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Research: Treatment

Effect of oral nutritional supplementation on wound healing in diabetic foot ulcers: a prospective randomized controlled trial

D. G. Armstrong[1], J. R. Hanft[2], V. R. Driver[3], A. P. S. Smith[4], J. L. Lazaro-Martinez[5], A. M. Reyzelman[6], G. J. Furst[7], D. J. Vayser[8], H. L. Cervantes[9], R. J. Snyder[10], M. F. Moore[11], P. E. May[12], J. L. Nelson[13], G. E. Baggs[13] and A. C. Voss[13] on behalf of the Diabetic Foot Nutrition Study Group

[1]Southern Arizona Limb Salvage Alliance (SALSA), Department of Surgery, University of Arizona College of Medicine, Tucson, AZ, [2]Doctors Research Network, South Miami, FL, [3]Boston University School of Medicine, Boston, MA, [4]APS Medcon, Scottsdale, AZ, [5]Universidad Complutense de Madrid, Madrid, Spain, [6]Center for Clinical Research Inc., Castro Valley, CA, [7]East Setauket, NY, [8]ILD Consulting Inc., La Jolla, CA, [9]Foot and Ankle Center, San Jose, CA, [10]South Florida Wound Care Group, Tamarac, FL, [11]Wound Institute and Research Center, Dunmore, PA, [12]VA Sierra Nevada Health Care System, Reno, NV and [13]Abbott Nutrition, Columbus, OH, USA

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Abstract

Aims Among people with diabetes, 10–25% will experience a foot ulcer. Research has shown that supplementation with arginine, glutamine and β-hydroxy-β-methylbutyrate may improve wound repair. This study tested whether such supplementation would improve healing of foot ulcers in persons with diabetes.

Methods Along with standard of care, 270 subjects received, in a double-blinded fashion, (twice per day) either arginine, glutamine and β-hydroxy-β-methylbutyrate or a control drink for 16 weeks. The proportion of subjects with total wound closure and time to complete healing was assessed. In a post-hoc analysis, the interaction of serum albumin or limb perfusion, as measured by ankle–brachial index, and supplementation on healing was investigated.

Results Overall, there were no group differences in wound closure or time to wound healing at week 16. However, in subjects with an albumin level of ≤ 40 g/l and/or an ankle–brachial index of < 1.0, a significantly greater proportion of subjects in the arginine, glutamine and β-hydroxy-β-methylbutyrate group healed at week 16 compared with control subjects (P = 0.03 and 0.008, respectively). Those with low albumin or decreased limb perfusion in the supplementation group were 1.70 (95% CI 1.04–2.79) and 1.66 (95% CI 1.15–2.38) times more likely to heal.

Conclusions While no differences in healing were identified with supplementation in non-ischaemic patients or those with normal albumin, addition of arginine, glutamine and β-hydroxy-β-methylbutyrate as an adjunct to standard of care may improve healing of diabetic foot ulcers in patients with risk of poor limb perfusion and/or low albumin levels. Further investigation involving arginine, glutamine and β-hydroxy-β-methylbutyrate in these high-risk subgroups might prove clinically valuable.

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Introduction

People with diabetes often develop hard-to-heal diabetic foot ulcers secondary to neuropathy, peripheral vascular disease with ischaemia, or both [1]. In addition, infection, wound depth, size and duration also negatively impact healing and are therefore factors associated with failure to heal and possible amputation [2–4].

Although wound healing is complex, the ultimate outcome of the healing process is repair of the defective tissue. Collagen synthesis is required for complete wound healing and resolution of muscle injury [5]. The clinical goal is to increase collagen deposition to enhance wound strength and integrity. Nutrition is important in the repair of soft tissue injuries and wound healing [6]. Specific nutrients have been shown to enhance wound healing [7].

Correspondence to: David G. Armstrong. E-mail: armstrong@usa.net

What's new?

- This manuscript constitutes, to our knowledge, the first multi-centre, multinational, randomized trial evaluating the potential efficacy of oral nutritional supplementation on wound healing in diabetes.

A number of stressors, including wounds, can increase the need for arginine, causing it to become conditionally essential (requiring intake from food or supplements). Arginine regulates pathways required for tissue cell growth, replication and repair and may be beneficial to enhance wound healing [8,9]. Supplemental arginine improved markers of wound healing such as greater protein and hydroxyproline in the wound bed, enhanced T-lymphocyte function and promotion of positive nitrogen balance [8–10].

Glutamine is a non-essential amino acid that can also become conditionally essential in circumstances such as illness or tissue injury. Glutamine is important for tissue repair, is used for cell proliferation and can stimulate collagen synthesis [11].

β-Hydroxy-β-methylbutyrate is a naturally occurring metabolite of the amino acid leucine, inhibits proteolysis and modulates protein turnover [12]. Only 5% of leucine is converted to β-hydroxy-β-methylbutyrate in a 24-h period. In stress-induced animals, supplementation with β-hydroxy-β-methylbutyrate increased lean mass, suggesting that β-hydroxy-β-methylbutyrate protects lean mass from stress-related damage and enhances protein synthesis [13,14].

In a study of healthy elderly subjects, supplementation with the combination of arginine, glutamine and β-hydroxy-β-methylbutyrate increased collagen deposition (as reflected by hydroxyproline content) at the wound site, suggesting that supplementation with arginine, glutamine and β-hydroxy-β-methylbutyrate may provide a safe, non-invasive, nutritional means for improving wound repair [5].

Although numerous attempts have been made over the past 50 years to improve wound healing, treatment provided for diabetic foot ulcers is often inadequate [15]. This study was designed to evaluate the effects of supplemental arginine, glutamine and β-hydroxy-β-methylbutyrate in people with University of Texas grade 1A diabetic foot ulcers [2].

Patients and methods

The study was approved by Institutional Review Boards at each clinical site before patient enrolment and was performed in accordance with the protocol, Good Clinical Practice, any applicable privacy regulations, and the ethical principles that have their origin in the Declaration of Helsinki.

This study was a prospective, double-blinded, randomized, controlled, multi-centre design that compared the effects of a specialized nutritional supplement with a calorically similar, low glycaemic control supplement in community-dwelling subjects with Type 1 or Type 2 diabetes undergoing pharmacological treatment for glycaemic control who had at least one University of Texas grade 1A foot ulcer [2]. Subjects were recruited from 38 hospitals and wound care centres in the USA (36), Europe (1) and Taiwan (1).

Written informed consent and Health Insurance Portability and Accountability Act (or other applicable privacy regulation) authorization were obtained before any study procedures were performed. Subjects were considered for study entry if they were men or non-pregnant women at least 6 weeks post-partum and non-lactating; were ≥ 18 years old; had at least one foot ulcer below the ankle and not on the lesser digits that was diabetic and neuropathic in origin (Semmes–Weinstein test, insensate in ≥ 1 site) and the ulcer was ≥ 30 days but ≤ 12 months in duration and was ≥ 1 and ≤ 10 cm^2 surface area at visit 1/baseline; had adequate perfusion as measured by ankle–brachial index ≥ 0.7 but ≤ 1.2; or dorsum transcutaneous oxygen test with results of ≥ 30 mmHg; or Doppler arterial waveforms that were biphasic or better at the ankle; if they agreed not to begin taking any new medications, dietary supplements or alternative therapies; and willing to wear an off-loading device including a DH Walker (Ossur, Irvine, CA, USA), Charcot Restraint Orthotic Walker, or total contact cast.

Subjects were excluded from the study for the following reasons: uncontrolled diabetes [HbA_{1c} > 108 mmol/mol (12%)]; collagen vascular disease or autoimmune disease; mild, moderate or severe wound infection according to the Infectious Diseases Society of America [16] or International Working Group on the Diabetic Foot [17] 2, 3 or 4; recent systemic steroids (exceptions for inhaled steroids for asthma or chronic obstructive pulmonary disease, topical or optical steroids); recombinant human platelet-derived growth factor or similar therapies; or bioengineered tissue use within 4 weeks prior to baseline; antibiotic use within 1 week prior to baseline; history of radiation treatment to the ulcer site; wounds resulting from burns, venous insufficiency or osteomyelitis; active Charcot neuroarthropathy or a chronic Charcot deformity that could not be effectively offloaded; known immunosuppression; active malignancy; renal function impairment (*blood urea nitrogen* < 21.4 mmol/l, creatinine < 247.5 umol/l); liver failure/cirrhosis (Child class B or C); myocardial infarction in the past 3 months; pre-albumin ≤ 17 mg/dl; alcohol/substance abuse, any mental or physiological condition that may interfere with dietary intake; taking arginine, glutamine, β-hydroxy-β-methylbutyrate and unwilling to discontinue; history of allergy to any of the ingredients in the supplement; received collagen within 7 days of visit 1/baseline and unwilling to discontinue use for the duration of the study; or received negative pressure wound therapy within 7 days of visit 1/baseline and unable to discontinue use for the duration of the study.

Subjects were prospectively randomized (1:1 ratio) within each site to receive either the control or experimental

supplement using a pseudo-random permutated blocks (size 2) algorithm. The randomization was stratified by wound size (≥ 1 to ≤ 3 cm^2, > 3 but ≤ 10 cm^2), duration of the ulcer (≥ 30 days to ≤ 6 months, > 6 but ≤ 12 months) and HbA_{1c} < 75 mmol/mol (9%) or ≥ 75 mmol/mol (9%) but ≤ 108 mmol/mol (12%).

The experimental drink (arginine, glutamine and β-hydroxy-β-methylbutyrate) contained 79 kcal, 7 g L-arginine, 7 g L-glutamine and 1.5 g calcium β-hydroxy-β-methylbutyrate (providing 1.2 g β-hydroxy-β-methylbutyrateβ-hydroxy-β-methylbutyrate) (Juven®/Abound®; Abbott Nutrition, Columbus, OH, USA). The control drink was a calorically similar (88 kcal), low glycaemic response supplement. Both products were identical in packaging, appearance, dissolving characteristics and weight. Subjects were instructed to drink the entire packet dissolved in 237 ml of water twice per day, for 16 weeks.

Prior to enrolment, inclusion/exclusion criteria were verified, demographics recorded, medical history and current medications were reviewed, and ankle–brachial index was assessed. During the study, the ulcer wound bed was prepared as per the clinical judgment of the principal investigator and was assessed by two-dimensional planimetry analysis using validated digital photography (Silhouette System™; ARANZ Medical, Christchurch, New Zealand). The ulcer was rated using the University of Texas Wound Classification system.

Following randomization to treatment, and at regular intervals, subjects were administered quality-of-life questionnaires, including the EuroQOL five dimensions questionnaire (EQ-5D) [18] and the Diabetic Foot Ulcer Scale-Short Form [19], and blood samples were drawn for routine testing. At the initial visit, subjects were offloaded as described. Subjects were assessed weekly for 16 weeks.

The primary endpoints were the proportion of subjects with total wound closure and time to complete healing at 16 weeks. Complete wound closure was defined as 100% re-epithelialization of the wound without drainage. Secondary endpoints included the proportion of subjects achieving ≥ 15% reduction in wound area at 1 week, the proportion of subjects achieving ≥ 50% reduction in wound area at 4 weeks, the change in area of the ulcer, quality of life, and the incidence of complications, including diabetic foot ulcer infections, the appearance of additional lesions, ulcer recurrence and amputation.

Using a 0.025 level one-sided χ^2- test, the power was 80% to detect a 20% absolute difference in response rates (39% vs. 59%) between groups with a sample size per group of 107 subjects. Accounting for 20% attrition, 134 subjects per group were required (total of 268). Using a 0.025 level one-sided log-rank test with 80% power, the sample size for the co-primary endpoint, time to healing, was 98 completers per group, or an observed 90 events for the two groups. Sample size calculation was made using nQuery® Advisor 5.0 (Statistical Solutions, Saugus, MA, USA).

Variables expressed as proportion of subjects were analysed using logistic regression, and the Cochran–Mantel–Haenszel test stratified by site. Time to complete healing was analysed using Cox regression. Factors for treatment, randomization strata and site were used in regression. Continuous outcomes were analysed using repeated-measures analysis of covariance, with baseline covariate or Wilcoxon rank sum test by visit for non-normal data. Adverse events were analysed using Fisher's exact test.

One-sided *P*-values ≤ 0.025 for the co-primary outcomes were considered significant. All other tests and post-hoc analyses were two-sided and considered significant at $\alpha = 0.05$.

Data from subjects who received any amount of study drink were included in an intent-to-treat analysis (SAS® version 9.1; SAS Institute, Cary, NC, USA).

Results

Demographics and clinical comparison of study groups

A total of 1052 subjects were screened. Of these, 271 (130 receiving arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation, 141 control subjects) were eligible and enrolled from 38 sites from July 2008 to August 2010. A total of 237 were enrolled from the USA, 28 from Europe and six from Taiwan. One subject did not receive the study product and was excluded from the analyses (Fig. 1). There were no significant differences between groups in baseline characteristics (Table 1).

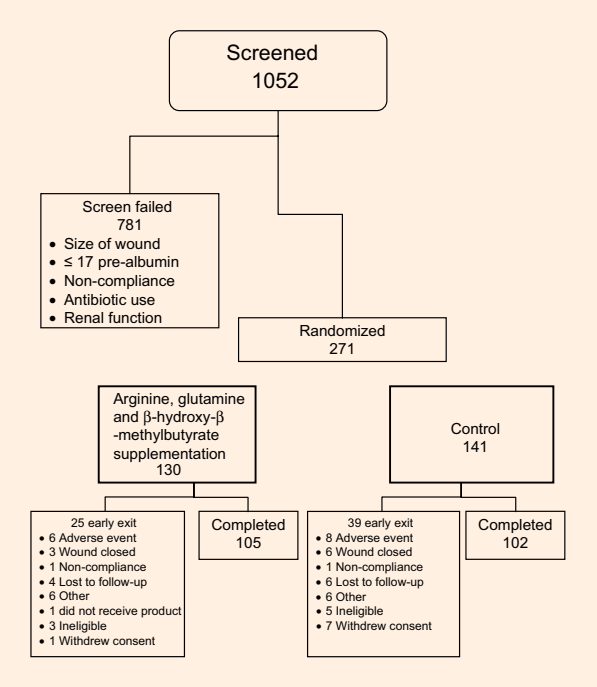

FIGURE 1 Subject disposition.

Table 1 Demographics and baseline characteristics

	Arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation *n* = 129	Control *n* = 141	Total	*P*-value
Gender, *n* (%)				0.157
Men	93 (72.1)	111 (78.7)	204 (75.6)	
Women	36 (27.9)	30 (21.3)	66 (24.4)	
Race (numeric), *n* (%)				0.295
Caucasian	111 (86.0)	116 (82.3)	227 (84.1)	
Black	10 (7.8)	18 (12.8)	28 (10.4)	
American Indian	3 (2.3)	0 (0.0)	3 (1.1)	
Asian	3 (2.3)	7 (5.0)	10 (3.7)	
Caucasian/American Indian	1 (0.8)	0 (0.0)	1 (0.4)	
Caucasian/other	1 (0.8)	0 (0.0)	1 (0.4)	
Ethnicity, *n* (%)				0.709
Hispanic	35 (27.1)	26 (18.4)	61 (22.6)	
Non-Hispanic	94 (72.9)	115 (81.6)	209 (77.4)	
Type of diabetes, *n* (%)				0.251
Type 1	7 (5.4)	17 (12.1)	24 (8.9)	
Type 2	122 (94.6)	123 (87.9)	245 (91.1)	
Location, *n* (%)				
USA	114 (88.4)	122 (86.5)	236 (87.4)	0.301
Europe	14 (10.9)	14 (9.9)	28 (10.4)	
Taiwan	1 (0.8)	5 (3.5)	6 (2.2)	
Subject had previous diabetic foot ulcers? *n* (%)				0.862
Yes	89 (69.0)	93 (66.4)	182 (67.7)	
Duration of diabetes (years)				0.074
Median (minimum, maximum)	13 (0, 45)	15 (1, 50)	15 (0, 50)	
Age (years) at randomization				0.308
Median (minimum, maximum)	58 (28, 86)	59 (29, 88)	58 (28, 88)	
BMI (kg/m^2)				0.185
Mean ± SD	33.06 ± 7.30	31.63 ± 7.07	32.32 ± 7.20	
Screening HbA_{1c} (mmol/mol)				0.737
Mean ± SD	64 ± 18	64 ± 17	64 ± 17	
Screening HbA_{1c} (%)				
Mean ± SD	8.0 ± 1.7	8.0 ± 1.5	8.0 ± 1.6	
Duration of study ulcer at entry in months				0.792
Median (minimum, maximum)	3 (1, 12)	3 (1, 11)	3 (1, 12)	
Baseline wound area				0.621
Median (minimum, maximum)	1.6 (0.4, 17.5)	1.8 (0.3, 9.9)	1.7 (0.3, 17.5)	

Primary and secondary outcomes

There were no statistically significant differences in the primary outcomes (proportion of subjects with total wound closure at 16 weeks; Fig. 2) and time to complete healing (data not shown). There was no statistical difference between groups in wound area (Fig. 3) and other secondary outcomes (Table 2).

Post-hoc analyses showed that, in subjects with a baseline albumin level ≤ 40 g/l (n = 127), there was a significantly greater proportion of subjects with total wound healing at 16 weeks in the supplementation group [31/61 (50.8%)] vs. the control group [23/66 (34.9%), P = 0.0325] (Fig. 4). In addition, in subjects with a baseline ankle–brachial index < 1.00 (n = 119), there was a significantly greater proportion of subjects in the supplementation group with wound closure at 16 weeks [35/58 (60.3%)] vs. the control group [24/61 (39.3%), P = 0.0079] (Fig. 5). In subjects with both a baseline ankle–brachial index of < 1.0 and an albumin level of ≤ 40 g/l (n = 62), there was a significantly greater proportion of subjects with total wound healing in the supplementation group [18/30 (60%)] vs. the control group [11/32 (34.4%), P = 0.0042].

Safety variables

There were no significant differences between the groups in the total number of adverse events reported. In the arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation group, 83 (63.8%) subjects reported 202 adverse events and in the control group, 88 (62.4%) subjects reported 190 adverse events. There were no clinically significant differences in blood coagulation variables, prothrombin time, *activated partial thromboplastin time* or international normalized ratio.

Discussion

The results of this study suggest that, overall, there is no significant improvement in healing of diabetic foot ulcers

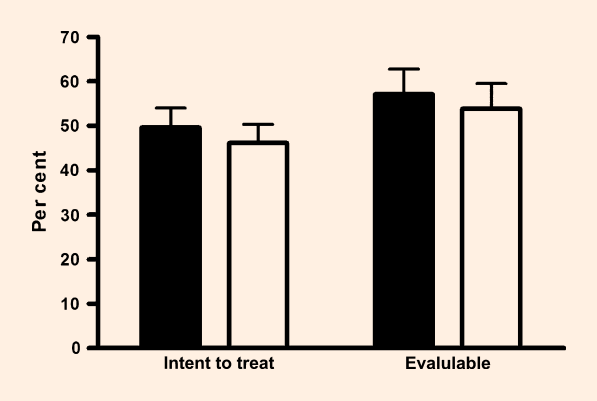

FIGURE 2 Proportion of subjects with total wound closure at 16 weeks; arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation (■) and Control (□).

following nutritional supplementation. There are a number of possible reasons that the results did not show a significant effect of arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation on the primary and secondary variables in the larger population. Clinical studies evaluating wound healing in people with diabetes are complicated to conduct. Patients who develop diabetic foot ulcers typically have neuropathy and poor limb perfusion, inappropriate diets, sedentary lifestyles, multiple medications, lack of good foot care and are frequently uncomfortable with offloading procedures. Thus, in the present study, perhaps no significant group differences were observed because of the large heterogeneity often seen in this population of patients.

Supplementation with arginine, glutamine and β-hydroxy-β-methylbutyrate as an adjunct to standard of care, however, may improve healing of diabetic foot ulcers specifically in patients with low albumin and/or poor limb perfusion. While nutrition is a factor associated with wound healing [7], following a comprehensive search of the literature, this is the first prospective randomized controlled trial identified that has addressed the effect of oral nutritional supplementation on healing in this population.

Analysis of the primary and secondary variables showed that, overall, the treatment was not effective in this group of patients. However, post-hoc evaluation suggested that, in subjects with low albumin and/or poor limb perfusion, there were a significantly greater proportion of subjects in the treatment group with wound closure at 16 weeks compared with the control group.

Malnutrition is a complex process that involves more than a decline or change in dietary intake. In people with diabetes, the disease itself, injuries, medications and other factors affect metabolism, leading to poor nutrient utilization, weight loss, skin breakdown and poor healing. Gluconeogenesis is used as the primary energy source in patients with protein–energy malnutrition [7]. This process is driven by the release of amino acids from muscle tissue breakdown [7]. The catabolism of protein is reflected in increased urinary nitrogen loss and decreased levels of albumin as well as skin breakdown [7]. In the patient with a diabetic foot ulcer, the wound consumes large quantities of energy during the healing process both by inflammatory cells and the fibroblasts' production of collagen and matrix. Albumin is a measure of protein status [7]. It is affected by hydration status, injury, infection and inflammatory response. Increased protein needs for malnourished persons have been correlated with depressed levels of albumin [7]. In disease states, albumin levels of ≤ 40 g/l are associated with

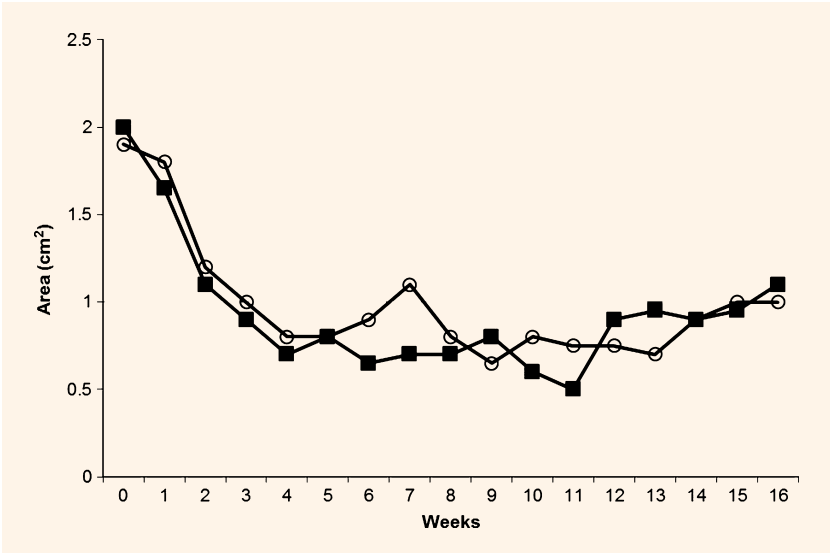

FIGURE 3 Median wound area (cm^2) by week; arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation (■) (n = 129) and Control (○) (n = 141).

Table 2 Secondary outcomes

	Treatment		
	Arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation *n* = 129	Control *n* = 141	*P*-value
Wound reduced ≥ 15% at 1 week, *n*/*N* (%)	71/112 (63.4)	79/128 (61.7)	0.789
Wound reduced ≥ 50% at 4 weeks, *n*/*N* (%)	56/95 (58.9)	46/101(45.5)	0.087
Wound complications			
Recurrence?, *n*/*N* (%)	11/129 (8.5)	12/141 (8.5)	0.996
Infection of wound?, *n*/*N* (%)	21/129 (16.3)	15/141 (10.6)	0.173
Additional lesions?, *n*/*N* (%)	38/129 (29.5)	40/141 (28.4)	0.844
Amputation?, *n*/*N* (%)	3/129 (2.3)	5/141 (3.5)	0.552
Wound area (cm^2),			
Median (Q_1, Q_3)*, *n*			
Week 1	1.65 (1.2, 3.1) 126	1.8 (1.2, 3.5) 139	0.621
Week 4	0.7 (0.4, 2.1) 102	0.8 (0.4, 2.0) 112	0.961
Week 8	0.7 (0.3, 1.85) 84	0.8 (0.2, 2.2) 79	0.732
Week 12	0.9 (0.2, 2.8) 67	0.75 (0.2, 2.15) 64	0.685
Week 16/exit	1.1 (0.3, 3.9) 61	1.0 (0.4, 2.2) 65	0.623
EQ-5D index score§,			0.338†
Mean ± SD (n)			
Week 1/baseline	0.69 ± 0.23 (113)	0.73 ± 0.19 (121)	
Week 4	0.71 ± 0.20 (103)	0.76 ± 0.19 (106)	
Week 8	0.70 ± 0.21 (105)	0.76 ± 0.20 (97)	
Week 12	0.70 ± 0.22 (97)	0.75 ± 0.21 (88)	
Week 16/exit	0.73 ± 0.20 (102)	0.76 ± 0.23 (100)	
DFS-SF subscale: leisure‡,			
Median (Q_1, Q_3), *n*			
Week 1/baseline	40 (12.5, 80) (108)	45 (25, 70) (113)	0.711
Week 4	50 (20, 75) (99)	50 (25,75) (100)	0.460
Week 8	47.5 (25, 80) (100)	50 (27.5, 80) (88)	0.873
Week 12	50 (25, 80) (92)	52.5 (37.5, 80) (80)	0.644
Week 16/exit	50 (25, 80) (96)	55 (37.5, 87.5) (92)	0.215
DFS-SF subscale: physical health,			
Median (Q_1, Q_3), n			
Week 1/baseline	65 (37.5, 80) (108)	60 (35, 85) (113)	0.737
Week 4	70 (45, 85) (99)	60 (35, 90) (100)	0.962
Week 8	70 (45, 87.5) (100)	70 (37.5, 85) (88)	0.976
Week 12	70 (40, 90) (92)	70 (47.5, 90) (80)	0.955
Week 16/exit	70 (42.5, 90) (96)	72.5 (50,90) (92)	0.607
DFS-SF subscale: dependence/daily life,			
Median (Q_1, Q_3), *n*			
Week 1/baseline	67.5 (32.5, 95) (108)	70 (50, 90) (112)	0.672
Week 4	75 (35, 90) (99)	70 (47.5, 90) (100)	0.670
Week 8	72.5 (35, 90) (100)	70 (47.5, 90) (88)	0.740
Week 12	75 (40, 92.5) (92)	80 (50, 95) (80)	0.577
Week 16/exit	75 (40, 95) (97)	75 (47.5, 90) (92)	0.686
DFS-SF subscales: negative emotions,			
Median (Q_1, Q_3), *n*			
Week 1/baseline	61.3 (31.3, 83.3) (108)	54.2 (37.5, 79.2) (112)	0.805
Week 4	58.3 (29.2, 83.3) (99)	66.7 (29.2, 85.4) (100)	0.651
Week 8	62.5 (35.4, 87.5) (100)	70.8 (37.5, 87.5) (88)	0.696
Week 12	60.4 (33.3, 85.4) (92)	70.8 (43.8, 89.6) (80)	0.535
Week 16/exit	66.7 (41.7, 87.5) (97)	70.8 (37.5, 87.5) (92)	0.390
DFS-SF subscales: worried about ulcers/feet,			
Median (Q_1, Q_3), *n*			
Week 1/baseline	43.8 (25, 71.9) (108)	40.6 (18.8, 65.6) (112)	0.541
Week 4	56.3 (25, 81.3) (99)	56.3 (25, 81.3) (100)	0.940
Week 8	50 (28.2, 75) (100)	50 (25, 81.3) (88)	0.927
Week 12	56.3 (25, 75) (92)	62.5 (31.3, 87.5) (80)	0.331
Week 16/exit	56.3 (25, 75) (97)	59.4 (25, 87.5) (92)	0.207
DFS-SF subscale: bothered by ulcer care,			
Median (Q_1, Q_3), *n*			
Week 1/baseline	56.3 (31.3, 81.3) (108)	50 (31.3, 75) (111)	0.467
Week 4	56.3 (37.5, 87.5) (98)	56.3 (31.3, 81.3) (99)	0.745
Week 8	68.8 (37.5, 81.3) (100)	62.5 (37.5, 84.4) (88)	0.950

Table 2 (*Continued*)

	Treatment		
	Arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation *n* = 129	Control *n* = 141	*P*-value
Week 12	65.6 (37.5, 87.5) (92)	68.8 (46.9, 84.4) (80)	0.474
Week 16/exit	62.5 (37.5, 81.3) (96)	68.8 (43.8, 87.5) (92)	0.324

*n/N, sample size; Q_1, first quartile; Q_3, third quartile; SD, standard deviation.
†Repeated measures (weeks 4, 8, 12, 16) treatment main effect with week 1 as covariate. No significant treatment × time interaction, $P = 0.884$.
‡Each Diabetic Foot Ulcer—Short Form (DFS-SF) subscale is scored from 0 to 100, with higher scores denoting better health-related quality of life.
§The EuroQOL five dimensions (EQ-5D) index score falls on a scale where 0.0 = death and 1.0 = perfect health

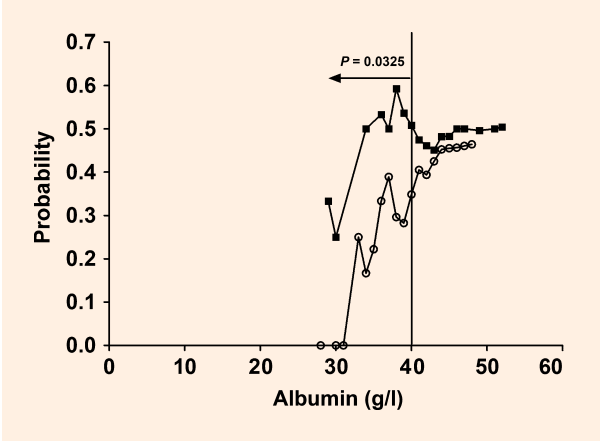

FIGURE 4 Cumulative probability of wound closure vs. albumin at entry by patient subgroups. Each point (x, y) represents the proportion, y, of subjects with total wound closure in the subgroup of subjects with baseline albumin ≤ x. Specifically, for the subgroup of subjects (N = 127) with albumin ≤ 40 g/l, proportion healed is higher in the arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation group (■) (n = 61) vs. the control (○) (n = 66) (P = 0.0325) Cochran–Mantel–Haenszel test stratified by site.

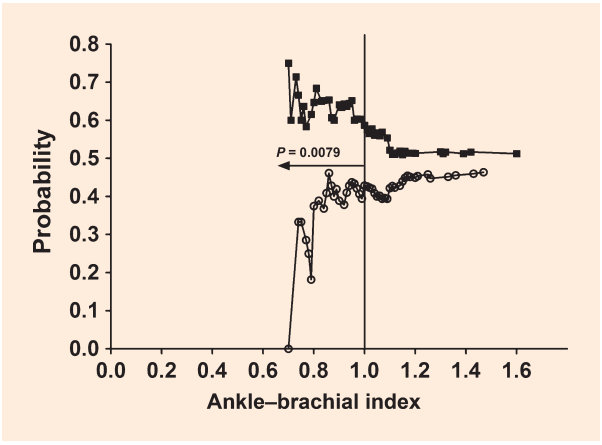

FIGURE 5 Cumulative probability of wound closure vs. ankle–brachial index at entry by patient. Each point (x, y) represents the proportion, y, of subjects with total wound closure in the subgroup of subjects with baseline ankle–brachial index ≤ x. Specifically, for the subgroup of subjects (N = 119) with ankle–brachial index < 1.0, proportion healed is higher in the arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation group (■) (n = 58) vs. the control group (○) (n = 61) (P = 0.0079) CMH test stratified by site.

increased mortality, with the hazard of death increasing incrementally, even with decreases as low as 2 g/l [20]. In the present study, subjects who were at risk of malnutrition (albumin level ≤ 40 g/l) had a better healing response after 16 weeks of supplementation with arginine, glutamine and β-hydroxy-β-methylbutyrate. Interestingly, albumin levels did not change across the 16 weeks, suggesting that the improvement was not a change in protein status but occurred by other mechanisms of action. Although speculative, serum albumin might be used as a biomarker for those who may respond best to treatment. Inflammatory status based on C-reactive protein was not different between the groups, although high-sensitivity C-reactive protein was not utilized. Although not measured in the present study, arginine, glutamine and β-hydroxy-β-methylbutyrate decrease muscle protein breakdown and/or increase muscle protein synthesis [9,13,21].

Impaired blood flow at the wound surface can result in oxygen and nutrient deficiency, limiting nutrient access for healing. In the present study, subjects had ulcers of neuropathic origin and their blood glucose under pharmacological control; however, peripheral neuropathies and arterial disease commonly coexist in patients with diabetic foot ulcers [22]. The ankle–brachial index is the ratio of the blood pressure in the lower legs to the blood pressure in the arms. In normal healthy people the pressure at the ankle is slightly higher than at the elbow. Thus, the normal range for

ankle–brachial index is recognized as being between 1.0 and 1.2. Interestingly, an ankle–brachial index value reduction of only 0.1 has been found to be associated with a 1.7 times risk of major amputation [23]. In the present study, subjects who were at risk of developing poor limb perfusion, defined by an ankle–brachial index < 1.00, had a better healing response after 16 weeks of arginine, glutamine and β-hydroxy-β-methylbutyrate supplementation. Arginine is the sole substrate for nitric oxide synthase that produces nitric oxide, which is critical to wound collagen accumulation and acquisition of mechanical strength. Nitric oxide production activates wound macrophages and neutrophils and leads to improved microvascular haemodynamic changes. Although speculative, the presence of arginine in the supplement may have had an effect on vascular deficiency.

Results from safety analyses showed that, in the evaluable group, but not in the intent-to-treat group, more subjects in the supplementation group developed mild wound infections. However, the rates of positive cultures were similar between groups and a mild infection was defined as a manifestation of inflammation. Therefore, it is possible that the mild infections reported were associated with the inflammatory response rather than a true infection. It is suggested that future studies should be designed using a definition that delineates better between inflammation and infection. While most of the subjects with reported infections received antibiotics, there were no group differences in antibiotic use, in contrast to a recent study showing that antibiotic treatment was reduced by 50% in people with diabetic foot ulcers who were treated with arginine, glutamine and β-hydroxy-β-methylbutyrate [24]. There were no group differences in the number of adverse events associated with infections.

Possible limitations of the present study include that only patients with non-infected University of Texas Ulcer classification grade 1A ulcers participated in the trial. Therefore, the results may not be generalizable to all patients with diabetic foot ulcers. In addition, the study was designed to evaluate wound healing after 16 weeks, consistent with other studies [25,26]. This, however, may not have been long enough to have identified an overall beneficial effect in the larger patient population.

The proportion of control subjects that had complete healing at week 16 was higher than expected based on the literature. This may have been attributable to the fact that, when subjects are in a clinical trial they receive good standards of care on a weekly basis and are more attentive to their home wound care, off-loading procedures and dietary intake. Thus, the results appear to show that good standards of care provided improved healing. Therefore, it is possible that in the present study the beneficial effects of supplementation with arginine, glutamine and β-hydroxy-β-methylbutyrate may have been blunted by the control subjects doing well. In the study design, sample size was determined assuming that 39% of subjects would have total wound closure in the control group, and that the experimental treatment would improve this rate by 20 absolute percentage points. Given that approximately 46% of control patients had total wound closure at 16 weeks, it appears that it was unrealistic to expect the treatment to improve this rate by 20 percentage points. Future studies should be designed using a more focused delta efficacy value, in addition to a concomitant a priori study focus on those with poor nutrition and impaired vascular status.

In summary, the results of this study show that those with diabetic foot ulcers without concomitant impaired blood flow and normal serum albumin levels may not benefit from supplementation. However, those patients with risk of poor limb perfusion and/or low albumin levels may benefit. Further investigation involving supplementation with arginine, glutamine and β-hydroxy-β-methylbutyrate in these high-risk subgroups should be conducted to confirm or refute these findings.

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Competing interests

JLN, GEB and ACV are employees of Abbott. There are no other conflicts of interest to declare.

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References

1 Mills JL, Conte MS, Armstrong DG, Pomposelli FB, Schanzer A, Sidawy AN *et al.* The society for vascular surgery lower extremity

threatened limb classification system: risk stratification based on wound, ischemia, and foot infection (WIfI). *J Vasc Surg* 2014; **59**: 220–234.
2 Armstrong DG, Lavery LA, Harkless LB. Validation of a diabetic wound classification system. The contribution of depth, infection, and ischemia to risk of amputation. *Diabetes Care* 1998; **21**: 855–859.
3 Oyibo SO, Jude EB, Tarawneh I, Nguyen HC, Harkless LB, Boulton AJ. A comparison of two diabetic foot ulcer classification systems: the Wagner and the University of Texas wound classification systems. *Diabetes Care* 2001; **24**: 84–88.
4 Oyibo SO, Jude EB, Tarawneh I, Nguyen HC, Armstrong DG, Harkless LB *et al*. The effects of ulcer size and site, patient's age, sex and type and duration of diabetes on the outcome of diabetic foot ulcers. *Diabet Med* 2001; **18**: 133–138.
5 Williams JZ, Abumrad N, Barbul A. Effect of a specialized amino acid mixture on human collagen deposition. *Ann Surg* 2002; **236**: 369–375.
6 Himes D. Protein–calorie malnutrition and involuntary weight loss: the role of aggressive nutritional intervention in wound healing. *Ostomy Wound Manag* 1999; **45**: 46–51.
7 Litchford M. Nutritional issues in the patient with diabetes and foot ulcers. In: Bowker JH, Pfeifer MA eds. *Levin and O'Neal's The Diabetic Foot*, 7th ed. Philadelphia: Mosby Elsevier, 2008: 199–217.
8 Barbul A, Lazarou SA, Efron DT, Wasserkrug HL, Efron G. Arginine enhances wound healing and lymphocyte immune responses in humans. *Surgery* 1990; **108**: 331–337.
9 Kirk SJ, Hurson M, Regan MC, Holt DR, Wasserkrug HL, Barbul A. Arginine stimulates wound healing and immune function in elderly human beings. *Surgery* 1993; **114**: 155–160.
10 Hurson M, Regan MC, Kirk SJ, Wasserkrug HL, Barbul A. Metabolic effects of arginine in a healthy elderly population. *J Parenter Enteral Nutr* 1995; **19**: 227–230.
11 Karna E, Miltyk W, Wolczynski S, Palka JA. The potential mechanism for glutamine-induced collagen biosynthesis in cultured human skin fibroblasts. *Comp Biochem Phys B* 2001; **130**: 23–32.
12 Nissen S, Sharp RL, Panton L, Vukovich M, Trappe S, Fuller JC Jr. Beta-hydroxy-beta-methylbutyrate (HMB) supplementation in humans is safe and may decrease cardiovascular risk factors. *J Nutr* 2000; **130**: 1937–1945.
13 Smith HJ, Mukerji P, Tisdale MJ. Attenuation of proteasome-induced proteolysis in skeletal muscle by beta-hydroxy-beta-methylbutyrate in cancer-induced muscle loss. *Cancer Res* 2005; **65**: 277–283.
14 Smith HJ, Wyke SM, Tisdale MJ. Mechanism of the attenuation of proteolysis-inducing factor stimulated protein degradation in muscle by beta-hydroxy-beta-methylbutyrate. *Cancer Res* 2004; **64**: 8731–8735.
15 Cavanagh PR, Lipsky BA, Bradbury AW, Botek G. Treatment for diabetic foot ulcers. *Lancet* 2005; **366**: 1725–1735.
16 Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG *et al*. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis* 2012; **54**: e132–173.
17 Lipsky BA, Peters EJ, Berendt AR, Senneville E, Bakker K, Embil JM *et al*. Specific guidelines for the treatment of diabetic foot infections 2011. *Diabetes Metab Res* 2012; **28**: S234–235.
18 Kind P, Dolan P, Gudex C, Williams A. Variations in population health status: results from a UK national questionnaire survey. *Br Med J* 1998; **316**: 736–741.
19 Bann CM, Fehnell SE, Gagnon DD. Development and validation of the diabetic foot ulcer scale-short form (DFS-SF). *Pharmacoeconomics* 2003; **21**: 1277–1290.
20 Kalantar-Zadeh K, Cano JN, Budde K, Chazot C, Kovesdy CP, Mak RH *et al*. Diets and enteral supplements for improving outcomes in chronic kidney disease. *Nat Rev Nephrol* 2011; **7**: 369–381.
21 Vinnars E, Hammarqvist F, von der Decken A, Wernerman J. Role of glutamine and its analogs in post-traumatic muscle protein and amino acid metabolism. *J Parenter Enteral Nutr* 1990; **14**: 125S–129S.
22 Reiber GE, LeMaster JW. Epidemiology and economic impact of foot ulcers and amputations in people with diabetes. In: Bowker JH, Pfeifer MA eds. *Levin and O'Neals The Diabetic Foot*, 7th ed. Philadelphia: Mosby Elsevier, 2008: 3–22.
23 Faglia E, Favales F, Aldeghi A, Calia P, Quarantiello A, Oriani G *et al*. Adjuctive systemic hyperbaric oxygen therapy in treatment of severe prevalently ischemic diabetic foot ulcer. A randomized study. Diabetes Care 1996; 19: 1338–1343.
24 Tatti P, Barber A. The use of a specialized nutritional supplement for diabetic foot ulcers reduces the use of antibiotics. *J Endocrinol Metab* 2012; **2**: 26–31.
25 Blume PA, Walters J, Payne W, Ayala J, Lantis J. Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial. *Diabetes Care* 2008; **31**: 631–636.
26 Armstrong DG, Lavery LA. Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomised controlled trial. *Lancet* 2005; **366**: 1704–1710.