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ARTICLE

The Comparison of Nutritional Supplementation on Wound Healing in Type 2 Diabetes Patients with Foot Ulcers: A Systematic Review

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Abstract:
Diabetic foot ulcers are a common occurrence impacting nutritional status and morbidity. Nutritional support improves wound healing for diabetic patients with foot ulcers/lesions. This review aims to evaluate nutritional interventions such as arginine, glutamine, and omega-3 fatty acids on the wound healing outcomes of foot ulcers/lesions in patients with diabetes. The literature search utilized PubMed and CINCHL for peer-reviewed randomized controlled trials, cohort studies, retrospective trials, and case-control studies between 2005-2023. We assessed eligible criteria for the relationship between nutritional interventions arginine, glutamine, and omega-3 fatty acids and wound healing in diabetic participants. Eighteen studies met the inclusion criteria and were included in the review. Overall, there is a relationship between arginine, glutamine, and omega-3 fatty acids nutritional supplementation and a significant increase in wound healing. However, further research with larger sample sizes, longer durations, and more data is needed to conclude the supplementation dosage.

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1 | INTRODUCTION

The prevalence of Type 2 Diabetes (T2D) affects over 37 million Americans and individuals diagnosed with T2D account for 90-95%. [1-3] The diagnosis rate continues to rise for Americans, especially among the youth and young adults. [(1), (2)] Managing T2D can be stressful due to lifestyle changes, medications, and screening tests and management to prevent diabetes-related complications. The development of diabetic foot ulcers is just one of the more frequent complications an individual can develop if they do not manage their diabetes, resulting in the highest admissions related to diabetic complications. [(1), (2), (3), (4)] Risk factors can generate T2D patients that are more susceptible to foot ulcers, for example, poor glycemic control, incorrect or inconsistent foot care, neuropathy, and peripheral vascular disease, can all initiate the development of a foot ulcer in an area where repeated trauma and pressure sensations occur, leading to the need for treatment. [(2), (4)] of Americans with diabetes, about 60% will develop neuropathy which puts them at risk for a foot ulcer to develop, and 15 to 25% at some point in their life will develop foot ulcers.[(3)] Foot ulcers from diabetes complications are the primary cause of non-traumatic limb amputations globally.[(4)] A diabetic foot ulcer consists of three stages and initially starts as a callus due to neuropathy, allowing physical deformity and sensory loss that advances to the formation of dry skin, allowing

continual trauma to the dry callus area. This finally allows a subcutaneous hemorrhage that erodes and develops into an ulcer. [4] Nutrition is a crucial part of care concerning preventing and caring for foot ulcers. The CDC found an estimated 10% of Americans have some sort of nutrient deficiency and risk factors like age and race/ethnicity could increase a patient's chances for more severe health complications. [3] The standard oral nutritional support (ONS) incorporates protein and immunonutrition containing vitamins A, C, E, zinc, copper, and manganese to enhance wound healing. {5-6] Patients can receive the ONS orally, if the gut is functioning, or through enteral and parental nutrition support if oral is inadequate. [5], [6] Nutritional support intends to maximize calories and resolve or prevent deficiencies commonly found to negatively impact wound healing, commonly affected such as protein, vitamin D, vitamin C, and zinc. [5], [6] Depending on the stage of healing the patient reaches, determines the nutritional requirement. For example, during the proliferation stage, protein is very important for collagen synthesis to improve tissue strength (1.5g/kg to 3g/kg per day of protein) allowing maximized tissue regeneration. [4] The patient's current nutritional status and requirements are important to determine the course of action regarding nutritional support. [7] Improving nutritional intake could improve wound healing by altering specific micronutrients in patients presenting with a diabetic foot ulcer.

The evaluation of the essential amino acids, such as arginine and glutamine, for immune system improvements also becomes critical for wound healing. Arginine regulates pathways essential for tissue cell growth, while glutamine is essential for tissue repair, specifically during the cell proliferation and stimulation of collagen synthesis phases. [8], [9], [10] Arginine supplementation has been shown to enhance markers for wound healing by increasing protein and hydroxyproline in the wound, attracting T-lymphocyte function, and creating a positive nitrogen balance for a desired wound-healing environment. [8], [9], [10] While glutamine is one of the amplest amino acids in the body, its properties play an important role in patients who are in critical conditions, which is why it is essential to decrease protein breakdown and increase protein synthesis to create optimal proper ties for wound healing. [8], [9] In addition, omega-3 fatty acids are a polyunsaturated fat, with beneficial properties for wound healing due to their affiliation with anti-inflammatory and antioxidant characteri stics. [11] Omega 3 fatty acids have been shown to increase the generation of pro-inflammatory cytokines in the wound area, allowing improvements in wound healing. [11] There is evidence providing results of omega-3 fatty acids engaging an anti-diabetic effect and increasing the wound healing process creating improved metabolic profiles, inflammation, and oxidative stress. [11] Improving wound healing is an ongoing subject and treatment for diabetic foot ulcers often comes up short and immunonutrition has been shown to improve wound strength and integrity by using specific nutrient supplementation.

Therefore, this systematic review's objective is to compare the different use of micronutrients, arginine, glutamine, and/or omega 3 fatty acid nutritional supplementation versus exclusively providing standard oral nutritional support or no nutritional support care at all for T2D adult patients with active foot ulcers and measuring their outcomes of full wound healing, time for completed wound closure and effects on decreasing wound surface area. Altering nutritional supplementation has shown positive results in other wound types and critically ill patients; however, there are gaps for diabetic foot ulcer treatment in the role of nutritional intervention. The review of the different types of nutritional supplementation will contribute to the evidence of treatment and outcomes and indicate a need for more future research and treatment improvements on diabetic foot ulcer treatment.

2 | METHOD

This paper will review articles on nutritional supplements containing either arginine, glutamine, or omega-3 fatty acids separately or combined. This review is in accordance with the Preferred

Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA). [12]

Protocol and Registration

This systematic review of randomized controlled trials (RCTs), cohort studies, retrospective trials, and case-control studies was registered with the International Prospective Register of Systematic Reviews, PROSPERO (ID: CRD42023406874) on March 23, 2023.

Search Strategy

A literature search for peer-reviewed literature was performed utilizing two online databases, PubMed and CINCHL for studies published between 2005 to 2023. A search of original research articles between 2005 to 2023 was achieved to collect as many quantitative studies as possible to compare the quality of early immunonutrition supplementation studies through the years and to acquire new findings and future improvements needed. Searches were confined to publications in the English language, and the following terms from medical subject headings included: (diabetics OR diabetes OR adult-onset diabetes OR type 2 diabetes OR type II diabetes) AND (arginine OR arginine supplementation OR L-arginine OR arginine supplements OR glutamine OR glutamine supplementation OR glutamine supplements OR omega 3 fatty acid OR omega 3 fatty acid supplementation OR omega 3 fatty acid supplements) AND (wound healing OR remodeling stage OR wound repair OR wound closing OR wound closure). The reference lists of systematic reviews were manually evaluated for any additional articles.

Eligibility Criteria

The eligibility criteria for this review were limited to studies evaluating the population, setting, intervention, outcome, study design, study group size, and human trials as shown in Table 1. Eligible studies evaluated the measurements of wound healing and micronutrient supplementation intake of T2D patients during their treatment for diabetic foot ulcers.

Table 1 *Inclusion and Exclusion Criteria*

Criteria	Inclusion	Exclusion
Age	≥ 18 years old	< 18 years old
Gender	Any gender	
Health Status/Condition	Type 2 diabetes with an active foot ulcer	Not diagnosed with type 2 diabetes, no active foot ulcer, or pregnant woman
Setting/Country	Healthcare setting (community, hospital, inpatient, and outpatient) All countries	Hospice care
Intervention/Exposure	Nutritional supplementation: oral, enteral, parenteral nutrition, or special diets, any dose or duration	Topical supplementations only or grafts only
Outcome	Ulcer healing, time completed to heal, and reduced surface area of the wound	No wound outcome measured
Study Design Preferences	Random Controlled Trials, cohort studies, retrospective trials, and case-control studies	Reviews, meta-analyses, case reports
Study Size	≥ 10 participants	< 10 participants
Language	English	Not translated into English
Publication Year Range	(2005-2023) For this systematic review, the review aimed to collect as many quantitative studies as I could, therefore needing a larger publication year range to allow a more quality comparison through	<2005

Data Extraction

The PRISMA flow diagram Figure 1 provides the review selection process used to select the final eligible articles by the primary author. Articles were independently reviewed, and duplicates were removed to further screen titles and abstracts to eliminate articles that did not meet the eligibility criteria. The articles selected were evaluated through full texts for inclusion criteria by the author for the final selection.

A data extraction table was created using the Cochrane data extraction template used to extract the data by the author. [13] Data extracted from the articles were: author, date of publication, country, study design, the study's aim, setting, participants' characteristics, intervention, intervention delivery, control variable (if available), outcomes measured, results, conclusion, and source of funding. No missing data required a request from the articles' authors.

Quality Assessment

The articles were assessed by the primary author using the Cochrane tool section to assess the risk of bias by proceeding through random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of potential bias. This allowed the articles to categorize the sources as having low, high, or unclear risk of bias. [13] The Academy of Nutrition and Dietetics (AND) Evidence Analysis Manuel Conclusion Grading table was used to grade the quality of the articles, compare consistency, the number of studies and subjects, clinical impact, and generalizability of the population. [14] The different grade levels included grade I= good, grade II= fair, grade III= limited, grade IV= expert opinion only, and lastly grades V= not assignable. [14]

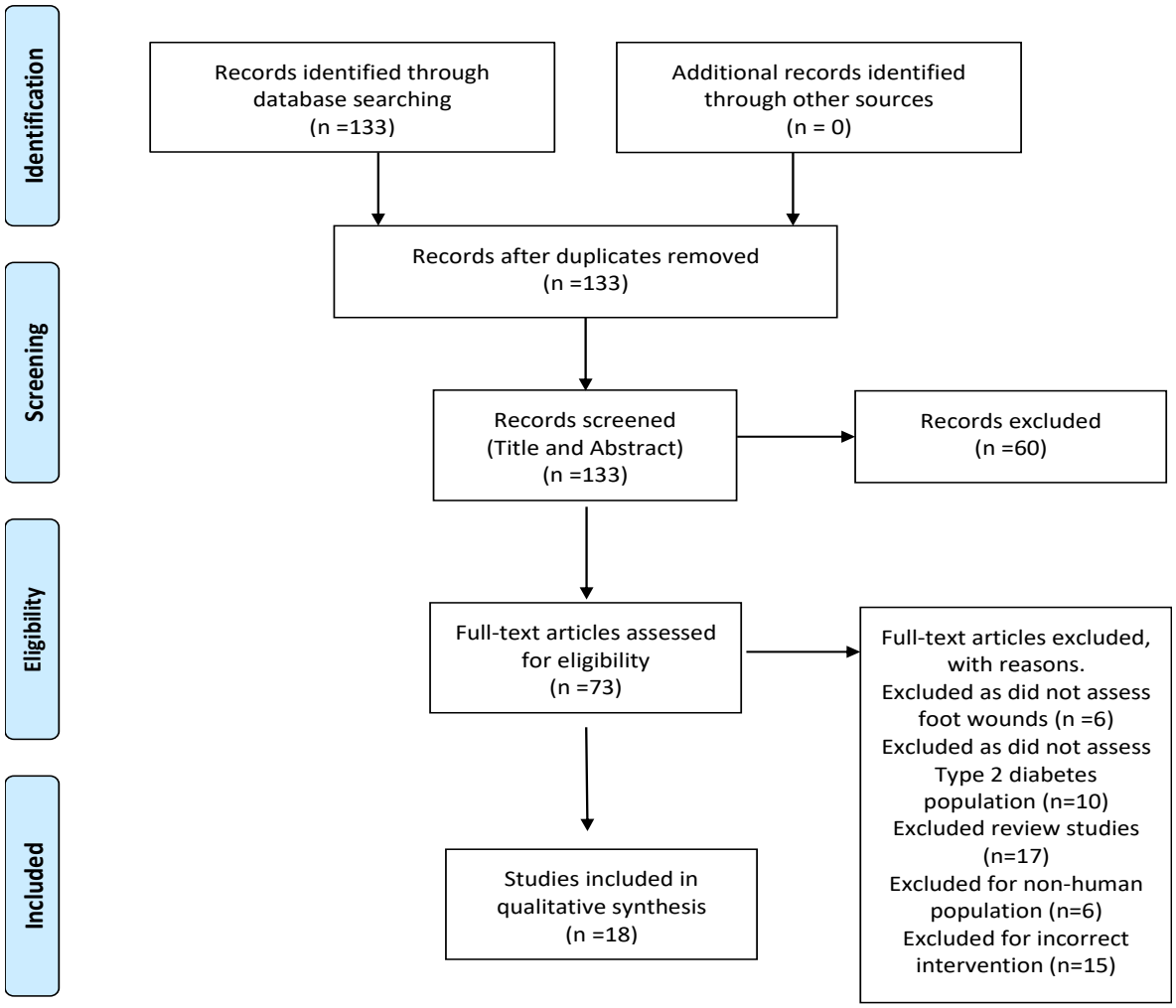

Figure 1: *Study Selection Process*

Data Synthesis

Article heterogeneity was assessed by grouping the article's use of interventions within each group to calculate a decrease in heterogeneity and to compare a difference between the usage of the three different interventions separately or in a combination of arginine, glutamine, and omega 3. The mean difference and p-values were collected and evaluated for the different primary outcomes to compare the statistical significance of the article's intervention usage. If a control group was provided in the study, it was compared with the intervention provided as no intervention or standard immunonutrition supplementation. If there was no primary outcome provided, the time of the intervention administered will be used as a primary outcome to allow the comparison of the intervention's effectiveness over time.

3 | RESULTS

Study Selection

Results of the search/flowchart were provided in Figure 1, outlining the process to select studies that met the inclusion criteria. There were 133 articles, and 60 articles were excluded after the title and abstracts reviewed, leaving 73 articles for full-text review. There were six excluded for no extremity wounds, nine excluded for not meeting the T2D population, 17 studies excluded for not meeting research design criteria, six excluded for non-human participants, and 15 excluded for contrasting interventions. This left 19 articles to include in this systematic review. During data extraction, one additional article was excluded due to accessibility. An access request was sent but not released, leaving 18 articles.

Included Studies

There were 12 randomized controlled trials [8], [11], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24]; five cohort studies; two were retrospective [25], [26], and two prospective studies. [27], [28] There were two pilot studies, one including a literature review at the end as shown in Table 1. [29], [30]

Demographics and characteristics

The 18 included studies had a total of 1,346 participants. Twelve studies included both females and males, and the remaining studies did not specify gender. The characteristics are summarized in Table 1 with several studies providing data on diabetes duration diagnosis listed in years and diabetic foot ulcer and/or lesion duration listed in weeks, months, or year.

Table 2: *Characteristics & Demographics of Studies*

Author	Study Design and sample size	Age (years)	Gender (M: F)	Duration of diabetes (years)	Duration of Ulcer/lesions
Armstrong et al., 2014	RCT, N= 271	28-88 y	M=75.6%, F= 24.4%		≥ 30 days but ≤ 12 months
Banks et al., 2020	RCT, N= 50	Intervention= 62.3 y, Control= 65.8			
Basiri et al., 2022	RCT, N= 29	Intervention= 52.9 y, control= 53.8 y	M=19, F= 10		Intervention= 10.97 months; Control= 10.58 months
Basiri et al., 2022	RCT, N-29	Intervention= 52.9 y, control= 53.8 y	M=19, F= 10		Intervention= 10.97-month, Control= 10.58 months
Basiri et al., 2020	RCT, N= 29	53.3 y		Intervention= 14.4 y, Control= 11.7 y	
Basyreva et al., 2021	Pilot Study, N= 14	Intervention= 59 y; Control= 69 y	M= 6, F= 8		
Bauer et al., 2013	RCT, N= 24	67.8 y	M= 11, F= 13		

Cereda et al., 2015	RCT, N= 200	81.7 y	M= 63, F= 137		
Clark et al., 2023	Retrospective study, N= 341	Intervention= 53.3y, Control= 52.7y	Intervention male= 78.9%, Control male= 70.0%		
Jones et al., 2014 Jude et al., 2010	Pilot study RCT, N= 9 RCT, N= 30	Intervention= 37-60 y, Control= 43-54y Intervention= 62.2y, Control= 64.2y	M=6, F= 3	 Intervention= 10.2y, Control= 8.5y	> 30 days
Leigh et al., 2012	RCT, N= 22	4.5 g group= 69.8y, 9.0 g group= 67.5y			
Mehl et al., 2021	RCT, N=30	64.7y			
Shih-Yuan et al., 2019	A prospective observational study, N=62	Unhealed group=76.0 y, Healed group= 72.9 y	F= 63.2%	10.0-20.0 y	
Sipahi et al., 2013	Retrospective study, N= 11	66 y	M= 81.8%		
Soleimani et al., 2017	RCT, N= 60	Intervention= 58.8 y, Control= 59.9 y	F=14		Intervention= 3.3 weeks, Control= 3.4 weeks
Tehan et al., 2023	Cohort study, N= 117	63 y	M= 82%	20 y	
Wong et al., 2014	RCT, N= 23	Intervention= 79.4 y, Control= 75.5 y			

Note. RCT= randomized control trial

Arginine and glutamine supplementation

There were five articles summarized in Table 2 that met the inclusion criteria and investigated arginine and glutamine supplementation together for participants diagnosed with T2D and suffering from a diabetic foot ulcer or lesion in their lower extremities. [8], [24], [25], [26], [30] The quantity and duration of arginine and glutamine supplementation varied among the articles.

The highest arginine dose tested in the studies was 7.4 g and was combined with glutamine at 7.4 g, twice a day for four weeks. [26] Researchers Wong and colleagues combined 7 g of arginine and glutamine twice a day for two weeks. [24] Armstrong et al. study, one of the most known studies on this topic, used 7 g of L-arginine and 7 g of L-glutamine twice a day but with a longer duration of 16 weeks. [8] Likewise, Jones et al. combined 7 g of arginine and only 1.5 g of glutamine for 14 days. [30] Clark et al. evaluated arginine and glutamine but did not disclose the quantity of either supplement. [25] Participants were provided ONS daily for 14 days or greater. [25]

All five studies highlighted improvements in wound healing and tissue viability regardless of the amount of combined arginine and glutamine. One study found high-risk participants with lower albumin levels of less than or equal to 4.0 g/L and ankle-brachial index < 1.0 responded more effectively to the intervention and had significantly greater (p=0.0042) total wound healing of 60% versus 34.4% in the control group. [8] Sipahi et al. noted significant (p=0.006) improvements in healing on wound depth score of 63.6% in patients. [26] In addition, significant results (p=0.001) in wound appearance score improved with 7.4 g of arginine and 7.4 g of glutamine by 72.7% in patients. [24] Another study providing a daily dosage of 7 g for both arginine and glutamine with only two weeks of evaluation found a significant increase (p= 0.007) in viable tissue within the first

week at 71.11% in the intervention group. [24] Lastly, Clark et al. study noted a significant reduction (p=0.01) of wound area during discharge at -61.8% compared to -33.8% for the control group. [25] Overall, Clark and colleagues had a reduction in wound area with arginine and glutamine supplementation intervention, with most notable healing in the first two weeks with an odd's ratio of 2.62 ($p < 0.05$). [25]

Table 3. *Articles using Arginine and Glutamine Supplementation Included Major Findings*

Author, years	Study Aim	Major Findings	Bias Rating
Armstrong et al., 2014	Investigate the supplements arginine and glutamine in wound healing of foot ulcers in adults with diabetes.	A significantly greater proportion of participants in the invention group had wound closure at 16 weeks than the control group (p= 0.0079). Participants with an ankle-brachial index of <1.0 and albumin level ≤ 40g/l had a greater total wound healing in the invention group versus the control group (p= 0.0042).	Low
Clark et al., 2023	Evaluate the effectiveness of the use of wound-specific ONS on inpatients in a rehabilitation hospital on improvements in different types of chronic wounds.	The wound reduction area in the intervention group versus the control group was 61.1% vs. 34.5%. the odds of wound healing in patients in the	High
Jones et al., 2014	Describe the effectiveness of arginine, glutamine, and beta-hydroxy-beta-methyl-butyrate for participants diagnosed with diabetic foot ulcers.	The intervention group's mean change of tissue hydroxyproline concentration of arginine and glutamine was +67.8% versus the control group -78.4%.	Unclear
Sipahi et al., 2013	Evaluate the nutritional supplementation of arginine and glutamine for improving wound healing in diabetic patients.	Healing was shown in a wound depth score of 63.6% (p=0.006) of patients, wound appearance score improved in 72.7% (p=0.001) in patients. No deterioration was shown, and tolerance of supplementation showed no side effects.	High
Wong et al., 2014	Investigate the effects of supplementation containing arginine and glutamine on wound healing rates of pressure ulcers.	There was an increase in viable tissue in two weeks for the ONS group 71.11% the first week, and in one-week PUSH scores (p=0.013) for the ONS group decreased from 12.25 to 10.81 and at 2 weeks to 9.63 for the ONS group.	Unclear

Note. ONS= oral nutritional supplementation

Arginine only supplementation

There were several studies that investigated the sole use of arginine supplementation versus placebo or standard oral nutritional support for wound healing in T2D participants see Table 3. One study compared differing dosages of arginine impacting wound healing rate while other researchers compared arginine with standard treatment.

Basiri and colleagues compared several different protein quantities containing L-arginine paired with different vitamins, minerals, and glucose control supplementation combinations to examine a difference in the rate of wound healing. [16], [17], [18]

The earlier random control trial from the Basiri et al. researchers' group had an intervention of two servings of a nutrient-dense formula containing 500 kcal, 28 g of protein with L-arginine present, with several flavor options available, and Boost Glucose Control for 12 weeks. [16] The energy at baseline for the intervention group was 53.2% and the control was 42.7%. [16] A t12 weeks the intervention group significantly increased (p= 0.03) to 70.5% for the intervention group. [16] The authors determined within the first four weeks, there was an 18.01mm/week healing compared to 1.4 mm/week in the control group resulting in 12.85- fold faster (p=0.01) than the control group healing rate for the intervention group. [16] Additionally, Basiri et al. tested 14 g of protein

containing L-arginine with 250 kcal and Boost Glucose Control formula twice a week for 12 weeks. [17] The RCT study had significant increases ($p < 0.05$) in protein intake for their intervention group from 54.5% to 84.9% versus the control group's 43.1% to 54.7% and protein synthesis significantly increased ($p < 0.05$) by 24.8% for the intervention group. [17] This study was focused on lean body mass and phase angle with participants not only diagnosed with T2D but also overweight or obese patients. The lean body mass was 3.8 kg for the intervention group compared to 4.9 kg for the control group. [17] The same authors also did a third similar study except increased the intervention supplement to 500 kcal and 28 g of protein containing L-arginine for 12 weeks. [18] The trial focused on inflammation and measured IL6 and found a significant decrease ($p < 0.05$) in the intervention group allowing a positive control on inflammation, and the control group resulting in a mean concentration of IL6 being 15 times higher. [18]

Cereda et al. provided two daily servings for eight weeks of 40 g protein, 500 kcal, and 1.5 g arginine between meals.[20] The intervention had significa ntly increased reduction (p=0.018) in ulcers by 69.9% (95% CI) versus the control 54.1% and a persistent reduction of around 40% or greater at 8 weeks for the intervention group. [20] Mehl colleagues provided 200 mL twice daily of ONS for four weeks containing high protein for both groups but only the intervention group contained arginine and the surface area significantly decreased (p=0.004) in the intervention group and patients diagnosed with diabetes mean weekly edge growth results were 1.85 mm compared to 3.0mm from participants not diagnosed with diabetes. [23]

Bauer et al. provided the intervention group with 10.5 g of protein including 4.5 g of arginine for four weeks and the control group received 9 g of protein not containing arginine twice daily. [19] This study by Bauer et al. was one of the only studies that had improvements in the pressure ulcer scale for healing (PUSH) score of 33.4% in their control group versus the intervention group of 4.3% (95% CI) wound healing rate. [19]

There were a few studies that evaluated the (PUSH score) for the measurement of the wound over time. Banks and colleagues' intervention group had a higher significant reduction (p=0.01) of the ulcer area at -86.0 compared to -74.0 in the control group. (15) Leigh et al. was the only study to compare different quantities of arginine supplementation. [22] The study compared the quantity of 4.5 g versus 9.0 g of arginine over the course of three weeks. [22] The findings were similar between the two different dosages for PUSH score changes or healing rates but a significant decrease ($p < 0.001$) in PUSH scores over time with both groups. [22] Lastly, the study found 52% of their participants were malnourished resulting in having more severe PUSH scores at baseline initially. [22] Jude and colleagues aimed to measure endothelial function, transcutaneous oxygen, and neuropathy by providing the intervention group with 3 g of L-arginine three times a day for 12 weeks. [21] This study did not have significant resulting (p=0.84) data on improvements in vibration perception threshold, and foot transcutaneous oxygen pressure. [21] Additionally, several of the studies noted protein intake was low when measured at baseline. [17], [22], [23]

Table 4: *Articles using Arginine Supplementation Only Major Findings*

Author, years	Study Aim	Major Findings	Bias Rating
Banks et al., 2020	Investigate intensive nutrition intervention on patients with pressure ulcers for wound healing.	The intervention had a higher reduction trend in the pressure ulcer area at -86.0 versus -74.0 for the control group (p=0.01).	High
Basiri et al., 2022a	Evaluate patients for 12 weeks providing adequate supplementation to meet the extra needs for energy and protein to reach at least 50% of the recommended dietary allowance	There was a significant increase in protein intake in the invention group (54.5% to 84.9%) compared to the control group (43.1% to 54.7%). The invention group lost less lean body mass and decreased phase angle versus the	Low

	in vitamins and minerals for wound healing.	control group, and protein synthesis increased by 24.8%.	
Basiri et al., 2022b	Examine the effects of nutritional intervention on controlling inflammation in diabetic foot ulcer patients.	The mean concentration of IL6 was 15 times higher in the control group due to infection. The TTP concentration in the intervention group was seven times higher than the control group improving better control over inflammation for the intervention group.	Low
Basiri et al., 2020	Test the effects of nutritional supplementation with nutrition education on accelerating wound healing in participants with diabetic foot ulcers.	The nutritional supplementation with education significantly improved the rate of wound healing (12.85-fold faster), wound appearance, and depth score in the intervention group with the most improvement occurring in the first four weeks of treatment.	Low
Bauer et al., 2013	Investigate the effectiveness of a wound ONS versus a standard ONS on wound healing and nutritional status.	The control group had a significant change in the rate of wound healing 33.4% improvement vs the intervention group 4.3% (P=0.044) and no significant differences in the secondary outcomes.	Unclear
Cereda et al., 2015	Test the effectiveness of arginine supplementation, zinc, and antioxidants in a high-calorie, high-protein formula that will improve pressure ulcer healing.	The intervention had increased reduction in ulcers by 69.9% (95% CI) vs control 54.1%. The intervention group had a persistent reduction of around 40% or greater at 8 weeks (p=0.018).	Low
Jude et al., 2010	To evaluate patients with type 2 diabetes and the effect L-arginine has on their endothelial function, transcutaneous oxygen, and clinical neuropathy suffering from peripheral neuropathy.	There was no significant data for improved VPT in the placebo group versus the intervention (3.97 vs. 1.74) p-value 0.26; blood flow decreased, but no significant differences and TcPO2 increase in control and intervention (4.75 and 6.24 mmHg; P=0.84).	Low
Leigh et al., 2012	Investigate 4.5 g of arginine as an ONS to increase the healing of pressure ulcers versus 9 g of arginine.	The PUSH scores were similar between 4.5 g at 8.9 and 9.0g at 8.1 (p=0.507). There were no significant differences in the treatments for healing rates or PUSH score, the 4.5 g group had complete healing at 8.7 weeks and the 9.0 g group was at 8.4 weeks. 52% of patients were considered malnourished having more severe PUSH scores at baseline.	Low
Mehl et al., 2021	Investigate ONS containing arginine on improving the healing of hard-to-heal wounds.	There was a significant decrease in wound surface area in the intervention group (p=0.004), and patients diagnosed with diabetes had a mean weekly edge growth of 1.85 mm and without diabetes a 3.0 mm.	Unclear

Note. ONS= *oral nutritional supplementation, PUSH= pressure ulcer scale for healing, VPT= vibration perception threshold, TcPO2= foot transcutaneous oxygen pressure*

Arginine and omega-3 fatty acid supplementation

There was only one RCT study that used both arginine and omega-3 fatty acids together see Table 4. This cohort research evaluated omega-3 fatty acids in the form of fish oil dietary supplements and arginaid. [28] The comparison of healed and non-healed groups using the calculation of the odds ratio from participants who received a supplement resulted in a significant increase (p= 0.02) in their total wound healing by 4.36 times versus participants who did not take any supplementation. [28] The baseline measurement for previous ulceration history was 88% (p=0.36), for patients who had no dietary supplementation advice for wound healing was 72% (p=0.20), and the participants who took any type of dietary supplementation for wound healing was 17% (p=0.01). [28] Most patients were not consulted on

wound healing or dietary supplementations leading to only 17% taking dietary supplementation for wound healing. [28]

Omega-3 fatty acid-only supplementation

Two studies in Table 4 solely used omega-3 fatty acid supplementation for wound healing in T2D participants. Basyreval and authors evaluated the quantity and delivery of the supplementation using omega-3 fatty acid in the form of an oral water-soluble form with vitamin D at 480 mg versus 240 mg of omega-3 fatty acid. [29] Soleimani and colleagues provide participants with omega-3 fatty acid as flaxseed oil measuring at 1000 mg of supplementation. [11]

In the comparison of wound healing area, there was a significant reduction (p=0.002) in ulcers in length -2.0 cm, width -1.8 cm, and -0.8 cm depth (p= 0.02) versus the control group's length -1.0 cm, width -1.0 cm, and -0.5 cm depth. [11] Soleimani et al. also had significant improvements (p=0.01) in the serum hs-CRP intervention group at -25.5 µg/mL versus the control group's -8.2 µg/mL and a significant increase (p=0.03) in plasma TAC +83.5 vs. -73.4 mmol/L (p<0.001), and GSH levels +60.7 vs. -15.5 µmol/L. [11] The Basyreva et al. study highlights significant results ($p < 0.001$) of nutritional supplementation preventing PMA-stimulated NET formation and cell death and allowing significant regulation ($p < 0.05$) of neutrophil function improving wound healing and decreasing the severity of complications. [29]

Table 5: *Articles on Arginine and Omega-3 Fatty Acid and/or Omega-3 Fatty Acid Only Major Findings*

Author, years	Study Aim	Major Findings	Bias Rating
Basyreva et al., 2021	Investigate the effects of vitamin D3 in combination with omega-3 fatty acid on NETosis in type 2 diabetic patients with necrotizing lesions on lower extremities.	Diabetic patients showed supplementation invention prevented PMA-stimulated NET formation and cell death and allowed regulation of neutrophil function improving wound healing and decreasing the severity of complications.	Low
Soleimani et al., 2017	To investigate the effects flaxseed oil omega-3 fatty acid supplementation has on diabetic foot ulcer wound healing.	There was a significant reduction in ulcer length (-2.0 cm), width (-1.8 cm), and depth (-0.8 cm) versus the control group's length (-1.0 cm), width (-1.0 cm), and depth (-0.5 cm). Also, improvements in serum hs-CRP (-25.5 vs. -8.2 µg/mL), an increase in plasma TAC (+83.5 vs. -73.4 mmol/L), and GSH levels (+60.7 vs. -15.5 µmol/L).	Low
Tehan et al., 2023	Evaluate clinical and social factors in wound healing of patients diagnosed with diabetic foot ulcers.	Methods used in the healed wound group were fish oil (n=3), Arginaid (n=7), no dietary intervention advice on wound healing (n= 84, 72%), took dietary supplements for wound healing (n=20, 17%, p=0.01). Healed group participants that took more than one supplement (n=13). With an odds ratio of 4.36, participants taking a nutritional supplement increased their wound healing by 4.36 times versus none.	Unclear

Note. hs-CRP= high sensitivity C-reactive protein, plasma TAC= plasma total antioxidant capacity, GSH= glutathione concentration

Measurements of arginine and/or glutamine serum levels

One prospective study measured the amount of arginine and/or glutamine serum levels in participants. This also provided a different

perspective on the indication of wound catabolism and inflammation present in participants whether the presence of arginine improved blood flow and collagen and if glutamine protected the wound from inflammatory injury and increased cell growth. [27] The comparison of the healed and non-healed group outcomes for participants from the Shih-Yuan et al. study in the healed group had significantly (p=0.045) higher levels of arginine serum levels 300.0 µmol/L versus 186.6 µmoL. [27] The participants had higher levels of arginine and showed improved wound healing outcomes for the follow-up see Table 5 below.

Table 6: *Articles on Arginine and Glutamine Serum Level Measurements*

Author, years	Study Aim	Major Findings	Bias Rating
Shih-Yuan et al., 2019	Examine amino acid serum levels in diabetic patients with foot ulcers.	The healed group had a mean serum level of arginine at 300.0 µmol/L compared to the unhealed group at 186.6 µmol/L (p=0.045) and similar levels of leukocyte (normal) and both groups had low levels of C-reactive protein levels (no infections).	Unclear

Risk of bias

The Cochrane tool section was used to assess the risk of bias in the studies and presented in the tables of major findings. There were nine low risks, six unclear, and three high risks. Studies that were low risk had adequate allocation sequence and concealment, blinding of participants, personnel, and outcome assessment, and no other risk of bias detected. [13]) The three high risks for biased articles were either due to allocation concealment unclear or incomplete data due to lack of clearly stating measurements, and lastly, one article clearly not stating criteria selection for T2D sample selection. [15], [25], [26])

4 | DISCUSSION

Treatment for diabetic foot ulcers and lesions has shown to be a complicated and potentially challenging journey. Treatment requires different types of procedures and patients are at an increased risk for complications, such as amputation. [4], [6] Due to this increased prevalence of T2D globally, reoccurrences, and severe complications of diabetic foot ulcers, altering nutritional supplementation specifically for patients diagnosed with T2D is essential for desired outcomes. [1] The review revealed each supplementation when used individually or in combination, improves wound healing in diabetic foot ulcers, pressure ulcers, or lesions.

Based on the findings of this review, 11 out of 14 studies concluded arginine supplementation was shown to improve foot ulcer/lesions wound healing outcomes and protein synthesis. [8], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [26] The study by Leigh et al. had significant results of improved wound healing, although the data presented suggested they did not have significant results when comparing the dosage of 4.5 g versus 9.0 g of arginine. [22] The exact dose of arginine to treat diabetic ulcers/lesions remains unclear despite several studies with varying dosages that have shown promising results.

In various studies that solely focused on arginine supplementation, it was found that there were significant improvements in the healing of foot ulcers and lesions. This led to a greater understanding of the benefits of nutritional supplements and highlighted the importance of evaluating current nutritional support protocols. Furthermore, it suggested a need for revising existing guidelines. [15], [17], [17], [18], [20], [21], [22], [23] The study by Bauer et al. was the only one that highlighted increased improvements in the standard protocol control group compared to arginine intervention for wound healing rate. [19] A few of the studies had small sample sizes but the relationship between wound healing and arginine supplementation was significant ($p < 0.001$) to propose a positive outcome. [22], [23] Therefore, the studies suggest sufficient evidence to support the

addition of arginine supplementation for treating diabetic foot ulcers/lesions, and future RCT studies should have larger sample sizes and durations.

Five studies included glutamine supplementation with arginine either measuring equal to or less than arginine's quantity. [8], [24], [25], [26], [30] All five studies had significant findings ($p < 0.001$) when adding glutamine for nutritional support, notably for wound appearance score improvement. [8], [24], [25], [26], [30] The interpretation of the findings reveals the increase in viable tissue and decrease in wound surface area was significantly improved ($p < 0.007$) in two studies by adding glutamine with arginine, allowing a decrease in complications and continued wound healing. [24], [25] Glutamine acted as a boost for wound healing when used with arginine and provided significant findings as a complementing treatment. The results suggest glutamine is essential to increase protein synthesis and create a desired wound-healing environment. [8] In addition to the use of glutamine, the ankle-brachial index measurement is of valuable use in the future due to the ability to act as an indicator of patients at risk of poor perfusion in their limbs, which in turn is associated with amputations. [8] The studies have shown that glutamine can be a helpful addition in enhancing the wound healing process when combined with arginine, especially in T2D patients with severe conditions. [8], [24],[25], [26], [30] This suggests that glutamine can be utilized as a boost to create optimal conditions for wound healing.

The study by Armstrong and colleagues focused on the participant's nutritional status at baseline and participants who were considered as having severe nutritional status data suggest that they had significant results ($p < 0.0042$) and benefitted more from arginine and glutamine supplementation compared to participants with albumin levels > 40g/L. [8] The studies commonly take baseline measurements; a few of the studies did not just measure standard weight, age, and/or height but went further into detail about the patient's nutritional status and diet history. Several studies determined patients' nutritional status was more severe, for example, malnourished, low protein intake, or no previous dietary supplementations practiced at baseline. [8], [22], [28] In addition, findings of early intervention should also be used due to exhibiting accelerated improvements occurring in the first few weeks of supplementation for wound healing. [24], [25], [28] The findings indicate the importance of establishing a patient's nutritional status and allowing early nutritional supplementation of arginine and glutamine to increase the wound healing rate, especially if deficiencies are detected during admission.

Additionally, omega-3 fatty acids have been considered as possible supplementation to improve wound healing. Omega-3 fatty acids highlight a different perspective of wound healing focusing on an anti-inflammatory agent allowing the healing process to initiate. [11] Significant findings ($p= 0.01$) for reducing the wound surface area and improving hs-CRP, plasma TAC, and GSH levels increased the capability to start the wound healing process. [11] Basyreva et al. study evaluated the neutrophil function of wound healing and its ability to potentially decrease the severity of complications. [29] The study discovered significant results ($p < 0.001$) of decreased cell death and allowed regulation of neutrophil function in return, improved wound healing, and decreased the severity of complications like infections. [29] There were significant findings from the Basyreva et al. study and Soleimani et al. study supplementing with omega-3 fatty acids, but there is limited data. [11], [29] In the future, studies should consider anti-inflammatory supplementations like omega-3 fatty acids due to the importance of inflammation, oxidative stress, and metabolic profile improvements in the wound healing process.

Improving diabetic care is vital, regardless of the increased prevalence of diabetic patients, the standard guidelines by the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) are still in place despite the gap between present outcomes and desired outcomes. [5] Standard ONS provides immunonutrition containing A, C, E, zinc, copper, and manganese with the intention of wound healing. [5], [6] There are several RCTs investigating arginine, glutamine, and omega-3 fatty acids but there is still a need to test the accurate efficacy of these supplementations and allow for the development of evidence-based practice

guidelines. [6], [31] For the time being, depending on the patient's clinical evaluation and the characteristics of the wound, it is important to use clinical judgment to assess the risk versus benefits of these enhancing interventions. [31]

Applications for intervention/practitioner

Based on the findings from this review, arginine increases the wound-healing rate and increases effectiveness when combined with glutamine to enhance the initiation of wound healing, accelerate wound-healing rate, and improve the nutritional status of the patient. Omega-3 fatty acids can potentially complement arginine and glutamine by an anti-inflammatory response to enhance and initiate wound healing. All three supplementations are relatively safe with no severe reactions reported but should be prescribed under medical supervision. [8], [11], [22] From this review, a baseline dosage of 7.0 g of arginine combined with 7.0 of glutamine through ONS and omega-3 fatty acid in an oral water-soluble form of 240 mg all three given twice a day for patients suffering from diabetic foot ulcers/lesions.

The nutritional status of participants in many of the studies was not considered in the inclusion/exclusion criteria. The supplements were provided randomly regardless of the existence or absence of malnutrition at baseline. Due to the established results of Armstrong and colleagues, it is essential to evaluate patients' and examine nutritional status for potential deficiencies to initiate the correct dosage of supplementations. [8]. Early intervention should also be used due to significant findings exhibiting accelerated improvement occurring in the first few weeks of supplementation for wound healing and the presence of deficiencies in patients before admission. [24], [25], [28]

Quality assessment

The quality and validity assessment of each article showed most of the results were consistently assigned good in the relevant questions. Validity only had a few fair results due to a lack of clearly stated measurements or clear criteria for the sample selection of T2D participants. There were no articles assigned to not assignable for validity, in other words, no articles were assigned six or more negative results on the validity questions. Overall, the results of quality showed good or fair results, and all articles included in the review were relevant.

Strengths and Limitations

This Review was conducted systematically to ensure that bias was limited when evaluating the inclusion criteria. One of the review's strong points is the large number of studies that were reviewed, including several RCTs. Additionally, the review followed a systematic approach.

There were also limitations of this review, with several studies excluded due to inaccessibility, and one was excluded due to not being able to translate into English. Although this systematic review concludes there is a relationship between arginine, glutamine, and/or omega-3 fatty acids and wound healing, the studies themselves present limitations. There were three studies performed by the same team Basiri et al. studies presenting concerns about the recruitment of participants due to homogeneity among participants recruited and outcomes between the studies. [16], [17], [18] There is limited data on race/ethnicity groups and the relationship between arginine, glutamine, and omega-3 fatty acid supplementations for diabetic foot ulcers and future studies are needed to increase knowledge and to reduce racial disparities in healthcare.

Many of the included studies had small sample sizes and short durations of treatment calling for future studies with larger sample sizes and longer treatment times, nevertheless, there were significant differences in increasing wound healing rate, decreasing wound surface area, and increasing in viable tissue impacted wound healing positively in the studies. Unfortunately, a few studies did not report complete healing and in future studies testing complete/total healing as outcomes is needed. Banks et al. study did not provide the percentage of participants who were diagnosed with T2D in their study, making it hard to generalize the outcome for the T2D population. [16]

5 | CONCLUSION

Most of the studies had a significant relationship between nutritional supplementation of arginine, glutamine, and/or omega-3 fatty acids in wound healing for T2D patients suffering from diabetic foot ulcers. [8], [11], [16], [17], [18], [22], [23], [24], [25], [26], [27], [28], [29] However, some studies had small sample sizes, and a few studies did not provide specific details for T2D participants on wound healing rates. Therefore, there is a need for future studies with larger sample sizes and longer study durations to be performed to evaluate specific nutritional supplements arginine, glutamine, and/or omega-3 fatty acids on foot ulcer wound healing in specifically T2D patients. Results showed the most significant wound healing were the studies providing arginine and glutamine combined as the intervention. [8], [24], [26], [30] In conclusion, the studies support arginine, glutamine, and omega-3 fatty acid supplementation improving wound healing for patients with diabetic foot ulcers/lesions, particularly when combined.

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