EFFECTS OF ARGININE CONTAINING ORAL NUTRITION SUPPLEMENTS MIXTURES ON PRESSURE ULCER HEALING: EVIDENCE ANALYSIS OF THE CURRENT LITERATURE

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ABSTRACT

Introduction

Pressure ulcers (PU) are a nationwide healthcare problem. The acquisition of PU in hospital institutions is penalized by the government-funded insurance. Thus PU care has become a quality indicator in the current healthcare industry. The purpose of this project is to evaluate the literature published from January of 2010 until April of 2015 regarding the effect of arginine-containing oral nutritional supplements on PU healing.

Methods

A systematic review followed the Evidence Analysis Process from the Academy of Nutrition and Dietetics. All articles selected for inclusion were analyzed through the Evidence Worksheets and Quality Criteria Checklists. Lastly a conclusion statement and the grade for the reviewed literature were provided.

Results

Four articles were left after the inclusion criteria were applied to the articles found in the systematic review. Two of them had a positive rating, and two had a neutral rating. Three studies were short-term studies analyzing rate of PU healing over set times; while one followed patients until full PU healing. All studies provided an overall 4.5 to 9 g of arginine in an oral supplement mixture containing varying amounts of calories and select micronutrients.

Conclusions

Arginine-containing supplements providing a total of 4.5 to 9 g of arginine were found to significantly increase the rate of PU healing when compared to controls not receiving supplementation or receiving a placebo. However, after analysis of the studies using the Evidence Analysis Process grading criteria, this conclusion statement obtained a grade III (limited/weak) because of the small number of studies, the variation in study population, inclusion criteria, and supplement use. Thus, there needs to be additional research to substantiate a change in current practice.

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Chapter 1: Introduction

Pressure ulcers (PUs) are a prevalent problem in the current healthcare system. In 2008, the Centers for Medicaid and Medicare Services made changes to their hospital admission re-imbursement guidelines that included making the finding of a hospital-acquired PU an automatic decline for hospital admission re-imbursement (Department of Health and Humans Services [DHHS], DHHS to State Medical Director, July 31, 2008). Thus, hospital acquired PUs are a healthcare-quality indicator that has since gained importance for the reason that healthcare facilities are left with an increased number of non-reimbursable admissions.

PUs affect all stages of care, from acute inpatient facilities to home care. Even though PUs are preventable, practitioners continue to report their development and regression, amidst all protective measures. Once a PU is found, current recommendations call for a set of extensive interventions in order to prevent its further deterioration. These interventions may include frequent skin care, turning schedules (when bed-bound), as well as nutrition assessment and interventions.

The nutritional screening and subsequent assessment should help identify people who are at risk or already have PUs, as well as develop interventions appropriate for each individual. The goal for patients at risk for PUs includes maintenance of adequate nutritional status, identification and treatment of the causes that may be contributing to poor oral intake, and routine monitoring of weight status (Academy of Nutrition and Dietetics [AND], 2015c). Current calorie and protein goal recommendations range from 30-40 calories per kilogram and from 1.5-2.0 gram per kilogram, respectively. The variation is dependent on the nutrition adequacy of the patient at the time the assessment is made. Very little is known in regards to supplementation

with amino acids, vitamins and minerals due to the lack of data showing these nutrients' effectiveness to decrease PU risk or improve PU healing.

Arginine is a conditionally essential amino acid, meaning that, the body can make it in adequate amounts under normal circumstances. However, the body cannot keep up with its demands in times of metabolic stress or poor oral intake (Crowe & Brockbank, 2009). Only two arginine studies are cited in the Academy's Nutrition Care Manual. Both evaluated the effectiveness of arginine-containing oral supplement mixtures on the healing of pressure ulcers (Benati, 2001; Desneves, 2005). Due to methodological and reporting flaws, there could not be a clear conclusion made from these two studies (AND, 2015c).

There have been multiple randomized controlled trials that have evaluated the use of a high calorie, high protein formula enriched with arginine, zinc and antioxidants. However, some of these studies had a small study population, did not standardize for the calorie content of the supplement, or had a very restricted inclusion/exclusion criterion, all of which impacted the generalizability of the findings. It is believed that the effect of these nutrients on PU healing is likely synergistic (Cereda, Klersy, Serioli, Crespi, & D'Andrea, 2015).

The Evidence Analysis Library (EAL) published a project on wound care between 2011 and 2012. The overall project was given a grade V (i.e. no evidence available to support or reject the conclusion). Only three questions within the project addressed supplemental arginine in the context of improving wound healing (AND, 2015e). Since 2011, there has been more research published addressing arginine mixture supplementation as an intervention towards the healing of PUs. The National Pressure Ulcer Advisory Panel, European Pressure Ulcer Advisory Panel, and Pan Pacific Pressure Injury Alliance have published recommendations in favor of

supplementation with high protein, arginine and micronutrients for adults with PUs stages III and IV (2014). Conflicting recommendations warrant this review. If the potential benefits have been deemed significant, current dietetics practice may change to provide evidence-based care.

Research Question

Spinal cord injury patients are amongst the greatest population at risk for PUs. At the beginning of this project, the proposed research question was: "What effect does arginine have on healing of pressure ulcers in patients with spinal cord injuries?" However, after the preliminary research, very few studies were found, showing a need to redefine the scope of the project. Therefore, the research question was modified to: "What effect do arginine-containing oral nutrition supplements have on the healing of pressure ulcers?"

Sub-problems

PU healing was in some cases measured as a decrease in PUSH (Pressure Ulcer Scale for Healing) scores (a tool used to measure the change in PU status over a period of time), or time to healing. Tissue granulation was also deemed a positive outcome in the context of pressure ulcer progression. Therefore, sub-problems analyzed within this review respond to the question: "What effect does an arginine-containing oral nutrition supplements have on healing of pressure ulcers, assessed by time, PUSH scores, or tissue granulation?"

Limitations

A major limitation to this EAL project is the small amount of new research that has been published since the last published review on 2011. Most of the research has focused on arginine-containing supplement mixtures, even before 2011. Thus, the individual effect of supplemental arginine is not being evaluated, but the combined effect of these mixtures. Using supplement mixtures that contain calories, as well as protein and other micronutrients, will be a confounding factor. Therefore, if the studies do not control for calorie, protein and micronutrients intake, this review may be seriously and negatively impacted.

Delimitations

These consist of the inclusion and exclusion criteria used to search and select the primary articles to be reviewed. All articles included in the final review were written in English, published in peer-reviewed journals between January 2010 and April 2015. Sample sizes are of at least 30 subjects or more. The primary intervention consisted of feeding study subjects with an oral arginine-containing supplement mixture.

Articles in which interventions included arginine supplementation via enteral or parenteral feeding were excluded. Additionally, animal studies were excluded because they do not comply with the requirements for an EAL project. Any study that included patients with diabetic, surgical wounds as well as PUs were excluded.

Assumptions

Throughout the review process, it was assumed that these oral arginine-containing supplement mixtures provide positive effects on the healing of PUs. Also, it was assumed that any vitamin or mineral would have provided a null effect on healing PU. The latter assumption stems from the expectation that each study had ensured that patients were maintaining adequate oral intake throughout the intervention.

Definitions

Wound supplement or oral arginine-containing supplement mixture: A liquid supplement meant for oral consumption rather than enteral nutrition/feeding, which may be enriched with arginine, zinc and vitamins C, E, A, or any other micronutrients.

PUSH scores: A tool developed by the NPUAP to help monitor the change in PUs over time. PUSH scores are determined by addition of the sub-scores obtained through estimation of the wound area, amount of exudate/drainage upon removal of the dressing, and the type of tissue present at the wound bed. The scores range from 0 to 17. The lowest score indicates the wound is closed, whereas 17 is the worst possible score (NPUAP, n.d.-b).

Pressure Ulcer (PU): An area where there is damage to the skin and the underlying tissues. The damage may be caused by pressure, friction, shear, moisture, or any combination of these factors (Stechmiller, 2010).

Arginine: A conditionally essential amino acid, which acts as a substrate for protein synthesis, collagen deposition and cellular growth. (Stechmiller, 2010).

Chapter 2: Literature Review

The mortality of PU complications, as reported by the US Joint Commission on Patient Safety, is estimated to be at about 60,000 people per year (Kruger et al., 2013). In-hospital mortality is 4.2% for patients who have PUs as their primary diagnosis, and 11.6% for those with PUs as a secondary diagnosis (Russo, Steiner, & Spector, 2008). Furthermore, 7 to 8% of patients with paraplegia are likely to die from PU complications. At least half of all patients with facility-acquired PUs are estimated to die within 12 months of their hospitalization. Patients who have chronic non-healing PUs usually have higher mortality rates (Richards, Waites, Ying Chen, Kogos, & Schmitt, 2004).

While the incidence of an event is usually used to demonstrate quality of care, incidence of PUs does not always reflect the point in time at which this PU may have developed or found. Thus, the quality of care or prevention of PUs, at any given facility, would be best evaluated by a variance of incidence (Thomas and Berlowitz, 2014, Chapter 2). Consequently, a more appropriate quality indicator would be the number of facility-acquired PU. This measure takes into account only those patients without a PU at baseline, but rather developed one during their hospital admission.

Yearly estimates indicate that in the United States, 1 to 3 million people develop PUs (Kruger et al., 2013). In 2010, about 0.05% of all older adults in the US ages 65-74 years old, 1.2% of those 75-84 years old, and 2.4% of those older than 85 years old were hospitalized with chief complaint related to PUs (United States Department of Health and Human Services [US DHHS], n.d.). There was an 80% increase in the number of patients hospitalized with PUs from 1993 to 2006. This increase in hospitalizations aggregated to a cost of about $11 billion (Russo,

Steiner, & Spector, 2008). Acute care hospitals treat about 2.5 million PUs each year, with about 15% of their patient population having a PU at any one time during their hospital stay. In addition, PUs may increase patient stay by 5-14 days, therefore increasing costs by about $16,000-20,000 (The Agency for Healthcare Research and Quality [AHRQ], 2014).

According to a National Pressure Ulcer Advisory Panel (NPUAP) review, a study conducted at a neurological intensive care unit reported a 12.4% incidence rate for PUs stage II and above. The International Pressure Ulcer Prevalence Surveys reported in 2009 that facility acquired rates ranged from 8.8% to 10.3% in intensive care units. General care patients are also at high risk for developing PUs. The survey also reported that there was a prevalence of 8-14% within acute care medical and surgical units; however, the rate for facility acquired PU ranged from 3-5%. Prevalence of PUs at nursing homes is similar to that of acute care facilities with an 8-12% range (Thomas & Berlowitz, 2014, Chapter 2).

In 2008, the Centers for Medicaid and Medicare Services (CMS) labeled hospital acquired stage III and IV PUs as “never events.” Never events are defined as preventable errors in medical care that could have been largely influenced by the policies and procedures of each healthcare organization. Furthermore, the CMS indicated that the treatment of never events was no longer covered by Medicare payments (Department of Health and Humans Services [DHHS], DHHS to State Medical Director, July 31, 2008). This change has placed the financial burden of hospital-acquired stage III and IV PUs on healthcare institutions (AHRQ, 2014), thereby heightening the importance of following policies and procedures of care. This burden is noticeably large, since about 75% of adult hospitalizations related to PUs had been reported as

having Medicare as its most common payer (Kruger et al., 2013). Due to the financial and public health burden, it is of prime importance to prevent the development of PUs.

PU prevention strategies involve nursing cares such as daily skin assessment and frequent pressure redistribution; as well as specific nutrition interventions such as maintaining fluid and adequacy of nutritional intake (Jarrett, Holt, & LaBresh, 2014). In a review published by the American Society for Parenteral and Enteral Nutrition (ASPEN), it was reported that undernourished patients were twice as likely to develop PUs when compared to adequately nourished patients. One of the research papers in this review showed that about 65% of patients who were identified as severely undernourished at baseline went on to develop PUs. Additionally, poor nutritional intake, body mas index of <18.5-20 kg/m^2 and unintentional weight loss of 5-10% of body weight were identified as nutritional factors linked to the development of PUs. However, a summary of systematic review from 2005 to 2009 revealed that correction of nutritional deficiencies via supplementation could be an appropriate intervention in the context of PUs; while the effect of disease-specific supplementation via parenteral, enteral or oral routes on the healing of PUs was unclear (Thomas, 2014).

As described above, PUs are a pervasive healthcare problem that reaches all levels of patient care, from acute to long-term care. It is widely known that adequate nutrition can positively impact PU prevention and treatment. Thus, the purpose of this literature review is to critically analyze the evidence regarding nutrition interventions, more specifically, the use of arginine-containing oral nutrition supplement mixtures on PU improvement. A background on wound healing will be discussed along with a description of how specific nutrients including arginine are involved in the wound healing process.

Background

Pressure Ulcers

A PU is an injury to the skin, and potentially the underlying tissues, caused by moisture, friction and pressure. PUs are common injuries in patients who lack mobility due to sedation, diminished strength and cognition, or injury. Disease states that affect blood flow and skin health also increase the patient susceptibility to PUs (US NLM, NIH, 2015). Spinal cord injury (SCI) patients are among those at highest risk for development of PUs. Their lack of sensory perception in combination with chronic comorbidities such as Diabetes Mellitus, obesity, and renal disease, places these patients at risk for recurrent PU development and downward progression (Kruger, Pires, Ngam, Streling, & Rubayi, 2013).

PU Staging

The NPUAP redefined the staging of PUs in 2007. The degree of damage to the tissues can vary from a stage I to IV, the latter being the deepest injury. Some PUs may be labeled as unstageable or as a suspected deep tissue injury (U.S. National Library of Medicine [US NLM], National Institute of Health [NIH], 2015; NPUAP, n.d.-a). A Stage I PU constitutes an area of intact skin that shows non-blanchable redness. A stage II refers to an area of the skin where there is partial thickness loss of the dermis or a ruptured serum-filled or sero-sanguineous blister. A stage III shows full thickness tissue loss; meaning the fatty layer may be visible. At this stage bone, tendons and muscle tissue are not exposed. Thus, a stage IV is represented by the full exposure of the latter three tissues. The two additional categories are unstageable and suspected deep tissue injury. The first is used when there is a full thickness tissue loss but the base of the

injury is covered by slough (yellow, gray, green, tan or brown) and/or eschar (tan, brown or black). The latter denomination is used when there is a purple or maroon yet intact area of skin or, when there is a blood filled blister (NPUAP, n.d.-a). In the clinical setting, a trained wound care nurse would be the one to evaluate and assign the appropriate staging to the wound.

PU Etiology

A host of extrinsic and intrinsic factors contribute to the overall development of PUs (Kruger et al., 2013). Pressure, shear forces, moisture, friction, and immobility are considered extrinsic factors; while comorbidities, such as sepsis and altered level of consciousness, as well as age are intrinsic factors.

Kirman and Geibel (2014) described that PUs are a result of constant unrelieved pressure that exceeds the arterial capillary pressure of 32 mm Hg or venous capillary closing pressure of 8-12 mm Hg; which produces an impairment of blood flow that leads to ischemia. This sustained external pressure is the initial insult that leads to a cascade of tissue degeneration. The pressure may be caused by mattresses, wheelchair pads, hospital bed rails; to name a few. However, not all skin in contact with the above named objects is exposed to the same amounts of pressure. The patients' highest pressure points are the sacrum, heel and occiput while in the supine position. In the sitting position, the ischial tuberosities are found to be under the highest pressure.

Kirman and Geibel (2014) further described that it may take as little as two hours of constant pressure to develop irreversible changes to the subcutaneous tissues. Nonetheless, the top layers of skin can sustain up to 12 hours of constant pressure. Kruger et al. (2013) pointed out that muscle tissue is more susceptible to pressure than the top layers of skin. Fat, being less tense

than skin, is more susceptible to irreversible changes. While injuries to these two bottom layers may be occurring, the top layer of skin may show insignificant changes. This is known as the "tip-of-the-iceberg" phenomenon.

Wound Healing Process

Wound healing can be summarized as a process consisting of four phases: hemostasis, inflammation, proliferation, and tissues remodeling (Guo & DiPietro, 2010). The first phase is characterized by vascular constriction, platelet aggregation, degranulation and fibrin formation. Within the inflammation phase neutrophils, monocytes and lymphocytes infiltrate the wound site. Monocytes differentiate into macrophages, which release cytokines that further promote the inflammation response by activating additional leukocytes. Macrophages also clear apoptotic cells towards the end of the inflammation phase. This latter function of macrophages leads to tissue regeneration, hence beginning the proliferation phase in which keratinocytes, fibroblasts, and angiogenesis are stimulated.

T-lymphocytes migrate to the wound during the late proliferative to early remodeling stages. The exact role of these cells is currently unknown. The two subgroups of T-lymphocytes cells, T-helper and T-suppressor, are thought to have opposing effects on wound healing; positive and inhibitory roles, respectively. Nonetheless, the proliferative phase consists of epithelial proliferation and the formation of a provisional matrix within the wound site. Fibroblasts and endothelial cells provide the support needed for collagen formation, capillary growth, and the formation of granular tissue. More specifically, fibroblasts make up the collagen and the two major components of the extracellular matrix; namely glycosaminoglycans and proteoglycans (Guo & DiPietro, 2010).

The last phase (i.e. tissue remodeling) starts when the extracellular matrix is fully built. This phase can last years. It is characterized by the retraction of vascular tissues and extracellular matrix and, thus leading to the new tissues taking on a cellular architecture that more closely resembles the pre-injury status. Oxygenation, infection, age, hormonal state, stress, Diabetes Mellitus, medications, BMI, alcohol consumption, smoking and nutrition are all factors affecting the wound healing process (Guo & DiPietro, 2010) by either delaying or improving the healing process. As previously mentioned, correction of nutritional deficiencies may be warranted in the context of PU treatment in order to provide sufficient nutrients to support their function in the wound healing process.

Nutrients in Wound Healing

PU Treatment: Energy, protein and micronutrients

The Academy of Nutrition and Dietetics (AND, 2015c) recommends that patients with PUs and are of healthy weight be prescribed 30-35 calories per kilogram daily. However, energy requirement for those who are considered underweight or losing weight should be increased to 35-40 calorie per kilogram daily. Protein intake recommendations for healthy adults are currently 0.8 to 1.0 grams per kilogram of body weight per day. For the elderly, the recommended protein intake is 1.0 to 1.2 grams per kilogram per day. However the European Pressure Ulcer Advisory Panel recommends that patients who are considered to be at risk for PUs should receive protein in amounts ranging from 1.5 to 2.0 grams per kilogram of body weight daily.

The Evidence Analysis Library (EAL) has more specific recommendations for populations at greater risk for PUs, particularly those with Spinal Cord Injuries (SCI). Energy

recommendations are dependent on the degree of their SCI, but protein recommendations are for patients with Stage II PUs are higher than the general population at 1.2-1.5 grams of protein per kilogram of body weight per day. Protein intake recommendations increase further for those patients with Stage III to IV to 1.5-2.0 grams of protein per kilogram of body weight per day (AND, 2015c). The Nutrition care Manual lacks specific recommendations for carbohydrate or dietary fat intake for patients with PUs.

Micronutrient status among patient with PUs should be monitored. The AND recommends a multivitamin/mineral supplementation containing the RDI for micronutrients in cases where deficiencies are known or suspected (2015c). Zinc is necessary for the granulation processes and the development on new epithelia due to its function as a co-factor for RNA and DNA polymerase. A deficiency can have severe deleterious effects on tissue rebuilding (Guo & DiPietro, 2010). Both the European Pressure Ulcer Advisory Panel (EPUAP) and the NPUAP recommend prescription of zinc only when there is a known or suspected deficiency (Sernekos, 2013). In agreement with the latter recommendations, AND added that zinc supplementation should be of no more than 50mg of elemental zinc twice per day, for no longer than 2 to 3 weeks (AND, 2015c).

Specific micronutrients recommendations have been made by the AND regarding patients with SCI and PUs. Vitamin A, for example, is recommended for enhanced wound healing in oral doses between 10,000 to 50,000 IUs per day. Additionally, intravenous doses of vitamin A of 10,000 IUs for 10 days can be given to malnourished patients, or those with moderate to severely injuries. Further research is needed, however, to determine optimal dosages of vitamin A (AND, 2015c).

Vitamin C is another micronutrient associated with wound care thus supplemental vitamin C is often recommended. Vitamin C is needed for the hydroxylation step of lysine and proline, which help stabilize the collagen structure; thus, deficiency has been linked to defects on tissue repair (Guo & DiPietro, 2010). There is currently a discrepancy on whether supplementation with vitamin C should be routine care. The EPUAP and the NPUAP do not recommend routine vitamin C prescription unless deficiency is suspected or known (Sernekos, 2013). However, very specific doses of Vitamin C are recommended by the Agency for Health Care Research and Quality. For SCI patients with Stage I and II PUs, 100 to 200 milligrams of vitamin C are recommended daily, while 1,000 to 2,000 milligrams of daily vitamin C are recommended for patients with Stage III and IV PUs. AND has determined there is insufficient evidence to determine an optimal dosage of vitamin C.

Amino acids may be an effective nutrition therapy for PUs. Arginine is considered essential in times of metabolic stress and poor oral intake (Crowe & Brockbank, 2009). Sernekos (2013) pointed out that this amino acid's functions include being a precursor to proline and polyamines. As stated before, proline is involved in the hydroxylation and formation of collagen, while polyamines are building blocks for proteins. Arginine is also the only substrate for nitric oxide production. This compound is toxic to bacteria, contributes to angiogenesis, and acts as a vasodilator (Sernekos, 2013). These latter characteristics make this amino acid a positive contributor to tissue regeneration.

In Sernekos (2013), average arginine intake of the general population is estimated to be between 5-6 g daily. According to the EPUAP, NPUAP and the Pan Pacific Pressure Injury Alliance 2014 guidelines, patients unable to meet their nutritional needs with high calorie high

protein supplements, should be given additional protein, arginine, and micronutrients. This recommendation applies to adults with stage III or IV PUs, or in cases where the patient has multiple PUs. However, the current AND's consensus surrounding arginine indicates that additional research is needed to make any recommendations in regards to arginine and micronutrient supplementation.

Arginine Containing Supplement's Role in Medical Nutrition Therapy for PUs: Current Evidence

Within the past decade many potential benefits have been cited regarding the use of arginine containing supplement mixtures as an intervention for patients with. This research has generally been done in various populations and within different healthcare settings. To date, there are no studies that solely evaluate the effect of supplemental arginine alone on PU resolution (Sernekos, 2013). Thus, this evidence review will primarily focus on arginine containing supplements designed to have a similar micronutrient profile while aiming to reduce the time to PU healing in a wide variety of patient populations.

Open label clinical trials

Frias Soriano, Lage Vazquez, Perez-Portabella, Xandri Graupera, Wouters-Wesseling, and Wagenaar (2004) conducted a small open-label clinical trial in Spanish hospitals. Their sample size consisted of 39 inpatients (mean age of 75 years old) with Stage III or IV PUs, without renal or hepatic insufficiency. Wound parameters were assessed at baseline. Wound healing rate was determined by assessing the difference between wound area at baseline and at week 3. Wound conditions were described categorically. Researchers assessed adequacy of

dietary intake by calculating the patient's needs (using the Harris Benedict equation) and comparing them to their actual intake (described in percentages).

Doses of the wound supplement (Cubitan, Nutricia) were given depending on the adequacy of patient's intake, their albumin values and PU grade. The number of packets needed was re-assessed weekly. Each 200 ml packet of Cubitan contained 250 kcal, 20 grams of protein of which 3 g was arginine, 28.4 g of carbohydrates, 7 g of fat, 250 mg of vitamin C, 37.6 mg of vitamin E, 9 mg of Zinc, among other nutrients. This supplement was given 1-3 times per day for a period of 3 weeks in addition to the hospital's standard diet, tube feeds, and PU care.

After 3 weeks, there was a significant reduction in the area PU from 23.6 cm^2 to 19.2 cm^2 ($P<0.001$). The researchers reported a 29% reduction in PU area or 0.34 cm^2 per day. Additionally, there were significant reductions in the incidence of exudate in infected PU from 38% to 15% ($P=0.012$), and in the incidence of necrotic tissue from 44% to 10% ($P=0.001$). Supplement intake averaged 1.9 ± 0.6 packages per day. At least 34% of all patients complied with meeting 50-75% of estimated nutritional needs. Twenty nine percent complied with 75-100% and 37% received over 100% of their needs. However, based on body mass index (BMI), triceps skin fold and albumin levels, 24 % of the study sample were considered nutritionally depleted.

The authors concluded that the use of a wound specific supplement resulted in a larger reduction of PU area and a faster rate of healing compared to a previous study reporting a rate of healing of 0.075 cm^2 per day (Soriano et al, 2004). Based on this comparison the authors suggest that Cubitan's additional nutrients, namely arginine, vitamin C and Zinc may have played a

pivotal difference in their study. Compared to other published studies, the study group was considered relatively well nourished.

The authors did not directly indicate any limitations or strengths within their study however; there are several limitations to note. The assessment of patient's nutritional status included assessment of albumin levels, which are known to decrease in times of inflammation and metabolic stress. The assessment of the need for supplementation was based on categorical estimation of intake rather than objective data (e.g. calorie count data). The assessment of wound conditions was subjective.

A much larger open clinical trial was performed by Heyman, Van De Looverbosch, Meijer, and Schols (2008) at 61 different long-term care facilities in Luxemburg and Belgium. The sample size consisted of 245 long-term care residents with Stage II, III, or IV PUs. The authors reported they did not use any exclusion criteria, thus the sample was reflecting of a nursing home environment within their geographic location.

The participant group had a mean age of 82 $\pm$ 10 years and a mean body weight of 61.3 $\pm$ 15.5 kg. Patients were given a wound-supplement (Cubitan, Nutricia) 3 times per day for 9 weeks. The supplement was given in addition to the facility's standard diet or tube feeding. The specific nutritional information of this supplement was explained in previous pages. PU care was standardized; however, the exact methods for PU care were not described in the publication.

After 3 weeks, there was a mean reduction in PU area from 1580 mm^2 to 1103mm^2 ($P<0.0001$), or 30% reduction. At 9 weeks the average PU area was noted 743mm^2, which the authors concluded, was a 53% reduction compared to baseline ($P<0.0001$). Additionally, wound

closure was achieved by week 3 in 6.6% of PUs, and increased to 19.8% by Week 9. The degree of exudate was assessed in categories of mild, moderate, and severe. At baseline 33% of PUs were reported as having mild exudate, 25% moderate, and 13% severe. At 9 weeks, it was reported as 22%, 14%, and 4%, respectively. Wound supplement intake was recorded as 2.3 $\pm$ 0.56 servings per day, which showed good compliance.

The authors concluded that after 9 weeks of supplementation, there was a significant reduction of PU area in the residents of these two particular long-term care facilities. Also, they pointed out that the reduction in PU area was accompanied by improvement in PU conditions.

Researchers discussed a few limitations and strengths. Limitations included the fact that this study was not a randomized, placebo-controlled blinded trial. Also, inter-rater reliability between centers could not be confirmed, the subjective observations of the wound exudate, the instrument used for measuring wound area was not optimal (ruler versus planimetry) were other limitations included in the publication. The strengths of this study included the size of the study's sample, the length of the study, and the fact that supplement compliance as well as practitioners' acceptance were assessed.

In another study of PU among spinal cord patients, Brewer, Desneves, Pearce, et al. (2010) performed a prospective study on community based spinal cord injured patients recruited through the Spinal Cord Outreach Risk Reduction Team (SpORRT), in Melbourne Australia. Patients included in the study had the following characteristics: 18 years or older, had a spinal cord injury, residing within the Melbourne metropolitan area, and had a Stage II, III, or IV PU. Patients were excluded based on disease states such as phenylketonuria, sepsis, chronic renal

failure, and metabolic disease. Additional criteria for exclusion were the presence of diabetic foot ulcers, osteomyelitis, and hydroxyurea or corticosteroids intake.

Researchers used a historical control from patients in the SpORRT database. Participants of the study consumed 2 sachets per day of Arginaid (Nestle) until full healing of PU (providing 4.5 g of arginine, 4 g of carbohydrate, 155 mg of vitamin C and 60 mg of vitamin E per sachet). The same spinal cord nurse assessed PU healing for all patients. If a study participant was admitted to the hospital the intervention was continued. Best practice protocols were used for PU care, however they were not described in the publication.

A total of 18 patients were entered into the intervention group, while 17 historical controls were found through chart review. Researchers reported a two-fold statistically significant faster rate of time to healing in the intervention group (10.5 $\pm$ 1.3 weeks) versus that of the control group (21.1 $\pm$ 3.7 weeks). Compliance with supplement prescription averaged 85%. About 94% of the patients in the intervention group were considered well nourished or overweight upon visual exam.

The authors concluded that this small-scale study leads to a potentially significant effect of arginine containing supplements on PU healing. Limitations of this study included its small sample size, the lack of blinding to the intervention, and the use of historical controls. Additionally, researchers pointed out that the community environment made it more difficult to control all aspects of care (e.g. rotation schedules). Researchers discussed that the use of the same nurse for PU care in both the intervention group and for most of the historical control patients was a strength of this study.

Another prospective observational study included 34 patients with stage II, III, or IV PUs from the acute ward of the Victorian Spinal Cord Service in Austin Health Melbourne, Victoria (Chapman, Mills, Pearce and Crowe 2011). The researchers' primary outcome was time to PU healing. Full healing was described as patients having a PUSH score of zero, completed mobilization program, and intact skin. Participants had to be older than 18 years, have at least 1 stage II, III, or IV PU, and able to receive oral nutrition support.

Patients were counseled to achieve optimal nutritional status via the standard hospital diet. Participants were also prescribed two servings of 237 ml each per day of a wound supplement (Resource Arginaid Extra) providing 500 kcal, 18 g of protein, 9 g of arginine, 500 mg of vitamin C, and 30 mg of zinc along with standardized wound and nutritional care.

A total of 34 patients were enrolled but only 20 patients (mean age 43.8 years) consumed the wound supplement until full PU healing. The other 14 patients (mean age 49.1 years) stopped consuming the supplement or were discharged before wound healing occurred. Discontinuation of supplement intake was due to gastrointestinal upset symptoms (n=6), dislike of taste (n=3) and non-compliance (n=5). This latter group was then considered a pseudo-control and was given an alternative standard high-energy high-protein supplement without any additional zinc, vitamin or arginine. For this later group, discharge date was set as the date of full wound healing for the research purposes.

Overall, researchers reported a 2.5 fold greater rate of wound healing in the group that consistently consumed the supplement until wound healing versus those who did not ($p= 0.04$). Both groups maintained adequate energy and protein intake based on the dietitian's assessment.

Wound supplement compliance was reported to be on average 97.7%; for the group who consumed the supplement until full wound healing.

Researchers concluded that the use of an arginine-containing supplement with added zinc and vitamin C increased PU- healing by 2.5 times the rate of those who did not continue with the supplement until full wound healing. Limitations reported include the use of a pseudo-control group. This pseudo control group was initially included in the intervention group, however ceased to take the supplement and a washout period was not noted. The authors briefly discussed the potential bias of using a group which was previously included as part of the intervention, however they pointed out that baselines characteristics were not significantly different. Other limitations include the observational nature of the study, and the presence of multiple etiologies of wounds (diabetes, osteomyelitis, surgical), the lack of specific guidelines regarding standards of nutrition care, the assumption that the pseudo-control's PUs had attained a score of zero (healed) upon discharge. Strengths were not discussed within this publication. However, the study setting can be considered strength; since it was performed in actual clinical practice and mimicked day-to day interventions.

Open clinical trials have an innate limitation of providing the participants with a potential placebo effect. However these four articles elucidated the potential benefits that an arginine containing oral supplemental mixture could have on the healing of PUs. Downfalls of these studies include the use of historical controls (Brewer et al., 2010) or the lack of a control group at all (Frias Soriano et al., 2004; Heyman et al., 2008).

Replicability is a characteristic of a strong research study. The lack of detailed description of the exclusion criteria (Frias Soriano et al., 2004, Heyman et al., 2008) makes the

results of these publications harder to verify. Additionally, detailed descriptions of standard methods for PU and nutritional care were not in Heyman et al. (2008) and Chapman et al. (2011). However, Frias Soriano et al. (2004) provided detailed accounts of their standard methods for PU and nutritional care. The latter included the use of the Harris Benedict equation with a stress factor of 1.1 and an infection factor of 1.3 (when infection was present).

Heyman et al. (2008) lacked baseline information regarding nutritional status and the adequacy of the patient's nutritional intake. Frias Soriano et al. (2004) provided information on the patient's compliance with the mean prescribed intake but there was no discussion on whether the differences in dietary intake were significant compared to the control group. Chapman et al. (2011) did report that their patients maintained adequate nutritional intake throughout the study.

Overall the conclusions of these first 3 studies are in agreement with each other in that PU healing or healing rate are noted to be higher on patients given an arginine-containing supplements versus those taking a standard supplement. Yet, there is uncertainty in regards to the size of the effect of the supplementation since the overall adequacy of nutritional intake of the patients was not discussed in 2 of the 3 studies.

Randomized Trials

A small-randomized controlled trial was performed by Benati, Delvecchio, Cilla, and Pedone (2001). There were 16 patients in this study; all with severe cognitive impairment. The only exclusion criteria noted involved patients who were unlikely to benefit from nutritional supplementation; no other details were given. The researchers assessed PU healing by using the

pressure sore status tool, ranging from 13 (best score) to 65 (worst score). Assessments and follow up assessments were done at baseline and then every 5 days until day 15.

Subjects were divided into three groups: one consuming standard hospital diet, other the hospital diet plus a high protein supplement, and the last group had the standard diet plus a wound specific supplement. The supplement was not identified by name in this publication. The researchers did report that 200 ml of it provided 500 kcal, and 37 g of protein (arginine amount was not specified). All patients received standard wound care, however standard protocols were not described. Patient's age ranged from 72 to 91 years. Results (presented in graph form only) indicated a tendency for increased PU healing in the group supplemented with the wound specific supplement compared to the other two groups. From the graphical results provided in the publication, one can estimate that most pressure sore status tool scores tended to either decrease or increase by 5-10 points for the non-supplemented group, decreased by about 10 points in the second group supplemented with high calorie/protein supplement, and decreased by as much as 25-30 points in the group supplemented a high calorie/protein supplement that included arginine, zinc and antioxidants.

In spite of only finding a trend towards improvement, the authors concluded that nutrients such as arginine, zinc and vitamin A, C, and E could be used to improving wound healing. Although authors did not review strengths and limitations, the sample size can be named as one limitation. Also the authors failed to describe what standardized protocols for PU treatment were used and did not identify specifically which wound supplement they used thus making it harder to design a replication of this study. However, the overall design of this study is a strength as they attempted to compare three treatments: general diet, general diet plus a high calorie

supplements, and a general diet plus a high calorie supplement that contained arginine, zinc and antioxidants. This design could help elucidate the real impact of arginine containing supplement versus standard high calorie supplements.

In another randomized trial, Desneves, Todorovic, Cassar and Crowe (2005) performed a small, randomized trial on studied 16 patients who suffered from spinal cord injury, and had stage II, III, or IV PUs. The study was performed in a geriatric hospital in Australia. Exclusion criteria was the presence of a diagnosis of osteomyelitis, diabetes mellitus, or receiving enteral/parenteral nutrition support, or taking hydroxyurea or more than 10 mg of steroids per day.

Patients were assigned to 3 different groups: a standard hospital diet (control), a standard hospital diet plus 2 high-calorie high-protein supplements providing 500 kcal, 18 g of protein, 0 g of fat, 72 mg of vitamin C, and 7.5 mg of zinc (Resource Fruit beverage, Novartis), and a third group which also had the same standard diet plus two tetrapaks of an arginine-containing supplement providing 500 kcal, 18 g of protein, 0 g of fat, 500 mg of vitamin C, 30 mg of zinc, and 9 g of arginine (Resource Arginaid Extra, Novartis).

PU care was standardized, including turning schedules, bed mattress, and dressing changes. The PUSH tool was used for measuring change in PU status; measured at baseline and every week thereafter. Patient's daily requirements were estimated using the Schofield equation and protein requirements by using the NPUAP recommended amounts. Food and fluid records were kept for the three weeks the study lasted. Diet recall was used to cross check intake and monitor patient's compliance.

Mean age for the three groups was reported as 63 ± 9.9 years, 75.6 ± 5.9 years, and 83.2 ± 1.1 years, respectively. By the end of the study, the patients drinking the arginine-containing supplement had a significant improvement in PUSH scores. Compared to PUSH scores at baseline (9.4± 1.2) by week 2 and 3, PUSH scores were significantly reduced (4.4 ±1.5, 2.6 ± 0.6, weeks 2 and 3 respectively). Additionally, week 3 PUSH scores for the group supplemented with arginine were significantly lower than those of the standard (7.0 ± 1.5) and the high calorie-supplemented diet (6.0 ± 1.2). Overall, patients on the arginine oral supplement showed a 2.5 fold improvement in PUSH scores compared to the other two groups. However, it is also noted that patients on the standard diet plus the high calorie high protein supplement consumed significantly lower protein (63% of estimated protein needs) than the other two groups who consumed 79% (standard diet) and 92% (arginine containing supplement diet). These latter two intakes were not considered significantly different.

The authors pointed out that the small sample size was a limitation even though the differences in results were large. Also, not having followed the patients until complete healing of PU was pointed as an additional limitation. The finding that the actual intake of protein, compared to the estimated protein needs, in the group taking the standard high calorie/protein supplement was significantly lower than the other two treatment groups added another limitation to this study, as protein intake can largely affect wound healing. On the other hand, researchers choose their patients sample to not include disease states that greatly affect wound healing, such as diabetes mellitus, which added strength to their findings.

Cereda, Gini, Pedroli and Vanotti (2009) conducted a randomized controlled trial in a long-term care setting in the province of Como, Italy. Twenty-eight residents (mean age 82.2 ±

10.1 years) with PUs ranging from stage II, III and IV were followed for 12 weeks. Patients were excluded if they had an acute illness or chronic disease that could affect the healing process, or if they were on immunosuppressive therapies, had the PU for longer than 1 month or a tendency for lack of dietary adherence.

There were two groups in this study. The treatment group (n=13) received a standard hospital diet plus 400 ml of an oral supplement containing 34 grams of protein, 6 grams of arginine, 500 milligrams of vitamin C, and 18 milligrams of zinc. Any patient in the treatment group who was being tube fed received 1 liter of supplement containing 55 grams of protein, 8.5 grams of arginine, 320 milligrams of vitamin C, and 20 milligrams of zinc. The control group received a standard hospital diet or tube feeds as indicated. Overall, nutrition support was standardized to provide at least 30 calories per kilogram per day.

PU care was standardized and described within the publication. A consistent nurse, blinded to the interventions provided PU care. Biochemical data and anthropometrics were collected at the beginning and end of the study. PU assessments were done at baseline and weeks 2,4,6,8, and 12.

By week 12 both groups showed improvement in PU healing (assessed by PUSH scores), and PU surface area ($p<0.0001$) when compared to baseline. The difference in PUSH scores at week 12 compared to baseline was significantly greater in the treatment group (-6.1 ± 2.7) than that of the control group (-3.3 ± 2.4, $P<0.05$). At week 8, there was a significant reduction of PU area in the treatment group (-1140 ± 669.2 mm^2) compared to the control group (-571 ± 391.3 mm^2, $P<0.05$). Participants receiving the wound supplement had a significantly higher mean

reduction in PU area of about 57% versus 33% at week 8, and a 72% reduction versus 45% by week 12 ($P<0.005$).

The authors concluded that wound specific nutrition support (oral as well as enteral) should be considered in long-term care population in order to help reduce the rates of PU healing. Some limitations addressed by the researchers include the small sample size, and the lack of a control group with supplemental protein.

Van Anholt, Sobotka, Meijer, Heyman, Groen, Topinkova, van Leen, and Schols (2010) performed an 8-week double-blind randomized study with 43 patients from European hospitals (multicenter, multi-country) and long-term care facilities with stage III or IV PUs. The patient's mean age was 76.2 $\pm$ 3.2 years. Patients included in this study were those following a standard hospital diet without nutritional supplements for at least 2 weeks before the study. Patients who had a BMI qualifying them as underweight were excluded (if older than 70 years BMI had to be over 21 kg/m^2). Additionally, patients with severe medical conditions, wounds not qualifying as PUs, on corticosteroids, palliative care or diet restrictions were excluded.

This study had two groups: the treatment group (n=22) received 3 doses daily consisting of 200 ml of a Cubitan, Nutricia (20 g of protein, 3 g of arginine, 238 mg vitamin A, 250 mg of vitamin C, 38 mg of vitamin E, 9 mg of Zinc, in addition to carotenoids, selenium, cooper, and folic acid). The control group (n=21) received a calorie-free flavored placebo 3 times daily.

There were no significant differences among patient's demographics, nutritional and biochemical parameters at baseline. At the end of the study, PU surface area was significantly reduced in the treatment group versus the control group over the entire period of the study (P=

0.016). However the size of the reduction was not explicitly reported but one can estimate that the PU area in the treatment group was half the sizes seen in the control group at the end of the study-only graphs were included. Additionally, researchers reported that PU scores improved significantly more in the treatment group versus control group (P= 0.033). For this latter result, the exact difference was not given within the publication, but it can be estimated that week 8 scores for both treatment were found to be just above 5.

Nutritional records showed that patients within the treatment group consumed on average 75.8% of the supplement, which was significantly lower than the average (86.5%) consumed by the control group (P= 0.042). Adequacy of nutritional intake was not assessed at the end of the study. The number of gastrointestinal complaints varied significantly between both groups. Constipation was significantly more frequent in the treatment group, occurring in 4 subjects (P= 0.029).

Researchers concluded that the high protein arginine and micronutrient enriched supplement speeded the healing of PUs. They reported an average healing rate of 0.26 cm^2 per day over the first 3 weeks of the study. By week 8 the average healing rate decreased to 0.16 cm^2 per day. The healing rates for the control group staying consistent and averaging 0.14 cm^2 per day by week 3 and 0.15 cm^2 per day by week 8.

Leigh, Desneves, Rafferty, Pearce, King, Woodward, and Brown (2012) randomized 23 Australian hospital and rehabilitation patients in with stage II, III, and IV PUs. Patients included in this study PUs, showing no signs of healing for at least 2 weeks, on oral diet, and had not yet begun taking arginine-containing supplements. Patients with sepsis, acute gastric surgery, receiving dialysis or taking corticosteroids or hydroxyurea were excluded.

There were two arms to this study: the first one (n=12) received 1 sachet of Arginaid (Nestle) containing 4.5 g of arginine, 4 g of carbohydrates, 155 g of vitamin C, and 40.5 mg of vitamin E; the second (n=11) received 2 sachets of Arginaid (9 g or arginine and twice as much of the other two micronutrients); for a period of 3 weeks. Those patients discharged from the hospital prior to the end of the study were sent with the number of sachets needed to complete the study. PU care was standardized and explained within the publication. A pseudo-control group was used for comparison, by using a historical control from a previous study done on a similar population.

The average age was 69.8 $\pm$ 5.2 years, and 67.5 $\pm$ 4.9 years old, respectively. At 3 weeks both groups had significant improvement in PUSH scores compared to baseline ($p < 0.001$); however, there was no difference in the rates of healing of both groups ($p = 0.991$). Both treatment groups were consuming less than their estimated energy and protein requirements at baseline. The group taking 4.5 g of arginine supplement had an intake that met less than 60% of their estimated calorie and protein needs. This amount was considered significantly less than the other treatment group.

The estimated time to full healing obtained by extrapolating results from pseudo-control group is about 15.6 weeks. Within this study, researchers calculated estimated time to full healing to be 8.7 weeks from the 4.5 g of arginine group, and 8.4 weeks for the 9 g of arginine group. Malnourished patients showed a trend for less improvement of PU when compared to well-nourished patients. Compliance with supplement intake was reported as an average of 92% for both groups

The authors concluded that there was no evident difference in the effect of dose-based arginine supplementation. Limitations included the use of food records to track actual intake, the difference in protein intake between the two groups, the unavailability of a true control group, the small sample size, and limiting PU healing observation time to 3 weeks, which lead to the need to extrapolate time to full healing. Strengths include the randomization of this study, the use of a hospital-based population, which allowed for close monitoring, and the in-depth description of standard protocols.

Cereda, Klersy, Serioli, Crespi, and D'Andrea (2015) performed a multicenter randomized controlled blinded trial on malnourished patients with stage II, III, or IV PUs for 8 weeks. Patients were either residing at home receiving home care services or in long-term care facilities. Only patients who were malnourished, able to drink oral nutrition supplements, and provide consent were included in this study. It is important to note that malnutrition was considered a BMI of less than 20 kg/m^2 or less than $21kg/m^2$ for those over 65 years, recent unintentional weight loss as per ASPEN guidelines, low serum albumin, or reduced food intake prior to the study. Any patient with poorly controlled diabetes, acute organ failure, advanced renal disease or hepatic insufficiency, moderate to severe heart failure or peripheral disease, current or previous neoplastic disease, with low hemoglobin, obesity or having an infected wound, cellulitis, sepsis or osteomyelitis or on artificial nutrition were excluded.

There were two groups in this study: treatment (n=101) and control (n=99). In both groups patients had liberal intake tailored to meet individual needs in regards to chewing or swallowing. All patients received two 200 ml bottles of an energy-dense, protein-rich oral formula (500 kcal, 40 g of protein). The supplements were administered in 100 ml increments

throughout the day in between meals. The supplement received by the treatment team was Cubitan (Nutricia). The control group received a combination of two supplements Fortimel and Fantomalt (Nutricia). The supplements differed significantly in the amount of arginine, zinc, copper, manganese, selenium, vitamin E and C. The nutritional information for Cubitan has been previously explained within this project. The control supplement contained 0 g of arginine, 2.3 mg of zinc, 338 mcg of copper, 0.63 mg of manganese, 11 mcg of selenium, 2.3 mg of vitamin E, and 19 mg of vitamin C.

Nursing PU care was standardized and described in detail within this publication. Nutritional interventions were also done according to current recommendations (e.g. protein intake was calculated by using 1.5 g/kg of body weight). The researchers provided training for standard procedure at least twice during the study and once before the beginning of the study. PU improvement was measured at week 4 and at the end of the study.

The mean age for the treatment group was 81.1 years whereas the mean age for the control group was 81.7 years. Adherence to the recommended supplementation was reported as 84.8% (SD, 15.2%) for the experimental formula group versus 83.7% (SD, 16.3%) in the control formula (P =0.65). The intake was able to provide adequate amounts of energy and protein. By week 8, the mean reduction in PU size was 60.9% (CI, 54.3% to 67.5%) in the treatment group versus 45.2% (CI, 38.4% to 52.0%) in the control group (P = 0.017). There was a greater however non-significant proportion of patients with fully healed PU by week 8 in the treatment group. Upon secondary analysis, by week 4, the treatment group also showed a significantly high rate of complete healing (P= 0.042) and reduction of PU area (p= 0.003). The researchers did not

specify the rate of amount of PU area reduction by week 4. Gastrointestinal intolerance was recorded only in 5 patients.

The authors concluded that in malnourished patients receiving standard PU and nutritional care, the administration of arginine-containing supplements with zinc and antioxidants provides benefit towards PU healing. Researchers pointed out that the specificity of micronutrients given, and the similarity of high-calorie high-protein supplementation were strengths of this study. Additionally, they mentioned that the exclusion of well-nourished patients matches the PU prevalence of malnutrition, thus lessening the effect of this limitation. In addition the maintenance of adequate caloric and protein intake throughout the study provided a strength to this study.

Bauer, Isenring, and Waterhouse (2013) performed an 8-week pragmatic open randomized trial with the purpose of comparing a wound-specific oral supplement versus a standard supplement in regards to its effects on wound healing (assessed by PUSH scores). The sample population was from an acute care hospital not specified within the publication. Eligible participants were those with chronic wounds (venous ulcers, pressure ulcer or surgical wounds), older than 18 years, and not receiving enteral or parenteral nutrition support.

There were 24 patients (mean age of 67.8 years) divided into two treatment groups: supplementation with a wound-specific supplement (n=12) or supplemented with a standard oral nutrition supplement (n=12). Patients received two servings per day of the open label wound supplement (237 ml each, 250 kcal, 10.5 g of protein, 4.5 g of arginine) or of a standard supplement (250 kcal and 9 g of protein). The exact name brand of the supplement was not provided within the publication.

At baseline, there was a significant difference in nutritional status, assessed by Patient Generated Subjective Global Assessment (PSGA) tool, between the control group and the wound supplement group (PSGA scores, 6.7 and 11, respectively; p= 0.025). Thus, researchers controlled the analysis for this variable. Mean change in PUSH scores between the control group (33.4% improvement) and the experimental group (4.3% improvement) were significantly different (P=0.00597). The mean change for the control group was reported as -4.8 (CI: -9.5, -0.1) and for the experimental group it was reported as -0.6 (CI: -2.9, 1.8). There were no significant differences in mean intake or compliance with the supplements within the two groups.

Researchers concluded that a standard supplement might be more effective on wound healing than a wound specific supplement, thus a change in practice was not recommended. Limitations mentioned in the publication include the small sample size and the heterogeneous nature of the wounds. The researchers pointed out that the fact that this study was done in a clinical care setting provided strength to the results and showed that the intervention can be applicable in the clinical acute care setting. Researchers standardized care throughout the study, which decreased bias. They documented adverse events and reported specific GI symptoms noticed with these specific supplements. This latter strategy may help elucidate exact doses to help prevent these disturbances.

The randomized trials previously discussed have many similarities. Cereda et al. (2009), Van Anholt et al. (2010), Leigh et al. (2013), and Bauer et al. (2013) described in detail the inclusion and exclusion criteria. However, only Cereda et al. (2009), Cereda et al. (2015), and Leigh et al. (2012) described standard PU and nutritional care given throughout the study. As

previously mentioned, replication of these studies will be key to verify their findings, but difficult to do if the exact methods are not described.

Only Leigh et al. (2012) performed a study without a control group. Results from this study are more difficult to compare with the rest of the randomized trials, as they attempted to see the dose based effect of arginine-containing supplements on wound healing. Contrary to the other studies, only one study proposed that there is no difference on healing rate effect between 4.5 g or 9 g of arginine given as part of a wound specific supplement (Leigh et al., 2012).

Cereda et al. (2011) assessed the effect on a malnourished population. Their study was performed on long-term care facility patients where prevalence of malnutrition ranges from 23% to 85%. The prevalence reported in this publication is not much different from that in the acute hospitalized facilities, thus making this study applicable in both populations.

Conclusion

PUs are a detriment to the health and quality of life of patients who develop them. Once a PU is identified, it is extensively recommended that wound care is started and maintained at regular intervals in order to prevent further destruction of the tissues. Dietary interventions for PU prevention should primarily focus on ensuring adequate caloric, protein and fluid intake and correcting any suspected or confirmed deficiencies. Even though clinical dietitians are widely aware of the exact populations at higher risk for PUs, tissue breakdown in acute, long term and community dwelling patients still occurs. Thus, more aggressive interventions may be needed to correct the nutritional inadequacies and address the higher needs seen in patients with PUs.

These nine articles have all demonstrated the advances in wound care and PU research in the last 10 years. The major gaps observed in the older research is the lack of description of methodology and the weakness of their study designs; namely open trials versus randomized controlled trials. Most results showed potentially beneficial effects of arginine-containing supplements as it related to PU healing. However, due to the small number of research found in this topic before 2010, the methodological nuances of older publications, and the few newer publications with strong statistical power, there is a need to review in detail the latter group of articles in order to decide whether a change in practice is warranted.

Chapter 3: Methodology

Registered Dietitians (RD) working in the clinical field pride themselves on being providers of Evidence Based Practice (EBP). The Evidence Analysis Library (EAL) is a synthesis of the most up-to-date nutritional research. The EAL was created in 2004, through the collaboration of Academy of Nutrition and Dietetics members (Academy of Nutrition and Dietetics [AND], 2015a).

The process of reviewing current research and incorporating it into the EAL is a rigorous process; however, it is also meant to be reproducible. This process is applauded by organizations such as the Joint Commission on Accreditation of Healthcare (JCAHO) and used as a model by the Food and Drug Administration. The JCAHO recognized the EAL process as a prime example on how to best bring about evidence based practice into day-to-day clinical care (AND, 2015b). Following, there will be a summary of the steps used to evaluate the articles within this project. These steps are based on the last edition of the Evidence Analysis Manual (AND, 2012).

The last Wound Care EAL project was published in 2011-2012. This project reviewed the evidence regarding conditionally indispensable amino acids (arginine, glutamine and cysteine) needs for adult patients with renal disease and Pressure Ulcers who were/were not on dialysis. Their recommendations make no mention of supplemental arginine, or oral nutrition supplements with arginine as one of its main components (AND, 2015d).

Step I: Formulation of the Evidence Analysis Question

In this step, it was important to ask centered questions that aim to resolve areas where knowledge is most needed to help guide professional practice. It is important to first establish the context in which the questions will be asked. For example, will the question concern a specific nutrition intervention and health outcome, or will it concern a specific assessment factor and its relationship with a nutrition outcome.

The EAL Manual recommends formulating questions using the PICO format [Population; Intervention, procedure, or approach; Comparison intervention; Outcome of interest] (AND, 2012). For this project, the type of population where the questions applies was determined first. Then, the type of intervention, reference comparison and the nutrition/healthcare outcome was selected. Table 1shows the PICO process for the current project.

The current EAL question is set in a population conformed of patients without critical illness or an illnesses that would impede wound healing. The intervention is a supplementation with wound supplement containing arginine among other nutrients. The comparison treatments or interventions are either a regular standard diets or the use of a commercial supplement meant to help patients reach their estimated calorie goal; not meant for wound healing. Lastly, the outcome of interest was selected as the healing of PUs.

Table 1. PICO process for EFFECTS OF PROTEIN CONTAINING ORAL NUTRITION SUPPLEMENTS

P	Patients with Pressure Ulcers
I	Arginine containing Oral Nutrition Supplements
C	Standard Nutrition Supplements
O	Pressure Ulcer Healing

The resulting EAL question for this project was: For a patient with pressure ulcers, what is the most effective nutrition intervention for increasing healing of pressure ulcers: Standard oral nutrition supplementation or arginine containing oral nutrition supplementation?

Step II: Gather and Classify Evidence

This step involved an initial development of the inclusion and exclusion criteria. Additionally, search words as well as databases used were identified. PubMed and EBSCOhost interfaces were used to find the most current research related to this project's EAL question. Citations and abstracts were reviewed and compared against the indicated inclusive/exclusive criteria. The results of this comparison were used to build a "Search Plan & Results" report. The articles remaining within the inclusion criteria were analyzed and evaluated based on the EAL classification guidelines as described below (AND, 2012).

Figure 1: *Hierarchy and Classification of Studies.* System used to evaluate research in order to provide information about the type of study and level of evidence it provides (AND, 2012).

Primary Reports		Secondary Reports	
A	Randomized Controlled Trial Cluster Randomized Trial Randomized Crossover Trial	**M**	Meta-analysis or Systematic review Decision analysis Cost-benefit analysis Cost-effectiveness study
B	Prospective Cohort Study Retrospective Cohort Study		
C	Non-Randomized Controlled Trial Non-Randomized Crossover Trial Case-Control Study Time Series Study Diagnostic, Validity or Reliability Study	**R**	Narrative review (Review article) Consensus statement Consensus report
D	Non-Controlled Trial Case Study or Case Series Other Descriptive Study Cross-Sectional Study Trend Study Before-After Study	**X**	Medical opinion

Step III: Critical Appraisal of Each Article

This step can be summarized as the process of abstracting the details of each study into the EAL worksheets. The information gathered through critically appraising each article was used to determine the quality of each study, and to summarize major findings. A Quality Criteria Checklist (QCC) was used to help the reviewer with assigning an overall rating to the study. More specifically, the rating addressed the applicability and scientific validity of each study. This information was compiled into a report named the Quality Criteria Summary, which can then be made available online as an effort to demonstrate the transparency of the EAL process (AND, 2012).

Step IV: Summarize the Evidence

A narrative version and a tabulated version of the evidence summary was created. The narrative versions are meant to be brief and easy to read, while not losing sight of relevant details. The tabulated version was built using the Overview Table tool, which is used to help reviewers assess how the research compares against each other. Each overview table was personalized to fit within the critical comparison factors of a specific EAL project. The results of each article were summarized. Lastly, a brief narrative summary was written to show general concordances among the articles within the context of the project's question.

Step V: Write and Grade the Conclusion Statement

The final step of the evidence analysis process included grading the strength of the research and writing a conclusion statement. The strength of the research can vary from grade I through V; the latter signifying that there is no evidence to directly support or refute the conclusion statement. A summary of the meaning of each grade is provided below.

Figure 2: *Conclusion Grading Table.* Criteria used to evaluate the strength of research during the evidence analysis process.

Conclusion Grading Table					
Strength of Evidence Elements	Grade I: Good/Strong	Grade II: Fair	Grade III: Limited/Weak	Grade IV: Expert Opinion Only	Grade V: Grade Not Assignable
Quality • Scientific rigor/validity • Considers design and execution	Studies of strong design for question Free from design flaws, bias and execution problems	Studies of strong design from question with minor methodological concerns, OR only studies of weaker study design for	Studies of weak design for answering the question OR Inconclusive findings due to design flaws, bias, or	No studies available Conclusion based on usual practice, expert consensus, clinical experience, opinion, or	No evidence that pertains to question being addressed

		question	execution problems	extrapolation from basic research	
Consistency Of findings across studies	Findings generally consistent in direction and size of effect or degree of association, and statistical significance with minor exceptions at most	Inconsistency among results of studies with strong design, OR consistency with minor exceptions across studies of weaker design	Unexplained inconsistency among results from different studies OR single study unconfirmed by other studies	Conclusion supported solely by statements or informed nutrition or medical commentators	NA
Quantity • Number of studies • Number of subjects in studies	One to several good quality studies Large number of subjects studied Studies with negative results have sufficiently large sample size for adequate statistical power	Several studies by independent investigators Doubts about adequacy of sample size to avoid Type I and Type II error	Limited number of studies Low number of subjects studied and/or inadequate sample size within studies	Unsubstantiated by published research studies	Relevant studies have not been done
Clinical impact • Importance of studied outcomes • Magnitude of effect	Studied outcome relates directly to the question Size of effect is clinically meaningful Significant (statistical difference) is large	Some doubt about the statistical or clinical significance of the effect	Studied outcome is an intermediate outcome or surrogate for the true outcome of interest OR Size of effect is small or lacks statistical and/or clinical significance	Objective data unavailable	Indicates area for future research
Generalizability To population	Studied population,	Minor doubts about	Serious doubts about	Generalizability limited to scope	NA

of interest	intervention and outcomes are free from serous doubts about generalizability	generalizability	generalizability due to narrow or different study population, intervention or outcomes studied	of experience	

Search Plan and Results

Evidence Analysis Question

What is the evidence regarding the use of arginine-containing oral nutrition supplements and the healing of pressure ulcers?

Inclusion Criteria

The following inclusion criteria was used to conduct this analysis: subjects were adults over 18 years old, were either well-nourished or malnourished nutritional status, given an arginine-containing oral nutrition supplement specific to the healing time of pressure ulcers. Additional criteria included: primary research articles published in peer-reviewed journals, English language, and articles published between January 2010 to April 2015.

Exclusion Criteria

Articles with subjects <18 years old were excluded. In accordance with EAL guidelines, articles using non-human subjects were not included. Case studies, review articles and meta-analysis were excluded in the comprehensive literature review. Also, articles were excluded if the interventions included supplements that contained other amino acids other than arginine,

non-arginine containing supplements. As mentioned above, any study published in any language other than English, or published before 2010 was excluded. Studies that included patients with multiple types of wounds (PU, surgical wound and Diabetic ulcers) were excluded.

Search Terms: Search Vocabulary

The term "pressure ulcer" was used as the primary health condition. All searches were performed by combining "pressure ulcer" with the following terms: healing, treatment, arginine, and supplements. The results of each search are displayed in the following paragraphs.

Electronic Databases

PubMed was used as one of the sources for peer reviewed articles. EBSCOhost was also used to review three databases simultaneously, which included: MEDLINE, Academic Search Premier, and CINAHL. The restrictions applied to all searches included: Peer-reviewed journal, English, human subjects, and dates between January 2010 and April 2015.

Tables 2 and 3 include the results from both database searches. They also include the number of articles found with each search using the restrictions explained in the inclusion/exclusion criteria.

Table 2. PubMED search term and results

PubMED		
Search	Search Terms	Results
S1	"pressure ulcer treatment and arginine"	12
S2	"pressure ulcer and arginine"	13
S3	"pressure ulcer healing and arginine"	10
S4	"pressure ulcer healing and arginine supplement"	9

S5	"pressure ulcer treatment and arginine supplement"	9

Table 3. EBSCOhost search terms and hits

EBSCOhost		
Search	Search Terms	Results
S5	"pressure ulcer treatment and arginine supplement"	7
S4	"pressure ulcer healing and arginine supplement"	10
S3	"pressure ulcer healing and arginine"	16
S2	"pressure ulcer and arginine"	22
S1	"pressure ulcer treatment and arginine"	15

List of Included Articles

Brewer, Desneves, Pearce, Mills, Dunn, Brown & Crowe (2010). Effect of an arginine-containing nutritional supplement on pressure ulcer healing in community spinal patients. *Journal of Wound Care, 19* (7), 311-316.

Cereda, E., Klersy, C., Serioli, M., Crespi, A., & D'Andrea, F. (2015). A nutritional formula enriched with arginine, zinc, and antioxidants for the healing of pressure ulcers. *Annals of Internal Medicine, 162*, 167-174. doi:10.7326/M14-0696

Leigh, B., Desneves, K., Rafferty, J., Pearce, L., King, S., Woodward, M. C., & Brown, D. (2012). The effect of different doses of an arginine-containing supplement on the healing of pressure ulcers. *Journal of Wound Care, 21*, 150-156. PMID:22399084

Van Anholt, R. D., Sobotka, L., Meijer, E. P., Heyman, H., Groen, H. W., Topinkova, E., Van Leen, M., & Schols, J. M. G. A. (2010). Specific nutritional support accelerates pressure ulcer healing and reduces wound care intensity in non-malnourished patients. *Nutrition, 26*, 867-872. doi:10.1016/j.nut.2010.05.009

List of Excluded Articles

Excluded for intervention on patients with multiple wounds:

Bauer, J. D., Isenring, E., & Waterhouse, M. (2013). The effectiveness of a specialized oral nutrition supplement on outcomes in patients with chronic wounds: a pragmatic randomized study. *Journal of Human Nutrition and Dietetics, 26*, 452-458. doi:10.1111/jhn.12084

Chapman, B. R., Mills, K. J., Pearce, L. M., & Crowe, T. C. (2011). Use of an arginine-enriched oral nutrition supplement in the healing of pressure ulcers in patients with spinal cord injuries: An observational study. *Nutrition and Dietetics, 68*, 208-213. doi:10.1111/j.1747-0080.2011.01536.x

Tatti, P., Barber, A. E., di Mauro, P., & Masselli, L. (2010). Nutrition supplement. *European Wound management Association Journal, 10* (3), 13-18.

Excluded for use of formulation containing Beta-hydroxy beta-methybutyrate (HMG), arginine and glutamine, as well as intervention given via enteral nutrition rather than oral feeds only:

Wong, A., Chew, A., Wang, C. M., Ong, L., Zhang, S. H, & Young, S. (2014). The use of a specialized amino acid mixture for pressure ulcers: A placebo-controlled trial. *Journal of Wound Care, 23* (5), 259-269. PMID: 24810310

Excluded for use of nutrition support mediated intervention:

Yatabe, J., Saito, F., Ishida, I., Sato, A., Hoshi, M., Sizuki, K., Kameda, T., Ueno, S., Yatabe, M. S., Watanabe, T., & Sanada, H. (2011). Lower plasma arginine in enteral tube-fed patients with pressure ulcer and improved pressure ulcer healing after arginine supplementation by Arginaid Water. *Journal of Nutrition Health and Agining, 15* (4), 282-286. PMID: 21437560.

Excluded for being a review article:

Sernekos, L. A. (2013). Nutrition treatment of pressure ulcers: what is the evidence? *Journal of* American Association of Nurse Practitioners, 25, *281-288. doi: 10.1002/2327-*6924.12025.

Blanc, G., Meier, M. J., Stocco, J. G. D., Roehrs, H., Crozeta, K., & Barbosa, D. A. (2015). Effectiveness of an enteral nutrition therapy in the healing process of pressure ulcers: a systematic review. *Revista da Escola de Enfermagem da USP, 49* (1), 150-159. PMID: 25789655.

Excluded for being a clinical trial update:

Slomski, A. (2015). Clinical trials update. *Journal of the American Medical Association, 313* (12), 1200.

Excluded for unrelated topic potentially added to list of results due to MeSH terms:

Barisic, I., Belnovic, D., Klicek, R., Radic, B., Nikitovic, B., Drmic, D., Strinic, D., Bardak, D., Berkopic, L., Djuzel, V., Sever, M., Cvjetko, I., Romic, Z., Sindic, A., Bencic, M. L., Seiwerth, S., & Sikiric, P. (2013). Mortal hyperkalemia disturbances in rats are NO-system relaed. The life saving effect of pentadecapeptide BPC 157. *Regulatory Peptides, 181*, 50-66. doi: 10.1016/j.regpep.2012.12.007.

Ciammaichella, G., Belcaro, G., Dugall, M., Hosoi, M., Luzzi, R., Ippolito, E., & Cesarone, M. R. (2012). Product evaluation of Ureadin Rx Db (ISDIN) for prevention and treatment of mild-to-moderate xerosis of the foot in diabetic patients. Prevention of skin lesions due to microangiopathy. *Panminerva Medica, 54* (1 suppl 4), 35-42. PMID:23241933.

Jude, E. B., Dang, C., & Boulton, A. J. (2010). Effect of arginine on the microcirculation in the neuropathic diabetic foot with Type 2 diabetes mellitus: a double-blind, placebo-controlled study. Diabetic Medicine, 27 (1), 113-116. doi: 10.1111/j.1464-5491.2009.02876.x.

Massumoto, K., Nagata, K., Oka, Y., Kai, H., Yamaguchi, S., Wada, M., Kusuda, T., Hara, T., Hirose, S., Iwasaki, A., & Taguchi, T. (2011). Successful treatment of an infected wound in infants by a combination of negative pressure wound therapy and arginine supplementation. *Nutrition, 27* (11-12), 1141-1145. doi: 10.1016/j.nut.2011.01.006.

Pinheiro, L. C., Montenegro, M. F., Amaral, J. H., Ferreira, G. C., Oliveira, A. M., & Tanus-Santos, J. E. (2012). Increase in gastric pH reduces hypotensive effect of oral solution nitrite in rats. Free Radical Biology and Medicine, 53 (4), 701-709. PMID: 22721923.

Scott, A. R. (2015). Management of hyperosmolar hyperglycemic state in adults with diabetes. *Diabetic Medicine, 32* (6), 714-724. doi: 10.1111/dme.12757.

Sikiric, P., Seiwerth, S., Rucman, R., Turkovic, B., Rokotov, D. S., Brcic, L., Sever, M., Klicek, R., Radic, B., Drmic, D., Ilic, S., Kolenc, D., Vrcic, H., & Sebecic, B. (2011). Stable gastric pentadecapeptide BCP 157: novel therapy in gastrointestinal tract. *Current Pharmaceutical Design, 17* (16), 1612-1632. PMID: 21548867.

Yatabe, M. S., Taguchi, F., Ishida, I., Sato, A., Kameda, T., Ueno, S., Takano, K., Watanabe, T., Sanada, H., & Yatabe, J. (2013). Mini nutritional assessment as a useful method of predicting the development of pressure ulcers in elderly patients. *Journal of the American geriatrics Society, 61* (10), 1698-1704. doi: 10.1111/jgs.12455.

Four articles resulted from this search to be used for the Evidence Analysis, which is detailed in Chapter 4, Results.

Chapter 4: Results

Four studies, that met the inclusion/exclusion criteria described previously, were identified using the evidence analysis process. Each of the articles is described at extent and summarized in the Literature Overview Table (Table 4.). As indicated in the methodology section, each article was evaluated by completing the Evidence Worksheets and Quality Criteria Checklists (Appendix A). A summary of the Quality Criteria Summary is shown in Table 5.

The first study identified that fit the inclusion/exclusion criteria was Van Anholt et al. (2010). Researchers performed a randomized multicenter, multi-country double-blinded parallel group trial. The study's purpose was to assess the potential effect of an oral nutritional supplement on PU healing and on decreasing the intensity of wound care on non-malnourished patients with stage III or IV PUs from 8 different health centers, hospitals and long-term care facilities in 4 different countries. Their primary endpoint was to determine PU healing, assessed by change in PU area. Their secondary outcome was the change in PUSH scores.

Patients aged 18 to 90 years old, with at least one stage III or IV PU, who had been given standard care without the use of an oral nutrition supplement for at least 2 weeks prior to the beginning of the study, were included in the study. Exclusion criteria was described as having a BMI <18.5 kg/m^2 if within 18-70 years old, or a BMI< 21 kg/m^2 if older than 70 years old, severe medical conditions, diabetes mellitus ulcers, prognosis of less than 6 months, on palliative care, on steroid treatment, or on dietary protein restrictions.

Dietary interventions consisted in 2 servings daily of an arginine-containing oral nutrition supplement (Cubitan, Nutricia-nutritional information previously described) for a period of 8

weeks. The second arm of the study received a non-caloric flavored placebo of similar taste and appearance. Disease appropriate standardized PU and nutritional care was maintained throughout the study.

PU area (assumed to be of elliptical form) was obtained at baseline and assessed weekly. For patients with multiple PUs, only one was chosen to follow throughout the study. Baseline measurements included anthropometrics; Malnutrition Screening scores and blood parameters such as vitamin C, zinc, alanine aminotransferase, hemoglobin and C-reactive protein, among others. Compliance was reported in quarter fractions and recorded in a diary. Gastrointestinal tolerance was assessed weekly. The number of dressing used was recorded weekly. The time spent on dressing changes was recorded at week 1, 4, and 8. Mobility and activity levels were also tracked at baseline, week 4 and week 8 of the study.

Researchers reported that a total of 47 patients were randomized in this study. However, only 43 were included in the intent-to-treat analysis. The experimental group was comprised of 22 patients, while the control group had 21. There were no statistically significant differences among baseline characteristics. After eight weeks, there was a significantly larger decline in PU size within the experimental group compared to the placebo group (P=0.006, treatment by time; P=0.016, treatment by time^2). The exact difference for this latter result was not found in the publication; only graphed results. A rate of 0.26 cm^2/day over the first 3 weeks for the wound supplement group was noted; which then leveled off to 0.16 cm^2/day by week 8. The average healing rate for the placebo group remained mostly consistent between week 3 (0.14 cm^2/day) and week 8 (0.15 cm^2/day).

In-group analysis showed that there was a significant (P=0.019, ANOVA) decrease in PU size by week 3 in the experimental group, while the statistically significant (P=0.019, ANOVA) decrease in PU size did not show until week 5 within the placebo group. PUSH scores were found to be significantly different between the supplemented group versus the placebo (P=0.011, treatment by time; P=0.033, treatment by time^2, RMMM). Actual change in PUSH scores was not described in the publication.

There were less dressing changes performed in the ONS group versus the placebo group, which proved to be statistically significant (P=0.003, treatment by time; P=0.045, treatment by time^2, RMMM, post hoc). Thus, statistically significant less time was spent in dressing changes in the ONS versus control group (P=0.006, treatment by time; P=0.022, treatment by time^2, RMMM, post hoc). It was reported that 22 minutes were saved per patient per week (within the treatment group) after week 4 versus the control group at the same time point.

About 86.5 % of the placebo was consumed versus 75.8% of the treatment supplement (P=0.042, ANOVA). There were no significant differences in patient's activity or mobility. There were 41 adverse events, reported within the treatment group and 35 in the control group. All adverse events were reported as being of mild-moderate intensity. Difference in blood parameters showed no statistical significance between both arms of the study.

The authors concluded that an arginine-containing oral nutritional supplement could have a positive impact on the healing of PUs in non-malnourished patients. Additionally, they postulated that supplementation could be a cost effective intervention, since they found supplementation showed a reduction in nursing care needed for dressing changes.

Limitations include the lack of explanation of diet adequacy throughout or at the end of the study. There was no indication of the criterion for the selection of which PU was followed when a patient had multiple PUs. Also, the standard protocol for nutritional and PU care was not described, although it was noted to have been done according to locally used protocols. The randomized controlled trials are the gold standard in research thus counted as a strength of this study.

This first study received a neutral rating based on the Quality Criteria Checklist. The questions regarding the relevance of the study matter and the feasibility of the intervention were answered positively. There are four questions in this checklist that needed to be answered positively in order for this study to be able to receive a positive rating. These questions inquire about the selection of subjects being free from bias, the comparability of the study groups, the validity and reliability of the outcome measures, and the depth of the description of intervention protocol. The first 3 questions were answered positively; however this study did not provide detailed descriptions of the protocols used for nutrition intervention, such as the method for calculating calorie and protein needs. Also there was no mention of maintenance of intake adequacy throughout the study. Therefore it was judged that the intervention and intervening factors were not described in sufficient detail.

Other questions influencing validity included whether the study question was clearly stated, whether blinding was used appropriately, whether the method for handling withdrawals was described, whether the statistical analysis used was appropriate for the study design, and if the conclusions were supported by the results. The latter group of questions was answered in a positive manner. However, it was unclear whether there was a bias due to study funding. Overall,

this study was deemed of positive relevance and unclear validity, which is the reason for the neutral rating.

A second study was a randomized, multicenter, parallel assignment; double blinded, controlled clinical trial performed in a long-term and home-care population by Cereda et al. (2015). The primary outcome was to evaluate the percent change in PU at 8 weeks. The secondary outcomes included complete PU healing, PU area reduction of 40% or more by 8 weeks, the incidence of wound infections, the number of dressing required for wound care, and percent change in PU area by week 4.

Inclusion criteria included ability to drink an oral nutrition supplement, the ability to be consented for the study, and the presence of malnutrition at baseline. The authors excluded patients with poorly controlled Diabetes, acute organ failure, advance renal or hepatic insufficiency, moderate to severe heart failure, COPD or PVD, connective tissues disease, history of or current neoplastic disease, low hemoglobin, obesity, immunosuppressant therapy, infected wounds, cellulitis, sepsis or osteomyelitis, and artificial nutrition. A total of 200 patients were randomized; 101 patients were randomized to the experimental group, and 99 were randomized to the placebo group.

The nutritional intervention consisted of general diet advice given to home-care patients. Diet advice to long-term patients was tailored to their chewing/swallowing abilities. In addition to unrestricted food intake, a total of 2 servings of Cubitan were given daily. The amount of supplement was divided into 4 boluses of 100 milliliters each. The control group received a product combination of Fortimel and Fantomalt, which contained no arginine and significantly different amounts of zinc and antioxidants. Standardized nursing care was provided.

Standardization of PU care was ensured through multiple clinician training sessions on providing PU care.

Patients were provided with standardized PU care, which included pressure relieving devices and repositioning programs. Topical treatment were done by the same clinician specialized in wound care. The type of dressing and frequency of change was dependent on PU severity.

Baseline characteristics collected included anthropometrics, main diagnosis, and presence of diabetes. Calories were calculated by using the Harris Benedict equation using 1.2 for the activity factor and 1.1 for PU stress factor. Daily protein requirements were set as 1.5 g/kg of actual body weight. Anyone with a BMI larger than 23 kg/m^2 had their protein requirements calculated by using their ideal body weight (IBW). Daily calorie/protein intake was assessed every 2 weeks with collection of 3-day quantitative food diaries. PU area was measured at baseline, week 4 and week 8. Site and stage of PU was also collected at baseline.

Patient attrition rates dropped between both groups by week 4, where 78% of patients remained in the experimental group and 79% in the control group. At the end of the study, there were 67 patients in the experimental group and 71 in the control group. Results showed that both treatment groups experienced significant improvements in PU healing ($P<0.001$). At 8 weeks, there was a 60.9 % reduction in PU size within the experimental group (CI: 54.3% to 67.5%). At the same time interval, the control group showed a 45.2 % reduction in PU size (CI: 38.4% to 52.0%). When adjusted for treatment effect, these differences remained significant ($P = 0.017$).

Patients within the experimental group had at least a 40% reduction in PU size at week 8, 69.9% (CI: 59.5% to 79.9%) versus the control group at 54.1% reduction in PU size (CI: 42.7% to 65.5%). This difference was reported as significant with a P value of 0.02. There was no significant difference identified in regards to complete healing at week 8, among the 2 treatment groups (OR, 2.16 [CI, 0.88 to 5.39]; P=0.097). For those patients who remained at week 4 of the study, it was determined that the experimental formula showed a statistically significant effect on the rate of wound healing and the reduction of PU area (P=0.042, P=0.003, respectively).

Overall, mean reduction in PU size at week 8 was significantly greater among the experimental group versus the control (P = 0.017). Adherence was high in the experimental and control groups (84.8% and 83.7%, respectively). The same observations were made in regards to calorie and protein; patients adhered to estimated energy requirement recommendations. Overall, treatment resulted in significantly increased body weight at 8 weeks for both groups (P<0.001). The most common adverse events included dyspepsia and diarrhea.

The authors concluded that malnourished patients would benefit from an oral nutrition supplement containing arginine, zinc and antioxidants to aid in the healing of PUs. They also recommended that nutritional interventions should remain as an integral part of PU care.

Limitations of this study were that patients on artificial nutrition were not considered within this study. They explained that there is a large amount of patients who are treated for PU, who are also on nutrition support (Cereda et al., 2015). Strengths found in this study include the author's description of the exact calculations used for generating the nutrition prescription, and their monitoring of adverse events.

This study was given a positive rating. It was found that the matter in questions as well as interventions were relevant to current practice and reproducible. It was also found that all validity questions were answered positively. This includes but is not limited to the comparability of the study groups (there were no significant differences at baseline), the unbiased selection of study groups, and the thorough description of the interventions and outcomes.

Additionally, the research question was clearly given, the method for withdrawals were well described, the statistical analysis was appropriate for the type of study, and the conclusions were supported by the results. Lastly, the researchers also provided information about their funding sources. This study was judged to have a strong study design.

The third study was a randomized pseudo-controlled trial set to evaluate whether a dose of 4.5 grams of arginine given via an oral supplement had equal effects to the benefits seen with a dose of 9g of arginine (Leigh et al., 2012). The primary outcome was PU healing rate, measured by the weekly change in size and severity until the end of the third week, thus reported as PUSH scores.

The inclusion criteria consisted of inpatients or rehabilitation patients within the 3 campuses of Austin Health (Melbourne, Australia). Participants had to have stage II, III, or IV PUs not showing any signs of improvement, be able to eat orally, and not have had any arginine containing supplements at least 2 weeks prior to the study. A non-healing status was determined by reviewing nursing and physician records over the 2 weeks prior to the study. The exclusion criteria were the presence of sepsis, acute gastric surgery, dialysis, or on hydroxyurea, prednisolone, and dexamethasone therapy.

Patients were randomly assigned to either arm of the study. The first arm, received 4.5 g of arginine via an arginine-containing mixed supplement (Arginaid, Nestle) in addition to the standard hospital diet. The second arm of the study received two sachets (9g of arginine) in addition to the standard hospital diet. Each sachet was dissolved in 200 ml of water. In addition to arginine, Arginaid contains 4 g of carbohydrates, 155 mg of vitamin C, and 40.5 mg of vitamin E per sachet. Patients discharged from the hospital were given the appropriate number of sachets to last until the end of the study. At this time the same infection control clinician reviewed the PU. The researchers used a pseudo-control group from a previous study to compare the treatment results with a non-supplemented group.

PU care was given throughout the study following the hospital protocol, which was individualized to each patient, but was not described in the methods. However some strategies for wound management were: pressure re-distribution, turning schedules, friction elimination, daily skin assessment and hygiene and referral to orthotist.

An independent infection control clinician, blinded, to the study intervention, evaluated PU healing. Dietary intake was collected for the first 3 days of the study, in addition to at the end of the 3 weeks, or when complete healing was achieved. For patients discharged prior to the end of the study, compliance was monitored through follow-up phone calls. Supplement compliance was monitored daily and written in on bedside-charts. Dietary intake adequacy was then obtained by comparing intake versus approximated needs calculated via the Schofield equation and protein needs from the recommended amounts given by NPUAP. Weight was measured at baseline and weekly, thereafter. Height and nutritional status were assessed at baseline.

Data was analyzed for 23 participants. Twelve patients from the 4.5 g of arginine group, and 11 from the 9 g of arginine group. There were no significant differences in baseline characteristics such as patient's age, gender, BMI, PUSH scores, hemoglobin or albumin levels, or diagnosis of diabetes. The authors reported a significant decrease in PUSH scores over time but failed to report how much the decline was ($p<0.001$). Nonetheless, there was a lack of significance when the authors evaluated the healing rate between both groups ($p=0.991$).

About 52% of patients were found to be malnourished according to the criteria of the Subjective Global Assessment (SGA). Compared to malnourished patients, well-nourished patients had less severe PUs. The different doses of arginine demonstrated a lack of significant effect based on arginine dosage alone ($p=0.393$). Although randomization was done prior to treatment assignment, researchers found that, significantly higher calories and protein were consumed, at baseline, by the group randomized to 9 g of arginine ($p=0.036$, $p=0.018$, energy and protein, respectively).

It was found that both treatment groups were consuming much lower energy and protein than their estimated needs. The group with the lowest intake of arginine consumed significantly less calories ($p= 0.008$) and protein ($p=0.008$) than the group randomized to 9 g of arginine. There were no significant in-group or between-group changes in body weight throughout the length of the study. Overall compliance with supplement consumption was reported as 92%.

The authors concluded that a dose of 4.5 g of arginine provided similar healing rates on PUs when compared to a dose of 9 g of arginine. When compared to non-supplemented historical controls, the healing rates of both arms were about 2-fold that of the controls. Limitations include the lack of an active control group, the small sample size and, the length of the study

being 3 weeks rather than monitoring participants to observe the actual time-to-healing. The authors described the randomization of patients into each study group and the use of the same nurse (who was blinded to the study intervention) to evaluate PU healing as strengths in this study.

This study was evaluated for relevance and validity. All relevance questions of the Quality Criteria Checklist were answered in a positive manner implying that the matter evaluated in this publication is of current interest to practitioners and feasible to reproduce.

The study groups who received supplementation were randomized. However, the group receiving 9 g of arginine had an overall significantly higher intake of calorie and protein, when compared to the group receiving 4.5 g of arginine. This difference made the two treatment groups not comparable at baseline. Also, both intervention groups were compared to a historical control. The use of historical controls made the question of comparability unclear. Additionally, the study subjects did not appear to be blinded to the intervention. Therefore it was unclear whether the authors prevented bias. Thus, overall the study received a neutral grade.

Lastly, Brewer et al. (2010) conducted a prospective observational trial through the Austin Health Spinal Outreach Risk Reduction Team (SpORRT) in Melbourne, Australia. Recruitment happened in the same order that patients were referred to SpORRT for PU management. The purpose of this study was to evaluate the size of the effect of an arginine-containing supplement on the rate and time-to-healing of PUs in spinal cord injury patients residing in the community versus a historical control group who did not receive supplementation.

Inclusion criteria were patients suffering from spinal cord injury, older than 18 years, residing in the Melbourne metropolitan area, with at least one stage II, III, or IV PU. Patients were excluded if they had phenylketonuria, sepsis, chronic renal failure, metabolic disease, with present or suspected diabetic foot ulcers, or receiving hydroxyurea, prednisolone, or dexamethasone therapy.

The historical control group was obtained by searching the SpORRT database for patients with PUs in the 3 years prior to the study. Fifty charts were reviewed, of which 17 contained enough detailed information on PUs to be used as controls. Information needed in these charts included time to full healing.

The study group was asked to consume two sachets of Arginaid (nutritional information previously described). Briefly, this volume of supplementation would have provided 9 g of arginine among other nutrients. Each sachet was mixed with 200-250 ml of water prior to consumption. If patients were admitted to the hospital during the study, supplementation was continued until full wound healing was achieved.

The same spinal nurse assessing PUs at baseline recorded the date of healing. PU care was done according to best practice standards; however these were not described in the publication. PU assessment was done at regular intervals but the exact frequency was not noted. PU evaluation of the observational group was done according to PUSH scores. Since calculation of PUSH scores was not routine care for patients in the spinal outreach service, PUSH scores were not available for the historical control group.

Nutritional status was assessed by use of the Subjective Global Assessment (SGA) tool, which evaluates dietary intake, weight change, gastrointestinal symptoms as well as observations on muscle/fat wasting and edema/ascites. Compliance with supplementation was monitored after 3 days of the start of the intervention, and then at each nursing visit. Compliance was assessed by comparing the number of sachets left, versus the number of prescribed. Anyone consuming less than 75% of the recommended amount was excluded from the analysis.

Eighteen patients were recruited into the intervention group and 17 medical charts were used for the historical controls. There were no significant differences in baseline characteristics such as age, gender, diabetes diagnosis, number of years since spinal cord injury, level of injury, PU area, and number of PU per patient. A total of 26 PUs were found in the intervention group compared to 30 in the control group.

The rate of healing in the intervention group was twice as fast as the control group ($p= 0.006$). Time-to-healing was not significantly different in subgroup analysis of paraplegic versus quadriplegics. Time-to-healing was compared to the expected time-to-healing found in Bennett, Dealey, & Posnett (2004). Briefly, the expected times found in this publication include 13.4 weeks for stage II PUs, 18.2 weeks for stage III PUs, and 22.1 weeks for stage IV PUs. Brewer et al. (2010) reported statistically and clinically significant faster rates in their intervention group, for all PU stages.

Patients in the intervention group consumed at least 85% of the prescribed supplement. According to PSGA, 94% of all patients in the intervention group were considered well nourished. The influence of diabetes on PU healing was evaluated. The only significance noted

was on stage II PUs, where patients who had diabetes showed slower healing rates than those who did not have the diagnosis. These results where not observed for stage II or IV PUs.

Researchers concluded that there is a potential for a positive clinical effect with the use of arginine containing supplements to help the healing of PUs. Additionally, they point to the need for larger randomized controlled studies to verify these results and provide stronger evidence. Limitations include the small sample size, the lack of blinding of participants to the intervention, and the use of a historical control. Also, the observational nature of this study added additional variables that could not be standardized. Lastly, the community environment added more uncertainty to the standardization of PU care; as turning schedules and pressure-redistribution could not be controlled. Strengths of this study include the use of the same spinal cord nurse to evaluate wound progression, and the inclusion of patients with diabetes, which allowed for the evaluation of the effect of diabetes on PU healing rate.

This study obtained a neutral grade. All questions regarding the relevance of this study were answered positively granting that the subject is relevant to the current practice and the intervention is feasible. Most validity questions were answered positively. It was unclear if the groups were comparable because of the use of a historical control, which added the limitation that some historical data was unavailable (e.g. PUSH scores were not routinely documented in the historical controls). The nurse assessing the PUs was blinded to the intervention, however the patients were not, thus increasing the bias. Also, there was no description of funding for this study. The questionable comparability, increased bias and lack of description of funding made the validity of this study unclear which lead to its neutral grade. Also, a study cannot receive a positive rating unless comparability is clearly evident in a publication.

Table 4. Literature Overview Table

Author, Year, Study Design, Class Rating	Study Purpose	Study Population	Intervention	Outcomes	Conclusions	Limitations
Van Anholt, R. D., Sobotka, L., Meijer, E. P., Heyman, H., Groen, H. W., Topinkova, E., Van Leen, M., & Schols, J. M. G. A. (2010). Study Design: Randomized placebo-controlled, double-blinded trial Class: A Rating: (Ø)	To assess the potential of a wound oral nutrition supplement to improve healing of PUs and decrease the intensity of wound care in non-malnourished patients.	Men and women, 18 to 90y with at least one Stage III and IV PU, with a BMI of at least 18.5kg/m2 for those 18-70 years old and BMI >21kg/m2 for those older than 70 years, without severe medical conditions, non-pressure-related ulcers, life expectancy shorter than 6 months, palliative care, corticosteroid therapy, or on restrictive diets. N= 43 **Intervention Group: (Cubitan, Nutricia)** N= 22, 8 men/14 women, mean age 76.2 y, mean BMI 23.7 kg/m^2, 17 with stage III, 5 with stage IV PU. **Control Group: (Non-caloric flavored placebo)** N= 21, 11 men, 8 women, mean age 73y, mean BMI 25.8 kg/m^2, 14 with stage III, 7 with stage IV PU.	Standard nutritional diet and wound care were maintained according to institutional protocols. 8 week treatment of 200 ml 3 times per day	PU size decreased significantly over 8 weeks (values not reported). An average healing rate of 0.26 cm^2/d over the first 3 weeks was noted. This rate leveled off to 0.16 cm^2/d. PUSH scores were significantly improved (decreased by about half, exact numbers not provided in publication) between the treatment and control groups (p=0.011). A significant number of dressings were used in the treatment group, compared to the control (p=0.045). The exact decrease in dressings used was not reported for either of these findings. Significant less time was spent in changing dressings in the treatment group versus the control group (p=0.006). After week 4, on average 22 minutes were saved per patient per week in the treatment group	PU healing can be accelerated in non-malnourished patients by providing an arginine-containing supplement also enriched with antioxidants and zinc. Supplementation may increase quality of life and decrease time spent on wound care.	There was no disclosure regarding diet adequacy without the supplement. Did not provide detailed information of protocols for standard nutritional or pressure ulcer care. Patients were not followed until discharge thus increase in healing rate could not be linked to decrease in length of stay. Outcome values were not numerically provided rather only significance was noted.

Brewer, S., Desneves, K., Pearce, L., Mills, K., Dunn, L., Brown, D., &Crowe, T. (2010). Study Design: Non-randomized Controlled Trial Class: C Rating: Neutral (Ø)	To evaluate the rate and time-to-healing of pressure ulcers (PUs) in community dwelling spinal cord injury (SCI) patients consuming an specialized arginine containing supplement, versus a historical control group who did note received arginine containing supplements	Men and women, 18y or older, SCI patients residing in the metropolitan area of Melbourne (Australia) presenting with a category II, III or IV PU. N=35 **Intervention Group (**Arginaid [Nestle Nutrition, MN, US])**:** N= 18, 17 males/1 female, mean age 52.2 y. **Historical Control group:** N= 17, 17 males/0 female, mean age 49.9 y.	Recruitment happened through the Austin Health Spinal Outreach Risk Reduction Team (SpORRT) database when patients were referred to the SCI nurse for help with their PUs. The SpOORT database was reviewed resulting in 50 charts being audited of which 17 had sufficient information about PUs. These patients were used as the historical controls. 2 sachets of Arginaid until full healing was confirmed by visiting SCI nurse. Supplementation was done even when participants were admitted to the hospital.	Subgroup analysis showed that there was no significant difference in time to healing when comparing paraplegic versus quadriplegic patients. The mean time to healing for paraplegic historical controls was 19.4 weeks versus 22.8 weeks for paraplegic historical controls. The same lack of significance was observed within the intervention groups; 14.7 weeks if paraplegic, 10.4 weeks if quadriplegic. The PUs within the treatment group healed twice as fast as those in the control group (10.5 weeks for treatment versus 21.1 weeks for the historical control, P=0.006). The rate of healing in the treatment group was significantly different from the literature's expected time to healing rates for each category of PU. Mean time to healing of the treatment was 5.5 weeks versus ~14 weeks for stage II, 12.5 weeks versus ~18 weeks for stage III, and 14.4 weeks versus ~22.5 weeks for stage IV PUs.	A potentially significant clinical improvement was observed in PU healing with arginine supplementation .	Small sample size and use of historical controls. Lack of study blinding. Community dwelling participants made standardization of procedures difficult adding additional cofounding factors. Information about nutritional status, stage of PU and PUSH scores could not be obtained in the historical control group thus a comparison could no be made against the intervention group in regards to these attributes. PSGA has not been validated in SCI patients. The degree of blood glucose control was not reported, nor was the time of Diabetes diagnosis.

Leigh, B., Desneves, K., Rafferty, J., Pearce, L., King, S., Woodward, M. C., & Brown, D. (2012). Study design: Randomized Controlled Trial Class: A Rating: Neutral (Ø)	Investigate whether a dose of 4.5 g of arginine in the form of a commercial oral nutrition supplement is able to show similar benefits to healing rate of PUs when compared with existing evidence for 9g of arginine supplementation	Men or women admitted to inpatient care or the rehabilitation center at Austin Health (Melbourne, Australia). Patients had at least one stage II, III, or IV PU without any signs of healing, were able to drink oral supplements and had not started on supplements prior to the study. n=23 **Treatment (4.5 g arginine):** n=12, mean age of 69.8 y, 8 male/4 female, mean BMI of 26.9 kg/m^2 **Treatment (9g of arginine):** n= 11, mean age of 67.5 y, 6 male/5 female, mean BMI of 26.7 kg/m^2	Three week long treatment plus standard hospital diets. Patients were given 1 or 2 sachets of Arginaid (each containing 4.5 g of arginine) depending on the arm of the study they were randomized to. PU care and measurements were standardized. Diet adequacy was assessed for inpatients as well as outpatients (over the phone). Nutritional assessment was done via SGA.	There was a significant decrease in PUSH scores overtime but this difference did not appear significant when healing rates were assessed ($p<0.001$, 0=0.991, respectively). About 52% of patients were malnourished. Regardless of the treatment group patients were randomized to, well-nourished patients had less severe PUs at baseline compared to the same time-point malnourished patients ($p=0.283$). There was no significance in rate of PU healing according to arginine intake ($p=0.393$, rate not reported).	A dose of 4.5 g of arginine given through an arginine containing oral nutrition supplement showed no statistical or clinical significance when compared to a dose of 9g of arginine. The healing rate observed over the period of 3 weeks was about twice as fast as that of a reported historical group. There is a need for larger studies to validate these findings.	The lack of a concurrent control group. The small sample size. The length of the observational period and need to extrapolate results. Ever after randomization, researchers found that significantly higher calories and protein were consumed, at baseline, by the group randomized to 9 g of arginine ($p=0.036$, $p=0.018$, energy and protein, respectively).

Cereda, E., Klersy, C., Serioli, M., Crespi, A., & D'Andrea, F. (2015). Study design: Randomized parallel controlled blinded clinical trial. Class: A Rating: (+)	Identify the benefits of an oral nutrition supplement enriched with arginine, zinc and antioxidants on the healing of Pressure Ulcers on malnourished patients.	Adult men and women belonging to long-term care or receiving home care services that had a stage II, III, or IV PU. Patients had to qualify under malnourished criteria, be able to drink oral nutrition supplements, and provide consent. n=200 **Treatment (400 ml daily of Cubitan, Nutricia, 6 g of arginine):** n= 101, mean age 81.1 y, 31.7 % males, mean BMI 20.2 kg/m^2. **Control (pharmaceutical mix of Fortimel and Fantomalt, Nutricia, 0 g of arginine):** n= 99, mean age 81.7y, mean BMI 21.1 kg/m^2.	Eight week long intervention in addition to standard diet and standard PU care. Boluses of 100 ml of the experimental and control formulas were given in between meals 4 times per day. Adherence was recorded daily.	Both treatment and control group showed improved in PU healing ($P<0.001$). There was an increased reduction in the mean PU area in the treatment group (60.9%) versus the control group (45.2 %) which showed to be significant when adjusting for treatment effect ($P= 0.017$). About 69.9% of patients in the experimental formula group had a 40% or more reduction in PU size at 8 weeks, versus 54.1% in the control group ($p = 0.097$).	For malnourished patients, there is an observed additional benefit in PU healing when using an oral nutritional supplement enriched with arginine, zinc, and antioxidants.	Exclusion of non-malnourished patients. The exclusion of patients receiving artificial nutrition, as the PU population is also largely formed by patients on enteral feeds. The limited variance in patient population (long-term care and home care) makes applicability to larger more general population questionable.

Table 5. Quality Criteria Summary

	Van Anholt et al., 2010	**Brewer et al., 2010**	**Leigh et al., 2012**	**Cereda et al., 2015**
Rating	∅	∅	∅	+
Relevance Questions				
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes
Validity Questions				
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	Yes	**Unclear**	**Unclear**	Yes
4	Yes	Yes	Yes	Yes
5	Yes	No	Unclear	Yes
6	**Unclear**	Yes	Yes	Yes
7	Yes	Yes	Yes	Yes
8	Yes	Yes	Yes	Yes
9	Yes	Yes	Yes	Yes
10	Unclear	Unclear	Yes	Yes

Conclusion Statement and Grade

The effects of arginine containing supplements were evaluated in 4 studies. Most interventions provided 9 g of arginine-containing supplements in the form of Cubitan (providing arginine and several micronutrients in addition to calorie and protein) or Arginaid (providing Arginine, insignificant macronutrients and a few micronutrients) for a period of either 3 to 8 weeks or until full wound healing in hospitalized, long-term or home-care patients. The interventions with Cubitan resulted in increased rate of healing of 0.26 cm2/day by week 3 which leveled off to 0.16 cm2/day by week 8 compared to a consistent 0.15 cm2/day for the control group throughout 8 weeks. Also, this intervention resulted in a mean reduction in PU size of 60.9% compared to 45.2% of the controls. The studies that used Arginaid for their intervention, where compared to the same historical control group, which resulted in a significant 2-fold effect

on time-to-healing of PUs. A comparison of the dose effect of an arginine-containing supplement providing either 4.5 g or 9 g of arginine was done. It was found that there is no difference in PU outcomes whether patients drink a supplement containing 4.5 g of arginine or if they drink twice as much.

Overall, all 4 studies found that there was a benefit of using arginine containing supplements to increase the rate of healing of PUs. However, as noted in the individual discussion of each study's rating, not all studies had a strong design. At least two studies were found to be of neutral rating due to their use of historical controls, finding significant differences at baseline, and lack of in-depth description of intervening factors (adequacy of intake and equations used for calculation of calorie/protein intake). Also two out of the four studies were not clearly unbiased due to funding sources.

Grade III: Limited/Weak. The evidence provided by the authors of each individual study was consistent, but came from a limited number of studies, three of which were rated as neutral, while one was rated as positive. Multiple design flaws were found.

Chapter 5: Discussion

Evidence Summary

Making use of the Evidence Analysis Protocol described in Chapter 3, four studies were evaluated to answer the research question. There were great variations in their study designs, as well as the supplements used in the intervention. However, the conclusions were similar among the studies agreeing that arginine-containing supplements provided some measure of improvement on PU healing. This conclusion does not mean that arginine alone provided a direct benefit to PU healing, rather that there was an observed benefit from the intake of supplements marketed for wound healing purposes. Arginine is highlighted in these supplements due to its known participation in the wound healing process.

Cereda et al. (2015) and Van Anholt et al. (2010) used the Cubitan supplement, which provides additional calories, protein and several micronutrients in addition to the amino acid arginine. These two studies were placebo controlled and the use of supplementation resulted in significant increases in the rate of PU healing versus placebo.

Meanwhile, Brewer et al. (2010) and Leigh et al. (2012) used a formulation designed to provide additional arginine and minimal macronutrients in addition to vitamin C, E and zinc. Both of these studies used the same historical control group. Leigh et al. (2012) aimed to compare the effect of different doses of arginine. Also, they used a historical control to evaluate the effect of supplementation. Brewer et al. investigated the effect of 9 g of arginine on PU healing and followed the patients until full wound healing. Then the researchers compared the

rate of healing of the supplemented group against the historical control and found it to be twice as fast.

Brewer et al. (2010) and Leigh et al. (2012) only included patients with spinal cord injuries. Because of their injuries, these patients may require additional non-nutritional interventions to continue to promote wound healing such as frequent turning schedules, dressing changes, etc. The populations used by these two groups of researchers are quite different. Brewer et al. (2010) chose to use acute inpatient and rehabilitation patients. Leigh et al. (2012) used a community-dwelling sample. The research by Van Anholt et al. (2010) was performed in an acute inpatient care setting while Cereda et al. (2015) used long-term or home care patients. The stark difference in the cares that can be provided (and controlled) for an inpatient population versus community-dwelling patients makes these studies less comparable. Therefore their results are harder to generalize because they may be dependent on how well controlled other risk factors were.

All of the studies had similar restrictions in regards to non-PU wounds such as diabetic ulcers. Septic patients and those on medication therapies that would slow wound healing were also excluded. Cereda et al. (2015) only included malnourished patients. This criterion was not accounted as a limitation by the authors of this study given that malnutrition is largely prevalent in the PU patient population. The researchers ensured that patients met their caloric and protein needs, however the effect of the supplementation on the potential micronutrient depletion that may go along with being malnourished cannot be discounted. If micronutrient repletion was needed in these patients, then a increase in the rate of wound healing would have been observed. This effect could not be placed on the use of additional supplementation but on an overall

adequate intake of micronutrients. Therefore from a population selection standpoint, the studies could have been better designed.

All studies used standardized PU and nutritional care, but only two of the four studies gave a detailed account of what equations they used to calculate calorie and protein needs and how they ensured adequate intake throughout the study. It is important to know whether patients in the interventions groups had an adequate intake of calories and, most importantly protein, since their protein requirements are increased at baseline in order to help support wound healing. If protein intake was not adequate then the goal of evaluating additional supplementation was not reached since, supplementation would have been used to cover the patient's estimated needs.

Future research should include larger populations that can provide more power to the statistical findings of future studies and are able to reduce statistical errors. Exclusion criteria could be limited to factors that affect wound healing without restricting the population to the point that the studies' generalizability is affected. For example, including malnourished populations without micronutrient deficiencies and who have reached adequate intake. Also they could include patients who are receiving enteral/parenteral feeds and supplemented with wound specific supplements to observe if the same effects on rate of healing of PUs are seen. The degree of control of PU cares (both nursing and nutritional) can be better assessed when the study population is not residing in the community receiving home care. A detailed description of nutrition support provided should be provided or referenced within the study publications in order to help the reproducibility of these studies. Lastly, arginine-containing supplements with significant calories such as Cubitan can be used carefully within the context of overall diet adequacy. The additional calories provided by the treatment should be taken into account in

order to truly assess the effect of additional supplementation. More studies are needed using arginine containing supplements without significant macronutrient content such as Arginaid. This design could help assess the effect of this wound mixture (containing arginine and micronutrients such as vitamin C, and zinc) on the rate of PU healing without adding another control variable.

In conclusion, these four studies provided valuable data on the potential positive effect of arginine-containing oral supplementation. However, as noted above these studies are small trials with have substantial flaws in their study designs and do not yet support a change in current recommendations for nutrition practice. However, PU care has become a healthcare quality indicator, thus affecting hospital reimbursement. In order to affect hospital reimbursement and add value to the dietetics profession, further research is needed in the area of arginine-containing supplementation designed to support PU healing.

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Appendix A

Evidence Worksheet for Primary Research Article

Citation:	Van Anholt, R. D., Sobotka, L., Meijer, E. P., Heyman, H., Groen, H. W., Topinkova, E., Van Leen, M., & Schols, J. M. G. A. (2010). Specific nutritional support accelerates pressure ulcer healing and reduces wound care intensity in non-malnourished patients. *Nutrition, 26*, 867-872. doi:10.1016/j.nut.2010.05.009
Study design:	Randomized controlled double blind trial
Study Class (A,B,C,D)	A
Research Quality Rating	**Neutral** (∅)
Purpose/Population Studied/Practice Studied	
Research purpose:	To assess the potential of an oral nutrition supplement to improve healing of PUs and decrease the intensity of wound care in non-malnourished patients.
Inclusion criteria:	• Age: 18-90 years old • At least 1 stage III – IV PU. • Have received standard care and standard institutional diet without any nutritional supplements for at least 2 weeks prior to the study. • BMI of at least 18.5kg/m2 for those 18-70 years old and BMI >21kg/m2 for those older than 70 years.
Exclusion criteria	• Anyone not matching the above given criteria: BMI of <18.5kg/m2 for those 18-70 years; BMI <21kg/m2 for those >70 years. • Patients of age <18 years or >90 years. • Severe medical conditions, non-pressure-related ulcers, life expectancy shorter than 6 months, palliative care. • Patients on corticosteroid therapy, on restrictive diets.
Recruitment	Patients were recruited from 4 different countries. Eight health centers, hospitals and long-term facilities participated.
Blinding used:	Double blind
Description of study protocol	• At baseline PU surface are was measured by assuming that area was elliptical. • For patients with multiple PUs, the investigator designated 1 PU to assess throughout the 8 weeks. • A secondary end-point was the change in PUSH scores over time. PUSH scores were assessed at baseline and then at weekly intervals until the end of the study. • Anthropometrics, Malnutrition Universal screening Tool and blood parameters (Vitamin C, Zn, alanine aminotransferase, gamma-glutamyl transpeptidase, creatinine, blood cell and platelet counts, hemoglobin, troponin, transthyretin, and C-reactive protein) were taken at baseline and at the end of the

	study. • The volume of supplement consumed was recorded in increments of 25%. • GI tolerance was assessed weekly via standardized questionnaires. • The numbers of dressings was recorded retrospectively at weekly intervals. The time spent per dressing was also recorded for week 1, 4 and 8; and multiplied byt the number of dressings. • Mobility and activity levels were also recorded at week 1, 4, and 8.
Intervention:	• Patients were randomly allocated to receive Cubitan (Nutricia, N.V, Zoetermeer, and The Netherlands). This oral nutrition supplement provides 250 kcal, 28.4 g of carbohydrates, 20 g of protein which includes 3 g of arginine, 7 g of fat, 238 mg of vitamin A, 250 mg of vitamin C, 38 mg of vitamin E, 1.5 mg of carotenoids, 9mg of Zn, 64 mcg of Se, 1.35 mg of copper, and 200 mcg of folic acid. • Controls received a non-flavored placebo for the same length of time (8 weeks). • Two hundred milliliters of Cubitan or placebo were allocated were served 3 times per day, within meals, to be consumed within 1 hour.
Statistical analysis:	Analysis was performed on an ITT group. Data was converted to a log-transformed in order to obtain a more normal distribution. Data for PU size was fixed; labeled as "0 cm2," "closed," and "no exudate." Dropout data was recorded as missing. Repeat measured mixed models were used to compare changes between groups. Baseline measurement as well as blood parameters were analyzed by ANOVA. Fisher's exact test was used for categorical values. Overall statistical significance was set as $p \leq 0.05$.
Timing of measurements:	PU size, PUSH scores, as well as GI tolerance were assessed weekly until the end of the study. PU zise and PUSH scores were also measured at baseline. Anthropometrics, Malnutrition Universal screening Tool and blood parameters were recorded at baseline and end of the study. Supplement intake was recorded for each day of study participation. The number of dressing changes and the time taken for dressing change were calculated at end of week 1,4, and 8. Mobility and activity levels were recorded at baseline week 4, and 8.
Dependent variables:	Pressure ulcer healing and PUSH scores.

Independent variables:	Arginine-containing supplement intake versus placebo.
Control Variables	Anthropometrics, blood levels, MST score, mobility, activity levels.
Initial n	N = 47 randomized.
Final n (attrition)	Only 43 patients included in the ITT analysis. The four patients excluded from the ITT were due to: death (1), hospitalization (1), BMI in excess of inclusion criteria (1), and withdrawal of consent (1).
Age	**Mean age: 76.2 + 3.2 (ONS); 73 + 3.3 (control)**
Ethnicity	Not mentioned.
Other relevant demographics:	None others were mentioned.
Anthropometrics:	Average BMI in ONS: 23.7 $\pm$ 1.0; within control 25.8 $\pm$ 1.1
Location:	Czech Republic, Belgium, The Netherlands and Curacao.
Summary of Results:	• Five patients dropped out from the treatment group and 6 from the control. • There were no significant differences in baseline characteristics (demographic, biochemical, nutritional or PU characteristics) between the two groups. • There was a significant difference in PU size, over 8 weeks, between the treatment and control group. However, the size measurements were not reported. An average healing rate of 0.26 cm^2/d over the first 3 weeks was noted. This rate leveled off to 0.16 cm^2/d after 8 weeks. • There was a significant different also in within group comparisons. For the treatment group, there was a significant change in PU size between baseline and week 3 and thereafter. A marked difference in PU size was noted between baseline and measurements beyond week 5 for the control group. • PUSH scores were significantly improved (decreased by about half) between the treatment and control groups (p=0.011). • Categorical distribution of PUs was significantly different between groups after week 4. There were fewer PUs scored as "necrotic" or "granulated" and more classified as "closed" or "epithelial." • There was a significant decrease in the number of dressings used in the treatment group, compared to the control group over the entire study period (p=0.045). More specifically, fewer dressings were required on week 3,5,6, and 7 compared to the control group. The exact decrease in dressings used was not reported for either of these findings. • Significant less time was spent in changing dressing in the treatment group versus the control group (p=0.006). After week 4, on average 22 minutes were saved per patient per

	week in the treatment group. • About 75.8 ± 3.7 % of the arginine-containing supplement offered was consumed. About 86.5 ± 2.3% of the placebo was consumed. • There were no significant differences in physical activity levels. • The only blood parameter found to be significantly different was vitamin C. It was 2.3 times higher in the treatment versus control groups (p=0.015). • There was a significantly higher rate of constipation (adverse event) in the treatment (4 cases) versus the control group (0 cases).
Author's Conclusions	
Author conclusion:	PU healing can be accelerated in non-malnourished patients by providing an arginine-containing supplement also enriched with antioxidants and zinc. Supplementation may increase quality of life and decrease time spent on wound care.
Reviewer comments:	***Strengths****:* • Used control group and randomization. ***Weaknesses****:* • Did not provide values overall decrease of nursing time, only noted it significantly different. • Did not follow patients until discharge, so researchers could not provide any insight on whether the faster healing time made a difference on length of stay. • There was no disclosure regarding diet adequacy without the supplement. • Did not provide detailed information of protocols for standard nutritional or pressure ulcer care.

Table 3.2.a. Quality Criteria Checklist: Primary Research

RELEVANCE QUESTIONS					
Citation: Van Anholt, R. D., Sobotka, L., Meijer, E. P., Heyman, H., Groen, H. W., Topinkova, E., Van Leen, M., & Schols, J. M. G. A. (2010). Specific nutritional support accelerates pressure ulcer healing and reduces wound care intensity in non-malnourished patients. *Nutrition, 26,* 867-872. doi:10.1016/j.nut.2010.05.009		YES	NO	UNCLEAR	NA
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1	√			
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					
1. Was the research question clearly stated?		YES √	NO	UNCLEAR	NA
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
2. Was the selection of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		YES √	NO	UNCLEAR	NA
2.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
2.2 Were criteria applied equally to all study groups?	2.2	√			
2.3 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were study groups comparable?		YES √	NO	UNCLEAR	NA
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1	√			
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√			
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3	√			

3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4					√
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5					√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6					√
4. Was method of handling <u>withdrawals</u> described?		YES √	NO	UNCLEAR	NA
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.2 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.4 Were reasons for withdrawals similar across groups?	4.4	√			
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
5. **Was <u>blinding</u> used to prevent introduction of bias?**		YES √	NO	UNCLEAR	NA
5.1 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?		√			
5.2 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2	√			
5.3 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3				√
5.4 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
6. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		YES	NO	UNCLEAR √	NA
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1	√			
6.2 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.3 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.4 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.5 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5			√	

6.6 Were extra or unplanned treatments described?	6.6		√		
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
7. Were outcomes clearly defined and the measurements valid and reliable?		YES √	NO	UNCLEAR	NA
7.1 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.3 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.4 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.5 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.6 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?		YES √	NO	UNCLEAR	NA
8.1 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.2 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.3 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.4 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4	√			
8.5 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6		√		
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
9. **Are conclusions supported by results with biases and limitations taken into consideration?**	**YES**	YES	NO	UNCLEAR √	NA
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2		√		
10. Is bias due to study's funding or sponsorship unlikely?	**YES**	YES	NO	UNCLEAR √	NA

10.1 Were sources of funding and investigators' affiliations described?	10.1		√		
10.2 Was there no apparent conflict of interest?	10.2	√			

Evidence Worksheet for Primary Research Article

Citation:	Brewer, S., Desneves, K., Pearce, L., Mills, K., Dunn, L., Brown, D., &Crowe, T. (2010). Effect of an arginine containing nutritional supplement on pressure ulcer healing in community spinal patients. *Journal of Wound Care, 19* (7), 311-316. PMID: 20616774
Study design:	Non-randomized Controlled Trial
Study Class (A,B,C,D)	C
Research Quality Rating	**Neutral** (∅)
Purpose/Population Studied/Practice Studied	
Research purpose:	To evaluate the rate and time-to-healing of pressure ulcers (PUs) in community dwelling spinal cord injury (SCI) patients consuming and specialized arginine containing supplement, versus a historical control group who did note received arginine containing supplements.
Inclusion criteria:	• SCI patients residing in the metropolitan area of Melbourne (Australia) aged 18 years old or more presenting with a category II, III or IV PU.
Exclusion criteria	• Patients with phenylketonuria, sepsis, chronic renal failure, metabolic disease, diabetic foot ulcers or with clinical suspicion of osteomyelitis. Patients on drug therapies including hydroxyurea or more than 10 mg of prednisolone or 1.5 mg of dexamethasone.
Recruitment	Patients were recruited through the Austin Health Spinal Outreach Risk Reduction Team (SpORRT) database in the same orders as they got referred to the SCI nurse for help with their PUs.
Blinding used:	This was not a blind study as the nurses where aware of patient's consumption of supplements, and participants were not blinded to the supplement.
Description of study protocol	• The SpOORT database was searched for the 3 years prior to the study. This resulted in 50 charts being audited of which 17 had sufficient information about PUs (e.g. PU healing was described, nursing visits continued until PU were healed). These 17 patients were used as the historical controls. • Nursing PU care (non-nutrition related) was standardized for all patients. Time-to-healing of PUs for the intervention group was assessed by PUSH scores and calculated at baseline and repeated approximately every 2 weeks. • Time to full healing was used within the control group, since PUSH scores were not available for the historical control group; as these scores are not routinely collected. • Patient's nutritional status was assessed through the Patient Subjective Global Assessment (PG-SGA) and recorded as

	nourished (A), mild/moderately malnourished (B), severely malnourished (C). • On day 3 after initial nursing visit, patients were called to assess compliance and tolerance of the supplement. Compliance was assessed by comparing the number of sachets of Arginaid remaining against the number given. All patients self-reported compliance and those who consumed less than 75% of the recommended supplementation were excluded from final cohort. • Diabetes diagnosis was monitored among the intervention group and noted in the historical controls. • Expected healing rates of PUs who were not consuming supplements were noted in the literature. This expected rates were used as a comparison standard. • The study was ethically approved by the Austin Health Human Research Ethics Committee and the Deakin University Human Research Ethics Committee.
Intervention:	• The intervention group consumed 2 sachets of Arginaid (Nestle Nutrition, MN, US) until full PU healing was confirmed by the visiting SCI nurse. Each sachet contained 4.5 g of arginine and also contained other nutrients such as carbohydrates, vitamin C and E. • If a participant was admitted to the hospital during the study they continued to take the recommended amount of supplement until the prescribed time. Wound healing for hospitalized participants was assessed by the same SCI nurse.
Statistical analysis:	• This statistical power of the participants of this study was compared to the N of a previous study deemed statistically and clinically significant. The present study had a larger group thus making it also adequately powered. • Continuous data corresponding to the control and intervention groups at baseline (age, PU area, years of injury) was compared by unpaired t-test. • Fisher's exact test was used to compared binomial outcome variables (e.g. gender, number of PU developed) between control and intervention groups. • Unpaired t-tests were used to compare time-to healing of PU between both arms of the study. • Significance of PU healing times in the intervention group (compared to expected rates) was determined through one-sample t-test. • A significance of $p<0.05$was used for all analysis. All results were presented as means $\pm$ SEM.
Timing of measurements:	PUSH scores of the intervention group at baseline and every 2

	weeks.
Dependent variables:	Time to PU healing and PUSH scores.

Independent variables:	Intake of arginine-containing supplement.
Control Variables	PU care individualized to meet standard practice in the community, nurse assessment, and nutritional status.
Initial n	Initially there were 18 participants in the intervention group and 17 historical controls.
Final n (attrition)	Attrition for the intervention group was high as 18 participants in the intervention group remained until the end of the study.
Age	Mean age: 49.9 (control), 52.2 (intervention), not statistically significant
Ethnicity	Not mentioned in the study.
Other relevant demographics:	• Control group: all were males, 3 had Diabetes Mellitus diagnosis, 11 were paraplegic, 6 quadriplegic, 26 PUs developed, no PUSH scores available, PU stages varied from I-IV. • Intervention group: 17 males, 1 female, 6 had a diagnosis of Diabetes Mellitus, 14 were paraplegic, 4 quadriplegic, 30 PUs developed, mean baseline PUSH scores for the 30 PUs was 7.5 (s.d: 0.7), mean (s.d.) PUSH scores: category 2, 4.6 (0.5); category 3, 7.6 (0.9), category 4, 12.1 (1.0).
Anthropometrics:	Reported only that 94% of the intervention group had a PSGA category A (well nourished). Per the researchers, visual examination lead them to conclude many of this participants were overweight, however no values of height, weight or BMI were found in the study.
Location:	Melbourne-Australia
Summary of Results:	• The most common PUs developed on the ischial tuberosities, lower half of the leg and sacral regions. • The PUs within the intervention group healed twice as fast as those in the control group (10.5 weeks for treatment versus 21.1 weeks for the historical control, P=0.006). • In general, the deeper the PU injury the longer the rate of healing. • Subgroup analysis showed that there was no significant difference in time to healing when comparing paraplegic patients versus quadriplegic patients. The mean time to healing for paraplegic historical controls was 19.4 weeks versus 22.8 weeks for paraplegic historical controls. The same lack of significance was observed within the intervention groups; 14.7 weeks if paraplegic, 10.4 weeks if quadriplegic. • The rate of healing in the intervention group was significantly different from the literature's expected time to healing rates for

	each category of PU. Mean expected time to healing versus intervention observed time to healing: stage II ~14 weeks versus 5.5 weeks, stage III ~18 weeks versus 12.5 weeks, stage IV ~22.5 weeks versus 14.4 weeks. • Supplement compliance was reported as 100% for 13/18 participants; at least 85% for the intervention group. • About 94% of participants within the intervention group were considered well nourished. • Time to healing was compared within two subgroups: patients with and without diabetes. There was no significant difference in time to healing within the historical or intervention groups. However when only the stage II PU patients were analyzed diabetic patient's PUs healed by week 9.5, whereas non-diabetic patients healing lasted only 3.8 weeks. This difference was considered significant as was not observed within category III or IV PUs.
Author's Conclusions	
Author conclusion:	A potentially significant clinical improvement was observed in PU healing with arginine supplementation.
Reviewer comments:	**Strengths**: • There was high supplement compliance. • A control group was used. • The nurse who had overseen most patients in the control group also took control of the care of patients within the intervention group. Protocols for care of SCI patients in both groups remained consistent. • Included comparison of time to healing of PUs for patients with Diabetes Melitus. **Weaknesses**: • Small sample size and the use of historical controls. • This was not a blinded study. • The study was done in the community where care could not be completely controlled for (e.g. turning schedules) • Information about nutritional status, stage of PU and PUSH scores could not be obtained in the historical control group thus a comparison could no be made against the intervention group in regards to these attributes. • PSGA has not been validated in SCI patients. • The degree of blood glucose control was not reported, nor was the time of Diabetes diagnosis.

Table 3.2.a. Quality Criteria Checklist: Primary Research

RELEVANCE QUESTIONS					
Citation: Brewer, S., Desneves, K., Pearce, L., Mills, K., Dunn, L., Brown, D., &Crowe, T. (2010). Effect of an arginine containing nutritional supplement on pressure ulcer healing in community spinal patients. *Journal of Wound Care, 19* (7), 311-316. PMID: 20616774		**YES**	**NO**	**UNCLEAR**	**NA**
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1	√			
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					
3. Was the <u>research question</u> clearly stated?		**YES** √	**NO**	**UNCLEAR**	**NA**
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
4. Was the <u>selection</u> of study subjects / patients free from bias?		**YES** √	**NO**	**UNCLEAR**	**NA**
2.2 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			

2.3 Were criteria applied equally to all study groups?	2.2	√			
2.4 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were study groups comparable?		**YES**	**NO**	**UNCLEAR** √	**NA**
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1				√
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√			
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3		√		
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4				√
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
8. Was method of handling withdrawals described?		**YES** √	**NO**	**UNCLEAR**	**NA**
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.3 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.5 Were reasons for withdrawals similar across groups?	4.4				

4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5					√
9. **Was <u>blinding</u> used to prevent introduction of bias?**		**YES**	**NO** √	**UNCLEAR**	**NA**
5.5 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?			√		
5.6 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2		√		
5.7 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3				√
5.8 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
10. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		**YES** √	**NO**	**UNCLEAR**	**NA**
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1				√
6.7 In observational study, were interventions, study settings, and clinicians / provider described?	6.2	√			
6.8 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.9 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.10 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5	√			
6.11 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7		√		
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
11. Were <u>outcomes</u> clearly defined and the measurements valid and		**Y**	**N**	**U**	**N**

reliable?		**ES** √		**NCLEAR**	**A**
7.2 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.7 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.8 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.9 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.10 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
11. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators?		**YES** √	**N**	**UNCLEAR**	**NA**
8.6 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
8.7 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
8.8 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
8.9 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
8.10 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5				√
8.6 Was clinical significance as well as statistical significance reported?	8.6	√			
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
12. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?**	**YES**	**YES**	**N**	**UNC**	**NA**

		√		**LEAR**	
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
13. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	**YES**	**N**	**UNCLEAR** √	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1		√		
10.2 Was there no apparent conflict of interest?	10.2	√			

Evidence Worksheet for Primary Research Article

Citation:	Leigh, B., Desneves, K., Rafferty, J., Pearce, L., King, S., Woodward, M. C., & Brown, D. (2012). The effect of different doses of an arginine-containing supplement on the healing of pressure ulcers. *Journal of Wound Care, 21* (3), 150-156. PMID: 22399084
Study design:	Randomized Controlled Trial
Study Class (A,B,C,D)	A
Research Quality Rating	**Neutral**
Purpose/Population Studied/Practice Studied	
Research purpose:	Investigate whether a dose of 4.5 g of arginine in the form of a commercial oral nutrition supplement is able to show similar benefits to healing rate of PUs when compared with existing evidence for 9g of arginine supplementation.
Inclusion criteria:	• Patient had category II, III, IV PU without any signs of healing-no signs of improvement over a period of 2 weeks. • Patient was able to consume an oral diet • Patient was not already taking arginine-containing supplements
Exclusion criteria	• Patient with sepsis, acute gastrointestinal surgery, receiving dialysis. • Patients receiving hydroxyurea, >10mg of prednisolone, 1.5 mg dexamethasone per day. • Patients with clinical suspicion of osteomyelitis.
Recruitment	Patients that matched the inclusion criteria who were admitted to Austin Health were approached for consent. The Deakin University Human Research Ethics Committee and the Austin Health Human Research Ethics Committee approved the study.
Blinding used:	Researchers were blinded to the participant's treatment assignment. The nurse consultant evaluating wound healing was blinded to treatment.
Description of study protocol	• Patients were randomized into 2 treatment groups: 4.5 g of Arginaid (Nestle Nutrition) versus 9g of Arginaid supplement. • Patients discharging from the hosptial were provided with the number of Arginaid sachets to complete the study. Their wounds were evaluated by the same nurse consultant in the nearest wound clinic at the end of the 3-week period of the study. • A pseudo control group was established from historical data used in a previous study that used the same nurse consultant as the current study. This group did not consume any supplemental arginine over a period of 3 weeks. • PU care was standardized and individualized to the depth of

	each wound. • Dietary intake was assessed at baseline and at the end of the study. Baseline 3-day food/fluid records were completed by the nursing staff and double-checked by a research dietitian. Dietary intake of patients who discharged before the end of the study was re-assessed through follow-up phone calls. Food record analysis was done through the Australian Food and nutrient Database (AusNut-1999), FoodWorks Professional 2007 and Xyris software (Australia). Arginine content of foods was done by use of the Australian database and complied manually. Dietary adequacy was determined by comparison of intake versus estimated requirements (using Schofield equation plus activity and/or injury factor). • Body weight for most patients was taken at baseline and then weekly until the end of the study. For patients with Spinal Cord Injury only baseline and end-of-study weights were taken. • Heights were taken using a stadiometer or ulna measurement. • Nutritional status was assessed using the Patient Subjective Global Assessment. Patients were allocated to 3 categories: well-nourished, mildly/moderately malnourished, or severely malnourished. • Pertinent information in regards to medical history, current medications and nutritional supplements were obtained as weel as baseline levels of albumin and hemoglobin. • Inpatient compliance was monitored daily by nursing and recorded in the patient's charts. Discharged patients were given a compliance form and filled them out (caregivers might have helped too). Unused supplements were brought back to the wound clinic at the end of the study.
Intervention:	This was a 3-week long study. It had two treatment groups. One group consumed 4.5 g of arginine via Arginaid in addition the standard hospital diet. The second group received 9g (two sachets of Arginaid) in addition to the hospital diet. Arginaid also contains 4 g of carbohydrates, 155 mg of vitamin C and 40.5 mg of vitamin E.
Statistical analysis:	• In-group and between group analysis of PU severity was done via repeated ANOVA. Each PU was analyzed separately even if they belonged to the same patient. • Baseline differences between age, weight, dietary intake, and biochemistry were determined by un-paired t-tests. • Two-way ANOVA was used to determine in-group differences between weight, dietary intake, and biochemistry over the 3 weeks of study period. • Significance was established at an alpha error of $p<0.005$

Timing of measurements:	PUSH scores were assessed at baseline and weekly until the end of the study at week 3.
Dependent variables:	PU healing rate, assessed by PUSH score improvement.

Independent variables:	Arginine containing supplement intake (1 sachet versus 2 sachets)
Control Variables	Nutritional intake, nutritional adequacy (malnourished-well nourished), stage of PU.
Initial n	There were 29 patients recruited into the study
Final n (attrition)	• Twenty-three patients entered into the final analysis. One of these patients passed away before the end of the three weeks but had records for the first 2 weeks, so the data was used. • There were 12 patients in the group receiving 4.5g of arginine, and 11 patients in the group receiving 9g of arginine.
Age	Ranged from 31-92 years. Mean age: 69.8 $\pm$ 5.2
Ethnicity	Not mentioned.
Other relevant demographics:	• There were 17 pressure ulcers in the 4.5 grams of arginine group, and 14 in the other group. • There were 4 patients with a Type 2 Diabetes diagnosis in the 4.5 g of arginine group, while 3 patients had this diagnosis in the other group.
Anthropometrics:	Mean BMI was 26.9 in the 4.5g of arginine group while it was 26.7 in the 9g of arginine group,
Location:	Austin Health (Melbourne, Australia), 3 campuses that provide acute inpatient and rehabilitation services.
Summary of Results:	• There were no significant differences in patient's age, gender, BMI, hemoglobin levels or diabetes diagnosis. • Mean baseline PUSH scores for the 2 treatment groups were 8.9 versus 8.1 for the 4.5 g arginine and 9g arginine groups, p=0.507. • There was a significant decrease in PUSH scores overtime but this difference did not appear significant when healing rates were assessed (p<0.001, 0=0.991, respectively). • About 52% of patients were malnourished. Regardless of the treatment group patients were randomized to, well-nourished patients had less severe PUs at baseline compared to the same time-point malnourished patients (p=0.283). Well-nourished patients showed a trend for faster rates of PU healing, however it was not significant (p=0.057) and the rate was not reported. • There was no significance in rate of PU healing according to arginine intake (p=0.393, rate not reported). • There was no significant interaction between treatment group and nutritional status on PU healing rates (p=0.727, rate was not reported). • There was no significant difference within the treatment

	groups in the consumption of energy, protein, arginine, vitamin C and Zinc from baseline to end of study. • There was significant higher consumption of energy (p=0.036) and protein (p=0.018) between the 2 treatment groups, which occurred by chance as the participants were randomized. • The 4.5 g of arginine group consumed 60% of less than their estimated protein (57.8 ± 4.2%)and energy (53.3 ± 5.4%) requirements. There was a significant difference in energy and protein consumption between the 4.5 and 9.0 g of arginine groups (p=0.008 energy; p=0.008 protein). • There was an average 2kg weight loss in the 4.5 g of arginine group between baseline and end of study, however this did not show statistical significance. The weights remained stable in the 9g of arginine group. • Supplement compliance averaged around 92% between the 4.5 and 9.0 g of arginine groups (90.3% versus 93.3% respectively). • Estimated time to full healing was 8.7 and 8.4 weeks for the 4.5 and 9.0 g of arginine groups respectively.
Author's Conclusions	
Author conclusion:	• Doses of 4.5 or 9.0 grams of arginine could provide the same benefit in regards to time to PU healing. • There was no significant difference in healing rates of PUs (assessed by PUSH scores) of malnourished patients who received twice as much arginine compared with the group that received 4.5 grams of arginine daily. • Compared to the historical control, patients in both treatment groups had an almost two-fold improvement in the expected healing time.
Reviewer comments:	***Strengths****:* • Independent wound evaluation done by a nurse consultant who was blinded to the treatment. • The participant randomization. • The used of a standard care protocol very comparable to previous studies done at the same location. This allowed for increase comparability with prior published findings. ***Weaknesses****:* • Use of food records, which may include under reporting of actual intake. • There was no active control group as it was standard practice of the hospital to provide arginine supplements for patients with stage II-IV PUs. • Relatively small n. • The use of a 3-week time period to assess rate of healing rather than time to full healing.

Table 3.2.a. Quality Criteria Checklist: Primary Research

RELEVANCE QUESTIONS					
Citation: Leigh, B., Desneves, K., Rafferty, J., Pearce, L., King, S., Woodward, M. C., & Brown, D. (2012). The effect of different doses of an arginine-containing supplement on the healing of pressure ulcers. *Journal of Wound Care, 21* (3), 150-156. PMID: 22399084		**YES**	**NO**	**UNCLEAR**	**NA**
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1	√			
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					
5. Was the <u>research question</u> clearly stated?		**YES** √	**NO**	**UNCLEAR**	**NA**
5.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
5.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
5.3 Were the target population and setting specified?	1.3	√			
6. Was the <u>selection</u> of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		**YES** √	**NO**	**UNCLEAR**	**NA**
6.1 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			

6.2 Were criteria applied equally to all study groups?	2.2	√			
6.3 Were health, demographics, and other characteristics of subjects described?	2.3	√			
6.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were study groups comparable?		**YES**	**NO**	**UNCLEAR** √	**NA**
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1	√			
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2		√		
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3		√		
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	3.4				√
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
12. Was method of handling withdrawals described?		**YES** √	**NO**	**UNCLEAR**	**NA**
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.4 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.6 Were reasons for withdrawals similar across groups?	4.4	√			
4.5 If diagnostic test, was decision to perform reference test not dependent	4.5				√

on results of test under study?					
13. **Was <u>blinding</u> used to prevent introduction of bias?**		**YES**	**NO**	**UNCLEAR** √	**NA**
5.9 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?				√	
5.10 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2	√			
5.11 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3				√
5.12 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
14. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?		**YES** √	**NO**	**UNCLEAR**	**NA**
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1	√			
6.12 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.13 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.14 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.15 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5	√			
6.16 Were extra or unplanned treatments described?	6.6				√
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√

15. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		**YES** √	**NO**	**UNCLEAR**	**NA**
7.3 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.11 Was the period of follow-up long enough for important outcome(s) to occur?	7.3			√	
7.12 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.13 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.14 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
14. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators?		**YES** √	**NO**	**UNCLEAR**	**NA**
6.4 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
6.5 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
6.6 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
6.7 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4				√
6.8 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6	√			
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
15. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?**	**YES**	**YE**	**NO**	**UN**	**NA**

		S √		**CLEAR**	
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
16. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	**YES**	**NO**	**UNCLEAR**	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			

Evidence Worksheet for Primary RESEARCH Article

Citation:	Cereda, E., Klersy, C., Serioli, M., Crespi, A., & D'Andrea, F. (2015). A nutritional formula enriched with arginine, zinc, and antioxidants for the healing of pressure ulcers. *Annals of Internal Medicine, 162*, 167-174. doi:10.7326/M14-0696
Study design:	Randomized, parallel, controlled blinded clinical trial
Study Class (A,B,C,D)	A
Research Quality Rating	**PLUS/POSITIVE (+)**
Purpose/Population Studied/Practice Studied	
Research purpose:	Identify the benefits of an oral nutrition supplement enriched with arginine, zinc and antioxidants on the healing of Pressure Ulcers on malnourished patients.
Inclusion criteria:	• Malnourished patients (BMI<20kg/m2 for patients <65 years old; BMI <21kg/m2 if age >65 years old; recent unintentional weight loss [>10% in 3 months or >5% in 1 month]; serum albumin levels of <35 if <65 years old, or <30 if >65 years old; reduced food intake [<60% of estimated needs in the week before the study). • Able to drink Oral Nutrition Supplements (ONS) • Able to provide consent (patient or legal guardian)
Exclusion criteria	• Poorly controlled Diabetes (A1C>7%) • Acute organ failure • Advanced renal or hepatic insufficiency • Moderate to severe heart failure • Chronic obstructive pulmonary disease or peripheral vascular disease • Connective tissue disease • Current or previous (<1 year) neoplastic disease • Hemoglobin <10 g/dL • Obesity • Current immunosuppressant therapy • Infected wounds • Cellulitis, sepsis, or osteomyelitis • On artificial nutrition
Recruitment	Registration was started on February of 2010, interrupted but completed on April 2010. Patients in long-term care or receiving home care who also had PU stage II, III or IV were screened for enrollment.
Blinding used:	Yes. One single person was allowed to know of the random assignments and this person was in charge of requesting pharmacy to remove labels from the experimental formula and to request preparation of the control formula. The oral formula was given in unlabeled bottles directly to the

	patient's residence. Patients as well as nurses and physicians were blinded to the treatment allocation.
Description of study protocol	• Computer generated randomization was used. • Adherence to the dietary intervention was monitored daily by the caregiver or dietitian and defined as the ratio of consumed to prescribed. • Baseline characteristics obtained include: care setting, main diagnosis, incidence of Diabetes, mobility, primary PU area, location, and stage, number of patients with multiple PUs, mean braden scale, BMI, number of patients with unintentional weight loss, albumin levels, mean requirements and intake. • PU care was given according to evidence-based guidelines. • Before the study the people involved in conducting the study attended training to standardize practice. This training was repeated twice during the study. • Energy and protein intake was assessed by the dietitian via a 3-day quantitative food diary. • The initial timing for the study's end point was set at 12 weeks, however an ethical review board decided that due to recent publications the assessment should be done earlier than 12 weeks. • Total energy requirement were calculated at baseline via Harris Benedict equation with a correction factor of 1.2 and 1.1 for PU. Daily protein requirements were set at 1.5 g/kg of actual body weight unless the patient had a BMI > 27kg/m2 in which case an IBW for BMI of 23kg/m2 was used.
Intervention:	Dietary advice given to every patient receiving home-care or at a long-term care facility. All patients received 2 bottles per day (400ml) of the oral nutritional formula (providing on approximately 500 kcal, 40 g of protein for at least 8 weeks or until full PU healing). The supplements were administered to patients in small boluses of 100 ml each, between meals. Cubitan (Nutricia) was the ONS containing arginine (6g/400ml), Zinc (18mg/400ml) and antioxidants (500mg of vitamin C and 76mg of vitamin E). The control supplement provided similar amounts of calorie and non-arginine protein as well as significantly less amounts of Zinc, and two antioxidants vitamins (E and C). Adverse events were monitored such as GI disorders of severe hypotension.

Statistical analysis:	Primary analysis compared all randomly assigned patients through and Intention to treat principle. A secondary analysis of the primary end point was done at week 4 for all patient remaining on the study. The change in PU area was assessed by unadjusted repeated measures in general linear regression models. The effect of treatment on the primary endpoint and all secondary end points on a continuous scale was analyzed via multivariate linear regression model. Secondary endpoints on a binomial scale were evaluated with multivariate logistic models. All endpoint were reported as means or OR with 95% CI.
Timing of measurements:	• Baseline information gathered regarding age, sex, setting of care, diagnosis and presence of Diabetes. • Retrospective collection of recent weight loss was gathered from medical records or from caregiver. • Body weight was measured at baseline and week 8. • Total energy and protein intakes were obtained at baseline and every 2 weeks. • PU area was measured by the nurses at baseline, week 4 and 8 of the study using the Visitrak wound measurement system. • At baseline, nurses collected information regarding PU stage and site and risk for PU by means of Braden scale.
Dependent variables:	Primary end point: Percentage of change in PU area at 8 weeks. Secondary end points: complete healing and reduction of at least 40% of the area of PU by 8 weeks, incidence of wound infections, the number of dressing required throughout the intervention, and the percentage change in the area of 4 weeks.

Independent variables:	Oral nutrition supplement with arginine intake versus control supplement.
Control Variables	Age, BMI, nutritional status, PU size, stage.
Initial n	There were 279 patients screened but only 200 were assigned to interventions (74 did not meet criteria, while 5 declined to participate).
Final n (attrition)	The final analysis was done on 200 patients however there were patients loss to follow up due to transfer of healthcare setting, hospitalization for sepsis, pneumonia, UTI, and stroke. Patients also withdrew from the study, reasons given include: not specified, desire to stop the intervention due to GI intolerance. This study used the intention to treat method.
Age	Mean age: 81.1 in the experimental group, 81.7 in the control group.
Ethnicity	Not mentioned.

Other relevant demographics:	Not mentioned.
Anthropometrics:	Mean BMI 20.2 kg/m2 (experimental), 21.2kg/m2 (control).
Location:	Multicenter (7 sites). Long term care residents or patients receiving home-care. Geographical location was not mentioned.
Summary of Results:	• Adherence to treatment was reported as 84.8% (sd, 15.2%) in the experimental group versus 83.7% (sd, 16.3%) in the control group. This difference was not considered significant (p=0.65). • The supplement helped achieve 97.1% (sd, 15.9%) of the protein and calorie requirements for the experimental group; while it covered 97.4% (sd, 12.5%) in the control group. This difference was not significant (p=0.72). • The intervention resulted in an increase of weight of 1.4 kg (sd, 2.4 kg) for the experimental group and 1.6 kg (sd, 2.6kg) in the control group. While the between group difference was not significant (p=0.75), the within group change over eight weeks was (p<0.001). • At week 8 the mean reduction in PU size in the experimental formula group was 60.9% (CI: 54.3%-67.5%), versus 45.2% (CI: 38.4%-52.0%) in the control group (P=0.017). • None of the covariates included in the model had a significant reduction in PU area. • The 4-week analysis on patients remaining on the treatment arm of the study showed significant differences with 17.1% (CI: 8.2%-26.5%) reduction in PU area. • About 69.9% (CI: 59.9%-79.9%) of patients in the experimental group had a 40% or more reduction in PU size by 8 weeks. This compared with a total of 54.1% (CI: 42.7%-65.5%) of patients in the control group; where OR 1.98 (CI, 1.12-3.48, p=0.018). • A larger number of patients had healed PU by week 8, however this difference was not significant (p=0.097). • No significant differences were found in the reduction in PU area at 4 weeks, incidence of wound infections, or number of dressing used throughout the study period. • The secondary study done on patients remaining in the study for at least 4 weeks showed a significant effect on the rate of complete healing (p=0.042) and the reduction of PU area (p=0.003) for the experimental group. • The proportion of patients that did not respond to reduction in PU area of at least 5% was similar across both groups (p=0.68).

	• GI intolerance occurred for 5 patients (3 in the experimental group, 2 in the control group). One patient required hospitalization for sepsis. Thirty-two patients died during the study but these deaths were not attributed to the intervention.
Author's Conclusions	
Author conclusion:	In the context of appropriate nutritional care (adequate intake of calorie and protein) malnourished patients had an increase in PUs healing with the use of a wound specific supplement (including arginine, zinc and antioxidants).
Reviewer comments:	***Strengths:*** Allowed some degree of comorbidity, which increases the applicability of this study to a similar-setting population suffering from PUs. The supplement was scheduled into small amount throughout the day which may have had a positive impact on the adherence to the treatment. ***Weaknesses:*** This study does not include various populations (ICU, acute hospitalized patients) therefore it is not applicable to the general population.

Table 3.2.a. Quality Criteria Checklist: Primary Research

RELEVANCE QUESTIONS					
Citation: Cereda, E., Klersy, C., Serioli, M., Crespi, A., & D'Andrea, F. (2015). A nutritional formula enriched with arginine, zinc, and antioxidants for the healing of pressure ulcers. *Annals of Internal Medicine, 162,* 167-174. doi:10.7326/M14-0696		**YES √**	**NO**	**UNCLEAR**	**NA**
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	1	√			
2. Did the authors study an outcome (dependent variable) or topic that the patients / clients / population group would care about?	2	√			
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	3	√			
4. Is the intervention or procedure feasible (NA for some epidemiological studies)?	4	√			
If the answers to all of the above relevance questions are "yes", the report is eligible for designation with a plus (+) on the Evidence Quality Worksheet, depending on answers to the following validity questions.					
VALIDITY QUESTIONS					

7. Was the <u>research question</u> clearly stated?		**YES** √	**NO**	**UNCLE**	**NA**
1.1 Was the specific intervention(s) or procedure (independent variable(s)) identified?	1.1	√			
1.2 Was the outcome(s) (dependent variable(s)) clearly indicated?	1.2	√			
1.3 Were the target population and setting specified?	1.3	√			
8. Was the <u>selection</u> of study subjects / patients free from bias? As per answers to subquestions below, selection was free from bias, but groups were not comparable (and thus study was biased)		**YES** √	**NO**	**UNCL**	**NA**
2.3 Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	2.1	√			
6.9 Were criteria applied equally to all study groups?	2.2	√			
6.10 Were health, demographics, and other characteristics of subjects described?	2.3	√			
2.4 Were the subjects /patients in a representative sample of the relevant population?	2.4	√			
3. Were <u>study groups comparable</u>?		**YES** √	**NO**	**UNCLEAR**	**NA**
3.1 Was the method of assigning subjects / patients to groups described and unbiased? (Method of randomization identified if RCT)	3.1	√			
3.2 Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	3.2	√			
3.3 Were concurrent controls used? (Concurrent preferred over historical controls.)	3.3	√			
3.4 If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted	3.4				√

for by using appropriate adjustments in statistical analysis?					
3.5 If case control study, were potential confounding factors comparable for cases and controls? If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.	3.5				√
3.6 If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g. "gold standard")?	3.6				√
16. Was method of handling <u>withdrawals</u> described?		**YES** √	**NO**	**UNCLEAR**	**NA**
4.1 Were follow up methods described and the same for all groups?	4.1	√			
4.5 Was the number, characteristics of withdrawals (i.e. dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group?	4.2	√			
4.3 Were all enrolled subjects/patients (in the original sample) accounted for?	4.3	√			
4.7 Were reasons for withdrawals similar across groups?	4.4	√			
4.5 If diagnostic test, was decision to perform reference test not dependent on results of test under study?	4.5				√
17. **Was <u>blinding</u> used to prevent introduction of bias?**		**YES** √	**NO**	**UNCLEAR**	**NA**
5.13 In intervention study, were subjects, clinicians / practitioners and investigators blinded to treatment group, **<u>as appropriate</u>**?		√			
5.14 Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	5.2	√			
5.15 In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	5.3				√
5.16 In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	5.4				√
5.5 In diagnostic study, were test results blinded to patient history and other test results?	5.5				√
18. Were intervention / therapeutic regimens / exposure factor or procedure and any comparison(s) described in detail? Were		**YE**	**NO**	**UN**	**N**

intervening factors described?		**S** √		**CLEAR**	
6.1 In RCT or other intervention trial, were protocols described for all regimens studied?	6.1	√			
6.17 In observational study, were interventions, study settings, and clinicians / provider described?	6.2				√
6.18 Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	6.3	√			
6.19 Was the amount of exposure and, if relevant, subject / patient compliance measured?	6.4	√			
6.20 Were co-interventions (e.g., ancillary treatments other therapies) described?	6.5	√			
6.21 Were extra or unplanned treatments described?	6.6	√			
6.7 Was the information for 6.4, 6.5, 6.6 and 6.7 assessed the same way for all groups?	6.7	√			
6.8 In diagnostic study, were details of test administration and replication sufficient?	6.8				√
19. Were <u>outcomes</u> clearly defined and the measurements valid and reliable?		**YES** √	**NO**	**UNCLEAR**	**NA**
7.4 Were primary and secondary endpoints described and relevant to the question?	7.1	√			
7.2 Were nutrition measures appropriate to question and outcomes of concern?	7.2	√			
7.15 Was the period of follow-up long enough for important outcome(s) to occur?	7.3	√			
7.16 Were the observations and measurements based on standard, valid, and reliable data collection instruments / tests / procedures?	7.4	√			
7.17 Was the measurement of effect at an appropriate level of precision?	7.5	√			
7.18 Were other factors accounted for (measured) that could affect outcomes?	7.6	√			
7.7 Were the measurements conducted consistently across groups?	7.7	√			
17. Was the <u>statistical analysis</u> appropriate for the study design and type of outcome indicators?		**YES** √	**NO**	**UNCLEA**	**NA**

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6.11 Were statistical analyses adequately described and the results reported appropriately?	8.1	√			
6.12 Were correct statistical tests used and assumptions of test not violated?	8.2	√			
6.13 Were statistics reported with levels of significance and/or confidence intervals?	8.3	√			
6.14 Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	8.4	√			
6.15 Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g. multivariate analyses)?	8.5	√			
8.6 Was clinical significance as well as statistical significance reported?	8.6	√			
8.7 If negative findings, was a power calculation reported to address type 2 error?	8.7				√
18. **Are <u>conclusions</u> supported by results with biases and limitations taken into consideration?**	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
9.1 Is there a discussion of findings?	9.1	√			
9.2 Are biases and study limitations identified and discussed?	9.2	√			
19. Is bias due to study's <u>funding or sponsorship</u> unlikely?	**YES**	**YES** √	**NO**	**UNCLEAR**	**NA**
10.1 Were sources of funding and investigators' affiliations described?	10.1	√			
10.2 Was there no apparent conflict of interest?	10.2	√			