- LE = lower extremity
- NR = not reported
- pol = polarity
- H = healing rate
- Tx = treatment
- V = volts
the first three days and positive polarity was used until wounds reached a healing plateau, defined as failure to show progress for several days. At that time, polarity was changed to negative for three days, and switched back to positive once the wound showed evidence of healing. Current levels were set empirically (Gault & Gatens, 1976). Subjects in the treatment group showed a 1.5 to 2.5 times faster healing rate when compared to matched controls.

Alon, Azaria and Stein (1986) conducted a pilot study of 15 subjects testing the effect of HVPMC at 80 pps on healing diabetic foot ulcers occurring below the ankle. Positive polarity was used for the primary electrode and the second electrode was negative. Subjects received one hour of stimulation per day, three times per week. The standard wound therapy included debridement following a five-minute foot soak daily, followed by dry sterile gauze. Measurement of wounds was accomplished by photographing wounds, then using a digitizer to enter the wound area from the photograph into a computer for analysis of wound surface area.

Twelve of fifteen subjects completely healed their wounds within 2.6 months. One ulcer reduced in size from 92.7 mm² to 4.2 mm², one ulcer did not respond after six weeks of treatment, and one subject died of unrelated causes during the study period. The statistical analyses are correlation coefficients of ulcer healing time to the total
time of the ulcer existence \( (r = 0.24) \); and initial ulcer size to healing time \( (r = -0.28) \). This study found a weak correlation of ulcer duration to healing time; that is, the longer an ulcer had existed prior to the study the greater the time it took to heal. Also, the larger an ulcer was, the more quickly it healed, but these findings were not statistically significant, possibly due to the small sample size.

Only an abstract is available to evaluate the study. This pilot study is significant because it is the only research in the literature which examines the use of ES solely on the healing of diabetic foot ulcers. The results suggest the use of HVPMC for stimulating wound healing might be helpful in this population (Alon, Azaria, & Stein, 1986).

Fakhri and Amin (1987) studied the use of LVCMDC, 10-25V (polarity is not reported), sufficient to produce paresthesia, for ten minutes per day, two times per week to promote wound healing in 20 burned Iraqi males who had been resistant to healing. Time to complete healing measured in days was the dependent variable. Burns were full-thickness, mixed thickness, and partial thickness burns, and of mixed etiologies. Subjects served as their own controls with comparisons made between nontreatment and treatment periods; however, the time period of existence prior to treatment is only reported as the range of three months to two years for subjects and no further analysis is given of the "control
period." All burns healed within three months except one, and that subject was anemic. Experience during the study indicated that LVCMDC assisted in debriding the wounds. The study supported the use of ES in burn wounds of varying depth and size (Fakhri & Amin, 1987).

Kloth and Feedar (1988) conducted a pilot study of the use of HVMPC to accelerate healing of Stage IV decubitus ulcers in 16 subjects. Subjects were randomly assigned to treatment (n = 9) or control/sham (n = 7) groups. Ulcers received 45 minutes of treatment, once per day, five times/week. Polarity was positive initially and changed only when ulcers reached a healing plateau. Necrotic tissue was debrided from all wounds as necessary.

Treatment wounds healed at an average rate of 44.8% per week and 100% healed over a mean 7.3 weeks. Ulcers of subjects in the control group increased an average of 11.6% per week or an average of 28.9% over a mean of 7.4 weeks. Three members of the control group were crossed-over to the treatment group after the study was completed and these wounds healed at a rate of 38.1% per week with 100% healing in 8.3 weeks. Although the sample size was small and continuous debridement of necrotic tissue confounded the findings, the findings of this study support the use of HVMPC in treating Stage IV decubitus ulcers.

Human wound healing studies using ES conducted since 1990 are listed in Table 2-6. Feedar, Kloth, and Gentzkow
(1991) used monophasic pulsed current of 29.2mA, and 128pps, 128us duration, with alternating polarity in a randomized, double-blind multi-center study to treat pressure ulcers of Stages II, III, and IV (Shea, 1975) in 47 subjects. Wounds were treated for 30 minutes twice daily. Negative polarity was prescribed if wounds were infected and positive polarity if wounds were not infected. Polarity was changed every three days until the wound was reduced to partial thickness, Grade II. At that time, the stimulus was changed to 64pps with polarity alternating daily until the wound was healed.

This protocol is based on previous studies by Wolcott, Wheeler, Hardwicke, and Rowley (1969) and Carley and Wainapel (1985) which showed plateaus of healing resolved with changes in polarity. Wounds were irrigated with normal saline each day prior to treatment and packed with normal saline gauze as a conductive medium for the wound. Wounds were larger on average (mean 16.93 cm²) in the control group versus the treatment group (mean 14.64 cm²). There was greater undermining of wounds in the treatment group versus the control group.

After four weeks, treated wounds were 44% of their original size; control wounds were 67% of their initial size (p<.02). Healing rates averaged 14%/week for the treatment group, 8%/week for the control group. No treatment wounds increased in size, but five control wounds became larger. These results support the use of this protocol with
Table 2-6. Recent Human Wound Healing Studies (1990 to Present)

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Subjects</th>
<th>Type of Current &amp; Polarity</th>
<th>Wound Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feedar, Kloth, &amp; Gentzkow (1991)</td>
<td>50 pressure ulcers</td>
<td>HVMPC -/+ pol</td>
<td>% original wound size</td>
<td>Tx: 44% Cont 67%</td>
</tr>
<tr>
<td>Griffin et al. (1991)</td>
<td>17 DU in SCI pts.</td>
<td>HVMPC - pol</td>
<td>% reduction in wound surface</td>
<td>Tx: 80% Cont: 52%</td>
</tr>
<tr>
<td>Unger, Eddy, &amp; Raimastry (1991)</td>
<td>17 pts./ DU</td>
<td>HVMPC -6d then + pol</td>
<td>% reduction in wound size</td>
<td>Tx 2.4X &gt; cont; 8/9 healed in Tx group; 3/8 healed cont group</td>
</tr>
<tr>
<td>Unger (1991)</td>
<td>154 pts. geriatric (223 DU)</td>
<td>HVMPC -/+ pol</td>
<td>% of wounds healed in group</td>
<td>200/223 healed in 54 days</td>
</tr>
<tr>
<td>Lundeberg, Eriksson, &amp; Malm (1992)</td>
<td>64 diabetic venous ulcers</td>
<td>TENS alt pol</td>
<td>wound tracing digitized</td>
<td>Tx 61% v. Cont 41%</td>
</tr>
<tr>
<td>Gentzkow, Alon, Taler, Eltorai, &amp; Montroy (1993)</td>
<td>78 DU in 68 pts.</td>
<td>HVMPC -/+ pol</td>
<td>Advanced 2 Wnd chars. or classes</td>
<td>14 Wounds healed, 57 impr.</td>
</tr>
<tr>
<td>Stefanovska et al. (1993)</td>
<td>150 SCI DU</td>
<td>HVMPC SBP +/-</td>
<td>% healing per day</td>
<td>SBP &gt;*DC DC &gt;*Cont</td>
</tr>
<tr>
<td>Wood, Evans, Schallreuter et al. (1993)</td>
<td>74 DU; 43 exp. and 31 control</td>
<td>PLIDC - pol</td>
<td>Wound tracings of surface area</td>
<td>25(58%) Tx healed; 1(3%) cont. healed</td>
</tr>
</tbody>
</table>

* statistically significant, p < .05

**Abbreviations and Symbols used in table:**

- > = greater than
- < = less than
- exp = experimental
- HR = healing rate
- V = volts
- d = day
- NR = not reported
- DU = decubitus ulcers
- pol = polarity
- Tx = treatment
- alt = alternating
- exp = experimental
- SBP = systolic blood pressure
- DC = digital control
alternating polarities to treat Grade II, III, and IV pressure ulcers. Statistical methods are questionable since more than one wound was included per subject and the t-test, used for analysis, requires independence of observations.

One difficulty with the measurement in the Feedar, Kloth, and Gentzkow (1991) study is the use of change in pressure ulcer grade as the dependent variable. Recent publication from the National Pressure Ulcer Advisory Panel addresses "the inappropriateness of restaging an ulcer to denote healing" (Maklebust & Margolis, 1995). There are no data to support a natural progression of pressure ulcers from one stage to the next. Pressure ulcer staging is intended as an initial assessment of an ulcer's anatomical depth and is a static measure. The panel also addressed the inappropriateness of basing treatment on ulcer stage alone. Thus, ulcer stage is not an outcome of treatment nor the sole determinant of treatment protocols.

Griffin, Tooms, Mendius, Clifft, Vander Zwaag, and El-Zeky (1991) studied the use of HVMPC (200V, 100pps, a total current of 500uA) applied for one hour over a 20-day period on healing pressure ulcers in 17 spinal-cord injured subjects who were randomly assigned to treatment groups. The treatment polarity was negative throughout the study. Percentage of change in wound surface area was the dependent variable measured by three photographs of the wound that were computer analyzed and averaged. The HVMPC group showed
an 80% (28%/week) reduction in wound surface area compared to 52% (18.2%/week) in the placebo group.

Comparison of wound measurements was difficult for a number of reasons. Some subjects in this study had multiple ulcers, and it is reasonable that subjects with multiple ulcers would heal at different rates than subjects with only one ulcer. Wound contour change due to positioning relative to gravity can also affect wound size measures depending upon wound type. Griffith and colleagues stratified the sample of subjects based on the grades of ulcers, but did not stratify based on anatomical location (ischial versus coccygeal). The study findings showed that these two types of wounds healed differently (Griffith, Tooms, Mendius, Clifft, Vander Zwaag, & El-Zeky, 1991). This study is representative of the problems clinicians face in assessing and treating pressure ulcers.

Implications of this study are that in designing wound healing studies, it is desirable to have as homogenous a group of subjects as possible. A matched pair grouping design (by wound site, type, and size) is not as strong as a randomized design with stratification based on ulcer location (such as truck or extremity), type and size. A randomized design would have provided a better comparison between the wounds studied; however, any design involving only 17 subjects is difficult. Problems with methods to measure and analyze wound healing still need to be resolved.
Unger, Eddy, and Raimastry (1991) studied the efficacy of HVMPC to augment healing of pressure ulcers. The intensity was 150V, 750mA, 50pps, negative polarity; after Day 6, voltage was changed to positive polarity of 100V, 80pps, 500mA. Subjects with pressure ulcers (n = 17) were randomly assigned to either a HVMPC group (n = 9) or a sham-treatment group (n = 8). All subjects were treated twice daily for 30 minutes until wounds were healed. The electrode used was aluminum foil, shaped to fit the wound applied over moistened saline gauze. Gauze was placed into the wound to include undermined areas.

The dependent variable was stated as reduction of wound size; however, results were given as the number and percent of subjects healed in each group [8/9 in the treatment group (88.9%), and 3/8 in the placebo group (37.5%)]. The average time to heal was 51.2 days for the treatment group and 77 days for the sham treated group. The reduction in healing time was statistically significant (p = 0.043). Average wound area healed in the treatment group was 460.0mm² compared to 118.5mm² for the sham treatment group (p = 0.034). The healing in the treatment group was 2.4 times greater than healing in the sham group. The fact that an average healing time is provided when subjects in both groups (1 in the treatment group and 5 in the control group) remain unhealed is statistically problematic.
Unger (1991) followed the trial with a large clinical study of pressure ulcers. The effect of the previously described protocol for HVMPC (initial treatment, 150V, 50pps, negative polarity changed on day 6 to 100V, 80pps, positive polarity using the aluminum foil active electrode) was observed in 154 geriatric subjects with 223 wounds that had demonstrated resistance to healing with routine nursing care. Wounds had existed without improvement under standard nursing care for a mean of 71 days prior to the ES treatment. The control was the time the wound existed prior to HVMPC ($M = 71.4$ days) with poor wound healing. Wounds were mechanically debrided "as necessary." Analysis showed that 200 of 223 wounds (89.7%) healed in an average of 54.3 days.

These results are significant, considering all wounds had demonstrated a resistance to healing prior to HVMPC treatment. The parameters, electrode, and protocol are typical of clinical practice (Sussman, 1993). The effects of accepted treatment need to be scientifically studied and efficacy of care needs to be documented.

Problems with this study are the confounding of results by differences in debridement of the various wounds and comparing HVMPC to prior treatment. The Hawthorne effect, the notice of improvement when staff know that they are being studied, may have been a strong factor in the therapeutic success. Greater attention to debridement
during the HVMPC phase may have promoted wound healing. Thus, it is difficult to attribute all of the positive response to the HVMPC. Lundeberg, Eriksson, and Malm (1992) tested the effect of alternating constant current (80Hz), intensity to evoke paresthesia, for 20 minutes twice per day with zinc-oxide-paste-impregnated bandage. The stimulating electrode was identified as being on healthy tissue adjacent to the wound. Placement of the electrode opposite in polarity to the stimulating electrode was not identified. The treatment group was compared to a control group treated only with the paste-impregnated bandages. The study involved 64 diabetic subjects with venous stasis ulcers, who were randomized to the control group (n = 32) or the treatment group (n = 32). Treatment was 12 weeks of outpatient therapy with subjects applying their own treatments. Evaluation of healing was by weekly wound tracings that were computer analyzed for change in area size.

Ulcers healed within 12 weeks in four subjects in the control group and ten in the treatment group. Wounds healed at an average rate of 61%/week for the treatment group and 41%/week for controls. Healing was significantly greater in the treatment group at 12 weeks (42% healed versus 15% healed in the control group, p < .05). These findings support the use of TENS to promote healing in diabetic subjects and demonstrate that subjects may be taught to
apply this therapy at home with good results (Lundeberg, Eriksson, & Malm, 1992).

Stefanovska, Vodovnik, Benko, and Turk (1993) explored whether wound healing was faster when electrically stimulated and whether there was a difference in the efficacy of treatment between LVPMC (600uA) versus pulsed biphasic alternating current (AC), approximately 25mA. The AC current referred to is a balanced wave with both positive and negative polarity of low intensity and a pulse duration of .25ms and 40Hz at an intensity to produce minimal contraction in the tissues (Stefanovska et al., 1993). This wave form is described by Baker (1988), as a symmetrical biphasic wave (SBP). Spinal cord injured subjects (n = 170 decubitus ulcers in 150 subjects) were randomly assigned to three groups: a control group (n = 50, which received "usual care"), a DC treatment group (n = 18), and an AC treatment group (n = 82). The number of subjects is stated as 150 "cases", but appears to be 142 from subject ages in the table. Results showed control wounds healed 2.89%/day ±3.12% (20.2%/week ±21.8%), wounds treated with DC current healed at a rate of 4.62%/day ±3.29% (32.3%/week ±23.0%), and AC treated wounds healed at a rate of 5.40%/day ±4.10% (37.8%/week ±28.7%). These data show that ES promoted wound healing and AC current (SBP) was more effective than DC. The investigators say this conclusion is premature, however.
Although there were comparable charge densities below the electrodes of both types of current, the amount of influence of either DC or AC is difficult to estimate within the wound when currents are applied lateral to the wound. The nature of each individual wound bed would have an influence on the electrical conduction, and there was considerable variation in wound characteristics.

Correlation analysis was done in the three groups to determine whether wound duration, wound depth, initial wound area, age, and the duration of the spinal cord injury were significantly related to healing time. Because of the multiple inequalities among group subjects, the p = 0.02 level was selected for significance. Wound depth was negatively correlated to healing time in all groups (Control, \( r = -0.34, p < 0.02 \); AC, \( r = -0.29, p < 0.02 \); DC, \( r = -0.73, p < .02 \)). Wound duration was negatively correlated with healing in the control group (\( r = -0.41, p < .001 \)), but was not significant in the other two groups. Wound size was negatively correlated with healing in the AC group (\( r = -0.38, p < 0.0001 \)), but was not significant in the other two groups.

There were no correlations between any of the parameters and the healing rate for all groups using multiple regression analysis. However, there was a weak correlation between subject age and healing rates (\( r = 0.29, p < 0.02 \)). The older the subject, the more likely AC
stimulation was effective in producing wound healing. There was a negative correlation between age and healing in the control group ($r = -0.18$, $p > .02$) such that the older the subject, the slower the healing.

Limitations of the study are the groups were not equal in size nor clinical measures. If all subjects were randomized to the various arms of the study, close to equal numbers and conditions in the groups would be expected. The DC group had deeper wounds on average and fewer subjects than the other two groups, which would have biased results against the effectiveness of DC current. The authors try to present a large volume of data within a relatively short article. Presentation of the data becomes cryptic and there are inconsistencies in the data reporting. The DC group is reported as having 18 subjects, but data are only given for 12. There were 82 subjects in the AC group and 50 subjects in the control group. Wounds in the AC group existed significantly longer prior to treatment than those in the DC group ($p < 0.02$). There is believed to be enzymes in chronic wounds that slow wound healing (Stacey, Lainez, Kender-Kalenas, & Morrison, 1995; Emara, et al., 1995). The study was conducted at multiple sites in Slovenia and the investigators themselves question the uniformity of treatment.

Although there are inconsistencies in the number of subjects in the treatment groups and the number of subjects
for whom data were reported, this study is significant because it provides data on a large number of wounds and it was the first study to document the differences between AC and DC current versus placebo in humans. The findings strongly support the hypothesis that ES promotes wound healing in difficult cases. However, the investigators stated that there is no indication for using ES in wounds that are healing normally.

Gentzkow, Alon, Taler, Eltorai, and Montroy (1993) conducted a quasi-experimental trial of the effect of HVMPC on the healing of 61 Stage III and IV decubitus ulcers. Treatment consisted of two 30-minute sessions/day, 128pps, monophasic 30mA current. Polarity was negative initially and until the wound showed a granulating base. Polarity was then changed to positive and kept that way unless a healing plateau was observed. When a plateau was observed, polarity was changed to negative until the wound began to advance, then returned to positive. Healing was measured by an improvement in the classification of the wound by one level, (i.e., Stage III to Stage II according to International Association of Enterostomal Therapists, 1988 classification system). Subjects acted as their own controls in a pretreatment-posttreatment design. Sixty-four percent of subjects were over 60 years (range 25-100 years), greater than 33% were from a veterans' hospital, and 95% were wheelchair bound. Diabetes was present in 23% of subjects.
Results showed that 73% improved one or more wound stages after treatment. Fourteen ulcers were completely healed in a mean of 8.4 weeks. No ulcers became worse. Most ulcers developed granulation tissue by the end of the study (Gentzkow, Alon, Taler, Eltorai, & Montroy, 1993).

Using improvement of wound stage as a method of measurement is a subjective measurement of questionable validity (National Pressure Ulcer Advisory Panel, 1995). In addition, confounding factors present were that more than one ulcer was included for some subjects (78 ulcers for 68 subjects initially). Data also were excluded from analysis if values were missing, or subjects missed treatments, possibly reflecting a bias toward subjects who were more compliant or less ill.

According to the journal, Physical Therapy, this is the same study as reported by another investigator Mulder (1991). A sub-population of the study is also reflected in the Feddar, Kloth, and Gentzkow (1991) study.

Mulder (1991) reports a two-phase study with percent of wound closure used to measure healing in the blinded-phase of the study. During the second, open-phase, excellent, good, and poor healing were measured. The treatment group showed a 56% decrease in wound size and the control group showed a 33% decrease in wound size in the first phase. The treatment group had a higher percentage of wounds in the excellent (treatment group, M = 38.5%; control group, M =
20.8%) and good categories (treatment, M = 53.8%, control, M = 33.3%) than poor category (treatment, M = 7.7%, control, M = 45.8%). Mulder compared the treatment and control groups before the study, demonstrating no statistically significant differences between them. Details of this analysis are not provided.

Wood, Evans, Schallreuter, Jacobson, Sufit, Newman, White, and Jacobson (1993) conducted a double-blind, multi-center randomized clinical trial of the effect of pulsed, low-intensity direct current (LVMPC), 600uA and 0.8Hz, applied three times per week on Stage II and Stage III decubitus ulcers which had been present for at least five weeks. Duration of each treatment is not reported. Groups were compared after randomization and found to have no statistically significant differences in age, ulcer chronicity, resistance to prior treatments, or degree of physical activity. Blinded wound measurement was done by individuals not otherwise involved in the study. The control consisted of a sham treatment group. In addition, ten guinea pigs were studied for tissue analysis.

Forty-three ulcers in 41 subjects were in the human experimental group and 25 ulcers (58%) healed completely within eight weeks. Thirty-one ulcers in 30 human subjects were in the control group and only one ulcer (3%) healed during the eight weeks. None of the experimental group
ulcers increased in size during the study; however, 10 of the 31 control ulcers increased in size.

Guinea pig skin analysis showed a statistically significant \( p < .001 \) increase of thioredoxin reductase activity (62%) above control levels in treated animals. Thioredoxin reductase is a specific, sensitive measure of calcium binding in epidermis, similar in activity to calmodulin (Wood et al., 1993).

These investigators conclude that LVMPC was more physiological than other forms of ES previously studied; however, the net charge to the tissues was in the range of previously conducted studies. This trial represented a large controlled human study with the added feature of an animal trial for tissue analysis. A limitation of the study report is the fact that duration of treatment was not reported, making it difficult to assess the efficacy of treatment and to generalize results to other groups.

**Electrical Stimulation and Perfusion in Humans**

Wound healing relies on an adequate source of nutrition for the tissues. Skin blood flow is governed by the sympathetic nervous system, which is impaired in many persons with chronic skin wounds, especially diabetes (Rutherford & Shannon, 1995; Ruderman, Gupta, & Sussman, 1992). Electrical stimulation offers a possible treatment to increase perfusion in chronic wounds. It is proposed that ES produces a temporary sympatholytic response similar
to that induced by surgical spinal cord sympathectomy
(Rutherford & Shannon, 1995; Jacobs et al., 1990).

Several investigators have demonstrated the effects of ES on skin perfusion in humans (Table 7 and 8). Evidence shows that negative polarity reduces edema. Albumin, which carries a negative charge, is repelled into and maintained in the circulation by a negative charge and the ES causes a muscle-pumping action, which serves to pump fluid into the lymphatics and veins (Low & Reed, 1993). The decreased "tissue pressure" allows for improved capillary circulation.

Many investigators have found that ES increased skin circulation in individuals with various types of neuropathy. Kaada and colleagues (1982, 1983, 1984) studied the mechanisms of ES and vasodilation. Wong and Jette (1984) showed that vasodilation of the skin occurs with ES irrespective of wave form in subjects with intact nerves.

Kaada (1982) found that low frequency TENS of 30 to 40 minutes duration resulted in peripheral vasodilation in four subjects with Raynaud’s disease and two subjects with diabetic neuropathy (3 males and 3 females). A constant square wave of 20-30mA, 0.2ms duration, 100pps, was used to achieve minimal muscle contraction in tissues adjacent to the stimulating electrode applied to the Hoku point (the web space between the thumb and index finger on the hand). Skin temperature continuously measured with an electronic digital thermometer was the dependent variable.
In all six subjects, TENS resulted in a temperature rise in cold extremities of 9 to 10°C. The rise in temperature of the stimulated hand was only 0.5 to 2°C. The duration of the response was four to six hours. In the six subjects, experiments were repeated 10 to 15 times each over a two-week to three-month period with similar results. Kaada attributed results to a mechanism similar to that induced by acupuncture of the Hoku point (Kaada, 1982).

Kaada and Eielsen (1983) investigated whether the mechanism for TENS to produce vasodilation was via serotonin mediated channels. The experiment performed in 1982 was repeated using the same subjects with Raynaud’s phenomenon and diabetic neuropathy while administering a known serotonin antagonist, cyproheptadine. The previously observed rise in skin temperature was blocked by cyproheptadine, confirming that the mechanism for action was mediated by serotonin. Kaada and Eielsen (1983) proposed that TENS causes central nervous system release of serotonin, which antagonizes the effect of norepinephrine in the sympathetic nervous system allowing vasodilation in the extremities.

Dodgen, Johnson, Baker, and Chambers (1987) studied the effect of three different forms of ES on skin circulation in
Table 2-7. Early Human Studies by Kaada and Colleagues of the Effect of ES on Skin Perfusion

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Subjects</th>
<th>Type of Current &amp; Polarity</th>
<th>Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaada (1982)</td>
<td>6 Subjects: 4 with Raynaud’s Dx; 2 with diabetic neuropathy</td>
<td>TENS, - pol</td>
<td>Skin temperature w/digital thermometer</td>
<td>All 6 subjects showed a rise 7-10°C</td>
</tr>
<tr>
<td>Kaada &amp; Eielsen (1983)</td>
<td>11 Subjects: 9 with Raynaud’s Dx 2 with diabetic neuropathy</td>
<td>TENS, - pol</td>
<td>Skin temperature w/digital thermometer</td>
<td>Response not blocked by multiple neurotransmitter blockers</td>
</tr>
<tr>
<td>Kaada &amp; Eielsen (1983)</td>
<td>6 Subjects: 4 with Raynaud’s Dx 2 with diabetic neuropathy</td>
<td>TENS, - pol</td>
<td>Skin temperature w/digital thermometer</td>
<td>All 6 showed no increase when taking serotonin blocker</td>
</tr>
<tr>
<td>Kaada, Olsen &amp; Eielsen (1984)</td>
<td>15 Normal subjects; 20 w/Raynaud’s Dx</td>
<td>TENS, - POL</td>
<td>Skin temperature w/digital thermometer; plasma VIP levels</td>
<td>Increase in plasma VIP after TENS in all subjects; control no change</td>
</tr>
<tr>
<td>Kaada, Hegland, Oktedalen, &amp; Opstad (1984)</td>
<td>9 normal subjects; 2 with ALS</td>
<td>TENS, - pol</td>
<td>Plasma VIP and CSF VIP</td>
<td>Increase in plasma VIP; no increase in VIP in CSF</td>
</tr>
<tr>
<td>Kaada &amp; Helle (1984)</td>
<td>4 Subjects: 3 with Raynaud’s Dx; 1 with diabetic neuropathy</td>
<td>TENS, - pol</td>
<td>Skin temperature w/digital thermometer Plasma VIP</td>
<td>Rise in plasma VIP &amp; other vasoactive substances had effect</td>
</tr>
</tbody>
</table>

Abbreviations used in table:
ALS = amyotrophic lateral sclerosis  TENS = transcutaneous electrical nerve stimulation
CSF = cerebrospinal fluid   VIP = vasoactive intestinal peptide
DU = decubitus ulcer
DX = disease
### Table 2-8. Recent Human Studies of the Effect of ES on Skin Perfusion

<table>
<thead>
<tr>
<th>Investigator &amp; Year</th>
<th>Subjects</th>
<th>Type of Current &amp; Polarity</th>
<th>Measurement</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodgen, Johnson, Baker, &amp; Chambers (1987)</td>
<td>10 Diabetic subjects &gt;40 yrs. old</td>
<td>HVMPC - pol, HVMPC + pol, SBP</td>
<td>TcPO₂</td>
<td>Increase in TcPO₂ for all waveforms: HVMPC neg. 4.9mmHg, pos. 5.8mmHg, TENS 5.1mmHg</td>
</tr>
<tr>
<td>Gagnier, Manix, &amp; Baker (1988)</td>
<td>10 paraplegic subjects</td>
<td>HVMPC + pol SBP &amp; AC</td>
<td>TcPO₂</td>
<td>Increase in TcPO₂ for all waveforms.</td>
</tr>
<tr>
<td>Jacobs, Jorning, Beckers, Ubbink, vanKleef, Slaaf, &amp; Reneman (1990)</td>
<td>20 subjects with rest ischemic pain of legs/feet</td>
<td>Epidural spinal cord ES</td>
<td>Paresthesia</td>
<td>No change in ABI’s. Pain relief for 18/20; 12 subjects healed ulcers</td>
</tr>
<tr>
<td>Kjartansson &amp; Lundeberg (1990)</td>
<td>20 subjects w/surgical skin flaps:</td>
<td>TENS &amp; sham treatment, cross-over design</td>
<td>Laser Doppler units of measured blood flow</td>
<td>Local blood flow incr. by TENS, but not placebo</td>
</tr>
<tr>
<td>Mulder, Dompeling, van Slochteren-van der Book, Kuipers, &amp; Smit (1991)</td>
<td>8 subjects Raynaud’s Dx</td>
<td>TENS</td>
<td>Skin temp., plethysmography, and TcpO₂</td>
<td>No change in any measurements</td>
</tr>
<tr>
<td>Mawson, Siddiqui, Connolly, Sharp, Stewart, Summer, &amp; Biundo (1993)</td>
<td>29 SCI DU</td>
<td>HVMPC</td>
<td>TcpO₂ levels</td>
<td>Incr. 17 mm Hg</td>
</tr>
</tbody>
</table>

**Abbreviations used in table:**
- ABI = ankle-brachial index
- SCI = spinal cord injury
- DU = decubitus ulcer
- TcPO₂ = transcutaneous oxygen
- TENS = transcutaneous electrical nerve stimulation
- Dx = disease
ten diabetic subjects, measured by transcutaneous oxygen levels (TcPO$_2$). It was not stated whether any of the subjects had wounds, although the purpose of the study was to identify a type of ES that would promote wound healing by increasing perfusion.

The waveforms used were identified as monophasic, paired-spike waveforms (HVMPC), both positive and negative polarity, and a symmetrical biphasic waveform (SBP). Symmetrical biphasic pulsed current is a current type not addressed by Kloth and Feedar (1990), but it is addressed in a book on ES by Low and Reed (1993). It consists of 300us phases of both positive and negative polarity.

All three of the waveforms resulted in an increase of TcPo$_2$ levels: HVMPC negative polarity, $M = 4.9$ mmHg; HVMPC positive polarity, $M = 5.8$ mmHg; and SBP, $M = 5.1$ mmHg ($p < .05$). Poststimulation values rose even higher in response to HVMPC negative polarity, $M = 9.5$ mmHg; HVMPC positive polarity, $M = 9.7$ mmHg; and SBP 9.0 mmHg. Dodgen and colleagues (1987) demonstrated that ES in these wave forms increases skin circulation in diabetic subjects. It is not possible to fully evaluate the methods of the investigation, since all that is available to evaluate this study is an abstract.

cord injury. Subjects received 30 minutes of ES of the same waveforms as described above. This is a followup of the previously reported study (Dodgen et al., 1987). Diabetic subjects began the baseline period with significantly lower transcutaneous oxygen levels than their age-matched counterparts. All subjects demonstrated a rise in transcutaneous oxygen levels regardless of the waveform, but it is not known whether the ES brought diabetics to a level equivalent to their age-matched counterparts.

Gagnier, Manix, and Baker (1988) examined the effect of ES on transcutaneous oxygen levels in ten paraplegic subjects using HVMPC positive polarity, SBP, and alternating current (AC). The waveform of the alternating current is 2,500 cycles per second pulsed 10ms on, 10ms off, at a frequency of 50pps with balanced positive and negative polarity (Baker, 1995). Transcutaneous oximetry readings were taken for 90 minutes with a 30 minute pre-stimulation period, a 30 minute stimulation, and a 30 minute post-stimulation period. Statistically significant increases were found with HVMPC and TENS, but not AC at the end of stimulation. However, the increase in TcpO₂ was significant for all waveforms at the end of the post-stimulation period. Level of spinal cord injury, age, time since injury, the degree of inability to obtain a muscle contraction, the presence of pressure ulcers, and the order in which waveforms were applied were recorded but not reported.
Jacobs and colleagues (1990) investigated the effect of implanted epidural spinal cord ES on perfusion of extremities in 20 subjects with chronic atherosclerosis producing lower limb threatening ischemia. Ankle-brachial indices (ABI), toe blood pressures, and nutritional capillary blood flow via sodium-fluorescein capillary microscopy with video recording were used as outcome measurements.

Seven of the twenty subjects had insulin-dependent diabetes, but not autonomic neuropathy. Four subjects had a contralateral above-the-knee amputation, which had been performed for atherosclerosis obliterans. Two subjects continued to smoke more than ten cigarettes per day. No subjects were on vasoactive drugs. All subjects had ischemic pain at rest, ischemic ulcers, and had previously undergone vascular reconstruction of the lower extremity. Prior to the investigation, subjects were being treated conservatively with bed rest, frequent dressing changes, gentle debridement, and systemic antibiotics when appropriate. Initially, the mean ABI was 30% and the mean toe blood pressure was 14 mmHg. A normal toe blood pressure is > 70 mmHg (Rutherford & Shannon, 1995). Amputation was likely for all subjects due to disease severity.

Stimulation produced analgesia with complete pain relief for 18/20 subjects within 12 hours of implantation. In the two subjects with a negative response, no paresthesia
could be achieved and thus no pain relief. These two subjects subsequently had amputations. The 18 subjects were followed for a range of three months to three years (M = 27 months). Ischemic pain returned 3 months after implantation for two subjects and in 10 to 12 months after implantation for another four subjects. Initially, 5 of these 6 subjects had ischemic ulcers > 3cm in diameter.

During the first postoperative months, these ulcers showed improved healing. After the recurrence of pain, however, the ulcers deteriorated rapidly and limb amputation could not be avoided. During the follow-up period, pain relief continued for 12 subjects and ischemic ulcers were healed in 8 of these 12 persons. Of these subjects, 7 ulcers had diameters <3 cm and 1 had a diameter >3 cm. Life-table analysis showed foot salvage rate by ulcer size of 80% and 56% after 1 and 2 years respectively. There were no differences between subjects with and without diabetes.

Data showed that subjects who were successfully treated with the stimulation had hospitalizations averaging 19 days, while subjects needing amputation had a mean of 91 days of hospitalization, including rehabilitation. Ankle-brachial indices did not change significantly, nor did toe pressure. However, microcirculatory data showed the number of perfused capillaries increased significantly after ES. Diameters of the capillaries did not change. Red-blood-cell (RBC) velocity increased from 0.088mm/s prior to treatment to
0.496mm/s one day following treatment. In the two subjects in which pain was not relieved, these parameters did not change. In the followup period, microcirculatory blood flow correlated with the clinical status of the subject. The 8 subjects who had recurrent pain or progressive ischemia were labelled as "nonrespondents" and the 12 with pain relief and improvement of RBC velocity after treatment were labelled as "respondents." In respondents, the peak RBC velocity remained significantly improved (p < .001) above pre-treatment values for the entire followup period.

This study is significant with measurements of blood flow being validated by the clinical status of the subjects. A considerable effect size of the treatment was shown by perfused capillary density increasing from 10mm² to 19mm². The investigators recommended that only subjects who are not candidates for vascular reconstructive surgery should be considered for this treatment. In addition, subjects with diabetes and autonomic neuropathy would not considered as good candidates. The high healing failure rate noted in the life table for ulcers >3cm suggests that subjects with ulcers >3cm should not receive this treatment, or that this treatment would need to be paired with adjunctive therapy; however, this was only one subject, so more data is needed.

This study provides evidence for a role for ES in healing ulcers in subjects with diabetes and demonstrates ABIs and toe-brachial indices (TBIs) may useful to assess
blood flow to the lower extremities. Vascular indices correlated with the clinical status of ulcer size. Both increased by 5mmHg in this study, however, statistically this was not significant. Since the effect of the lumbar ES is a sympathectomy that produces increased blood flow in the lower extremities evidenced by foot paresthesia, topically applied ES may provide a similar result at less expense, less risk of infection, and less inconvenience for persons with this problem.

Kjartansson and Lundeberg (1990) conducted a randomized, cross-over trial in which 20 subjects were treated with electrical nerve stimulation (TENS) (monophasic, square wave pulses with a duration of 20us, 90Hz, intensity four times perception threshold) in order to assess the effect of ES on perfusion of skin flaps. Subjects had received skin flaps from reconstructive surgical procedures, which included skin flaps for subcutaneous tumors of the face and trunk, mammary carcinoma, and avulsion trauma of lower extremities. Eight subjects started with placebo electrical nerve stimulation and twelve subjects started with TENS. Since internal validity may be an issue when treatments are performed on various regions of the body with different blood flow and innervation characteristics, researchers randomized and did a cross-over design to provide the strongest scientific evidence for a study limited to 20 subjects. Perfusion was
measured by capillary refill, ranked in four categories, and laser Doppler flow (Laser Doppler Units). There were no group differences in blood flow.

In general, blood flow increased 10 to 15 minutes after the commencement of treatment, with a gradual improvement in blood flow over three consecutive days. However, four subjects were unaffected by the TENS treatment. The only side effect was allergic dermatitis in five subjects due to the adhesive tape holding the electrodes. The investigators attributed the therapeutic vasodilation to segmental inhibition of the sympathetic nervous system mediated by substance P and calcitonin gene-related peptide. This study demonstrated that ES is relatively safe for use on wide areas of the body. The high occurrence of allergic dermatitis in response to adhesive tape may be decreased by the use of hypoallergenic tape or self-adhering electrodes.

Mulder, Dompeling, van Slochteren-van der Boor, Kuipers, and Smit (1991) studied the effect of TENS on skin blood flow in eight subjects with Raynaud's syndrome. Skin temperature of the extremities, photoplethysmography, and transcutaneous oxygen levels were used to measure change in perfusion in response to TENS. Vasoactive drugs and foods were limited for 24 hours prior to the study.

The investigators found a gradual rise in skin temperature during the first hour of the study, from 32.8°C to 33.4°C. No differences were observed in TcpO₂ or
photoplethysmography readings. The authors concluded that TENS is of no clinical value in the treatment of Raynaud's syndrome, although some marginal increase in circulation did occur.

This study used a very small sample (n = 8) with subjects providing their own controls on a separate day. The study report does not provide much detail of the study. Authors did not report the number of subjects, effect size, needed for statistical power at alpha equal to 0.05 to reach the conclusions drawn, even though the possibility exists that their conclusions are valid. The findings are different from those of Kaada (1982), who studied both Raynaud's syndrome and diabetes patients and found an increase in circulation for all subjects.

Mawson, Siddiqui, Connolly, Sharp, Stewart, Summer, and Biundo (1993) investigated whether HVMGS (75V, 10pps) could provide a stimulus to increase sacral transcutaneous oxygen levels in 32 spinal-cord-injured subjects (3 lying prone and 29 lying supine on egg-crate mattresses). The purpose was to find a mechanism to increase resistance to the formation of pressure ulcers on the back and buttock. Since persons with spinal cord injury have lower sacral oxygen levels than persons without spinal cord injury, the ES was applied at the sixth thoracic vertebra. Transcutaneous oxygen levels were measured by TcpO₂ in response to stimulation. The
sacrum was the point of measurement, since it is the most frequent site of pressure ulcer development.

In subjects lying prone, a sustained, dose-related increase in \( T_{cpO_2} \) was seen after ES with the most dramatic rise in those with the lowest baseline \( T_{cpO_2} \) levels (42mmHg). This increase was greater at 75V (+23mmHg, 57% above baseline) than 50V. Neither level of stimulation produced a visual contraction in the underlying muscles and no subjects reported discomfort with the treatments. Stimulation at 100V had no additional incremental effect on \( T_{cpO_2} \) levels above that produced at 75V. In subjects lying supine, a 35% improvement (+17 mmHg) in \( T_{cpO_2} \) was achieved (p<.001) which fell only slightly during a 15 minute post-stimulation period. The above studies were repeated with sham treatments and no improvement in \( T_{cpO_2} \) levels were seen. An additional observation was performed on two separate occasions in ten subjects to determine whether results were reproducible and data showed they were.

The study of Mawson et al. (1993) provides evidence of the potential for ES to increase perfusion in persons with nerve injury. It is not known how similar the mechanisms are between subjects with spinal cord injury and subjects with diabetic neuropathy, however, both types of neuropathy are abnormalities involving the autonomic nervous system, that have demonstrated changes in blood flow patterns.
Summary of the Literature Review

A wealth of experimental data has been obtained from the past thirty years through controlled investigations of electrical phenomena demonstrating the "skin battery" and its effect on the growth and healing of soft tissues (Becker, 1960-1985; Foulds & Barker, 1983; Jaffe & Vanable, 1984). Observations of naturally occurring electricity in animals, its relationship to wound healing, and influence on cells involved in the healing process have provided a basis for human studies. Though seminal work has resulted in safe and effective protocols to stimulate healing in animals and humans, ES for wound healing is still in its infancy (Kloth & Feedar, 1990).

Electrical stimulation offers a possible adjunct to good wound care to increase healing in difficult cases. A number of investigators have sought to explain the mechanism by which this might occur. From animal studies, strong evidence has indicated that influences on perfusion may explain how ES promotes wound healing.

Results of individual studies are difficult to compare. Many different levels of stimulation, durations of treatment, and configurations of electrodes are reported in the literature. However, overall evidence shows that many forms of ES can positively influence wound healing. Fish and colleagues (1990, 1991) provide strong evidence for the use of HVMPC, negative polarity to reduce edema. Mertz et
al. (1983 to 1994) and Brown and colleagues (1986 to 1988) found the greatest increase in wound healing in pigs and rabbits using HVMPC negative polarity initially, changing to positive polarity for subsequent treatments.

Conflicting evidence is presented regarding efficacy of various wave forms to promote wound healing. Chu, et al. (1990, 1991, 1995) have shown the best results with LVCMDC in rats and guinea pigs. Convenience of treatment in humans favors HVMPC, as treatment times can be shorter. Humans are generally unable to remain connected to an ES device for days. Stefanovska, et al. (1993) found both AC and DC wave forms significantly promote wound healing. Unger (1991) applied ES to over 200 pressure ulcers in the clinical setting in a population resistant to healing and achieved 87% healed ulcers in a two month period.

Mawson and others (1993) show that HVMPC around 75V achieves a significant increase in skin blood flow in spinal-cord-injured subjects with sacral pressure ulcers. Dodgen et al. (1987), Baker (1988), Jacobs et al., (1990), Lundeberg et al. (1992) provide evidence that ES is as effective in increasing TcpO₂ levels for diabetic subjects as it is in other subjects.

Arterial and neuropathic ulcers are the primary ulcers that lead to amputation in persons with diabetes (Pecoraro, Reiber & Burgess, 1991). However, Lundeberg and colleagues (1992) did not address the influence of various levels of
neuropathy and other parameters on the response to treatment.

Studies are conspicuously absent from the literature that systematically investigate the effect of ES in the healing of diabetic foot ulcers exclusively. Variables that need to be addressed in such studies include Wagner Classification, level of peripheral neuropathy, level of peripheral vascular disease, level of glucose control, medications, age, gender, and ethnicity.

Since ES has shown a consistently favorable effect on healing and TcPO₂ levels, more research is needed to assess its usefulness in persons with diabetic foot ulcers. Other variables that influence perfusion and healing should be taken into consideration in these studies.
CHAPTER 3

METHOD

Research Design

A repeated measures, one group design was used to explore the study hypotheses. Subjects received a 30-minute treatment of HVMPC. Five readings of skin perfusion (TcpO₂) were recorded: baseline, after 15 minutes of treatment, at the end of treatment, after 15 minutes of recovery, and after 30 minutes of recovery.

Research Hypotheses

Primary Null Hypothesis:

There will be no difference in foot skin perfusion before, during, and after high-voltage monophasic pulsed current (HVMPC) electrical stimulation in diabetic persons with or at risk for foot ulcers.

Secondary Null Hypotheses:

The four secondary null hypotheses are as follows:

Controlling for gender, ethnicity, and age as covariates, there will be no differences in skin perfusion at baseline, end of treatment, and end of recovery in subjects with or at risk for diabetic foot ulcers by:

1) Levels of glycohemoglobin (high and very high)
2) Peripheral neuropathy (slight and insensate)
3) Peripheral vascular disease (slight and moderate-to-severe)
4) Wagner Grade [0 or 1,2 (ulcer no or yes)]
Assumptions

This study had four assumptions. They were:

1) Transcutaneous oxygen (TcpO₂) readings reflected true capillary oxygen readings within ±5mm of mercury.

2) The TcpO₂ reading adjacent to the stimulator was representative of general foot tissue oxygen readings.

3) The TcpO₂ reading after treatment reflected the effect of the ES treatment.

4) Subjects report of their health status, including medications prescribed, was accurate.

Definitions of Terms

Electrical Stimulation

Electrical stimulation is the application of an electrical current by an external generator to the skin via electrodes. In this study, ES is operationalized as high-voltage, monophasic, pulsed current (HVMPC) set at 100 volts of paired-wave, monophasic, pulsed current at 100 pulses pairs per second lasting 5 microseconds (μs) with no delay between waves provided by the GV II High Voltage Galvanic Stimulation System Model 7000S via carbon-rubber electrodes (Medical Devices Incorporated, Minneapolis, MN).

Carbon-Rubber Electrodes

Carbon-rubber electrodes are conductive adhesive pads that provide a uniform distribution of the applied current
to the tissue via a contact adhesive gel applied by the manufacturer.

Electrical Connection Cables

GVII cables (Medical Devices, Inc., MN) are insulated cables appropriately color-coded for the stimulating negative electrode (red) and the nonstimulating positive electrode (black).

Electrical Power Source

The electrical power source for the stimulator is current from the AC wall current of the facilities used for data collection.

Skin Perfusion

Skin perfusion is operationalized as transcutaneous oxygen readings (TcpO₂) obtained with Novametrix 840-VFD Transcutaneous O₂/CO₂ Monitor sensors. One sensor was used for the foot and the one for the chest. An increase in transcutaneous oxygen readings from baseline indicates an increase in skin perfusion. A fall in TcpO₂ readings from baseline indicates a decrease in perfusion of the skin.

Transcutaneous Oxygen/Carbon Dioxide Levels

Transcutaneous oxygen and carbon dioxide levels were the values indicated on the Novametrix 840 after 20 minutes of stabilization, and for each point of data collection. Sensor temperature was 44°C for subjects with TBIs > 0.40, 43°C for subjects with TBIs between 0.30 and 0.40, and 42°C for subjects with TBIs < 0.30.
Gender

Gender was male or female, indicated by the subject.

Age

Age is how old the subject says s/he is in years. Categories of age are: 40 to 49 years, 50 to 59 years, 60 to 69 years, 70 to 79 years, and 80 years of age or older.

Ethnicity

Ethnicity is the ethnic group the subject says s/he belongs to. The categories of ethnicity are Black and Non-Black.

Diabetic Persons

Persons with diabetes mellitus included persons with either insulin-dependent diabetes (IDDM) or non-insulin-dependent diabetes (NIDDM). This was determined by asking subjects whether they had been told by a doctor that they have diabetes. If the subject was not sure of the type of diabetes they had, they were asked questions about how the diabetes was managed initially (insulin, tablets, or diet alone). Insulin dependent diabetes mellitus was defined as diabetes which presented with diabetic ketoacidosis and required insulin for initial management. Non-insulin dependent diabetes (NIDDM) was defined as diabetes which presented with a gradual onset, and was managed by diet or tablets initially. Subjects with NIDDM could rely on insulin injections for control of blood sugar at the time of the study.
Management of Diabetes

The therapeutic regime used to manage diabetes was defined as how the subject said it was managed. Categories of management were diet alone, tablet, insulin plus tablet, and insulin alone.

Blood Glucose Level (Glycohemoglobin, HemoglobinA1c)

Blood glucose level is operationalized as glycohemoglobin (HgbA1c) determined by a random blood specimen assessed for A1c-fraction performed by a licensed professional laboratory. Levels to 7.0% (150mg/dl or below) were categorized normal, 7.1 to 9.0% (151 to 230 mg/dl) were categorized high, 9.1 to 11.0% (231 to 350 mg/dl) were very high, and > 11.0% (> 350mg/dl) were extremely high. When a blood specimen could not be obtained due to unsuccessful venipuncture, a random finger-stick blood glucose was used with the corresponding HgA1c estimated for that individual.

Duration of Diabetes

The subject was asked how long s/he has had diabetes, e.g. when was s/he first told by a physician that s/he had diabetes? This was accepted as the duration of diabetes.

Medications

Medications were operationalized as those medications which the subject reported as being prescribed for him/her and which the subject was taking. These were recorded simply as the number of total medications taken, except
calcium-channel blocking agents and nitrate-containing drugs were specifically listed.

Concurrent Diseases

The subject was asked during the interview period which conditions s/he had in addition to diabetes. These were recorded as the number of conditions (diseases) the subject had in addition to diabetes. This provided a gross estimate of level of severity of illness for the subject.

Peripheral Neuropathy

Neuropathy was measured by two categories of sensory detection, touch and vibration. The ability to feel touch was operationalized as the detection of Semmes-Weinstein Monofilaments (SWMF) (Research Designs, Inc., Houston, TX) based on light touch (until the filament just bends) when applied for 1 second in a random pattern across 10 areas of the foot: great toe (hallux), middle toe, little toe, ball, arch, lateral heel, ankle (medial malleolus), and dorsum of the foot (above the toes over the metatarsals). Individuals without neuropathy feel the 4.21 SWMF (1 gram of pressure).

Levels of peripheral neuropathy were operationalized as ability to feel <5.07 = minimal neuropathy, able to feel the 5.07 SWMF (but not lighter touch filaments) on all or part of the foot = moderate neuropathy, and unable to feel the 5.07 SWMF anywhere on the foot = severe neuropathy.

The ability to feel vibration (vibratory perception threshold) was operationalized as the point at which the
subject just detected vibration on the foot by the probe of an electric aesthesiometer touched lightly to the ball of the foot (Biothesiometer, Newbury, Ohio). For the purposes of this data analysis, values <20 volts = minimal neuropathy; 20 to 39 = moderate neuropathy; 40 and above = severe neuropathy.

**Peripheral Vascular Disease**

The level of peripheral vascular disease was operationalized as both the ankle-brachial index (ABI) and the toe-brachial index (TBI). These indices were obtained by dividing the systolic pressure of the extremity by the higher of the 2 systolic pressures of the brachial arteries. An index of less than 0.3 indicates severe disease. Values between 0.31 and 0.91 indicated moderate disease. Values above 0.92 indicated minimal disease. Values greater than 1.2 indicated Moenckeberg’s sclerosis (Harrelson, 1989).

**Severity of Ulceration (Wagner Classification)**

Severity of ulceration was operationalized as the categories identified in the Wagner Scale, which uses a 0-5 grading system (Wagner, 1981) for classifying diabetic foot ulcers. The scale identifies Grade 0 for persons with intact skin and preulcerous conditions that include foot bone deformities such as hammertoes, bunions, partial amputations, and Charcot foot. These subjects are considered to be at risk for a foot ulcer. Only those with
ulcer grades less than Grade 3 were included in the study sample. Wagner grades are listed in Table 9.

**Table 3-1. Wagner Class Scale**

<table>
<thead>
<tr>
<th>Skin Symptoms</th>
<th>Ulcer Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin intact, bony deformity, Charcot foot, partial amputations</td>
<td>0</td>
</tr>
<tr>
<td>Ulcer penetrating to subcutaneous tissue (shallow ulcer)</td>
<td>1</td>
</tr>
<tr>
<td>Ulcer penetrating to tendon, ligament, joint, or bone (deep ulcer)</td>
<td>2</td>
</tr>
<tr>
<td>Ulcer with infection of deep tissues (tendon, ligament, joint capsule, or bone)</td>
<td>3</td>
</tr>
<tr>
<td>Gangrene of part of the foot</td>
<td>4</td>
</tr>
<tr>
<td>Gangrene of the entire foot</td>
<td>5</td>
</tr>
</tbody>
</table>

**Variables**

**Independent Variable:**

The independent variable was ES applied for 30 minutes as HVMPC (100V, 100pps, negative polarity), equaling 200uA/s.

**Dependent Variable:**

The dependent variable was skin perfusion defined as transcutaneous oxygen (TcpO₂) level in millimeters of mercury (mmHg).

**Moderator Variables:**

Moderator variables were: 1) glucose control, 2) peripheral neuropathy, 3) peripheral vascular disease,
4) Wagner Class, 5) gender, 6) ethnicity, 7) age, and 8) medications.

Glucose control was normal (<7%), high (7.1% to 9.0%), very high (9.1% to 11.0%), or extremely high (>11.0%).

Peripheral neuropathy was minimal, moderate, or severe.

Peripheral vascular disease was minimal, moderate or severe operationalized as the toe-brachial index when available on the stimulated foot and the ankle-brachial index when the toe-brachial index was not available.

Wagner Class was Wagner Grade 0, 1 or 2 determined by comparing the subject’s feet with the descriptions of the Wagner Scale, Table 9.

5) Gender was male or female.

6) Ethnicity was Black or Non-Black.

7) Age was 40 years and over expressed in years.

8) Medications were the number of medications a subject was taking. This provided a rough severity of illness index. Calcium-channel blockers and nitrate-containing medications were specially categorized to identify primary vasoactive drugs.

Research Setting

Three large diabetic foot centers on the West Coast were used to recruit subjects eligible for this study. A total of 400 subjects per month attended the diabetic foot clinic at a podiatry college (Altman, 1993). Of these, 100
subjects had active ulcers and another 100 had foot deformities placing them at risk for ulceration. About 1,000 diabetic subjects attended the internal medicine clinic of the university medical center. Of these, 350 either had foot wounds or foot deformity. Approximately 150 subjects attended the amputation prevention clinic at the veterans hospital. Of these, approximately 100 had foot wounds or foot deformity.

Two sites were used to actually conduct the study, the podiatry college and the veterans hospital. At the podiatry college, a standard three-bed hospital room with adjustable Hill-Rom beds was used. At the veterans hospital, a podiatric clinic room with an adjustable podiatry chair including a reclining backrest was used.

**Sample**

The target population was subjects 40 years of age or older with diabetes and Wagner Foot Classification 0, 1, or 2. Consecutive sampling was used to accrue subjects.

1) **Inclusion criteria**: Subjects were a) persons with diabetes; b) with Wagner 0, 1, or 2 foot classification; c) 40 years of age or older; d) who have a great toe on at least one foot; e) were able to give written, informed consent in English, and f) not tobacco users.

Limiting the sample to those over 40 years of age represented the majority of individuals who have diabetes and foot ulcers. The great toe was needed to provide an
accurate measurement of peripheral vascular disease. Limiting the sample to English-speaking individuals was necessary so that individuals would understand the consent procedure. The nicotine in tobacco could have caused peripheral vasoconstriction and cigarette smoke could have decreased oxygen in the blood by the binding of carbon monoxide in cigarette smoke to hemoglobin to form methemoglobin (Levin, 1993). Methemoglobin is resistant to giving up oxygen to the tissues. The vasoconstrictive effect of nicotine is present in the vascular system for about two weeks after the last use and continues to exert an effect on the vasculature (Rutherford, 1995).

2) **Exclusion criteria**: Subjects were excluded if they: a) were pregnant, b) had cardiac pacemakers, c) had Charcot foot, d) had a neoplasm of the foot or site of ES, e) had acute respiratory distress. The effects on an unborn fetus are unknown. It was desirable to reduce any possible risk of interference with the electrical signals received and sent by cardiac pacemakers, as cardiac asystole could have resulted. Charcot foot is an inflammatory process that is poorly understood; therefore, the risk is unknown, also lack of circulation is not a problem in persons with Charcot foot. Since ES is believed to promote growth in tissue, cancer could have grown faster (Kloth & Feedar, 1990). Subjects were required to lie down with the head slightly
elevated at 30°, which might not be safe for subjects experiencing severe respiratory difficulty.

Sample Size Calculation (Power Analysis)

A sample size estimate was performed to determine the number of subjects needed to answer Hypothesis I. Assuming a 9% difference in perfusion (± 5mmHg) before and after treatment based on Baker (1988), a sample size of 120 was needed. A power analysis for Hypothesis II with all moderator variables required a sample size of between 140 and 160. These numbers ensured a power of 0.8 with a beta of 0.2 and a two-tailed alpha of 0.05. Hypothesis II required the larger sample size and 160 was used, which was the more conservative sample size. If no difference between baseline and after treatment were detected in a sample of 160, it would not be due to lack of power in detecting a meaningful difference.

Protection of Human Subjects

The primary risk to subjects was that the treatment could be painful when first applied. To mitigate this risk, a parameter was selected that was below the threshold for pain perception and the intensity of the treatment was adjusted to a comfortable level if pain occurred.

Informed consent was provided by an explanation of the study written in English at approximately the fifth grade level (Appendix B), asking diabetic subjects, at risk for or with foot ulcers, to participate in a study to describe the
effect of HVMPC on transcutaneous oxygen levels. Subjects were provided a layperson explanation of why it is believed the treatment could be helpful, such as, "We are interested in knowing if ES increases blood flow in the skin of people with diabetes and foot ulcers."

Subjects were told they might feel a slight tingling, slight pain, or no sensation from the treatment depending upon individual differences. Subjects were informed if pain were felt, the intensity of the treatment would be decreased to a level where pain was no longer felt. After reading the explanation, a verbal explanation was given to the subject by the nurse. The subject was asked whether s/he had questions and those questions were answered to the subject's satisfaction. Subjects were provided the "Experimental Subject's Bill of Rights," and written, informed consent in the form as prescribed by the University of California, San Francisco, Committee on Human Research (Appendix A).

The research protocol was approved by the research committees at the California College of Podiatric Medicine; University of California, San Francisco; the San Francisco Veterans Affairs Medical Center; and Madigan Army Medical Center, Ft. Lewis, Washington.

Techniques and Instruments

Glycohemoglobin

Glycohemoglobin, hemoglobin$_{A1C}$ (Hgb$_{A1C}$), is a measure of nonenzymatic glycation of proteins, which decreases their
functional ability. Glycation causes a decrease in the cellular membrane flexibility of red blood cells leading to rupture of red blood cells, and decreases oxygen carrying capacity of the blood. Glycated red blood cells are also less able to deform to move through capillaries. Oxygen binds more tightly to glycated hemoglobin, resulting in less release of oxygen to the tissues (Abraham, 1985).

Glycohemoglobin is the standard for measuring glucose control (Abraham, 1985). It reflects an average blood glucose of a subject over the previous 12 weeks, based on the lifespan of a red blood cell. This measure has a reliability of 0.97 by affinity chromatography, up to 21 days after the specimen is drawn. Standard error of measurement is approximately 0.6% (Abraham, 1985).

Neuropathy

The Semmes-Weinstein Monofilaments (SWMF) and vibrating aesthesiometry are the best ways to measure peripheral neuropathy in diabetic feet (Birke & Sims, 1986). Threshold tests show gradual changes in chronic neuropathic conditions because of their sensitivity.

Semmes Weinstein Monofilaments

Semmes-Weinstein monofilaments are nylon filaments calibrated to exert a specific pressure load to the skin (6.10, 75 grams; 5.07, 10 grams; 4.31, 1 gram; 3.61, 0.1 gram; 3.22, 0.01 gram) when touched to the skin at a right-angle and pressed just until they bend (Research Designs
Inc., Houston, TX) (Holewski, Stess, Graf, & Grunfeld, 1988). Subjects are asked to close their eyes to exclude visual cues. A pattern is followed touching the toes, ball of the foot, arch, heel, ankle, and dorsum of the foot.

Pressure detection measures the function of small sensory fibers in the skin. The loss of the ability to detect ten grams of external pressure has been highly correlated to the occurrence of foot ulcers in diabetic subjects (r=0.76) (Sosenko, Kato, Soto, & Bild, 1990).

The SWMF has a sensitivity of 0.84 with a specificity of 0.96. Persons with foot ulcers are usually unable to detect the 5.07 SWMF anywhere on the foot (Levin, 1993). Semmes-Weinstein Monofilament detection was shown to have 0.89 reliability by triplicate repetition in 720 trials by podiatrists (Holewski, Stess, Graf, & Grunfeld, 1988). The primary investigator established consistency in testing while conducting the pilot study.

**Vibrating Aesthesiometry**

Vibratory aesthesiometry measures the vibratory perception threshold (VPT) of the large sensory fibers of the skin (Biothesiometer, Clifton, NJ). The probe is placed on the foot lightly and the subject asked to identify when vibration is felt. It has a sensitivity of 0.83, a specificity of 0.87, and a positive predictive value of .49 in diabetic subjects (Sosenko, Kato, Soto, & Bild, 1990). In this study, the Biothesiometer was touched to the ball of
the subjects' feet because not everyone had toes bilaterally. The hallux is the preferred site to measure vibration perception threshold; however, it was not available on all subjects.

The electric Biothesiometer Model PVD-1 (Biomedical Instrument Company, Newbury, Ohio) is designed to vibrate at a calibrated intensity between 1 and 50 volts at 120 Hertz, and measurement is by voltage level first detected. Persons without neuropathy feel vibrations less than 15 volts intensity (Grunfeld, 1991).

A significant correlation (p < 0.001) has been found between abnormal foot vibratory perception threshold using the Biothesiometer and diabetic foot ulceration (Boulton, Kubrusly, Bowker, Gadia, Quintero, Becker, Skyler, & Sosenko, 1986). The likelihood of ulceration increased with worsening vibratory perception, especially levels above 25 volts (p < .001).

The Biothesiometer has been a standard for measurement of vibratory perception threshold (VPT) for years and was recently compared with the Neurothesiometer, a new device to measure VPT (Young, Every, & Boulton, 1993). The Biothesiometer had a correlation of 0.93 (p<.001) with the Neurothesiometer. The coefficient of variation was 8.6% for the Biothesiometer and 8.1% for the Neurothesiometer. Individual Biothesiometers do not correlate well between each other (Baker, 1995). However, the same Biothesiometer
shows fair reliability (approximately 0.7) when used by the same operator (+15 volts) (Young, Every, & Boulton, 1993). The same Biothesiometer was used throughout the study by the primary investigator.

**Photoplethysmography**

Photoplethysmography (Parks Podia-Lab 1081A, Parks Medical Company, Aloha, OR) uses a transducer that transmits and receives an infrared light reflected from red blood cells in the microcirculation. The waveform is converted to a volume tracing on grid paper, which moves at either 5mm/second or 25mm/second, and mirrors blood flow in the extremity. The transducer diode is attached to the toe with cellophane tape or a velcro strap. The detected blood flow is used to measure segmental blood pressures in the digits and extremities. For this study, blood pressures were taken on both ankles and the big toes, when available.

The toe is considered more reliable in diabetic subjects because Moenckeberg's sclerosis in diabetic subjects results in a falsely elevated ankle blood pressure in some subjects, so the ABI could be inaccurate. However, ABIs were used when the TBI could not be reliably obtained. Toe-brachial indices greater than .92 are normal (Harrelson, 1988; Kerner, 1992; Hoffman, 1992).

Stevens, Goss, Foster, Pitei, Edmonds, and Watkins (1993) report a coefficient of variation of 5% in normal subjects, 9.2% in ulcerated non-diabetic neuropathy
subjects, and 9.6% in ulcerated diabetic neuropathy subjects. Diabetic subjects with recurrent ulceration have reduced toe pressures and, therefore, reduced TBIs when compared with non-ulcerated diabetic subjects or controls.

Rooke and Osmundson (1989) report that reproducibility of ankle-brachial indices is 0.8. Jones and Rutherford (1991) state the test has a well-established reliability, a positive predictive value of 100%, and a negative predictive value of 43%. These values vary by the criteria accepted as pressures sufficient for wound healing (Jones & Rutherford, 1991).

**Transcutaneous Oxygen Monitor**

Transcutaneous oximetry measured skin oxygen tension (TcpO$_2$), skin carbon dioxide tension (TcpCO$_2$), and sensor temperature (Novametrix Medical Systems, Wallingford, CT). Two Novametrix Model 840 Transcutaneous Oxygen Monitors (Wallingford, CT), with a Clark-type polarographic combination oxygen/carbon-dioxide sensors, were used to obtain the capillary oxygen level on the foot and chest of each subject. The sensors provided both oxygen and carbon dioxide readings from a single sensor. Continuous display of TcpO$_2$ and TcpCO$_2$ allowed the researcher to manually record levels from the screen to the data collection form.

The Novametrix 840 contained a heating section with thermistors for controlling sensor temperature within 0.1°C. The sensor was heated to facilitate the diffusion of oxygen
and carbon dioxide through the skin to the sensor by liquefying the skin cuticle. Recommended temperatures for adults are 44° to 45° C, although 37° to 45° may be used (Novametrix, Wallingford, CT). In this study, 44°C was used for subjects with TBIs/ABIs > 0.40, 43°C was used for subjects with TBIs/ABIs between 0.30 and 0.40, and 42°C was used for subjects with TBIs/ABIs < 0.30. The higher temperature was used for subjects without severe vascular disease because the machine reliability is greater at the higher temperature. Lower temperatures were used to protect subjects with decreased blood flow from potential burning of tissue due to inability to dissipate heat from the heated sensor.

Transcutaneous oxygen levels have been shown to be predictive of ability to heal. Readings above 40mmHg are associated with successful healing in 98% of cases. Readings between 20 and 40mmHg show variable outcomes, and values below 20mmHg are associated with healing failure in 89% of cases. This method has a 70% sensitivity and an 88% specificity with a positive predictive value of healing of 64% and a predictive value of healing failure of 91% (Bacharach, Rooke, Osmundson, & Gloviczki, 1992).

The Novametrix Model 840-VFD TcpO₂/TcpCO₂ Monitor has an accuracy of ±1%, stability of 1mmHg/hour for both oxygen and carbon dioxide. The temperature control is accurate within 0.1° C. Coefficients of variation are approximately
10% for oxygen and 5% for carbon dioxide (Wimberley, Burnett, Covington, Maas, Mueller-Plathe, Siggaard-Anderson, Weisberg, and Zijlstra (1990). Rooke and Osmundson (1989) report that the reproducibility of TcpO₂ measurements is approximately 0.8. Rooke (1992) states reliability can be improved by the subject breathing supplemental oxygen; however, supplemental oxygen was not used in this study.

This method of measurement is limited in that readings of TcpO₂ are generally lower and TcpCO₂ generally higher than arterial blood. However, approximating arterial blood was not a goal of this study; rather, a measurement of the level of oxygen which approximates capillary oxygen in the periwound area was sought.

Accuracy varies in adults, particularly those with poor tissue perfusion; therefore, increases or decreases within subjects are more significant than absolute values (Rithalia, 1991). Accuracy of measurements depends on proper maintenance and calibration of the machine. The manufacturer’s guidelines were followed for these procedures.

Medical Devices, Inc. GV II Stimulator

The Medical Devices, Inc. GV II "Galvanic" Stimulator is a 110 volt, AC/DC convertible stimulator which delivers between 1 and 700 microamperes (uA) of charge to the skin via carbon-rubber, gelled self-adhesive electrodes at rates of 1 to 100 pulsed, monophasic exponential paired spikes per
second. The current delivered at the prescribed setting of 100 volts, 100 pulse pairs per second is 200uA/cm² (Medical Devices, Inc., Minneapolis, MN). Electrode size was negative polarity, 2-inches x 2-inches, and positive polarity, 4-inches x 6-inches. The treatment selected represents a treatment which has demonstrated positive results on venous ulcers in diabetic subjects (Lundeberg, Eriksson, & Malm, 1992).

Procedure

Subject Recruitment and Informed Consent

Subjects were recruited, screened, and scheduled for the protocol. When the subject arrived for the appointment, informed consent was obtained and a blood sample drawn for glycohemoglobin. Subjects were asked to remove their shoes and socks and lie supine with the lower extremities bare. At the podiatric college, a hospital bed was used. At the veterans hospital, a podiatric chair, which reclines, was used. In both settings, subjects were placed in a supine position with the head raised approximately 30 degrees and made comfortable.

Subject Medical History

Subjects were asked to bring a list of their medications to the treatment appointment so that they could be recorded during pre-treatment data collection. When subjects forgot to bring a list of their medications, they were called at home that same evening 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 as concomitant conditions.

**Transcutaneous Oxygen Monitor Sensor Application**

The transcutaneous oxygen machine was calibrated, the skin cleansed with rubbing alcohol, and sensors applied with the double-backed tape adhering ring. The procedure for calibration, site preparation, and application of the sensor outlined in the manufacturer’s instructions was followed.

The reference sensor was placed one inch beneath the clavicle on the left side of the chest at the mid-clavicular line and the foot sensor was placed adjacent to the stimulating electrode on the foot. Twenty minutes were allowed for the sensors to stabilize. Stabilization time ranges from 10 to 30 minutes with an average stabilization time of 15 minutes.

**Subject Assessment**

During the 20 minute stabilization time, demographic information were obtained, vital signs were taken, neurological and vascular assessments were performed. Blood pressures (Tyco, Moorestown, NJ) on both arms and an apical heart rate were taken. Oral temperature was taken to rule out systemic infection (Diatek, San Diego, CA). In the event of elevated temperature, the procedure was rescheduled. Room temperature, barometric pressure and humidity were recorded.
Ulcer Measurement

The ulcer was measured using transparent plastic wrap and a fine point transparency marker. This was later traced onto a grid marked in square millimeters which was manually counted to determine surface area.

Wagner Classification Grade was determined. For subjects in Wagner Grade 0 (intact skin), a table of random numbers was used to select the study extremity. For those in Wagner Grades 1 and 2, the foot with the ulcer was utilized. For some subjects, the toe of the ulcerated foot had been amputated, preventing toe-brachial measurement of blood flow. For these subjects, the nonulcerated foot was used.

Neuropathy Measurement

Neuropathy testing was performed using SWMF. Testing began with the 5.07 (10 gram load) and became progressively lighter until the subject could not identify when s/he was touched. Ability to feel the SWMF was documented according to anatomical landmarks and dermatomes on the foot. The most distal dermatome where touch was felt was recorded as the level of sensation. The more proximal the sensory dermatome of detection indicated a greater the degree of neuropathy. Detection was noted according to anatomical landmarks and dermatome. This was accomplished beginning with the hallux and progressing proximally until the monofilament was detected (Holewski, Stess, Graf, &
Grunfeld, 1988). Progression of the test was from the hallux to lateral toes, first metatarsal head to lateral metatarsal heads, across the sole of the foot to the heal and then the dorsum of the foot. A minimum of ten locations on the foot were tested.

The Biothesiometer (Newbury, Ohio) was used to assess vibration perception threshold (VPT). A demonstration of the probe, with and without vibration, was provided to the subject's hand so the subject would know what to expect on the foot. The probe was then touched to the fleshy part of the ball of the foot, avoiding heavily calloused areas, and the dial gradually turned up. The primary investigator's hand was steadied by bracing on the investigator's knee or the bed, whichever was convenient due to positioning. The subject was asked to identify when s/he first felt vibration (vibration perception threshold). Three trials were recorded on each lower extremity and averaged to obtain a mean VPT.

**Vascular Measurement**

Vascular assessment was done using the Parks Podia-Lab 1081A with the head of the bed at a consistent height, approximately 30 degrees. Having the head of the bed up 30 degrees slightly elevates the pressure reading, but some subjects were unable to lie flat due to breathing problems, back problems, etc. A slightly elevated head of the bed/chair provided for a uniform height for all subjects.
A 10cm wide cuff was used for the ankle blood pressure. A 2.5cm wide cuff was used for the toe blood pressure. The toe diode site was used to detect blood pressures in both the ankle and the toe. The cuffs were inflated to a level where the pulsatile wave form was obliterated, but never greater than 200mmHg. Manufacturer recommend that higher pressures are to be avoided (IMEX Procedure Manual). The pressure was then gradually decreased until the return of the blood flow was recognized as a regular pulse form. This is the systolic pressure reading.

The subject’s posterior calf was shaved lightly with a dry safety razor or electrical razor when significant hair was present to prevent pain from pulling the hair when the electrode was removed. The skin was cleansed with an alcohol pad prior to the electrode being attached to assure good adhesion of the electrode. A carbon-rubber electrode with an adhesive conductive gel provided by the stimulator manufacturer was used (Medical Devices, Inc., Minneapolis, MN). The negative electrode was placed on the metatarsal area of the foot (or the lowest area where the 5.07 SWMF was felt) and the positive electrode was placed on the calf muscle or 15 centimeters above the active electrode over a large muscle (Kloth & Feedar, 1990). If a subject was unable to feel the 5.07 SWMF anywhere on the foot, the stimulating electrode and TcpO₂ foot sensor were placed at the lowest dermatome where the 5.07 SWMF was detected.
Baseline transcutaneous oxygen readings were recorded and the ES treatment was begun. The controls were set for the proper ES settings. The treatment duration was 30 minutes. During the treatment, the nurse monitored the subject and decreased the intensity of the stimulation, if necessary. Decreases in intensity were noted on the data collection sheet.

Transcutaneous oxygen level readings were taken and recorded at baseline, 15 minutes, 30 minutes (end of treatment), 45 minutes, and 60 minutes (30 minutes after the end of ES). At the conclusion of the treatment, wounds were redressed in the same manner as when the subject came for study. Table 10 summarizes the data collection procedure. A diagram of the foot is provided in Appendix B for the readers reference.

Pilot Work

A pilot study was conducted during the summer of 1994 on five subjects from the San Francisco Veterans Affairs Medical Center Diabetic Foot Clinic prior to this study. The study procedure reported here was used, except glycohemoglobin was not drawn. The pilot study data were not included in the final data analysis of this study, but are included in Appendix C.
Table 3-2. Data Collection Procedure

<table>
<thead>
<tr>
<th>DATA TO BE COLLECTED/TIME</th>
<th>Baseline</th>
<th>After 15 Mins. of ES</th>
<th>After 30 Mins. of ES (End)</th>
<th>15 Mins. After End of ES</th>
<th>30 Mins. After End of ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature, Humidity, &amp; Barometric Pressure</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Demographics</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachial</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toe</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound Size/Location</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wagner Classification</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral Neuropathy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semmes-Weinstein Monofilament</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibratory Aesthesiometry</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcutaneous Oxygen:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Chest</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sensor Temperature</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

The purposes of the pilot work were: 1) To identify problems with the design, such as the time period required and level of discomfort from the waveform selected. 2) To give the researcher practice in using the research protocol and to assure a smooth protocol for study subjects. 3) To test data collection instruments and refine the data analysis plan.
The pilot study determined that the design protocol was feasible and the level of ES was acceptable to subjects. The primary investigator gained proficiency in using the methods of the study; thus, the goals of the pilot study were realized.

Statistical Methods

Sample characteristics and variables were analyzed using descriptive statistics with measures of central tendency and variance. A one-way repeated-measures, analysis of variance was used to answer the primary hypotheses. A one-way repeated measures analysis of covariance was used to answer the second hypotheses, controlling for age, gender, and ethnicity. Logistic regression analysis determined whether moderator variables were predictive of changes in skin perfusion by group in response to ES.
CHAPTER 4
RESULTS

This study investigated the effect of transcutaneous electrical stimulation (100 volts, 100 pulses per second) on foot skin perfusion in persons with diabetes who were 40 years of age and older with or at risk for foot ulcers. Moderator variables examined were glucose, peripheral neuropathy, peripheral vascular disease, Wagner Class, gender, and ethnicity. Other clinical variables assessed were age, type of diabetes, years diagnosed with diabetes, number and type of medications, and number of concurrent diseases.

In this chapter, sample characteristics are first presented. The study hypotheses are described and tested. Descriptive data for variables associated with each hypothesis are presented followed by inferential analysis.

Sample Characteristics

The sample consisted of 135 persons with diabetes and risk for foot ulceration. Three subjects were eliminated from analysis due to incomplete data leaving a total of 132. One of these subjects removed from analysis had leg cramps believed to be unrelated to the treatment and the data collection could not be completed. The second subject was omitted because stable TcpO₂ sensor readings could not be
obtained. The third subject was determined to be an outlier with TcpO₂ values above 100mmHg; this was deemed to be measurement error.

Of the 132 subjects, 72 (55%) were male and 60 (45%) were female. By ethnicity, 55 (42%) were Black and 77 (58%) were non-Black. Non-Black subjects included: Caucasian, 71 (53%); Hispanic, 4 (3%); Middle Eastern, 1 (0.5%); Native American, 2 (1.5%); Philippino, 2 (1.5%), Chinese, 2 (1.5%). Age ranged from 42 to 84 years [Mean, (M) = 65.7, SD = 9.6]. The number of ulcers ranged from 1 to 3 per subject, 24 subjects (18% of the sample) had foot ulcers (3 subjects had 3 ulcers, 3 subjects had 2 ulcers, and 18 subjects had 1 ulcer). Multiple ulcers included ulcers that were on the study foot and bilateral ulcers. Mean number of years since diagnosis with diabetes was 15 years (SD 11). Sample characteristics presented in Table 4–1 include mean age, years since diagnosis with diabetes, type of diabetes, gender, ethnicity, and number of foot ulcers.

**Diabetes**

Seven subjects (5.5%) had IDDM and 125 (94.5%) subjects had NIDDM. The number of years diagnosed with diabetes ranged between 1 year and 52 years (M=15.3, SD=10.6; diagnosis within the past 10 years, 56; 11-20 years, 40; 21-55 years, 36). Glycohemoglobin ranged from 5.6% to 14.3% (M = 9.2%, SD 2.0, normal <6.2%).
**Table 4-1. Sample Characteristics (n = 132)**

<table>
<thead>
<tr>
<th>Mean Age (SD)</th>
<th>Mean Yrs. Dx with Diabetes (SD)</th>
<th>Type Diabetes</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>Presence of Foot Ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>66 (9.6)</td>
<td>15</td>
<td>7, IDDM</td>
<td>72 Males</td>
<td>55 Blacks</td>
<td>33 in 24 Subjects</td>
</tr>
<tr>
<td>125, NIDDM</td>
<td>60 Females</td>
<td>77 Non-Blacks</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations in Table:**
- Dx = diagnosed
- NIDDM = non-insulin dependent
- IDDM = insulin-dependent diabetes mellitus
- SD = standard deviation
- Yrs. = Years

Strategies for diabetes management were broken into 4 groups: diet alone, 20; tablets alone, 52, insulin alone, 50; and insulin plus a hypoglycemic tablet, 10. Diabetes is considered more severe the greater the dependence upon a source of exogenous insulin. Table 4-2 shows diabetes management by group, mean Hgb\textsubscript{A1c}, and mean age in each category. Worsening pancreatic function is indicated from top to bottom on the table.

**Table 4-2. Type of Diabetes Management, Hgb\textsubscript{A1c}, and Age (n = 132)**

<table>
<thead>
<tr>
<th>Type of Control of Diabetes</th>
<th>Number</th>
<th>Mean Hgb\textsubscript{A1c} (SD)</th>
<th>Mean Age (Yrs.) (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet Alone</td>
<td>20</td>
<td>7.9% (2.3)</td>
<td>66.3 (19.7)</td>
</tr>
<tr>
<td>Tablet Alone</td>
<td>52</td>
<td>9.1% (1.9)</td>
<td>66.1 (9.5)</td>
</tr>
<tr>
<td>Insulin + Tablet</td>
<td>10</td>
<td>9.7% (2.1)</td>
<td>58.9 (10.4)</td>
</tr>
<tr>
<td>Insulin Alone</td>
<td>50</td>
<td>9.6% (1.9)</td>
<td>65.0 (8.7)</td>
</tr>
</tbody>
</table>
Peripheral Neuropathy

Among 132 subjects, 71 right feet and 61 left feet were stimulated and measured for response. Table 4-3 lists the places on the foot stimulated. The ball of the foot was stimulated most frequently (54% of cases), the midfoot was stimulated next most frequently (23%).

Table 4-3. Site of Electrical Stimulation (n = 132)

<table>
<thead>
<tr>
<th>Site of Foot Stimulation</th>
<th>Number of Subjects</th>
<th>Percent of Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ball</td>
<td>73</td>
<td>54</td>
</tr>
<tr>
<td>Midfoot</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>Heel</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Achilles Tendon</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Dorsum</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Calf</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

Level of neuropathy ranged from no detectable neuropathy to unable to feel the 5.07 SWMF below the level of the upper calf. Mean vibration perception thresholds (VPT) on the right and left sides were 28V (SD 17) with a range from 4 to not detectable. Those VPTs which were not detectable were above the vibration that the instrument could generate.

Peripheral Vascular Disease

Table 4-4 lists sample vascular measurements with brachial, ankle, and toe blood pressures, ankle-brachial
index (ABI), and toe-brachial index (TBI). Minimal atherosclerosis (TBI  0.92) was present in 41 persons; moderate atherosclerosis (TBI = 0.31 to 0.91) in 85 subjects; and severe atherosclerosis (TBI  0.30) in 6 subjects.

Heart rate range was 48 to 108 beats per minute (M = 74, SD 12.1), body temperature range was 35.0 to 37.5°C (M = 36.7°C). The subject with a heart rate of 48 beats per minute took multiple cardiac drugs, including digoxin. The subject with a rate of 108 beats per minute had recently had heart valve surgery. Neither of these factors was an exclusion criterion.

Table 4-4. Mean Vascular Measurements, All Subjects (n=132)

<table>
<thead>
<tr>
<th>Group (TBI)</th>
<th>Arm BP mmHg (SD)</th>
<th>Ankle BP mmHg (SD)</th>
<th>ABI (SD)</th>
<th>Toe BP mmHg (SD)</th>
<th>TBI (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right (R)</td>
<td>148 (21)</td>
<td>148 (37)</td>
<td>.97 (.25)</td>
<td>115 (37)</td>
<td>.77 (.26)</td>
</tr>
<tr>
<td>Left (L)</td>
<td>148 (22)</td>
<td>151 (42)</td>
<td>.99 (.23)</td>
<td>116 (42)</td>
<td>.77 (.25)</td>
</tr>
<tr>
<td>Minimal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt; .92) R</td>
<td>142 (22)</td>
<td>155 (40)</td>
<td>1.0 (.20)</td>
<td>142 (43)</td>
<td>.96 (.22)</td>
</tr>
<tr>
<td>n = 41 L</td>
<td>142 (22)</td>
<td>159 (35)</td>
<td>1.1 (.17)</td>
<td>142 (31)</td>
<td>.98 (.16)</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(.31-.91) R</td>
<td>149 (19)</td>
<td>150 (41)</td>
<td>.98 (.24)</td>
<td>106 (33)</td>
<td>.70 (.19)</td>
</tr>
<tr>
<td>n = 85 L</td>
<td>149 (20)</td>
<td>143 (36)</td>
<td>.94 (.23)</td>
<td>105 (32)</td>
<td>.70 (.22)</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(  .30) R</td>
<td>169 (35)</td>
<td>126 (68)</td>
<td>.73 (.38)</td>
<td>38 ( 8)</td>
<td>.23 (.05)</td>
</tr>
<tr>
<td>n = 6 L</td>
<td>168 (30)</td>
<td>133 (44)</td>
<td>.80 (.30)</td>
<td>75 (32)</td>
<td>.45 (.18)</td>
</tr>
</tbody>
</table>

Abbreviations in Table:
ABI = ankle-brachial index
BP = blood pressure
R = right
L = left
SD = standard deviation
TBI = toe-brachial index
mmHg = millimeters of mercury

Because the group with severe peripheral vascular disease was too small for sound statistical comparisons
(6 subjects), groups were collapsed into Slight and Moderate-to-Severe (Table 4-5). A toe blood pressure \( \geq 70\text{mmHg} \) was the criterion for the Slight category and a toe blood pressure \(< 70\text{mmHg} \) was the criterion for the Moderate-to-Severe category. In subjects in which toe blood pressures were not obtainable, the ankle blood pressure was used for categorization. The scientific basis for this classification is found in the literature which indicates that subjects with toe blood pressures \( \geq 70\text{mmHg} \) generally heal foot ulcers (Rutherford & Shannon, 1995).

### Table 4-5. Mean Vascular Measurements, All Subjects Recategorized (n=132)

<table>
<thead>
<tr>
<th>Group (n, TBP)</th>
<th>Arm BP mmHg (SD)</th>
<th>Ankle BP mmHg (SD)</th>
<th>ABI (SD)</th>
<th>Toe BP mmHg (SD)</th>
<th>TBI (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Subjects (132)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>148 (21)</td>
<td>148 (37)</td>
<td>.97 (.25)</td>
<td>115 (37)</td>
<td>.77 (.26)</td>
</tr>
<tr>
<td>L</td>
<td>148 (22)</td>
<td>151 (42)</td>
<td>.99 (.23)</td>
<td>116 (42)</td>
<td>.77 (.25)</td>
</tr>
<tr>
<td>Slight (109, ( \geq 70\text{mmHg} ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>148 (20)</td>
<td>156 (38)</td>
<td>1.0 (.21)</td>
<td>126 (37)</td>
<td>.83 (.22)</td>
</tr>
<tr>
<td>L</td>
<td>148 (21)</td>
<td>152 (34)</td>
<td>.99 (.20)</td>
<td>125 (37)</td>
<td>.83 (.22)</td>
</tr>
<tr>
<td>Moderate-to-Severe (23, (&lt; 70\text{mmHg} ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>147 (27)</td>
<td>124 (49)</td>
<td>.85 (.34)</td>
<td>61 (22)</td>
<td>.43 (.19)</td>
</tr>
<tr>
<td>L</td>
<td>147 (26)</td>
<td>128 (46)</td>
<td>.87 (.32)</td>
<td>68 (26)</td>
<td>.45 (.13)</td>
</tr>
</tbody>
</table>

**Abbreviations in Table:**

- ABI = ankle-brachial index
- BP = blood pressure
- L = left
- R = right
- SD = standard deviation
- TBP = toe-brachial pressure
- mmHg = millimeters of mercury

The mean baseline TcpO₂ readings for all subjects and the results of treatment are presented in Table 4-6.
### Table 4-6. Mean TcpO₂ Readings (mmHg) Over Time, All Subjects (n = 132)

<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline (SD)</th>
<th>30 mins. (SD)</th>
<th>60 mins. (SD)</th>
<th>Change Baseline to End Tx (SD)</th>
<th>Change Baseline to End Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot TcpO₂</td>
<td>45.8 (16.2)</td>
<td>41.4 (21.3)</td>
<td>41.1 (23.7)</td>
<td>-5 (17)</td>
<td>-5 (19)</td>
</tr>
</tbody>
</table>

**Abbreviations in table:**
- O₂ = oxygen
- SD = standard deviation
- Tx = treatment

**Medications**

The number of medications taken by subjects ranged from 0 to 18 (M = 5.0, SD = 3.0). Medications with the greatest potential for influencing response to ES were calcium-channel blocking medications and nitrate-containing medications. Subjects who took calcium-channel blockers were 45/133 (34%) and 21/133 (16%) subjects took long-acting nitrates. The number of concurrent diseases ranged between 0 and 8 (M = 2.2, SD = 1.5).

**Environmental Monitoring**

Both rooms where the study was conducted were part of the regulated temperature control of the facilities and maintained temperatures close to 70°F. Environmental monitoring showed mean room temperature was 72°F (SD 1.8). Mean humidity was 41% (SD 8%); mean barometric pressure, 29.80mmHg (SD 0.22). Mean time of the day was 1:00 p.m., which reflected subject preference for afternoon appointments.
Testing of Study Hypotheses

Analysis of Hypothesis I (Primary Null Hypothesis):

There will be no difference in foot skin perfusion before, during, and after high-voltage monophasic pulsed current (HVMPC) electrical stimulation in diabetic persons with or at risk for foot ulcers.

When all subjects were considered together, there was a significant difference between the three measurements (Table 4-7). Post-hoc analysis using Scheffe’s criterion indicated that initial foot oxygen levels were statistically significantly higher ($M = 45.8$, $SD = 16.27$) than end treatment foot oxygen levels ($M = 41.4$, $SD = 21.35$, $p = 0.02$) and end recovery foot oxygen levels ($M = 41.1$, $SD = 23.67$, $p = 0.01$).

However, end treatment foot oxygen levels were not statistically significantly different from end recovery foot oxygen levels ($p > 0.05$). Based on these data, the null hypothesis was rejected ($F = 5.7$, $p = 0.0039$).

Table 4-7. Repeated Measures Analysis of Variance for Difference in $TcPO_2$ in Response to ES Treatment, Within All Subjects ($n = 132$)

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>129</td>
<td>124187.477</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>2</td>
<td>42818.667</td>
<td>1800.067</td>
<td>900.033</td>
<td>5.661</td>
</tr>
<tr>
<td>Error 1</td>
<td>258</td>
<td>41018.600</td>
<td>158.987</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheffe’s Criterion post-hoc analysis (pair-wise comparisons):

Baseline $TcPO_2 >$ End Treatment $TcPO_2$ $p = 0.020$
Baseline $TcPO_2 >$ End Recovery $TcPO_2$ $p = 0.011$
End Recovery $> $ End Treatment $TcPO_2$ $p = >0.05$
Secondary Null Hypotheses:

The four secondary null hypotheses are as follows:

Controlling for gender, ethnicity, and age as covariates, there will be no differences in skin perfusion at baseline, end of treatment, and end of recovery in subjects with or at risk for diabetic foot ulcers by:

1) Levels of glycohemoglobin (high and very high)
2) Peripheral neuropathy (slight and insensate)
3) Peripheral vascular disease (slight and moderate-to-severe)
4) Wagner Grade [0 or 1,2 (ulcer no or yes)]

In preparation for the ANOVAs, the moderating variables were collapsed into two levels per variable to provide group sizes appropriate for statistical analyses. The scientific bases for the categories was drawn from the literature. Age was recategorized as < 65 years or > 65 years based on data that report diabetes and atherosclerosis become worse with age, with the greatest differences seen after 65 years (American Diabetes Association, 1993). Glucose was categorized as high [$Hgb_{Alc} \leq 9.0\% (<225mg/dl)$] and very high [$> 9.0\% (>225mg/dl)$]. Data show that neuropathic symptoms improve somewhat in individuals who keep blood glucose < 200mgdl (Weber & Cardile, 1990). Peripheral neuropathy was recategorized based on the ability to detect the 5.07 (10g SWMF) on all of the foot or not. The ability to detect the 5.07 on all of the foot was categorized as slight neuropathy and being unable to detect the 5.07 on all of the foot was categorized as insensate an foot (Coleman, 1993). Peripheral vascular disease was categorized as slight (toe blood
pressure ≥ 70mmHg) and moderate-to-severe (toe blood pressure < 70mmHg). Wagner Classification was reclassified as no ulcer (Wagner Class 0) or ulcer present (Wagner Class 1 or 2).

While controlling for age, ethnicity, and gender, repeated measures ANOVAs showed that skin perfusion was not significantly influenced by glycohemoglobin (Table 4-8), peripheral neuropathy (Table 4-9), peripheral vascular disease (Table 4-10), and Wagner Class (Table 4-11).

Therefore, the second null hypotheses was not rejected at the p = 0.05 level. There are no statistically significant difference in TcpO2 between baseline, end of treatment and end of recovery based on any of the seven moderator variables glucose level, peripheral neuropathy, peripheral vascular disease, Wagner Class, when controlling for age, ethnicity, and gender.

Table 4-8. Repeated Measures Analysis of Variance of TcpO2 in Response to ES Treatment, Within All Subjects by Glucose Level with Age, Ethnicity, and Gender as Covariates (n = 132)

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>128</td>
<td>121663.767</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariates</td>
<td>3</td>
<td>1437.340</td>
<td>479.113</td>
<td>0.496</td>
<td>0.686</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>174.820</td>
<td>174.821</td>
<td>0.181</td>
<td>0.671</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1</td>
<td>1188.235</td>
<td>1188.235</td>
<td>1.229</td>
<td>0.270</td>
</tr>
<tr>
<td>Gender</td>
<td>1</td>
<td>0.167</td>
<td>0.168</td>
<td>0.000</td>
<td>0.990</td>
</tr>
<tr>
<td>Glucose Level</td>
<td>1</td>
<td>442.152</td>
<td>442.152</td>
<td>0.457</td>
<td>0.500</td>
</tr>
<tr>
<td>Error 1</td>
<td>124</td>
<td>119856.004</td>
<td>966.653</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td>258</td>
<td>40686.000</td>
<td>1071.666</td>
<td>7.092</td>
<td>0.001</td>
</tr>
<tr>
<td>Foot TcpO2</td>
<td>2</td>
<td>2143.332</td>
<td>1071.666</td>
<td>7.092</td>
<td>0.001</td>
</tr>
<tr>
<td>Gluc X FtTcpO2</td>
<td>2</td>
<td>125.523</td>
<td>62.761</td>
<td>0.415</td>
<td>0.661</td>
</tr>
<tr>
<td>Error 2</td>
<td>254</td>
<td>38383.594</td>
<td>151.117</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4-9. Repeated Measures Analysis of Variance of \( \text{TcpO}_2 \) in Response to ES Treatment, Within All Subjects by Peripheral Neuropathy Level with Age, Ethnicity, and Gender as Covariates (\( n = 132 \))

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td></td>
<td>121663.767</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariates</td>
<td>3</td>
<td>1173.060</td>
<td>391.020</td>
<td>0.404</td>
<td>0.751</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>204.390</td>
<td>204.390</td>
<td>0.211</td>
<td>0.647</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1</td>
<td>943.109</td>
<td>943.109</td>
<td>0.974</td>
<td>0.326</td>
</tr>
<tr>
<td>Gender</td>
<td>1</td>
<td>8.575</td>
<td>8.575</td>
<td>0.009</td>
<td>0.925</td>
</tr>
<tr>
<td>Neuropathy Level</td>
<td>1</td>
<td>241.543</td>
<td>241.543</td>
<td>0.249</td>
<td>0.618</td>
</tr>
<tr>
<td>Error 1</td>
<td>124</td>
<td>120065.614</td>
<td>968.271</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td>258</td>
<td>40686.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot TcpO(_2)</td>
<td>2</td>
<td>1993.283</td>
<td>996.642</td>
<td>6.643</td>
<td>0.002</td>
</tr>
<tr>
<td>Neur X FtTcpO(_2)</td>
<td>2</td>
<td>399.227</td>
<td>199.613</td>
<td>1.330</td>
<td>0.266</td>
</tr>
<tr>
<td>Error 2</td>
<td>254</td>
<td>38109.889</td>
<td>150.039</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4-10 Repeated Measures Analysis of Variance of \( \text{TcpO}_2 \) in Response to ES Treatment, Within All Subjects by Peripheral Vascular Disease Level with Age, Ethnicity, and Gender as Covariates (\( n = 132 \))

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>128</td>
<td>121663.767</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariates</td>
<td>3</td>
<td>1976.989</td>
<td>658.996</td>
<td>0.713</td>
<td>0.546</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>470.720</td>
<td>470.720</td>
<td>0.509</td>
<td>0.477</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1</td>
<td>1444.423</td>
<td>1444.423</td>
<td>0.562</td>
<td>0.213</td>
</tr>
<tr>
<td>Gender</td>
<td>1</td>
<td>65.994</td>
<td>65.994</td>
<td>0.071</td>
<td>0.790</td>
</tr>
<tr>
<td>Peripheral Vasc</td>
<td>1</td>
<td>5630.534</td>
<td>563.534</td>
<td>6.088</td>
<td>0.015</td>
</tr>
<tr>
<td>Error 1</td>
<td>124</td>
<td>114676.622</td>
<td>924.812</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td>258</td>
<td>40686.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot TcpO(_2)</td>
<td>2</td>
<td>2587.434</td>
<td>1293.717</td>
<td>8.661</td>
<td>0.000</td>
</tr>
<tr>
<td>Vasc X FtTcpO(_2)</td>
<td>2</td>
<td>570.597</td>
<td>285.299</td>
<td>1.910</td>
<td>0.150</td>
</tr>
<tr>
<td>Error 2</td>
<td>254</td>
<td>37938.519</td>
<td>149.364</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4-11. Repeated Measures Analysis of Variance of TcpO₂ in Response to ES Treatment, Within All Subjects by Wagner Class Level with Age, Ethnicity, and Gender as Covariates (n = 132)

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariates</td>
<td>3</td>
<td>1094.813</td>
<td>364.938</td>
<td>0.377</td>
<td>0.770</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>180.058</td>
<td>180.058</td>
<td>0.186</td>
<td>0.667</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>1</td>
<td>900.391</td>
<td>900.391</td>
<td>0.930</td>
<td>0.337</td>
</tr>
<tr>
<td>Gender</td>
<td>1</td>
<td>2.464</td>
<td>2.464</td>
<td>0.003</td>
<td>0.960</td>
</tr>
<tr>
<td>Wagner Class</td>
<td>1</td>
<td>217.671</td>
<td>217.671</td>
<td>0.225</td>
<td>0.636</td>
</tr>
<tr>
<td>Error 1</td>
<td>124</td>
<td>120889.486</td>
<td>968.464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foot TcpO₂</td>
<td>2</td>
<td>40686.000</td>
<td>484.882</td>
<td>3.205</td>
<td>0.0422</td>
</tr>
<tr>
<td>WagnerX FtTcpO₂</td>
<td>2</td>
<td>79.955</td>
<td>39.978</td>
<td>0.264</td>
<td>0.768</td>
</tr>
<tr>
<td>Error 2</td>
<td>254</td>
<td>38429.161</td>
<td>151.296</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Subgroup Analysis: "Responders" vs. "Nonresponders"

Upon closer examination of the data, three distinct groups were evident: 35 subjects (26%) showed a significant increase in TcpO₂ (M = +14.0mmHg, SD 19.0; F = 19.8, p < .001); 97 subjects (72%) showed a significant decrease in TcpO₂ (M = -12.1mmHg, SD 9.2; F = 171.6, p < .001); and 3 subjects (2%) were unchanged. Unchanged subjects were reclassified based on differences in TcpO₂ between baseline and end of the recovery period. The group of subjects who showed a rise in TcpO₂ has been labelled "Responders," and the group that showed a decrease has been labelled "Nonresponders." Changes in TcpO₂ and TcpCO₂ levels by the two categories are presented in Table 4-12.
Table 4-12. Responders vs. Nonresponders: Mean TcpO₂ and TcpCO₂ Readings (mmHg) in Response to ES

<table>
<thead>
<tr>
<th>Response Group (n = no.)</th>
<th>Baseline Foot O₂/CO₂ mmHg (SD)</th>
<th>30 minutes Foot O₂/CO₂ mmHg (SD)</th>
<th>60 minutes Foot O₂/CO₂ mmHg (SD)</th>
<th>Change from Baseline to End Tx mmHg</th>
<th>Change from Baseline to End Recovery (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders (n = 36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂</td>
<td>43.2 (17.3)</td>
<td>58.3 (28.2)</td>
<td>53.7 (25.7)</td>
<td>14.8 (18.4)</td>
<td>10.5 (15.8)</td>
</tr>
<tr>
<td>CO₂</td>
<td>50.3 (17.4)</td>
<td>47.3 (20.2)</td>
<td>46.9 (19.4)</td>
<td>-2.9 (16.6)</td>
<td>-3.4 (15.6)</td>
</tr>
<tr>
<td>Non-responders (n = 95)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂</td>
<td>47.0 (15.7)</td>
<td>34.8 (13.3)</td>
<td>36.3 (21.1)</td>
<td>-12.2 (9.2)</td>
<td>-10.8 (17.4)</td>
</tr>
<tr>
<td>CO₂</td>
<td>44.9 (14.2)</td>
<td>55.6 (9.2)</td>
<td>55.7 (9.9)</td>
<td>+10.7 (11.0)</td>
<td>10.8 (14.3)</td>
</tr>
</tbody>
</table>

Abbreviations used in table:
CO₂ = transcutaneous carbon dioxide  SD = standard deviation
mmHg = millimeters of mercury  Tx = treatment
O₂ = transcutaneous oxygen

Differences between Responders and Nonresponders in VPT were statistically significant on the right (Responder, M = 33.5, SD 17.7; Nonresponder, M = 25.6, SD 16.1; F = 6.0, p = 0.016), but not statistically significant on the left by one-way ANOVA (Responder, M = 32.3, SD 18.0; Nonresponder, M = 25.9, SD 16.0; F = 3.8, p = 0.053). Ulcer characteristics, VPT, Hgbₐₐc, and vascular levels of the two groups, Responders and Nonresponders, are presented in Table 4-13.

A statistically significant difference was seen in response to ES treatment between subjects taking nitrite-containing drugs and those who did not take nitrate-containing drugs in this study (F = 9.31, p = 0.003).
**Table 4-13. Ulcer Number, VPT, Hgb<sub>Alc</sub>, and Vascular Levels: Responders vs. Nonresponders (n = 132)**

<table>
<thead>
<tr>
<th>Group (n, Mean Age)</th>
<th>Mean Ulcer Size (SD)</th>
<th>Mean VPT (SD)</th>
<th>Hgb&lt;sub&gt;Alc&lt;/sub&gt; (SD)</th>
<th>Vascular Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Arm BP</td>
</tr>
<tr>
<td>Responders (35, 66 yrs.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>35 (99)</td>
<td>34 (18)</td>
<td>9.4% (1.9)</td>
<td>144 (19)</td>
</tr>
<tr>
<td>L</td>
<td>32 (18)</td>
<td>32 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-responders (97, 65 yrs.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>15 (47)</td>
<td>27 (16)</td>
<td>9.1% (2.0)</td>
<td>149 (22)</td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>27 (16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different p < .05

Abbreviations used in table:
- ABI = ankle-brachial index
- SD = standard deviation
- BP = blood pressure
- TBI = toe-brachial index
- Hgb<sub>Alc</sub> = glycated hemoglobin
- VPT = vibratory perception threshold

However, there were NSS differences in response between subjects who took calcium-blocking medications and those who did not take calcium-blocking medications in this study (F = 0.85, p = 0.36).

**Nitrate Containing Drugs**

Nitrate containing drugs were logged for all subjects. Of 132 subjects, 21 subjects (16%) took nitrate-containing medications and 112 did not. Nitrite-containing drugs are listed in Table 4-14.

A significant difference was seen by one-way ANOVA in response to ES (change in TcpO<sub>2</sub> readings from baseline to end of treatment) between subjects that took nitrate-containing medications (M = +2.8mmHg, SD 24.0) and subjects that did not take nitrate containing medications.
### Table 4-14. Nitrate-Containing Medications

<table>
<thead>
<tr>
<th>Nitroglycerin</th>
<th>Isosorbide dinitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitro-Patch</td>
<td>Isordil</td>
</tr>
<tr>
<td>Nitro-Dur</td>
<td>Apo-ISDN</td>
</tr>
<tr>
<td>Nitrodisc</td>
<td>Cedocard-SR</td>
</tr>
<tr>
<td>Nitro-Bid</td>
<td>Coronex</td>
</tr>
<tr>
<td>Nitrocap</td>
<td>Dilatrate R</td>
</tr>
<tr>
<td>Nitocine</td>
<td>Iso-Bid</td>
</tr>
<tr>
<td>Nitradisc</td>
<td>Isorbid</td>
</tr>
<tr>
<td>Nitro-Dur</td>
<td>Isonate</td>
</tr>
<tr>
<td>Nitogard</td>
<td>Isotrate Novosorbide</td>
</tr>
<tr>
<td>Nitrol</td>
<td>Sorbitrate</td>
</tr>
<tr>
<td>Nitrolate ointment</td>
<td></td>
</tr>
<tr>
<td>Nitrolin</td>
<td>Pentaerythritol tetranitrate</td>
</tr>
<tr>
<td>Nitrolingual</td>
<td>Dilar</td>
</tr>
<tr>
<td>Nitrol TSAR</td>
<td>Ductate</td>
</tr>
<tr>
<td>Nitronet</td>
<td>Naptrate</td>
</tr>
<tr>
<td>Nitrong</td>
<td>Pentritol</td>
</tr>
<tr>
<td>Nitrospan</td>
<td>Pentylan</td>
</tr>
<tr>
<td>Nitrostat</td>
<td>Peritrate</td>
</tr>
<tr>
<td>Transderm Nitro</td>
<td>Pertrate Forte</td>
</tr>
<tr>
<td>Tridil</td>
<td>Pertrate SA</td>
</tr>
<tr>
<td>Erythrityl tetranitrate</td>
<td>PETN</td>
</tr>
<tr>
<td>Cardilate</td>
<td></td>
</tr>
</tbody>
</table>

(M = -6.8mmHg, SD 14.4, F = 6.0, p = 0.02). There was no statistically significant change from baseline to end of recovery for subjects taking nitrates (M = -2.7mmHg, SD 15.4) and subjects not taking nitrates (-5.9mmHg, SD 19.3) (F = 0.52, p = 0.47). Analysis of variance tables for differences in change in TcpO₂ in response to ES are listed in Table 4-15 and 4-16. Six subjects did not have recovery TcpO₂ readings for various reasons (one monitoring period had to be curtailed to accommodate the scheduling of another subject, sensors came loose, etc.).
Table 4-15. One-Way ANOVA, Change in TcpO₂, Baseline to End of Treatment, by Subjects Taking Nitrate-Containing Medications vs. Subjects Who Did Not (n= 132)

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>131</td>
<td>39023.729</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate-Medication</td>
<td>1</td>
<td>2588.381</td>
<td>2588.381</td>
<td>9.306</td>
<td>0.003</td>
</tr>
<tr>
<td>Error 1</td>
<td>130</td>
<td>36435.348</td>
<td>278.132</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4-16. One-Way ANOVA, Change in TcpO₂, Baseline to End of Recovery, by Subjects Taking Nitrate-Containing Medications vs. Subjects Who Did Not (n= 127)

<table>
<thead>
<tr>
<th>Source of Error</th>
<th>DF</th>
<th>SS(U)</th>
<th>MSS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>126</td>
<td>39574.992</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error 1</td>
<td>125</td>
<td>37588.632</td>
<td>300.709</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calcium-Channel Blocking Medications

Because of the mechanism of action of calcium-channel blocking medications, it is possible that the medications could influence the effect of ES, most likely blocking or decreasing the response to ES (Cooper & Schliwa, 1985). In this sample, 45 subjects (34%) took calcium-channel blocking medications and 87 subjects did not. Calcium-channel blocking drugs are listed in Table 4-17.

These drugs were logged for all subjects in this study. A one-way ANOVA indicated no statistically significant differences between subjects who took calcium-channel-blocking medications and those who did not in foot perfusion at baseline (p = 0.14), at the end of treatment (p = 0.68), and at the end of recovery (p = 0.68). The responses to ES
(change in TcpO₂ from baseline to end of treatment) \( (p = 0.36) \) and in the change in TcpO₂ from baseline to end of recovery were also not statistically significant \( (p = 0.32) \).

Table 4-17. Calcium-Channel Blocking Drugs

<table>
<thead>
<tr>
<th>Bepridil hydrochloride</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascor</td>
<td>Adalat</td>
</tr>
<tr>
<td></td>
<td>Apo-Nifed</td>
</tr>
<tr>
<td>Diltiazem hydrochloride</td>
<td>Novo-Nifed</td>
</tr>
<tr>
<td>Cardizem</td>
<td>Procardia</td>
</tr>
<tr>
<td>Isradipine</td>
<td>Verapamil hydrochloride</td>
</tr>
<tr>
<td>Dynacirc</td>
<td>Calan</td>
</tr>
<tr>
<td></td>
<td>Cordilox</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>Isoptin</td>
</tr>
<tr>
<td>Cardine</td>
<td>Veradil</td>
</tr>
</tbody>
</table>

Logistic Regression Analysis

Data were subjected to logistic regression analysis to determine which, if any, of the moderator variables (glucose level, level of neuropathy, level of vascular disease, Wagner Class, gender, ethnicity, age, or medications) might predict who would respond to ES (Table 4-18). The results from the logistic regression analysis are given as odds ratios (OR) with 95% confidence intervals around the OR. Note, the null hypothesis tests whether OR = 1, that is there is no difference between those who have the predictor present (1) or not present (0).
### Table 4-18 Logistic Regression Odds Ratios and 95% Confidence Intervals for 8 Moderator Variables to Predict Nonresponse to ES

<table>
<thead>
<tr>
<th>Category &amp; Criteria</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Age (1 = old, &gt;65 years, 0 = young, ≤65 years)</td>
<td>0.68</td>
<td>0.29</td>
</tr>
<tr>
<td>Gender (1 = male, 0 = female)</td>
<td>1.71</td>
<td>0.68</td>
</tr>
<tr>
<td>Ethnicity (1 = Black, 0 = Non-Black)</td>
<td>1.13</td>
<td>0.48</td>
</tr>
<tr>
<td>Glucose (&gt;9.1% Hgb&lt;sub&gt;A1c&lt;/sub&gt; = 1, ≤ 9.0% Hgb&lt;sub&gt;A1c&lt;/sub&gt; = 0)</td>
<td>0.55</td>
<td>0.24</td>
</tr>
<tr>
<td>Peripheral Neuropathy (&gt; 5.07 = 1, &lt; 5.07 = 0)</td>
<td>0.58</td>
<td>0.23</td>
</tr>
<tr>
<td>Peripheral Vascular Disease (TBP &lt; 70mmHg = 1, TBP ≥ 70mmHg = 0)</td>
<td>3.22</td>
<td>0.85</td>
</tr>
<tr>
<td>Wagner Class (No Ulcer = 1, Ulcer = 0)</td>
<td>0.39</td>
<td>0.13</td>
</tr>
<tr>
<td>Nitrate Taker (NTG No = 1, NTG Yes = 0)</td>
<td>2.16</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**Abbreviations in Table:**
- 1 = Risk of being a nonresponder
- 0 = Risk of being a responder
- Hgb<sub>A1c</sub> = Glycohemoglobin
- mmHg = Millimeters of mercury
- NTG = Nitrate-containing medication
- TBP = Toe blood pressure

The 95% confidence interval is interpreted in the following manner:

- If the OR < 1, the effect is protective.
- If the OR = 1, there is no effect.
- If the OR > 1, there is a harmful effect.

If the 95% confidence interval contains 1, the finding is not statistically significant. The width of a confidence interval is informative, as it provides information about the precision of the estimate of the OR.

Although not statistically significant and recognizing that results must be interpreted with caution, logistic
regression analysis indicated that having a high glucose level ($\text{Hgb}_{\text{A1C}} > 9.0\%$) was associated with an odds ratio of 0.55 of being a nonresponder. That is, having a high glucose is protective of being a nonresponder; 45% less likely to be a nonresponder. The 95% confidence interval indicates that the true value lies between 0.24 and 1.28 (not statistically significant).

Having a higher level of neuropathy is associated with an OR of 0.58, an effect of 0.58 (not statistically significant). Thus, a subject with a higher level of neuropathy was 42% less likely to be a nonresponder in this sample. The 95% confidence interval indicates that the true value may be as low as 0.23 or as high as 1.43 (not statistically significant).

Peripheral vascular disease measured by toe blood pressure less than 70 mmHg was associated with an OR of 3.3; or being 3.3 times more likely to be a nonresponder. The 95% confidence interval indicates that the true measure may be as low as 0.85 (protective) or as high as 12.29 times the risk of being a nonresponder (not statistically significant). This variable has low precision, given the large confidence interval.

Wagner Class of 1 or 2 was associated with an OR of 0.39, indicating that individuals with foot ulcers have a 61% lesser likelihood of being a nonresponder. The 95%
confidence interval is that the true value may be as low as 0.13 or as high as 1.19 (not statistically significant).

Not taking nitrate-containing medications is associated with an OR of 2.2. Hence, non-nitrate takers were more likely to be nonresponders. The 95% confidence interval indicates that the true value may be as low as 0.68 or as high as 6.86 (not statistically significant).

Being older (greater than 65 years of age) was associated with an OR of 0.68 (not statistically significant). This indicates that being older may provide a 32% less likelihood of being a nonresponder. The 95% confidence interval indicates that the true value may be as low as 0.29 or as large as 1.61; the latter would not be protective.

Being male was associated with an OR of 1.13. The 95% confidence interval indicates that the true value may be as low as 0.48 or may be as high as 2.68 times as likely to be a nonresponder.
CHAPTER V: DISCUSSION

This study used a repeated-measures design to investigate whether one 30-minute treatment of HVMPC, 100V, 100pps, negative polarity could produce an increase in skin perfusion in persons with diabetes who either have or at risk for foot ulcers. The purpose of this study was to examine the effect of ES on skin perfusion in the feet of persons with diabetes who have or are at risk for foot ulcers to determine whether this treatment might offer a means to speed wound healing in foot ulcers in diabetic subjects. Secondary purposes were to examine the effects of glucose control, peripheral neuropathy, peripheral vascular disease, Wagner Class, gender, ethnicity, age, and medications on skin perfusion in response to ES.

This chapter discusses the major findings of this study with regard to the related literature. The effect of electrical stimulation on foot skin perfusion will be discussed with consideration of factors that influence skin perfusion in diabetes. Discussion will include limitations of this research, significance of results, and implications for future research. Issues of validity and factors influencing conclusions will be addressed.

Findings in Relation to Hypotheses and Related Literature

The "skin battery" (Foulds & Barker, 1983) has been demonstrated to have an influence on wound healing in animal
and human subjects (Tables 1-6). The resting cell membrane has an energy potential that is specific to the tissue in which it resides (approximately -70mV). Depolarization of the cellular membrane, by impulses from the nervous system and other forces, causes events to happen specific to the stimulus and nature of the cell type (Stryer, 1988). It is hypothesized that ES improves circulation in persons with diabetes via the "axonal response" or an unknown mechanism which liberates vasoactive substances (Low & Reed, 1993) and by its influences on cellular types involved in wound healing.

Investigators have found skin perfusion increases in some subjects as a result of transcutaneous electrical nerve stimulation (Tables 7 and 8). Because a microvascular defect exists in diabetic subjects resulting in reduced capillary perfusion, ES was proposed as a means for increasing skin perfusion in persons with, or at risk for, diabetic foot ulcers.

**Findings with Regard to Hypothesis I**

**Primary Null Hypothesis:**

There will be no difference in foot skin perfusion before, during, or after HVMPC electrical stimulation in diabetic persons with or at risk for foot ulcers.

When all subjects were considered together, the null hypothesis was rejected by one-way analysis of variance (ANOVA). The mean response to ES of 30 minutes duration was
a decrease of approximately 5mmHg. Post-hoc analysis using Scheffe's criterion provided evidence that initial foot oxygen levels were significantly higher ($M = 45.8$, $SD = 16.27$) than end treatment foot oxygen levels ($M = 41.4$, $SD = 21.35$, $p = 0.02$) and end recovery foot oxygen levels ($M = 41.1$, $SD = 23.67$, $p = 0.01$). Thus, there was empirical evidence that a change in the tissues resulted from ES in this sample of subjects. However, the change was not an increase in perfusion, but a decrease in perfusion evidenced by a lower $TcP_O_2$.

A large subset ($n = 97$), 72% of the sample, showed a decrease in $TcP_O_2$ in response to ES using these parameters. Therefore, Null Hypothesis I was rejected at the .05 level. A very small group ($n = 3$, 2%) demonstrated no difference in $TcP_O_2$ in response to ES. These findings are inconsistent with the findings of Baker (1988) and Kaada (1982, 1983, & 1984) who reported a rise in $TcP_O_2$ by electrical stimulation irrespective of waveform used.

Baker (1988) does not elaborate on the characteristics of the sample studied, but reports that subjects are 30 diabetic patients, 40 patients with spinal cord injury, and 20 age-matched normal subjects. Persons with diabetes demonstrated a measurable increase in $TcP_O_2$ at the end of a 30 minute stimulation period using both positive and negative polarity of HVMPC and a symmetrical, biphasic waveform (Russian-type, medium frequency) (Low & Reed, 1993).
Baker (1988) reports that minimal-muscle contraction, "blunted the effect." The actual amount of current delivered to the tissues is not specified; therefore, amperage per cm² comparisons cannot be made with the current used in the present study. It is quite possible that the stimulus used in this study exceeded the stimuli of the three wave forms reported by Baker (1988).

Kaada and colleagues (1982, 1983, & 1984) primarily studied subjects with Raynaud's syndrome, although two subjects with diabetic neuropathy were included. Through a series of experiments on the same subjects, Kaada (1982–1984) demonstrated that the increase in blood flow was due in part to increase in plasma vasoactive intestinal peptide (VIP) and central nervous system serotonin. These two substances were not measured in the present study.

Burnstock (1993) describes 18 known vasoactive substances that are secreted by the perivascular nervous system or the vascular endothelium. Each one of these substances modulates blood flow. To the dismay of many wishing to study their effects, the response is not consistent between tissues (Kaada, 1984). Some, such as adenosine triphosphate (ATP), cause vasoconstriction in some tissues and vasodilation in others, depending upon the subcategory of receptor affected (Burnstock, 1993).

Kaada (1982) reported using a current of square-wave pulses with 0.2ms duration, up to 100 cycles per second (Hz)
resulting in 20–30 milliamperes being delivered to the tissue until minimal local contraction of the muscles was achieved without pain. This was done using 12cm² electrodes to the Hoku point on the hand. Skin temperature of both the fingers and toes were measured as the dependent variable. Although the amount of current delivered to the tissues was about the same, different sized electrodes were used. Thus, the design of Kaada and colleague’s studies was quite different from the design of the present study. Their stimulation site (Hoku point) was remote from the foot and TcpO₂ was not measured in that study.

**Subset Analysis**

Subset analysis in the present study showed that some subjects benefited from transcutaneous ES as evidenced by an increase in skin oxygen; 26% (n = 35) showed a mean rise in foot TcpO₂ of 14mmHg (p < 0.001). The percentage of subjects who benefitted in this study is comparable to the percentage of subjects (35%) that Rutherford and Shannon (1995) predicted benefit from lumbar sympathectomy.

Responders were different from nonresponders by the following: older (M = 66.4 years, SD 9.5; nonresponders, M = 65.4 years, SD 9.6; NSS), had a higher level of peripheral neuropathy (VPT M = 33.5, SD 17.7; nonresponders, VPT M = 25.6, SD 16.1; p = 0.016), had more adequate peripheral blood flow (Toe BP M = 121, SD 41; nonresponders, Toe BP M = 113.8, SD 42.7; NSS), had more foot ulcers in the group (12
ulcers among 36 subjects; nonresponders, 22 ulcers among 97 subjects; NSS), had larger ulcers (M = 35.5mm², SD 98.9; nonresponders, M = 14.6mm², SD 46.5; NSS), and had lower mean TcpO₂ levels at baseline (M = 43.2mmHg, SD 17.3; nonresponders, M = 47.0mmHg, SD 15.7; NSS). Twenty percent of responders took nitrate-containing medication.

Logistic regression was used to examine the eight variables (glucose control, peripheral neuropathy, peripheral vascular disease, Wagner Class, gender, ethnicity, age, and medications) that the literature indicates may be effect modifiers between ES and perfusion. This study failed to confirm that any of the modifiers influences the effect of ES on skin perfusion. Alternatively, the distribution of these moderator variables may be such that it was not possible to detect their effect in this sample.

Proposed Mechanism for ES to Increase Perfusion

One proposed mechanism for the influence of ES is an induced electrical sympathectomy of a temporary nature which interrupts vasoconstriction. The labelling of subjects with a positive response as "Responders" and those with a negative response as "Nonresponders" is consistent with reports of increases in perfusion related to surgical lumbar sympathectomy (Rutherford & Shannon, 1995).

Rutherford and Shannon (1995) theorize that there should be a 100% response of improved blood flow following
lumbar sympathectomy. However, that was not evidenced by the clinical results of surgery. Only 35% of subjects with vascular disease respond to surgical sympathectomy with an improvement in blood flow. Improvement in blood flow due to sympathectomy was short lived, lasting only 5 to 7 days. Since the nerves repair slowly, it is believed that vasoactive signalling messengers are responsible for return of vasoconstriction (Rutherford & Shannon, 1995). Thus, it appears biofeedback mechanisms regulate vasoactive substances to control the altered blood flow returning it toward "normal" for that individual.

Findings with Regard to Secondary Null Hypotheses:

The four secondary null hypotheses are as follows:

Controlling for gender, ethnicity, and age as covariates, there will be no differences in skin perfusion at baseline, end of treatment, and end of recovery in subjects with or at risk for diabetic foot ulcers by:

1) Levels of glycohemoglobin (high and very high)
2) Peripheral neuropathy (slight and insensate)
3) Peripheral vascular disease (slight and moderate-to-severe)
4) Wagner Grade [0 or 1,2 (ulcer no or yes)]

Controlling for age, ethnicity, and gender, analyses of variance indicated that there were no significant differences in foot skin perfusion based on any of the moderator variables. In this study, there was no support found that gender, ethnicity, glucose level, peripheral vascular disease, peripheral neuropathy, and Wagner Class moderate the effect of ES on TcpO₂. Therefore, the
secondary null hypotheses are not rejected at the $p = 0.05$ level. There were no statistically significant differences in perfusion based on the moderator variables by ANOVA. These results were not anticipated, based on the literature.

**Human Subject Safety**

To date, there are no serious side effects listed in the literature with regard to the application of ES for clinical use. The most common side effect is allergic dermatitis in response to various tapes that may be used to secure electrodes. No difficulties were encountered in this study with regard to allergic dermatitis primarily because hypoallergenic adhesive electrodes were used.

However, one male subject complained two months after the protocol that he felt peripheral neuropathy symptoms had increased in his hands after participation in the study. The subject was examined by a neurologist, who determined that the symptoms were being experienced due to degeneration of a condition in the neck vertebrae for which the subject was under treatment prior to the protocol. Thus, symptoms were determined to be unrelated. Therefore, the particular parameters used in this study have been shown to be safe for a large number of subjects who were carefully screened to preclude problems identified by the exclusion criteria.

**Limitations of the Study**

Data reported by subjects is assumed to be accurate. The limitation is that for most subjects, there was not a
medical confirmation of diagnoses and medications prescribed. People with Type II diabetes are often assumed to have diabetes for 10 years prior to diagnosis; therefore, the duration of diabetes is a rough estimate. Individuals' have varying abilities to remember how long s/he has been diagnosed with diabetes, names of medications, and medical histories.

Some TcpO₂/TcpCO₂ values obtained were clearly measurement problems. Difficulties were experienced from time to time in maintaining adequate seals around sensors due to a number of unanticipated factors: lotion on the subject's skin which was not completely removed by wiping with rubbing alcohol, lack of skin integrity (peeling skin) due to tinea pedis, foot curvature problems due to bone deformities, excessive amount of electrolyte solution placed on the TcpO₂ sensor causing inadherence of the sealing ring, and nervous movement of some subjects' feet causing the sensor to loosen the seal. One subject's data were rejected because stable values could not be obtained.

The question is raised to what extent warming of the TcpO₂ sensor to 44°C (which should maximally dilate the capillaries underneath it) confound the treatment effect of the stimulating electrode. Literature on TcpO₂ measurement suggests that it is most reliable at the 44°C temperature to allow for the easier diffusion of the oxygen through the skin into the sensor (Rooke & Osmundson, 1989). However,
the warmer electrode maximally dilates the capillaries under the sensor, thus blunting the treatment effect. The extent of this effect is unknown in the present study.

In addition, a systematic error could have been entered into the data when cooler electrodes were used for subjects with severe vascular disease, because measurement was not as sensitive to change in the underlying tissue. Cooler electrodes were only used in six subjects with severe vascular disease (TBI < 0.30). This number of subjects would not significantly influence the overall findings of this study, as the mean temperature reading was 43.9°C in this study in 133 subjects. Change was based on measurements with subjects compared to their own baselines; the differences are generalizable.

Multiple medications could have interacted with the treatment effect of the stimulating electrode to unknown degrees. For subjects taking long-acting nitrates, it was expected that the skin capillaries would already be maximally dilated. Calcium-channel blocking drugs produce a similar effect in the tissues to lower blood pressure. Some subjects took many drugs and interactions could not be identified.

Rutherford (1995) suggests that skin capillaries in subjects who have severe peripheral vascular disease are always maximally dilated because the tissue demand is high. However, the input of blood is severely restricted in these
individuals and cannot keep up with demand. These individuals would most likely demonstrate a decrease in response to ES due to the increased metabolism in the tissue and inability to increase perfusion to maintain skin perfusion and TcpO₂.

The head of the bed was kept elevated at approximately 30 degrees throughout the measurement and treatment process. This would have falsely elevated perfusion indices and made a subject’s vascular status appear better than it actually was. This might also have interfered with venous return from the legs resulting in a decrease in blood flow to the legs due to the restriction of outflow as described by Rutherford and Shannon (1995).

Despite these limitations, this study has provided important new information about persons with diabetes, with or at risk for foot ulcers, who might benefit from improved skin perfusion induced by ES treatment. In addition, the extensive descriptive statistics have provided valuable information on this subpopulation of subjects, which may enlighten clinicians for treatment of this complex problem.

Recommendations for Further Research

While this study was unable to identify a uniform positive effect for all subjects, it did show a statistically significant increase in TcpO₂ for 26% of the subjects. The logistic regression analysis has helped to predict which individuals are most likely to benefit by
increased skin perfusion and these data offer guidance for the conduct of future research into the use of ES treatment for the purpose of increasing perfusion in persons with diabetes and foot ulcers.

The treatment offers the possibility for helping a significant number of people to more rapidly heal foot ulcers. Additional controlled studies will need to be done to determine whether there is a realistic possibility that ES will significantly augment standard treatment to promote ulcer healing in subjects with diabetes. Additional studies should be pursued with changes made to eliminate the sources of error identified by the investigator.

Supplemental Oxygen

Hunt (1995) and Rooke (1992) indicate that supplemental respiratory oxygen improves the reliability of transcutaneous oxygen as a measurement of perfusion. Therefore, in future studies with TcpO₂ as a dependent measure researchers should evaluate whether or not to use supplemental oxygen according the subject’s ability to tolerate it.

Explore Other Electrical Stimulation Parameters

Different treatment parameters should be explored. Mawson et al. (1993) suggest that 75V, at 10 pulses per second would be more effective for producing vasodilation and an increase in TcpO₂ than 100V. It may be worthwhile to explore the effect of this setting on TcpO₂ in diabetic
subjects at risk for foot ulcers. This is reinforced by the work of Goldman and Pollack (1995) and Chu, et al. (1991), who state that ES must be within a low range to be biologically effective.

Many subjects stated that they did not feel the treatment (even as paresthesia) because settings were "too low," despite detecting the 5.07 SWMF at the stimulated dermatome. A higher level of stimulation may be optimal. Therefore, a design in which several electrical parameters could be compared using the same subjects would be beneficial to clarify these questions.

Four subjects demonstrated a rise in TcPO₂ at 15 minutes, but not at 30 minutes; so perhaps a shorter treatment of 20 minutes would be more effective. A design in which length of treatment could be compared using the same subjects also would be beneficial.

**Sample Selection**

There was wide variability in the sample evidenced by large ranges in all of the variables measured. Greater effort should be exerted to obtain a sample that is more homogenous. This could be achieved if the sample was limited to subjects with active ulcers of neuropathic origin, with adequate blood flow to the extremities, and again included subjects who take either nitroglycerin or calcium-channel blockers. The sample should reflect gender, ethnicity, and age groups of the population who have the
problem. A longer data collection time (at least a year) would be helpful to achieve a sample of approximately the same as this study or greater.

A repeated treatment study over the time period of several months would be helpful to determine whether subjects are consistent in their response to treatment and to determine whether there may be additive effects of additional treatments. A study of repeated treatment design would help to identify the optimal parameters.

In this study, there were only 6 subjects with severe peripheral vascular disease compared to 41 in the minimal subgroup and 85 in the moderate subgroup. The possibility exists that these subjects would not be able to benefit from this treatment due to limited blood inflow and outflow (Rutherford, 1995); however, this small sample is not sufficient from which to base future recommendations for research or treatment.

Laboratory Tests

Other laboratory analyses could add important information. Among these are radioimmunoassay for various vasoactive substances (Kaada, et al., 1982, 1983, 1984), especially VIP.

Implications of This Study for Nursing

Electrical stimulation is still in its infancy as an adjunctive therapy to wound healing, and optimum electrical dose and delivery are still to be identified. This study
has contributed to clarifying those by extensively describing the response of subjects with diabetes and foot problems, especially those at risk for foot ulceration. Problems in measurement and possible solutions have been identified. Areas for future research were identified.

It is important to acknowledge that only 132 of the targeted 160 subjects were obtained; however, a larger effect size than anticipated (+14mmHg) was seen in "responders" (-12 in "nonresponders"). A larger effect size would increase the power to detect a difference and reduce the sample size required. Statistical significance was found in relation to Hypothesis I; therefore, failure to detect a significant difference was not due to lack of power.

No complications from this treatment have been reported in the literature and from this treatment, other than the migraine headaches in response to treatment in Raynaud's patients post-sympathectomy (Kaada, et al., 1982), allergic dermatitis (Kjartansson & Lundeberg, 1990) and no complications occurred during this study related to ES treatment. Evidence suggests that this is a low risk treatment. The treatment is economical and no harm is done as long as the subjects meet safety criteria. Therefore, it is worth performing "test" treatments on volunteer subjects with non-healing wounds while monitoring their skin oxygen levels to see whether they might benefit from the treatment.
It is likely that no damage would be done and there is a 25% chance that the subject could benefit. Treatment is simple and subjects can be taught to perform the regimen at home (Lundeberg, Eriksson, & Malm, 1992).

This study examined the potential for ES as an adjunctive treatment for wound healing through identifying the effect on skin perfusion in diabetic subjects with neuropathy. About one-fourth of subjects (26%) were found to benefit from treatment.

If these short-time responses would lead to long-term increases in improved skin perfusion, ES offers the potential for providing a valuable adjunct to wound healing in difficult to heal wounds. Improving wound healing in 25% of subjects with diabetic foot ulcers might achieve a significant reduction in human suffering and healthcare costs.
References


Baker, L.L. (1995). Personal communication concerning the wave forms used in the 1987 and 1988 studies performed at Rancho Los Amigos Rehabilitation Hospital, Downey, CA.


University of California, San Francisco
Experimental Subjects Bill of Rights

The rights below are the rights of every person who is asked to be in a research study. As an experimental subject, I have the following rights:

1) To be told what the study is trying to find out.

2) To be told what will happen to me and whether any of the procedures, drugs, or devices is different from what would be used in standard practice.

3) To be told about the frequent and/or important risks, side effects, or discomforts of the things that will happen to me for research purposes.

4) To be told if I can expect any benefit from participating, and, if so, what the benefit might be.

5) To be told of the other choices I have and how they may be better or worse than being in the study.

6) To be allowed to ask any questions concerning the study both before agreeing to be involved and during the course of the study.

7) To be told what sort of medical treatment is available if any complications arise.

8) To refuse to participate at all or to change my mind about participation after the study is started. This decision will not affect my right to receive the care I would receive if I were not in the study.

9) To receive a copy of the signed and dated consent form.
10) To be free of pressure when considering whether I wish
to agree to be in study.

If I have other questions, I should ask the research or the
research assistant. In addition, I may contact the
Committee on Human Research, which is concerned with
the protection of volunteers in research projects. I may
reach the committee office by calling: (415)476-1814 from
8:00 a.m. to 5 p.m., Monday to Friday, or by writing to the
Committee on Human Research, Box 0962, University of
California, San Francisco, San Francisco, CA 94143.

Research Subject/Date
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO BE A RESEARCH SUBJECT
Electrical Stimulation and Diabetic Foot Skin Perfusion

A. PURPOSE AND BACKGROUND
Dr. Nancy Stotts, RN, and Darlene Gilcreast, RN, MSN from the UCSF School of Nursing, in conjunction with Dr. Morton Altman from California College of Podiatric Medicine and Dr. Kathryn Moss of the San Francisco Veterans Affairs Medical Center, are conducting a study to explore the effects of electrical stimulation on blood flow in the feet of diabetic patients. Because I am a diabetic at risk for foot problems, including foot ulcers, I am being asked to participate.

B. PROCEDURES
If I agree to be in this study, the following will happen:
1. My temperature will be taken, my blood pressure will be measured in my arm and toe, sensation in my foot will be evaluated with a nylon filament used to apply pressure and a vibration machine probe touched to the skin. The skin will not be broken.
2. I will have my wound (if I have one) traced on clear plastic for measurement, my blood will be drawn to test my blood glucose level, and blood flow to my foot will be measured with a sensor applied to my foot. The blood will be drawn by Darlene Gilcreast from a vein in my arm. The total amount of blood to be drawn is about 2 tablespoons.
3. Electrical stimulation will be applied to my foot using electrodes placed on the ball of the foot and the calf muscle with adhesive. The stimulation will last 30 minutes.
4. Every 15 minutes during the electrical stimulation and 15 and 30 minutes after the stimulation stops, blood flow to my foot will be measured with the sensor applied to my foot.
5/1/94
5. Participation in the study will take a total of about 2 hours and all study procedures will be done at the California College of Podiatric Medicine or the San Francisco Veterans Affairs Medical Center.

C. RISKS/DISCOMFORTS

1. Venipuncture: The risks of drawing blood include temporary discomfort from the needle stick, bruising, and rarely, infection.

2. Wound Measurement: There is no discomfort involved in having the wound examined. There is minimal discomfort from having the wound measured as the plastic merely touches the surface of the wound.

3. Confidentiality: Participation in research may involve loss of privacy. No individual identities will be used in any reports or publications resulting from this study.

4. Electrical Stimulation may cause tingling, and in some cases, slight discomfort. The level of stimulation will begin at zero and gradually turned up to see how I feel. If I feel discomfort, the level of electrical stimulation will be decreased immediately to eliminate the discomfort. I just need to tell Darlene Gilcreast and she will turn the stimulator down until I no longer feel discomfort.

D. TREATMENT AND COMPENSATION FOR INJURY

If I am injured as a result of being in this study, treatment will be available. The cost of such treatment may be covered by the University of California depending upon a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, I may call the office of the Committee for Human Research at (415) 476-1814.

5/1/94
E. BENEFITS
There is no benefit to me for participating in this study. It is hoped that the information gained from this study will help in the treatment of future diabetic patients at risk for or with foot ulcers.

F. ALTERNATIVES
If I chose not to participate in this study, I will receive the standard treatment for my condition prescribed by my doctor.

G. COSTS
I will not be charged for any of the study treatments or procedures. The costs of the laboratory tests, instrument to measure blood flow, measurement of wound size, and electrical stimulation will be paid by the study.

H. REIMBURSEMENT
In return for participating in the study and for my time, travel expenses will be reimbursed in the amount of $10. A check will be mailed to me approximately 6 weeks after my participation in the study has ended.

I. QUESTIONS
This study has been explained to me by Darlene Gilcreast, RN, MSN, and my questions were answered. If I have any other questions about the study, I may call Darlene Gilcreast at (415)387-0297 or pager 1-800-803-0543.

_________________________  _________________________
       Date                        Patient

5/1/94                        _______________________

                                    Person Obtaining Consent
Electrical Stimulation and Diabetic Foot Skin Perfusion
Subject Data Collection Form

Name: _______________________________ ID#: __________ Date: __________
Address: ___________________________________________________________________
Telephone: (____) __________ M F Age____ Caucasian__ Black__
Number of Yrs. Dx with Diabetes: ___
Medications: __________________________________________________________________
Concurrent Diseases

Ulcer No./Location: ___________ Ulcer Size: ________
Wagner Classification: _____ Description: ________________

Baseline Measurements: Humidity______ Barometric Pressure_______

Blood Pressures: Brachial (Sys/Dias): R____ Toe (Sys)R____ Ankle R:____
                L______ L____ L____
Toe/Brachial Index: R:____ L:____ Ankle Brachial Index: R:____ L:____
Apical Heart Rate:______ Oral Temperature:__________
SWMF Detection:________ Dermatome:____________
Aesthesiometry Detection: R:____/____ L:____/____

Measurements for Electrical Stimulation

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Time:</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensor Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TcpO₂: Chest/Foot</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>TcpCO₂: Chest/Foot</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>
APPENDIX B
Foot Diagram.

1 = Hallux
2 = second toe
3 = 3rd toe
4 = fourth toe
5 = fifth toe
6 = first metatarsal head
7 = second metatarsal head
8 = third metatarsal head
9 = fourth metatarsal head
10 = fifth metatarsal head
11 = arch of foot
12 = plantar aspect
13 = medial side
14 = lateral side
15 = dorsum
16 = heel
17 = medial malleolus
18 = lateral malleolus
19 = Achilles tendon
PILOT STUDY DATA:

Of 35 subjects (15 male, 20 females), means were: age, 66 years (SD 8.2); TcPO₂, 49 (SD 14.8); years with diabetes, 16 (SD 12); hemoglobin A₁c, 9.5 (SD 9.8). Ten had insensate feet. Nine subjects had a rise in TcPO₂ (mean 8mmHg, SD 1.3), two subjects showed no change, and 24 subjects had a decreased TcPO₂ (mean -15, SD 8.3). After 30 minutes of recovery, the mean TcPO₂ was 40 (SD 17.6) and not significantly different (p<.05) than after stimulation. When responders (those with increased TcPO₂) are compared with nonresponders (those with decreased TcPO₂), responders tended to be older (mean 74 years) than nonresponders (mean 63 years) and have a lower toe-brachial index (mean right 0.70, left 0.73) than nonresponders (mean right 0.80, left 0.82). These preliminary data indicate that some subjects respond to electrical stimulation by increased blood flow and that response continues through 30 minutes of recovery.