Effects of Supplemental Immunonutrition on Healing of Chronic Non-Healing Lower Extremity Ulcers in Diabetic Patients: A Pilot Study

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1) Background/Justification for Study

Diabetes mellitus is one of the most prevalent chronic diseases in the world and its incidence is expected to increase over the next 20 years. In 2018, approximately 34.2 million Americans (~10%) were affected with the disease. By 2050, the number of Americans diagnosed with diabetes is expected to exceed 48 million.[1,2] Diabetes mellitus represents a group of metabolic disorders characterized by hyperglycemia and changes in the metabolism of other macronutrients. There are three main types of diabetes mellitus: Type 1, Type 2, and Gestational. Type 1 diabetics are unable to make insulin and are considered insulin-dependent, Type 2 diabetics are insensitive to insulin, and gestational diabetics develop insulin insensitivity, transiently, during or shortly after pregnancy.[2] The etiology of disease may vary between types, yet the resulting pathophysiology is the same: poor peripheral blood flow, decreased cellular response at the injury site, elevated glucose levels, and poor nutrient transport.[3] In addition, due to these mechanisms, some patients develop progressive neurologic dysfunction, called peripheral neuropathy, which affects the most distal nerves first. The late effects of peripheral neuropathy can lead to an insensate foot that is prone to ulceration and subsequent infection.[4,5]

A common complication of diabetes-induced peripheral neuropathy is the development of diabetic foot ulcers (DFUs). While estimates vary, studies suggest that the average annual incidence of DFUs among diabetic patients may be as high as 6%.[6,7] DFUs can have a devastating effect on patients' lives, often leading to debilitating injuries, amputations, and even death. It is estimated that foot ulcers are one of the major sources for hospitalization among diabetic patients and precedes up to 84% of lower limb amputations in these patients. In addition to the drastic impact to affected patients, DFUs represent a significant economic burden to the health care system and payers. Estimates place the total cost for management of diabetic foot ulcers between $9-$13 billion in addition to treatment and management of diabetes mellitus alone.[6]

Conventional treatment of DFUs including shoe wear modification, self-monitoring, local wound care, brace and shoe offloading, and surgical intervention. More sophisticated treatments including bioengineered cellular technologies and hyperbaric oxygen therapy have been used with limited success. However, these modalities are time consuming, burdensome, costly, and often do not treat the root cause of the problem. Additionally, even with proper care, wounds can take a significantly longer time to heal in diabetic patients. This is due to the altered physiology which does not allow for the proper nutrients and factors to mobilize to the site of injury, leaving wounds at high risk for infection and possible amputation.[6]

In contrast to traditional treatment modalities, nutrition therapy has recently been shown to aide in healing of chronic wounds by providing essential nutrients which were not previously present in necessary amounts. Nutrition therapy has proven useful in modulating inflammation and the immune response, optimizing glucose control, and attenuating the hypermetabolic response to injuries, ultimately improving healing and recovery.[8] Multiple studies have produced strong evidence that nutrition therapy reduces the incidence of complications including poor healing, infections, and development of hypertrophic scars.[9] Thus, supplemental immunonutrition may offer a viable, low cost, rapidly scalable, and widely available approach to enhance the body's ability to heal itself.

Common immunonutrients include arginine, Omega-3 fatty acids, and vitamin C, each of which offer unique properties to enhance self-healing. Arginine is an amino acid that is important for protein metabolism and nitric oxide production; during times of extreme physiologic stress or malnutrition, it is considered an essential amino acid. Studies have suggested that arginine supplementation should be implemented to maintain a proper nitrogen balance for regulation of protein synthesis and to regulate nitric oxide levels for appropriate cellular signaling.[8,10] Most commonly found in fish, Omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are long chain polyunsaturated fats not produced in the human body, making their dietary intake essential. In addition to their well-known cardioprotective effects, Omega-3s compete with arachidonic acid as a substrate for cyclooxygenase and therefore serve as regulators of immune function.[11] Vitamin C is a water-soluble vitamin and a potent antioxidant and has an essential role in collagen biosynthesis. The antioxidant properties of vitamin C help to decrease the release of reactive oxygen and nitrogen species, contributing to the attenuation of a cell's inflammatory state. Vitamin C is also a key cofactor in collagen cross-linking, the process that gives collagen its strength.[12]

Various studies have been conducted with the described nutrients to evaluate their effects in specific patient populations. A multicenter, randomized, controlled, blinded trial evaluated the effect of a nutritional formula enriched with arginine, zinc, and antioxidants in 200 adult malnourished patients with stage 2-4 pressure ulcers. Improved wound healing was observed in patients who received up to 6 grams of arginine daily for 8 weeks compared to the control group.[13] A 2012 study examining various doses of arginine supplementation on wound healing demonstrated that patients with stage 2-4 pressure ulcers receiving either 4.5 or 9 grams of a daily arginine supplement achieved a 2-fold improvement on healing time compared to the control group, illustrating that a lower dose of arginine supplementation is sufficient to elicit clinical benefit.[14] A combination of arginine and vitamin C was shown to be effective for wound healing in a 2005 study involving inpatients with stage 2-4 pressure ulcers. Patients who were randomized to receive a standard diet plus two high-protein/energy supplements containing additional arginine (9 grams), vitamin C (500 mg), and zinc (30 mg) demonstrated a clinically significant improvement in pressure ulcer healing compared to a control group who did not receive the additional supplementation.[15] Additionally, vitamin C (500 mg), in conjunction with vitamin E, has been shown to reverse oxidative stress-induced morphological changes in RBCs, improve anemia, and reduce blood flow to the ulcer area in a randomized trial including 60 adult patients with diabetic foot ulcers following 12 weeks of daily supplementation.[16] The effects of omega-3 fatty acids, specifically 10 grams per day of EPA for 4 weeks, were illustrated in a 2012 study in which patients with pressure ulcers stage 2 or higher were randomized to receive a nutritional formula enriched with fish oil and other antioxidants versus an isonitrogenous nutritional formula. Patients receiving the formula enriched with fish oil and other antioxidants showed decreased progression of pressure ulcers and a decrease in concentrations of C-reactive protein.[17]

While several studies have been conducted on the beneficial effects of L-arginine, omega-3 fatty acids, and vitamin C over a range of subjects and disease conditions, there has yet to be a study involving a combination of these specific nutrients to help better heal chronic wounds in the diabetic population.[13-18] This study is designed to address this knowledge gap by assessing and comparing the effectiveness of oral immunonutrition versus standard of care wound therapies on diabetic patients, using nutrients that have already been proven to improve wound healing in other

patient populations. If proven to have significant effects, this study will provide an added strategy that may decrease rates of infections, wound complication, amputations, and possibly death in the diabetic population. Additionally, this treatment strategy may decrease resource utilization and cost of care for the treatment of DFUs. Positive results, given the wide availability of these nutrients, would be rapidly translatable, scalable, and deployable in other healthcare centers around the country.

2) Objectives / Research Aims

Chronic, non-healing foot ulcers are a common complication of diabetes that can threaten loss of limbs, shorten life expectancy, and require resource-intensive treatment. Patients who exhibit these late effects of diabetes are often medically complex and have not only a shortened life expectancy but also a lower quality of life than their non-ulcer peers.[2,3] Despite the prevalence of obesity in these patients, many exhibit metabolic signs of malnutrition, malabsorption, or both. Even when direct treatment for the ulcer is ideal, if the patient does not have the metabolic building blocks or the correct macronutrient healing environment, then treatment will be futile.

To this end, we believe focused immunonutrition supplementation consisting of L-Arginine, Omega-3 fatty acids, and Vitamin C combined with quality wound care will lead to an improvement in treatment success, satisfaction, patient reported outcome measures, and limb salvage.

Specific Aim 1: To compare the healing process of diabetic patients with chronic, non-healing, lower-extremity wounds receiving oral immunonutrition supplementation to patients receiving traditional care.

a. This will be addressed by performing a prospective, randomized pilot study including 30 diabetic patients with chronic lower extremity wounds, Wagner stages 1-2. Fifteen patients will receive oral immunonutrition supplementation (2 grams omega-3 fatty acids + 500 mg vitamin C + 4.5 grams L-arginine per day) in addition to current standard of care and 15 patients will receive the current standard of care only. Study participation will continue for 6 weeks.

b. Effect of wound healing will be assessed utilizing the Pressure Ulcer Scale for Healing (PUSH) Tool 3.0 which scores ulcers based on size (cm^2), exudate amount, and tissue type present. Plantar pressure profiles will be recorded using a Tekscan Pressure Mat and plantar weight-bearing foot images will be recorded. Additionally, patient charts will be monitored over the course of the study to identify rate of infection, surgical intervention, and amputation and for one year following completion of the study to assess ulcer recurrence rates.

Specific Aim 2: To assess compliance and patient satisfaction with an oral immunonutrition supplementation regimen in diabetic patients with chronic, non-healing, lower-extremity wounds.

a. Patients randomized to receive the oral immunonutrition supplementation in addition to standard of care will complete a daily compliance log documenting their intake of the provided supplements.
b. All patients will complete a weekly survey rating their pain score (VAS) and any symptoms/side effects experienced during their study participation.
c. At the final visit, subjects randomized to receive the oral immunonutrition supplementation will complete a satisfaction survey in which they detail their thoughts on their treatment plan.

3) Setting

Subjects visiting the Prisma Health Wound Healing Center – Richland will be recruited to participate in this study.

4) Resources Available

Documentation will be comprised of demographic information, medical histories, rates of infection/surgical intervention/amputation included in patient electronic medical records. Additionally, patient reported outcome measurements and compliance logs provided by the participants and PUSH Tool 3.0 assessments recorded by the provider will be analyzed.

A fellowship trained foot and ankle surgeon will lead the research team.

The research team will be comprised of practicing orthopaedic surgeons, wound center physicians and staff, research staff, orthopedic residents, and medical students.

5) Prior Approvals

None

6) Study Design

a. Recruitment Methods

Study participants will be recruited from the pool of patients visiting the Prisma Health Wound Healing Center – Richland. A research coordinator within the Prisma Health Orthopedics department will review weekly clinic schedules of participating providers at the Wound Healing Center to flag patients who may qualify for the study and are scheduled for an office visit. The provider who sees the patient will inform the patient of the research study during their office visit, screen to ensure eligibility criteria are met, and, if appropriate, consent the patient to participate in the study.

b. Inclusion and Exclusion Criteria

Inclusion Criteria:

- ≥ 18 years of age at the time of informed consent
- Toe blood pressure (TBP) >40 mmHg
- Hemoglobin A1c <10% (measured within the previous 6 months). If a patient has not had an A1c within 6 months of study enrollment, one will be performed at the time of enrollment to confirm study eligibility.
- Diagnosis of diabetes mellitus
- Presence of at least one new chronic, non-healing (present for ≥4 weeks), lower extremity wound (Wagner stage 1-2)
- Documented lipid panel within 3 months of study enrollment. If a patient has not had a lipid panel within 3 months of study enrollment, one will be performed at the time of enrollment to confirm study eligibility.
- Documented CMP within 3 months of study enrollment. If a patient has not had a CMP within 3 months of study enrollment, one will be performed at the time of enrollment to confirm study eligibility.
- Documented INR/PT within 3 months of study enrollment. If a patient has not had an INR/PT within 3 months of study enrollment, one will be performed at the time of enrollment to confirm study eligibility.
- Documented normal ECG within 3 months of study enrollment. If a patient has not had an ECG within 3 months of study enrollment, one will be performed at the time of enrollment to confirm study eligibility.
- Receiving standard of care defined as sharps debridement, appropriate dressing, and offloading.

Exclusion Criteria:

- Allergy to fish
- History of hypersensitivity reactions to LOVAZA or any of its components
- Current smoker
- Currently taking any OTC supplements containing Omega-3 fatty acids, L-Arginine, or Vitamin C, or antibiotics
- Presence of at least one of the following diseases or conditions:
    - End stage renal disease as defined by patients who have been diagnosed with Stage 4 kidney disease and are not on hemodialysis or who are on hemodialysis with a GFR consistently < 15L/min and BUN > 60.
    - Hepatic impairment as defined by patients with a Child-Turcotte-Pugh score ≥2 based on a CMP/INR/PT performed within 3 months of study enrollment.
    - Paroxysmal or persistent atrial fibrillation
    - Untreated deep bone infection (osteomyelitis)
    - Currently pregnant or breastfeeding
- Women of child-bearing potential
- Prisoners and other institutionalized individuals
- Any patients who have a legal representative to make medical decisions on their behalf or any individuals who are otherwise deemed as medically incompetent.

c. Local Number of Subjects

This is a pilot study which aims to enroll 30 patients locally, 15 per arm.

d. Study Timelines

We expect this study to be completed within one year of receiving IRB approval. We aim to achieve complete enrollment of 30 participants in a 6- to 9-month period. Following the completion of participant enrollment and data collection, the obtained results will be reviewed and statistically analyzed over a 3-month period. Finally, a manuscript detailing the study results will be written and submitted for publication.

e. Study Endpoints

Primary Outcome: Complete wound closure of a chronic, non-healing wound at 6 weeks.

a. Complete wound closure will be defined as skin reepithelialization without drainage or dressing requirements confirmed at two consecutive study visits 2 weeks apart. Incidence of complete wound closure in the control group will be compared to that of the treatment group.

Secondary Outcome: Rate of wound closure over 6-week study period.

a. PUSH Tool 3.0 scores and clinical photos will be compared between the control group and treatment group to evaluate the rate of wound closure over the course of the 6-week study period.

Tertiary Outcome: Compliance and patient satisfaction with immunonutrition supplementation regimen.

a. Patient satisfaction surveys and compliance logs will be evaluated to assess feasibility of implementing an immunonutrition regimen into standard wound care.

f. Procedures Involved

1. A research coordinator within the Prisma Health Orthopedics department will review weekly clinic schedules of participating providers at the Wound Healing Center to flag patients who may qualify for the study and are scheduled for an upcoming office visit. The provider scheduled to see the patient will be informed of potential subjects for inclusion.

2. At the office visit, the provider seeing the patient will inform the patient of the research study, screen to ensure eligibility criteria are met, and, if appropriate, consent the patient to participate in the study. The study will be thoroughly explained to the subject and all questions/concerns will be addressed. Research team members will be available to assist if the patient agrees to participate with the provider.

a. Note: If the patient has not had an ECG (within 3 months of study enrollment), a lipid panel (within 3 months of study enrollment), a CMP (within 3 months of study enrollment, an INR/PT (within 3 months of study enrollment), and/or an A1c lab (within 6 months of study enrollment) these will be performed to confirm normal parameters prior to continuation of study activities. If any labs or ECG are required, results will be reviewed by the provider and the patient will resume study activities at their next scheduled visit.

3. The provider will document wound characteristics via the PUSH Tool 3.0 (Appendix 2). Plantar pressure profile readings will be obtained using a Tekscan Pressure Mat and clinical plantar weight-bearing images will be captured. The provider will verify and document concomitant medications in the patient's chart. The provider will visualize and evaluate any additional wounds that are present and record characteristics in the patient's chart.

4. The patient will be randomized to the control group or treatment group via a pre-defined randomization table in which subject IDs are already assigned as belonging to either group.
   a. If randomized to the control group (SOC only), patients will receive standard of care defined as sharps debridement, appropriate dressing, and offloading. If randomized to the treatment group (SOC + Immunonutrition), subjects will receive standard of care defined as sharps debridement, appropriate dressing, and offloading in addition to aa 6-week supply of Omega-3 fatty acids (soft gel form, 2 pills per day, total of 1.68 grams) and L-Arginine/Vitamin C (powder form, to be mixed with 8 oz liquid, 1 scoop once daily, total of 4.5 grams L-arginine and 500 mg vitamin C). Subjects will also be provided a compliance log with detailed instructions for taking and tracking supplements.

5. Patients will be scheduled for biweekly wound checks with their provider (weeks 2, 4, 6). Wound characteristics will be documented at each follow up visit using the PUSH Tool 3.0, images of the wound will be obtained, and an interval history and physical exam will be documented. The provider will verify and document concomitant medications in the patient's chart. The provider will visualize and evaluate any additional wounds that are present (new or present at time of enrollment) and record characteristics in the patient's chart. Patients will complete a questionnaire detailing their pain level (VAS), symptoms experienced during the treatment phase, and any unscheduled visits to emergency departments/urgent care centers. This will be completed via a paper survey in clinic during their office visit (Appendix 4). On weeks where an office visit is not scheduled (weeks 1, 3, 5), a research coordinator will call the patient and collect survey responses (Appendix 3).

6. At the final office visit (week 6), plantar pressure profiles will be recorded via the Tekscan Pressure Mat, and clinical plantar weight-bearing images will be captured. Patients randomized to receive the oral immunonutrition supplementation will complete an additional survey detailing their satisfaction with the treatment plan and any additional thoughts they would like to share (Appendix 5) and return the completed compliance log

(Appendix 6). Patients in the treatment arm will have CMP and lipid panel labs drawn at this timepoint or within 3 months of study completion.

7. A follow-up safety visit will be conducted by the provider for patients randomized to the treatment group 6-8 weeks after the week 6 office visit. This visit is more than 5 half-lives after supplementation discontinuation and will allow for capture of adverse events that may occur after completion of dosing. The wound will be visualized during this visit. Adverse events and wound details will be documented in the patient's chart. This visit will be conducted in-office if the patient is already scheduled to see their provider within the visit window. If the patient is not scheduled to see their provider, this visit may be conducted virtually through EPIC.

8. Patient charts will be monitored throughout the patient's participation in the study for any signs of infection, unscheduled visits, calls to the provider, and/or onset of severe adverse events. Patient charts will continue to be monitored for one year following the completion of the study to assess ulcer recurrence rates.

The schedule of events is outlined below and is pictured in Appendix 1:

Visit 0: Previously scheduled office visit:

- Provider reviews study with patient, confirms that inclusion/exclusion criteria are met, and obtains informed consent.
  a. Note: If any labs or ECG are required, results will be reviewed by the provider and the patient will resume study activities at their next scheduled visit.
- Provider documents characteristics of wound via PUSH Tool 3.0, obtains plantar pressure profile reading, and captures clinical photos.
- Provider verifies and documents concomitant medications in the patient's chart.
- Provider visualizes and evaluates any additional wounds that are present and records characteristics in the patient's chart.
- Patient is randomized to receive either oral immunonutrition supplementation with standard of care or standard of care alone.
- Patient is provided oral immunonutrition supplements and compliance log, if appropriate.

Phone Call 1: (1 Week Call, first call after completing 7 days of oral immunonutrition):

- Patient completes questionnaire over phone with research coordinator (VAS, experienced symptoms since enrollment, review of supplement compliance).

Visit 1: 2 Week Follow-up (First visit after completing 14 days of oral immunonutrition):

- Provider documents characteristics of wound via PUSH Tool 3.0, captures clinical photos, obtains interval history, performs interval physical exam, and verifies and documents concomitant medications in the patient's chart.
- Provider visualizes and evaluates any additional wounds that are present and records characteristics in the patient's chart.
- Patient completes questionnaire in clinic (VAS, experienced symptoms since enrollment, review of supplement compliance).

Phone Call 2: (3 Week Call, second call after completing 21 days of oral Immunonutrition):

- Patient completes questionnaire over phone with research coordinator (VAS, experienced symptoms since enrollment, review of supplement compliance).

Visit 2: 4 Week Follow-up (Second visit after completing 28 days of oral immunonutrition):

- Provider documents characteristics of wound via PUSH Tool 3.0, captures clinical photos, obtains interval history, performs interval physical exam, and verifies and documents concomitant medications.
- Provider visualizes and evaluates any additional wounds that are present and records characteristics in the patient's chart.
- Patient completes questionnaire in clinic (VAS, experienced symptoms since enrollment, review of supplement compliance).

Phone Call 3: (5 Week Call, third call after completing 36 days of oral immunonutrition):

- Patient completes questionnaire over phone with research coordinator (VAS, experienced symptoms since enrollment, review of supplement compliance).

Visit 3: 6 Week Follow-up/Completion Visit (Third visit after completing 42 days of oral immunonutrition):

- Provider documents characteristics of wound via PUSH Tool 3.0, obtains plantar pressure profile reading, captures clinical photos, obtains interval history, performs interval physical exam, and verifies and documents concomitant medications.
- Provider visualizes and evaluates any additional wounds that are present and records characteristics in the patient's chart.
- Patient completes questionnaire in clinic (VAS, experienced symptoms since enrollment, review of supplement compliance).
- Patient completes satisfaction survey detailing their experience with supplementation (if applicable).
- Patient returns compliance log (if applicable).
- Provider orders CMP and lipid panel labs to be drawn at this timepoint or within 3 months of study completion.

Phone Call 4: (Final Safety Follow-up, 6-8 weeks after completing oral immunonutrition, if randomized to treatment group):

- Provider reviews and records adverse events experienced after supplement discontinuation and visualizes wound.

g. Data and Specimen Banking

Data collected from this study will be stored in the Prisma Health REDCap. Images will be stored on a password-protected network. No specimens will be collected as part of this research study. Only authorized research members of this study can access this information.

h. Statistical Analysis

Summary statistics will be used to summarize measurements and assessed for unadjusted differences across treatment condition. Student's t-tests will be used for continuous measures, exact binomial tests for dichotomous measures, and chi-square tests for categorical measures.

The main outcome of interest is the proportion of wound closed at the end of 6 weeks which we will model in a generalized linear models framework[15]

$$E[g(y_i)] = \beta_0 + Trt_i\beta_1 + Z_i\gamma$$

where y_i is the outcome measure of interest of the ith participant, Trt_i is an indicator variable of whether the participant was assigned to the treatment condition, Z_i is a vector of covariates for which we will adjust the model (i.e., initial wound size, diabetes etiology, sex, race, and age), and γ is a vector of regression parameters. We have written the outcome in terms of a link function (y_i) to emphasize that we will estimate a linear regression model (identity function) as well as a zero-one augmented beta regression model for which we will choose from among several link functions (logit, log, log-log, and complementary log-log). Anticipating that we will have outcomes of exactly 0% or 100% reduction of the wound size, we will model the transformed outcome[16]

$$y_i^* = \frac{99y + 0.5}{100}$$

Our focus is on the null hypothesis $H_0: \beta_1 = 0$. We will evaluate this null hypothesis using a Wald test of the parameter based on an appropriate variance estimate – chosen from model-based, sandwich, or sandwich based on an evaluation of the implied dispersion properties of the model-based variance estimator.

Other secondary outcome variables to be modeled include the time to complete healing (Cox proportional hazards regression), rate of closure (Cox proportional hazards regression), presence of infection (binary regression), and need for surgery (binary regression). Cox proportional hazards models will be used to interpret the relative hazards between treatment conditions. The proportional hazards assumption will be checeked using Schoenfeld residuals and accelerated failure time (parametric) regression models will also be evaluated. Binary outcomes will be modeled using various link functions (logit and log). Exponentiated coefficients for logit-link models will be interpreted as odds ratios, and exponentiated coefficients from log-link models will be interpreted as risk ratios. Binary outcomes may be relatively rare, and so we will also

estimated binary models using exact statistics. In addition, we will investigate bias through estimation of bias-corrected models.[17]

i. Data Management

Data will be maintained in the Prisma Health REDCap and on a secure server with password protection. Study data and signed consent documents will be stored in a locked office in Prisma Health Orthopedics. Only authorized research members of this study can access this information. The researchers involved in this study commit to handle all information with care and to protect research subjects' identities. Study data will be de-identified after data collection and before sending to the biostatistician for data analysis. No PHI data will be published or presented.

Key data points include:

- BMI
- Demographic info (age, gender, race, ethnicity)
- ABI
- Hb-A1c
- Diabetes mellitus diagnosis
- Wound characteristics such as size, exudate amount, and tissue type (as documented by PUSH Tool 3.0) and Wagner stage
- Lab results (CMP, lipid panel, INR/PT)
- ECG results
- Plantar pressure profile readings
- Clinical photos of wound
- Current medications (throughout study participation)
- Treatment received as standard of care
- Rate of infection
- Rate of surgical intervention
- Rate of amputation
- Rate of ulcer recurrence
- Comorbidities
- VAS scores
- Compliance with supplementation
- Supplementation side effects

j. Confidentiality

Signed consent documents will be stored in a locked office at Prisma Health Orthopedics. Data will be maintained in the Prisma Health REDCap and on a secure server with password protection. Only authorized research members of this study can access this information. The researchers involved in this study commit to handle all information with care and to protect research subjects' identities. Study data will be de-identified after data collection and before sending to a biostatistician for data analysis. No PHI data will be published or presented.

k. Withdrawal of Subjects

Subjects may withdraw from the study at any time. All data recorded to that point may be kept and used for data analysis.

The provider has the authority to remove any subject from the study at his/her discretion, at which time the provider will inform the subject of withdraw. Circumstances for withdraw include, but are not limited to:

a. Failure to meet inclusion/exclusion criteria (labs, A1c, ECG parameters)
b. Lack of compliance with supplementation
b. Rapid progression of wound(s) which is expected to result in surgery during the 6-week study period
c. Severe adverse effects which are possibly related to supplementation

All data recorded to the point of withdraw may be kept and used for data analysis.

l. Provisions to Monitor the Data to Ensure the Safety of Subjects

Data will be maintained on the Prisma Health REDCap and a secure server with password protection. Study data and signed consent documents will be stored in a locked office in Prisma Health Orthopedics. Only authorized research members of this study can access this information. The researchers involved in this study commit to handle all information with care and to protect research subjects' identities.

7) Risks to Subjects

Potential risks to subjects include adverse reactions to the oral immunonutrition supplementation:

<u>Omega-3 fatty acids (LOVAZA), 1.68 grams/day:</u>
The FDA states that ≤3.0 grams/day of EPA + DHA is safe for humans. The daily supplementation provided to patients per this protocol is 1.68 grams/day (0.93 grams/day EPA + 0.75 grams/day DHA), well below the tolerable threshold.
Possible side effects associated with omega-3 fatty acid supplementation (LOVAZA) include eructation, dyspepsia, taste perversion, constipation, gastrointestinal disorder, vomiting, increased ALT, increased AST, pruritus, rash, hemorrhagic diathesis, urticaria.

<u>Vitamin C, 500 mg/day:</u>
The upper limit suggested for daily vitamin C intake is 2,000 mg/day. The daily supplementation provided to patients per this protocol is 500 mg/day, well below the upper threshold.
Possible side effects associated with vitamin C supplementation include diarrhea, nausea, vomiting, heartburn, abdominal cramps, headache, insomnia.

L-Arginine, 4.5 grams/day:
There is no recommended daily amount established for arginine supplementation. The observed safe level for oral administration is 9 grams/day and levels used in research vary from 2 to 30 grams/day. The daily supplementation provided to patients per this protocol is 4.5 grams/day, well below the upper threshold.
Possible side effects associated with L-arginine supplementation include abdominal pain, nausea, diarrhea, dyspepsia, palpitations, headache, numbness, bitter taste, and hypotension.

Noted above are the known side effects of the individual supplements. However, this specific combination of supplements has not been studied together in humans and therefore, the risks are unknown.

8) Potential Benefits to Subjects

Patients in either treatment arm may benefit from increased monitoring by research staff with no additional expense to the patient. Research staff will be in constant contact with providers, meaning that providers will be alerted of changes in the patient's medical status potentially sooner than if the patient was not enrolled in the study.

Patients who are randomized to receive the oral immunonutrition supplementation may potentially benefit by receiving a new regimen of medical care for diabetic foot ulcers in addition to standard of care.

Indirectly, patients may benefit by providing new information for medical professionals to better treat similar patients in the future.

9) Safety Monitoring Plan

Laboratory Monitoring:
Periodic laboratory and ECG monitoring will be performed to assess safety of the oral supplement as detailed below:

Lipid panel monitoring: patients are eligible for study participation if they have had a lipid panel performed within 3 months of study enrollment. An additional lipid panel will be performed at study conclusion (6-week timepoint or within 3 months of study completion) for patients enrolled in the treatment arm.

Blood glucose monitoring: blood glucose is routinely monitored in diabetic patients at our institution. This data will be reviewed in the patients' charts as they become available, and the investigators will be alerted to any abnormalities.

Serum chemistry parameters and hepatic enzymes: patients are eligible for study participation if they have had a CMP performed within 3 months of study enrollment. An additional CMP will be performed at study conclusion (6-week timepoint or within 3 months of study completion) for patients enrolled in the treatment arm. Patients will have an INR/PT

performed at the time of study enrolled (if not within the past 3 months) to confirm no hepatic impairment.

Continuous Patient Monitoring:
Patients enrolled in this study will be flagged in EPIC as a participant in a research study. If a patient presents to an Emergency Department and/or is admitted to a Prisma Health hospital, the study team will be notified via EPIC. The patient's chart will be reviewed to determine the cause of the admission and promptly reported to the study investigators.

Adverse Event (AE) and Serious Adverse Event (SAE) Reporting:
Adverse events will be recorded at in-office visits, via study coordinator phone calls, and through manual chart review. All AEs will be reported to the wound care provider, if they are not already aware of the event. Serious adverse events will be immediately reported to all study investigators.

Patients who experience an adverse event (AE) assessed as ≥ Grade 3 (and ≥ Grade 2 for bone marrow and cardiovascular events) per NCI-CTCAE version 5.0[19] will discontinue use of the oral supplement, if in the treatment arm. These subjects will continue to be monitored until the AE resolves or stabilizes. AEs and SAEs will be promptly reported to the IRB and FDA, as required.

Adverse Event/Outcomes Scheduled Monitoring:
An independent Data Safety Committee has been assembled for scheduled monitoring of adverse events. The committee is comprised of:

Nicholas Strasser, MD
Assistant Professor, Department of Orthopedic Surgery
Foot and Ankle Surgeon
Vanderbilt University Medical Center

Samuel E. Ford, MD
Foot and Ankle Surgeon
OrthoCarolina

William Huntington, MD
Foot and Ankle Surgeon
Prisma Health - Upstate
Steadman Hawkins Clinic of the Carolinas

Following enrollment of 5 subjects to the study (17%), the Data Safety Committee will meet to review data collected to date, including an in-depth review of all recorded AEs and SAEs. The Data Safety Committee will make a determination on the continuation of the study at this point. If the study is allowed to proceed, the Data Safety Committee will continue to meet following the enrollment of each set of 5 patients to repeat this process. If the Data Safety Committee determines that the study should not proceed, all study activities will be

immediately halted. Patients in the treatment arm will be instructed to immediately discontinue the immunonutrition supplements.

Plan for management of unplanned and/or emergent surgery:

If a wound of a patient enrolled in the treatment arm is progressing more rapidly than expected, as determined by the wound care provider, the provider will withdraw the patient and instruct them to discontinue supplementation immediately in preparation for surgery. Additionally, all patients will be flagged as participating in this research study in EPIC with details on the supplements involved. There will be an explicit note that the PI of the study should be contacted prior to any surgical procedure to discuss the timing of the surgery and discontinuation of the supplements. If it is not possible for the patient to discontinue the supplementation 2 weeks prior to surgery, all relevant parties (PI, surgeon, anesthesia) will be notified of the patient's involvement in the study and discuss additional potential risks of surgery and a patient-specific strategy for management of these risks. The study coordinator will review the charts of patients included in the supplement arm daily and will facilitate this communication between parties, if necessary.

10) Provisions to Protect the Privacy Interests of Subjects

Members of the research team will have access to data contained in medical records and data provided by the patients. Only authorized members of the research team will be able to access this information. The research study will be thoroughly explained to potential subjects in a private manner. Consenting will be done also in a private setting, away from other subjects.

11) Compensation for Research-Related Injury

There is no compensation for research-related injury in this study.

12) Economic Burden to Subjects

There will be no additional economic burden to subjects outside of the typical costs for treatment of chronic, non-healing, lower extremity wounds in the diabetic population.

Patients will attend bi-weekly visits with their provider, which is the current standard of care for patients meeting eligibility requirements of this study and is billable to insurance.

Study funds will cover an ECG, A1c lab, CMP, INR/PT, and/or lipid panel lab if needed at the time of enrollment.

The oral immunonutrition supplements will be covered by study funds and provided to subjects free of charge. Study funds will cover a CMP and a lipid panel (at 6-week timepoint or within 3 months of study completion) for study participants in the treatment arm.

If the final safety follow-up visit is an additional visit solely for the purposes of participating in the research study, then study funds will cover the cost of this visit.

13) Consent Process

Subjects who meet eligibility criteria will be consented during their previously scheduled office visit by a participating provider. The study will be thoroughly explained to the subject and all questions/concerns will be addressed. Research team members will be available to assist if the patient agrees to participate with the surgeon. Signed consent documents will be stored in a locked office within Prisma Health Orthopedics and a copy will be uploaded to the subject's electronic medical record.

Subjects must speak English and understand instructions. Subjects that do not speak English will not be enrolled in this study. We will follow the IRB Written Documentation of Consent.

14) Process to Document Consent in Writing

We will follow the IRB Written Documentation of Consent.

15) References

1. National Diabetes Statistics Report, 2020. CDC. https://www.cdc.gov/diabetes/library/features/diabetes-stat-report.html
2. Deshpande, Anjali D et al. "Epidemiology of diabetes and diabetes-related complications." *Physical therapy* vol. 88,11 (2008): 1254-64. doi:10.2522/ptj.20080020
3. Allen L, Powell-Cope G, Mbah A, Bulat T, Njoh E. A Retrospective Review of Adverse Events Related to Diabetic Foot Ulcers. Ostomy Wound Manage. 2017 Jun;63(6):30-33. PMID: 28657897.
4. Corriere M, Rooparinesingh N, Kalyani RR. Epidemiology of diabetes and diabetes complications in the elderly: an emerging public health burden. *Curr Diab Rep*. 2013;13(6):805-813. doi:10.1007/s11892-013-0425-5
5. Hicks CW, Selvin E. Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. *Curr Diab Rep*. 2019;19(10):86. Published 2019 Aug 27. doi:10.1007/s11892-019-1212-8
6. Rice, J Bradford et al. "Burden of diabetic foot ulcers for medicare and private insurers." *Diabetes care* vol. 37,3 (2014): 651-8. doi:10.2337/dc13-2176
7. Margolis DJ, Malay DS, Hoffstad OJ, et al. Incidence of diabetic foot ulcer and lower extremity amputation among Medicare beneficiaries, 2006 to 2008: Data Points #2. 2011 Feb 17. In: Data Points Publication Series [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2011-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK65149/
8. Evans, David C et al. "Nutrition optimization prior to surgery." *Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition* vol. 29,1 (2014): 10-21. doi:10.1177/0884533613517006
9. Alexander JW, Supp DM. Role of Arginine and Omega-3 Fatty Acids in Wound Healing and Infection. *Adv Wound Care (New Rochelle)*. 2014;3(11):682-690. doi:10.1089/wound.2013.0469
10. Zhang, Xiao-jun et al. "The anabolic effect of arginine on proteins in skin wound and muscle is independent of nitric oxide production." *Clinical nutrition (Edinburgh, Scotland)* vol. 27,4 (2008): 649-56. doi:10.1016/j.clnu.2008.01.006

11. Meredith, Dennis S., et al. “Erratum to: The effect of omega-3 fatty-Acid supplements on perioperative bleeding following posterior spinal arthrodesis.” European Spine Journal, vol. 21, no. 12, 12 May 2012, pp. 2664–2664., doi:10.1007/s00586-012-2412-y.
12. Traber MG, Stevens JF. Vitamins C and E: beneficial effects from a mechanistic perspective. *Free Radic Biol Med*. 2011;51(5):1000-1013. doi:10.1016/j.freeradbiomed.2011.05.017
13. Cereda, Emanuele et al. “A nutritional formula enriched with arginine, zinc, and antioxidants for the healing of pressure ulcers: a randomized trial.” *Annals of internal medicine* vol. 162,3 (2015): 167-74. doi:10.7326/M14-0696
14. Leigh, B et al. “The effect of different doses of an arginine-containing supplement on the healing of pressure ulcers.” *Journal of wound care* vol. 21,3 (2012): 150-6. doi:10.12968/jowc.2012.21.3.150
15. Desneves, Katherine J et al. “Treatment with supplementary arginine, vitamin C and zinc in patients with pressure ulcers: a randomised controlled trial.” *Clinical nutrition (Edinburgh, Scotland)* vol. 24,6 (2005): 979-87. doi:10.1016/j.clnu.2005.06.011
16. B., Vasanthi; T. J., Ainsy Goldlin. Efficacy of Vitamin C and Vitamin E as an add on therapy in diabetic foot ulcer and red blood cell morphology as a biomarker of oxidative stress: a randomized, open label, comparative pilot study. International Journal of Basic & Clinical Pharmacology, [S.l.], v. 7, n. 3, p. 548-555, feb. 2018. ISSN 2279-0780. Available at: <https://www.ijbcp.com/index.php/ijbcp/article/view/2329>. Date accessed: 12 aug. 2021. doi:http://dx.doi.org/10.18203/2319-2003.ijbcp20180672.
17. Theilla, M., Schwartz, B., Cohen, J., Shapiro, H., Anbar, R., & Singer, P. (2012). Impact of a nutritional formula enriched in fish oil and micronutrients on pressure ulcers in critical care patients. *American journal of critical care : an official publication, American Association of Critical-Care Nurses, 21 4*, e102-9 .
18. J.C.L. Neyens, E. Cereda, E.P. Meijer, C. Lindholm, J.M.G.A. Schols. Arginine-enriched oral nutritional supplementation in the treatment of pressure ulcers: A literature review. Wound Medicine, Volume 16, 2017, Pages 46-51, ISSN 2213-9095.
19. NCI-CTCAE v5.0 Available at: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Appendix 1: Schedule of Events

Visit 0: Previously scheduled office visit

- Provider reviews study with patient, confirms that inclusion/exclusion criteria are met, and obtains informed consent
  a. Note: If the patient has not had an ECG (within 3 months of study enrollment), a lipid panel (within 3 months of study enrollment), a CMP/INR/PT (within 3 months of study enrollment), and/or an A1c lab (within 6 months of study enrollment) these will be performed to confirm normal parameters prior to continuation of study activities. If any labs or ECG are required, results will be reviewed by the provider and the patient will resume study activities at their next scheduled visit.
- Provider documents characteristics of wound via PUSH Tool 3.0, obtains plantar pressure profile readings via Tekscan Pressure Mat, and captures clinical photos
- Provider verifies and documents concomitant medications.
- Provider visualizes and evaluates any additional wounds that are present and records characteristics in the patient's chart.
- Patient is randomized to receive either oral immunonutrition supplementation or standard care
- Patient is provided oral immunonutrition supplements and compliance log, if appropriate

Biweekly Office Visits

- Document characteristics of wound via PUSH Tool 3.0, obtain pressure profile reading (visits 0 and week 6), capture clinical photos, obtain interval history, perform interval physical exam, verify and document concomitant medications, visualize and record characteristics of additional wounds present.
- Patient completes questionnaire (VAS, experienced symptoms since enrollment, review of supplement compliance (if applicable))

Biweekly Phone Calls

- Patient completes questionnaire over phone with research coordinator (VAS, experienced symptoms since enrollment, review of supplement compliance (if applicable))

Phone Call #1
Week 1
After 7 days of oral immunonutrition

Office Visit 1
Week 2
After 14 days of oral immunonutrition

Phone Call #2
Week 3
After 21 days of oral immunonutrition

Office Visit 2
Week 4
After 28 days of oral immunonutrition

Phone Call #3
Week 5
After 35 days of oral immunonutrition

Office Visit 3
Week 6/Completion visit
After 42 days of oral immunonutrition
*Patients in treatment arm return compliance log, complete satisfaction survey, and have lipid panel and CMP labs drawn (or within 3 months of study completion) *

Final Safety Follow-up Visit
6-8 Weeks after Office Visit 3/discontinuation of supplement

Appendix 2: PUSH Tool 3.0

NATIONAL PRESSURE ULCER ADVISORY PANEL

Pressure Ulcer Scale for Healing (PUSH)
PUSH Tool 3.0

Patient Name_____ Patient ID# _____

Ulcer Location _____ Date _____

Directions:
Observe and measure the pressure ulcer. Categorize the ulcer with respect to surface area, exudate, and type of wound tissue. Record a sub-score for each of these ulcer characteristics. Add the sub-scores to obtain the total score. A comparison of total scores measured over time provides an indication of the improvement or deterioration in pressure ulcer healing.

LENGTH X WIDTH (in cm²)	0 0	1 < 0.3	2 0.3 – 0.6	3 0.7 – 1.0	4 1.1 – 2.0	5 2.1 – 3.0	Sub-score
		6 3.1 – 4.0	7 4.1 – 8.0	8 8.1 – 12.0	9 12.1 – 24.0	10 > 24.0	
EXUDATE AMOUNT	0 None	1 Light	2 Moderate	3 Heavy			Sub-score
TISSUE TYPE	0 Closed	1 Epithelial Tissue	2 Granulation Tissue	3 Slough	4 Necrotic Tissue		Sub-score
							TOTAL SCORE

Length x Width: Measure the greatest length (head to toe) and the greatest width (side to side) using a centimeter ruler. Multiply these two measurements (length x width) to obtain an estimate of surface area in square centimeters (cm²). Caveat: Do not guess! Always use a centimeter ruler and always use the same method each time the ulcer is measured.

Exudate Amount: Estimate the amount of exudate (drainage) present after removal of the dressing and before applying any topical agent to the ulcer. Estimate the exudate (drainage) as none, light, moderate, or heavy.

Tissue Type: This refers to the types of tissue that are present in the wound (ulcer) bed. Score as a "4" if there is any necrotic tissue present. Score as a "3" if there is any amount of slough present and necrotic tissue is absent. Score as a "2" if the wound is clean and contains granulation tissue. A superficial wound that is reepithelializing is scored as a "1". When the wound is closed, score as a "0".

- 4 – **Necrotic Tissue (Eschar):** black, brown, or tan tissue that adheres firmly to the wound bed or ulcer edges and may be either firmer or softer than surrounding skin.
- 3 – **Slough:** yellow or white tissue that adheres to the ulcer bed in strings or thick clumps, or is mucinous.
- 2 – **Granulation Tissue:** pink or beefy red tissue with a shiny, moist, granular appearance.
- 1 – **Epithelial Tissue:** for superficial ulcers, new pink or shiny tissue (skin) that grows in from the edges or as islands on the ulcer surface.
- 0 – **Closed/Resurfaced:** the wound is completely covered with epithelium (new skin).

NATIONAL PRESSURE ULCER ADVISORY PANEL

Pressure Ulcer Healing Chart

To monitor trends in PUSH Scores over time

(Use a separate page for each pressure ulcer)

Patient Name_____ Patient ID# _____

Ulcer Location _____ Date _____

Directions:

Observe and measure pressure ulcers at regular intervals using the PUSH Tool.
Date and record PUSH Sub-scores and Total Scores on the Pressure Ulcer Healing Record below.

Pressure Ulcer Healing Record														
Date														
Length x Width														
Exudate Amount														
Tissue Type														
PUSH Total Score														

Graph the PUSH Total Scores on the Pressure Ulcer Healing Graph below.

PUSH Total Score	Pressure Ulcer Healing Graph													
17														
16														
15														
14														
13														
12														
11														
10														
9														
8														
7														
6														
5														
4														
3														
2														
1														
Healed = 0														
Date														

Appendix 3: Biweekly Clinic Survey (Weeks 2, 4, 6)

Effects of Supplemental Immunonutrition on Healing of Chronic Non-Healing Lower Extremity Ulcers in Diabetic Patients: Clinic Survey

Name: _____Date:_____

1. Which follow-up visit are you being seen for today?
   - ☐ 2-week visit
   - ☐ 4-week visit
   - ☐ 6-week visit

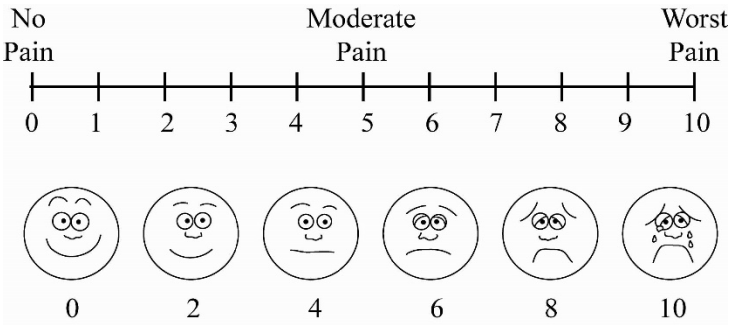
2. Did you receive immunonutrition supplements (Omega-3 fatty acids, Vitamin C, L-Arginine)?
   - ☐ Yes (proceed to question 3) _____
   - ☐ No (proceed to question 4)

3. Have you experienced any difficulty taking the supplementation according to the instructions?
   - ☐ Yes, please explain:_____
   - ☐ No

4. Have you experienced any of the following symptoms since your surgery? Check all that apply:
   - ☐ Abdominal Pain
   - ☐ Heartburn
   - ☐ Nausea
   - ☐ Vomiting
   - ☐ Diarrhea
   - ☐ Headache
   - ☐ Insomnia
   - ☐ Rash
   - ☐ Indigestion
   - ☐ Palpitations
   - ☐ Numbness
   - ☐ Bitter Taste
   - ☐ Low Blood Pressure
   - ☐ Burping
   - ☐ Taste Perversion
   - ☐ Constipation
   - ☐ Gastrointestinal Disorder
   - ☐ Itchy Skin
   - ☐ Easy bleeding or bruising
   - ☐ Red, itchy welts
   - ☐ Other, please explain: _____ _____

5. Have you made any trips to Emergency Departments/Urgent Care Centers since your last visit related to your foot ulcer?
   - ☐ Yes, please explain: _____
   - ☐ No

6. Circle your current level of pain related to your foot ulcer on the scale below:



Appendix 4: Biweekly Phone Survey (Weeks 1, 3, 5)

Effects of Supplemental Immunonutrition on Healing of Chronic Non-Healing Lower Extremity Ulcers in Diabetic Patients: Phone Survey

1) **Intro: "**Hello (*Patient's Name),* this is *(research staff member's name)* calling from the office of_____. I am calling regarding the research study you previously agreed to be enrolled in. Is now an okay time to speak?"
   1. **If No:** "Okay, when would be an acceptable time to call you back?"
   2. **If Yes:** *proceed to number 2*

2) **VAS Pain score:** "On a scale of 0-10, with 0 being no pain, and 10 being the worst pain possible, how would you rate your foot ulcer pain currently?"

If the subject is enrolled in the treatment arm (received oral immunonutrition supplementation), proceed with questions 3 and 4. If not, skip to question 5:

3) **Verification of supplementation:** "Did you receive supplementation and instructions on how it should be taken?
   1. **If No:** "May I ask why not?"
   2. **If Yes:** *proceed to number 3*

4) **Medication compliance: "**Have you been able to take the supplementation daily as instructed?"
   1. **If No:** "May I ask why not?"
   2. **If Yes:** *proceed to number 3*

5) **Experienced symptoms:**
   1. "Have you experienced any of the following symptoms during your participation in the study. Please answer yes or no to each"
      - ☐ Abdominal pain, heartburn, nausea, vomiting, diarrhea, headache, insomnia, rash, indigenstion, palpitations, numbness, bitter taste, low blood pressure, burping, taste perversion, constipation, gastrointestinal disorger, itchy skin, easy bleeding or bruising, red itchy welts
      - ☐ "Have you experienced any other symptoms"
        - ☐ **If yes:** "can you please explain?"
        - ☐ **If no:** *proceed to number 5*

6) **Emergency Department / Urgent Care Visits:** "Since beginning this study, have you had any unscheduled office visits, or have you been to any emergency departments or urgent care centers related to your foot ulcer?"
   1. **If Yes:** "Can you please explain?"
   2. **If No:** *proceed to number 6*

"Thank you for your time (*Patient name),* I hope you have a good day."

Appendix 5: Patient Satisfaction/Completion Survey

Effects of Supplemental Immunonutrition on Healing of Chronic Non-Healing Lower Extremity Ulcers in Diabetic Patients: Completion Survey

This survey should only be completed at the 6-week follow-up visit if you received the immunonutrition supplementation as a part of the research study

Name: _____ Date: _____

1. How satisfied were you with the immunonutrition therapy?
   - ☐ Very Unsatisfied
   - ☐ Unsatisfied
   - ☐ Neutral
   - ☐ Satisfied
   - ☐ Very Satisfied

2. Would you choose to follow the same therapy plan again, if you were to development another foot ulcer?
   - ☐ Yes
   - ☐ No

3. Would you recommend this therapy to friends/family?
   - ☐ Yes
   - ☐ No

4. Do you feel the therapy aided in the healing of your ulcer?
   - ☐ Yes
   - ☐ No

5. How would you rate the difficulty in taking the supplementation?
   - ☐ Very Difficult
   - ☐ Somewhat Difficult
   - ☐ Neutral
   - ☐ Easy
   - ☐ Very Easy

6. How often did you forget to take the supplementation?
   - ☐ Never
   - ☐ Sometimes
   - ☐ Often
   - ☐ Almost Always

7. Did you experience any negative side effects from the supplementation therapy?
   - ☐ Yes, please explain: _____
   - ☐ No

8. Is there anything else you would like us to know about your experience with this therapy?

_____

Effects of Supplemental Immunonutrition on Healing of Chronic Non-healing Lower Extremity Ulcers in Diabetic Patients
Compliance Log

Date Consented:	
Date Supplement Received: (Record the date that you were provided your supplements from Hawthorne Pharmacy)	

Supplement Instructions:
Lovaza capsules
Take 2 gel capsules by mouth daily.

Arginine/Vitamin C Powder
Mix one scoop of powder into 8 oz water, juice, or gatorade and drink once daily.

Instructions for completing compliance log: Week 1, Day 1 should be recorded as the first day AFTER you receive your supplements from Hawthorne Pharmacy. Indicate with a check mark when you take each supplement (2 Lovaza capsules and 1 Arginine/Vitamin C scoop) daily. Days should be consecutive. Do not skip days between doses. You will take these supplements every day for 6 weeks. Please be accurate in your reporting - ex. If you only take 1 Lovaza capsule on day 3, only record one check mark for this supplement.

Week 1

Day 1 Date:_____	Day 2 Date:_____	Day 3 Date:_____	Day 4 Date:_____	Day 5 Date:_____	Day 6 Date:_____	Day 7 Date:_____
Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___
Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___

Week 2

Day 1 Date:_____	Day 2 Date:_____	Day 3 Date:_____	Day 4 Date:_____	Day 5 Date:_____	Day 6 Date:_____	Day 7 Date:_____
Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___
Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___

Week 3

Day 1 Date:_____	Day 2 Date:_____	Day 3 Date:_____	Day 4 Date:_____	Day 5 Date:_____	Day 6 Date:_____	Day 7 Date:_____
Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___	Lovaza capsule (2 capsules/day): ___ ___
Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___	Arginine/Vitamin C Powder (1 scoops/day): ___

Week 4						
Day 1 **Date:**________________	**Day 2** **Date:**________________	**Day 3** **Date:**________________	**Day 4** **Date:**________________	**Day 5** **Date:**________________	**Day 6** **Date:**________________	**Day 7** **Date:**________________
Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____

Week 5						
Day 1 **Date:**________________	**Day 2** **Date:**________________	**Day 3** **Date:**________________	**Day 4** **Date:**________________	**Day 5** **Date:**________________	**Day 6** **Date:**________________	**Day 7** **Date:**________________
Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____

Week 6						
Day 1 **Date:**________________	**Day 2** **Date:**________________	**Day 3** **Date:**________________	**Day 4** **Date:**________________	**Day 5** **Date:**________________	**Day 6** **Date:**________________	**Day 7** **Date:**________________
Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____	Lovaza capsule (2 capsules/day): ____ ____ Arginine/Vitamin C Powder (1 scoops/day): ____