HYPERBARIC OXYGEN THERAPY FOR DIABETIC FOOT ULCER

Pasquale Longobardi, MD
Medical Director OutPatient Problem Wound Care and Hyperbaric Centre, Ravenna (Italy)
Address: Centro iperbarico, via Augusto Torre 3, 48124 Ravenna (Italy), phone +39.0544.500152,
email: direzione@iperbaricoravenna.it; web: www.iperbaricoravenna.it

P L A I N L A N G U A G E S U M M A R Y

HYPERBARIC OXYGEN THERAPY FOR DIABETIC FOOT ULCER

BACKGROUND

Foot ulcers are a major complication of diabetes and are associated with a substantial burden for the patients and the entire health care system[1]. It has been estimated 1.0–1.4 million patients with DFU at any time in the 27 leading European countries and 21–29 million patients worldwide. Multiple factors are involved in the aetiology of diabetic foot ulcerations (DFUs), the main ones being peripheral neuropathy, external trauma and peripheral vascular disease[2,3]. DFUs are notoriously prone to complications and resistant to therapy. Several therapies have been proposed as adjuncts to traditional wound care (dressing changes, offloading, and debridement) to improve tissue oxygenation and enhance the healing process. Even with the best conventional treatment, many wounds remain unhealed. There are many reasons why DFUs do not heal, including oedema, anaemia and poor perfusion, all of which impede normal wound healing.

The effectiveness of HBO therapy in healing of DFU has remained controversial[4,5,6]. There are several reasons for the varied responses observed in research and practice. First, a common misconception is that HBOT works alone. HBO is an adjunctive therapy that should be delivered after an individualized approach with other wound care standards. Second, DFUs have multiple causes that affect how we should treat them and how they respond to HBOT. Third, HBOT is approved by Society guidelines[7,8,9,10] and is funded by payers for Wagner III or higher ulcers that have failed 30-day standard therapy[11]. In reality, only recalcitrant cases that have persisted for a much longer time are referred to HBOT (with 1.2% of matched Medicare and 2.3% of matched privately insured DFU patients receiving HBOT)[3]. This report intends to assess the effect of HBOT on patient-important outcomes[12] such as rate of complete wound and major amputation in patients with DFU.

REVIEW QUESTION

Assess the effect of HBOT on patient/important outcomes such as rate of complete wound and major amputation in patients with DFU.

WHAT WE FOUND

Eleven studies have been included in this report: 8 randomised trials, 1 retrospective controlled study, 1 observational cohort study (for a total of 6995 participants) and 1 decision model. We found that HBOT in DFUs seemed to improve the chance of healing in the short term (up to six weeks), but there are insufficient data to confirm that this benefit is statistically valid with longer term follow-up. HBOT may reduce the number of major amputations in people with DFUs.
This plain language summary is up-to-date as of April 07, 2016

BACKGROUND

Diabetes is a complex chronic disease and its management is further complicated by other common comorbidities[3]. Foot ulcers are a major complication of diabetes and are associated with a substantial burden for the patients and the entire health care system[1]. It has been estimated 1.0–1.4 million patients with DFU at any time in the 27 leading European countries and 21–29 million patients worldwide. Multiple factors are involved in the aetiology of diabetic foot ulcerations (DFUs), the main ones being peripheral neuropathy, external trauma and peripheral vascular disease[2,3]. DFUs are notoriously prone to complications and resistant to therapy. Several therapies have been proposed as adjuncts to traditional wound care (dressing changes, offloading, and debridement) to improve tissue oxygenation and enhance the healing process. Even with the best conventional treatment, many wounds remain unhealed. There are many reasons why DFUs do not heal, including oedema, anaemia and poor perfusion, all of which impede normal wound healing. Hyperbaric oxygenation therapy (HBOT) has been reported to decrease tissue hypoxia and is proposed as treatment for delayed wound healing. However the effectiveness of HBO therapy in healing of DFU has remained controversial[4,5,6]. There are several reasons for the varied responses observed in research and practice. First, a common misconception is that HBOT works alone. HBO is an adjunctive therapy that should be delivered after an individualized approach with other wound care standards (e.g., debridement, revascularization, off-loading). In practice, once patients are referred to HBOT, other wound care methods usually stop or decrease in intensity. Second, DFUs have multiple causes (e.g., ischemic, neuropathic, infectious) that affect how we should treat them and how they respond to HBOT. Third, HBOT is approved by Society guidelines[7,8,9,10] and is funded by payers for Wagner III or higher ulcers that have failed 30-day standard therapy[11]. In reality, only recalcitrant cases that have persisted for a much longer time are referred to HBOT. This report intends to assess the effect of HBOT on patient-important outcomes[12] such as rate of complete wound and major amputation in patients with DFU.

PREVALENCE / ANNUAL INCIDENCE

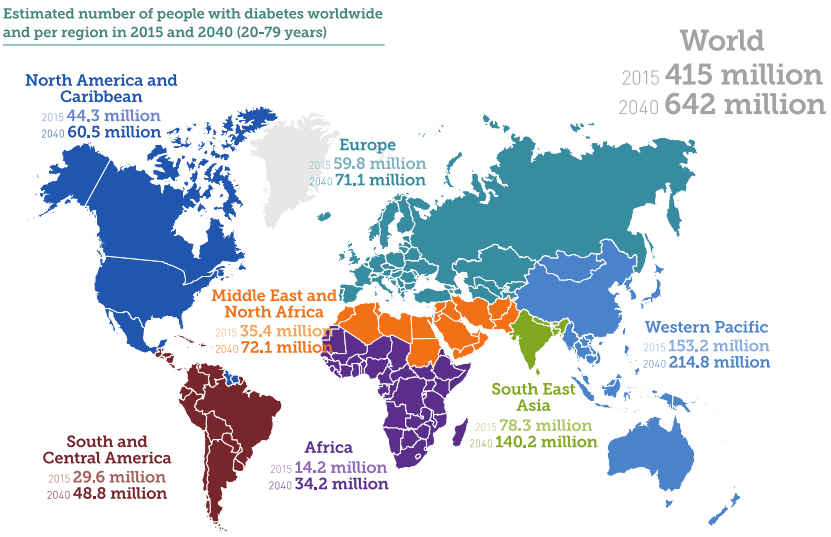
Diabetes has a prevalence of 8,8% (7,2-11,4%). It is one of the leading causes of chronic disease and limb loss worldwide currently affecting 415 million people (1 in 11 adults have diabetes). Every 6 seconds a person dies from diabetes (5.0 million deaths). It is predicted that by 2040 the number of reported diabetes cases will soar to 642 million (1 in 10 adults)[13] (Table 1 and Figure 1)

Table 1 Diabetes around the world[13] (© International Diabetes Federation http://www.diabetesatlas.org/, accessed by web on April 6, 2016)

The world at a glance

	2015	2040
Total world population	7.3 billion	9.0 billion
Adult population (20-79 years)	4.72 billion	6.16 billion
Child population (0-14 years)	1.92 billion	-
Diabetes (20-79 years)		
Global prevalence	8.8% (7.2-11.4%)	10.4% (8.5-13.5%)
Number of people with diabetes	415 million (340-536 million)	642 million (521-829 million)
Number of deaths due to diabetes	5.0 million	-
Health expenditure due to diabetes (20-79 years)		
Total health expenditure, R=2* 2015 USD	673 billion	802 billion
Hyperglycaemia in pregnancy (20-49 years)		
Proportion of live births affected	16.2%	-
Number of live births affected	20.9 million	-
Impaired glucose tolerance (20-79 years)		
Global prevalence	6.7% (4.5-12.1%)	7.8% (5.2-13.9%)
Number of people with impaired glucose tolerance	318 million (212.2-571.6 million)	481 million (317.1-855.7 million)
Type 1 diabetes (0-14 years)		
Number of children with type 1 diabetes	542,000	-
Number of newly diagnosed cases each year	86,000	-

Figure 1 Estimated age-adjusted prevalence of diabetes in adults (20-79), 2015[13]. Europe is not in Top Ten Countries / territories for number of people with diabetes (20-79 years) for both 2015 and for 2040 forecast. (© International Diabetes Federation http://www.diabetesatlas.org/, accessed by web on April 6, 2016)



In Europe, the number of people with diabetes is estimated to be 59.8 (45.1-85.6) million (9.1% [6.8-13.0%] of the population aged 20-79, including 23.5 million undiagnosed cases. While the Europe Region has the second-lowest age-adjusted comparative diabetes prevalence rate of any IDF region (after the Africa Region) there are still many countries with relatively high diabetes prevalence rates. Turkey has the highest age-adjusted comparative prevalence (12.8% comparative prevalence, 12.5% raw prevalence) and the third-highest number of people with diabetes in the Europe Region (6.3 [5.7-7.5] million), after Germany (6.5 [5.9-7.5] million) and the Russian Federation (12.1 [6.2-17.0] million). By 2040, it is predicted that there will be 71.1 million adults living with diabetes in the Europe Region. (Table 2).

It is reported that Europe has the highest number of children with type 1 diabetes compared with the other IDF regions – approximately 140,000. The European countries making the largest contribution to the overall numbers in type 1 diabetes in children are the United Kingdom, the Russian Federation and Germany[13]

Table 2 Diabetes in Europe : statistics in 2015 and forecast for 2040[13] (© International Diabetes Federation http://www.diabetesatlas.org/, accessed by web on April 6, 2016)

At a glance	2015	2040
Adult population (20-79 years)	660 million	663 million
Diabetes (20-79 years)		
Regional prevalence	9.1% (6.8-13.0%†)	10.7% (8.2-14.9%†)
Age-adjusted comparative prevalence	7.3% (5.5-10.9%†)	7.6% (5.7-11.2%†)
Number of people with diabetes	59.8 million (45.1-85.6 million‡)	71.1 million (54.4-98.7 million‡)
Number of deaths due to diabetes	627,000	-
Health expenditure due to diabetes (20-79 years)		
Total health expenditure, R=2*, USD	156 billion	174 billion
Impaired glucose tolerance (20-79 years)		
Regional prevalence	4.8% (3.1-11.4%†)	5.5% (3.6-11.9%†)
Age-adjusted comparative prevalence	4.1% (2.6-10.6%†)	4.3% (2.7-10.4%†)
Number of people with impaired glucose tolerance	31.7 million (20.3-75.2 million‡)	36.6 million (23.9-79.1 million‡)
Type 1 diabetes (0-14 years)		
Number of children with type 1 diabetes	140,000	-
Number of newly diagnosed children each year	21,600	-

The management of diabetes is complicated by other common comorbidities. Economic analysis potentially understate the incremental burden of DFU, because the matching process removed from the analysis relatively high-cost DFU patients with higher rates of comorbidities and medical resource use and relatively low-cost control patients (diabetic patients without DFU) with lower rates of comorbid conditions and medical resource use, because these outliers could not be matched.[3] (Table 3 for Medicare and Table 4 for private insurances)

Table 3 Patient characteristics, resource utilization and health care costs during the 12 months prior to the index date for Medicare (January 2007-September 2011)[3]. © 2014 by the American Diabetes Association

	Prematch characteristics			Postmatch characteristics		
	DFU *n* = 29,681	Controls *n* = 201,757	*P**	DFU *n* = 27,878	Controls *n* = 27,878	*P*†
Demographic characteristics						
Age, years, mean (SD)‡	77.7 (7.3)	75.7 (6.8)	<0.0001	77.6 (7.3)	77.7 (7.1)	0.5568
Male, %	43.3	44.2	0.0042	43.0	43.0	1.0000
Year of index date, %						
2007	30.7	31.2	0.0732	30.9	30.9	1.0000
2008	33.8	32.4	<0.0001	33.8	33.8	1.0000
2009	35.5	36.4	0.0031	35.3	35.3	1.0000
Select comorbid conditions, %						
Hypertension	86.4	82.4	<0.0001	85.7	86.4	0.0334
Hyperlipidemia	62.1	66.4	<0.0001	62.2	63.0	0.0533
Diabetes with complications	45.0	24.0	<0.0001	43.1	43.8	0.1225
PVD	34.7	16.3	<0.0001	32.5	32.5	0.9207
CHF	33.3	17.9	<0.0001	31.0	31.5	0.1600
Cerebrovascular disease	22.8	14.5	<0.0001	21.5	22.2	0.0463
COPD	22.5	15.5	<0.0001	21.2	21.7	0.1268
Renal disease	20.9	10.8	<0.0001	18.9	19.3	0.2475
End-stage renal disease	4.2	1.3	<0.0001	3.4	2.8	<0.0001
Infections	19.5	5.0	<0.0001	16.9	15.1	<0.0001
Depression	15.8	10.2	<0.0001	14.7	14.9	0.4747
Any malignancy	12.9	12.3	0.0024	12.7	13.5	0.0047
Obesity	11.0	7.0	<0.0001	10.2	10.3	0.4844
Myocardial infarction	9.9	6.2	<0.0001	9.0	9.3	0.3462
Smoking	6.8	5.9	<0.0001	6.5	7.0	0.0118
Metastatic solid tumor	1.4	1.1	0.0002	1.3	1.4	0.4019
Medical resource use, mean (SD)						
Inpatient days	2.0 (7.1)	0.9 (4.4)	<0.0001	3.5 (8.1)	3.5 (8.0)	0.1907
ED visits	1.0 (1.7)	0.6 (1.3)	<0.0001	0.9 (1.5)	0.9 (1.9)	0.6453
Outpatient days	49.6 (39.6)	33.4 (29.0)	<0.0001	46.1 (33.4)	46.7 (35.2)	0.0696
Total health care costs, mean (SD)§	$22,147 ($33,704)	$11,022 ($21,274)	<0.0001	$17,744 ($24,242)	$17,744 ($24,242)	0.0490

*Calculated using Wilcoxon rank sum tests for continuous variables and x2 tests for categorical variables. †Calculated using Wilcoxon signed rank tests for continuous variables and McNemar tests for categorical variables. ‡Estimated in quarter 4 of the year preceding index quarter. §Only medical costs were used, as Medicare data do not contain prescription drug information. US Dollar (USD) values were inflated to the 2012 U.S. dollar using the medical care component of the Consumer Price Index.

Table 4 Patient characteristics (aged 18-64 years), resource utilization and health care costs during the 12 months prior to the index date for private insurance (January 2007-September 2011)[3]. © 2014 by the American Diabetes Association

	DFU *n* = 5,681	Controls *n* = 113,337	*P**	DFU *n* = 4,536	Controls *n* = 4,536	*P*†
Demographic characteristics						
Age, years, mean (SD)‡	55.3 (7.1)	52.8 (8.7)	<0.0001	55.0 (7.3)	54.5 (7.7)	0.0026
Male, %	59.7	55.0	<0.0001	59.0	59.0	1.0000
Year of index date, %						
2007	24.0	25.6	0.0093	24.1	24.1	1.0000
2008	25.5	23.6	0.0010	26.1	26.1	1.0000
2009	27.4	27.9	0.4106	27.4	27.4	1.0000
2010	23.0	22.9	0.8003	22.5	22.5	1.0000
Select comorbid conditions, %						
Hypertension	65.4	56.0	<0.0001	62.4	61.5	0.3779
Hyperlipidemia	52.1	56.5	<0.0001	53.2	55.0	0.0757
Diabetes with complications	43.8	14.2	<0.0001	36.3	36.5	0.8201
PVD	15.2	3.5	<0.0001	9.6	9.3	0.5988
CHF	15.9	5.1	<0.0001	10.9	9.6	0.0380
Cerebrovascular disease	10.5	4.4	<0.0001	7.6	6.9	0.1976
COPD	7.9	3.4	<0.0001	5.9	5.5	0.3853
Renal disease	14.1	3.1	<0.0001	8.6	8.2	0.4930
End-stage renal disease	6.0	0.9	<0.0001	2.9	1.9	0.0010
Infections	27.3	2.7	<0.0001	16.7	15.4	0.0078
Depression	10.7	7.7	<0.0001	9.6	10.0	0.5144
Any malignancy	6.6	4.9	<0.0001	6.1	6.0	0.8927
Obesity	9.5	6.2	<0.0001	8.4	8.3	0.8485
Myocardial infarction	3.5	1.7	<0.0001	2.1	2.1	0.9397
Smoking	1.7	1.6	0.6880	1.6	1.5	0.7335
Metastatic solid tumor	0.8	0.7	0.0899	0.7	0.9	0.4126
Medical resource use, mean (SD)						
Inpatient days	13.8 (31.5)	5.2 (15.1)	<0.0001	8.4 (20.2)	7.7 (19.6)	0.0002
ED visits	1.3 (3.9)	0.5 (1.4)	<0.0001	0.8 (1.7)	0.8 (1.8)	0.0223
Outpatient days	16.7 (17.3)	11.1 (11.4)	<0.0001	14.2 (12.9)	14.2 (12.5)	0.9112
Prescription drug use						
Unique medications, mean (SD)§	13.2 (11.5)	9.5 (8.5)	<0.0001	12.3 (10.5)	11.7 (10.0)	0.0026
Immunosuppressants, %‖	2.4	0.8	<0.0001	1.9	1.5	0.2160
Total health care costs, mean (SD)¶	$31,844 ($84,565)	$12,790 ($36,898)	<0.0001	$14,761 ($22,149)	$14,766 ($22,153)	0.2328

*Calculated using Wilcoxon rank sum tests for continuous variables and x2 tests for categorical variables. †Calculated using Wilcoxon signed rank tests for continuous variables and McNemar tests for categorical variables. ‡Estimated at index date. §Calculated as the number of prescriptions with unique National Drug Code codes according to the first nine digits filled. ‖Includes prescriptions with supply in the 12 months prior to the index date, whether or not the prescription itself was filled in these 12 months. ¶Calculated as the sum of medical and prescription drug costs. Dollar values were inflated to the 2012 U.S. dollar using the medical care component of the Consumer Price Index.

The prevalence of Diabetic Foot Ulcer (DFU) is 50-70 per 1000 patients with diabetes. Applying these rates to the estimated diabetic population of the 27 leading European countries (20.2 million)[14] suggests that 1.0–1.4 million patients have a DFU at any time[15] (Table 5). Applying these prevalence rates to the worldwide diabetic population in 2015, 20.75–29 million patients have a DFU at any time.

Table 5: prevalence and annual incidence of Diabetic Foot Ulcer in Europe-27 Countries[13] (calculated in March 2016)

	Range	**Total EU-27 Countries**
Population with diabetes		20,2 million[16]
Diabetic foot ulcers:		
• Prevalence	50-70/1000 diabetics[17]	1,0-1,4 million
• Incidence	20-30/1000 diabetics [18]	400,000-600,000

Approximately 80% of diabetes-related lower extremity amputations are preceded by a foot ulcer. The patient demographics related to DFU are typical for patients with long-standing diabetes. Risk factors for ulceration include neuropathy, PAOD, foot deformity, limited ankle range of motion, high plantar foot pressures, minor trauma, previous ulceration or amputation, and visual impairment[19] Once an ulcer has developed, infection and PAOD are the major factors contributing to subsequent amputation [20,21]
Approximately 12% of people with DFU progress to lower extremity amputation[2,16,22]. The annual average incidence of amputation is 2.5 to 18 per 1,000 diabetics[2,16]. While toe, foot,

and below-knee amputations have declined over the last 15 to 20 years, there are still 65,000-70,000 amputations performed annually[22]. DFU contribute to over half of lower extremity amputations in the United States in a group at risk representing only 3 per cent of the population[16,2,22]. In 54 to 73 percent of these cases, there is no angiogram performed despite the fact that angiograms can reduce the odds of amputation by 90 percent[22,23] The relapse rate for DFU is 66% over five years.

CLINICAL PRESENTATION

One difficulty in analysing the existing body of literature lies in the heterogeneity of the patient populations being studied, the interventions being used and the outcomes being compared. In the arena of classification of a wound infection and the severity and outcome of treatment of a Diabetic Foot Ulcer, there is no empirical evidence that one classification system is better than any other[9]. Table 6 shows three different classifications of DFU (classic Wagner Graging System; University of Texas Health Centre at San Antonio, USA; Infectious Disease Society of America – IDSA).

Modern use of the Wagner classification system grades wounds on observations such as deformity, depth, infection, gangrene and location. The Wagner system is the primary method of assessment used in the orthopedic literature and grades ulcers with respect to depth and presence of necrosis, not taking into account vascular perfusion of the foot Despite consensus between foot and ankle surgeons and hyperbaric physicians that the Wagner grade is archaic and inadequate, most of the historical and contemporary studies and most reimbursement determinations, especially in the USA, regarding the use of HBOT for DFUs are based on the Wagner DFU wound appearances.

The University of Texas classification system is a better predictor of outcome because it combines the presence or absence of infection plus perfusion in a vertical scale and the depth of the wound on a horizontal scale to generate a 16-choice matrix.

The Infectious Disease Society of America (IDSA) bases its classification system on the severity of diabetic foot infections and has shown an increased trend for more frequent and higher levels of amputation with the seriousness of infection.

A recent guideline by the Society for Vascular Surgery[25] (Table 7) published a risk stratification based on three major factors that impact amputation risk and clinical management – Wound, Ischemia and foot Infection (WIfI) – to generate a matrix of 32 permutations of wound categories that generally have worse outcomes as one moves down and to the right.

Table 8 shows the International Working Group on the Diabetic Foot (IWGDF) classification system based on five key categories that address all of the relevant comorbidities contributing to the pathology of a DFU: Perfusion; Extent/size; Depth/tissue loss; Infection and Sensation (PEDIS)[24].

Table 6 Three different classifications of DFU: Wagner Graging System; University of Texas Health Centre at San Antonio, USA; Infectious Disease Society of America – IDSA. (Huang et al[10]. Copyright © 2015 Undersea & Hyperbaric Medical Society, Inc.

GRADING CLASSIFICATION	GRADE TIER	GRADING SYSTEM
Classic Wagner Grading System	6 grades based on anatomy and presence of infection	Grade 0 no open lesion, may have healed lesions Grade 1 superficial ulcer without penetration to deeper layers Grade 2 deeper ulcer, reaching tendon, bone, or joint capsule Grade 3 deeper tissues are involved, and there is abscess, osteomyelitis, or tendonitis Grade 4 there is gangrene of some part of the toe, toes, and/or forefoot Grade 5 gangrene involves the whole foot or enough of the foot that no local procedures are possible and BKA is indicated
University of Texas Health Science Center at San Antonio	4 stages based on absence or presence of ischemia and infection	Stage A no infection Stage B infection Stage C ischemia Stage D infection and ischemia
	4 grades based on extent and depth of wound	Grade 0 epithelialized wound Grade 1 superficial wound Grade 2 wound penetrating tendon or capsule Grade 3 wound penetrating bone or joint
IDSA (Infectious Disease Society of America)	4 grades 4 IDSA levels of based on severity of severity of infection	Grade 1 infection with at least two of following criteria: localized swelling, erythema, pain, warmth, purulent discharge; PEDIS 1; IDSA infection severity: uninfected Grade 2 local infection involving only skin and subcutaneous tissue with erythema >0.5 cm and < 2 cm around ulcer; PEDIS 2; IDSA infection severity: mild Grade 3 local infection with erythema > 2 cm or involving structures deeper to skin and subcutaneous tissue with no signs of systemic inflammation; PEDIS 3: IDSA infection severity: moderate Grade 4 local infection with systemic inflammation response signs (SIRS) with two or more of the following criteria: temp > 38 degrees or < 36 degrees, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or $PaCO_2$ < 32 mm Hg, white blood count (WBC) > 12,000 or < 4000 cells/microliter or > 10% immature band forms; PEDIS 4: IDSA infection severity: severe

Table 7 The Society for Vascular Surgery[25] published a risk stratification based on three major factors that impact amputation risk and clinical management: Wound, Ischemia and foot Infection (WIfI), to generate a matrix of wound categories that generally have worse outcomes as one moves down. Huang et al[10]. Copyright © 2015 Undersea & Hyperbaric Medical Society, Inc.

Society for Vascular Surgery Wound Ischemia Foot Infection (WIfI) System	4 grades for each of three criteria of wound, ischemia and foot infection (WIfI)	**Wound**	
		Grade 0	no ulcer or gangrene
		Grade 1	shallow ulcer; no gangrene
		Grade 2	deeper ulcer with exposed joint or tendon; gangrene limited to digits
		Grade 3	deep ulcer involving forefoot, midfoot, heel; extensive gangrene involving forefoot, midfoot, or heel
		Ischemia	
		Grade 0	ABI ≥ 0.80; arterial systemic pressure >100 mm Hg; and/or $TcPO_2$ ≥ 60 mm Hg
		Grade 1	ABI 0.6-0.79; arterial systemic pressure 70-100 mm Hg; and/or $TcPO_2$ 40-59 mm Hg
		Grade 2	ABI 0.4-0.59; arterial systemic pressure 50-70 mm Hg; and/or $TcPO_2$ 30-39 mm Hg
		Grade 3	ABI ≤ 0.39; arterial systemic pressure 50-70 mm Hg; and/or $TcPO_2$ <30 mm Hg
		Infection	
		Grade 0	uninfected: no signs or symptoms
		Grade 1	local infection: erythema > 0.5 cm and ≤ 2 cm with pain, warmth, purulent discharge (mild)
		Grade 2	local infection with > 2 cm erythema; involves deeper structures (moderate)
		Grade 3	local infection with signs of SIRS (refer to IDSA definition) (severe)

Table 8 International Working Group on the Diabetic Foot (IWGDF) classification system based on five key categories that address all of the relevant comorbidities contributing to the pathology of a Diabetic Foot Ulcer: Perfusion; Extent/size; Depth/tissue loss; Infection and Sensation (PEDIS). Huang et al[10]. Copyright © 2015 Undersea & Hyperbaric Medical Society, Inc.

GRADING CLASSIFICATION	GRADE TIER	GRADING SYSTEM
International Working Group on the Diabetic Foot	Five categories, scored based on different criteria	**Perfusion** Grade 1 no signs/symptoms of PAD Grade 2 symptoms or signs of PAD, but not of critical limb ischemia (CLI) Grade 3 critical limb ischemia as defined by systolic ankle blood pressure <50 mm Hg or systolic toe blood pressure <30 mm Hg or $TcPO_2$ < 30 mm Hg.
		Extent/size No scoring system was provided. Recommendations were that wounds should be measured after debridement and that the frequency distribution of the size of the ulcers should be reported in each study as quartiles.
		Depth/tissue loss Grade 1 superficial full-thickness ulcer, not penetrating any structure deeper than the dermis. Grade 2 deep ulcer, penetrating below the dermis to subcutaneous structures, involving fascia, muscle or tendon Grade 3 all subsequent layers of the foot involved, including bone and/or joint (exposed bone, probing to bone)
		Infection Grade 1 no symptoms or signs of infection Grade 2 infection involving the skin and the subcutaneous tissue only (without involvement of deeper tissues and without systemic signs) Grade 3 erythema >2 cm plus one of the items described above (swelling, tenderness, warmth, discharge) or infection involving structures deeper than skin and subcutaneous tissues such as abscess, osteomyelitis, septic arthritis, fasciitis. No systemic inflammatory response signs, as described below Grade 4 any foot infection with the following signs of a systemic inflammatory response syndrome. This response is manifested by two or more of the following conditions: temperature >38°C or <36°C; heart rate >90 beats/minute; respiratory rate >20 breaths/minute; $PaCO_2$ <32-mm Hg; white blood cell count >12.000 or<4.000/cu mm; 10% immature (band) forms.
		Sensation Grade 1 no loss of protective sensation on the affected foot detected, defined as the presence of sensory modalities described below. Grade 2 loss of protective sensation on the affected foot is defined as the absence of perception of one of the following tests in the affected foot: • Absent pressure sensation, determined with a 10-g monofilament, on two out of three sites on the plantar side of the foot, as described in the International Consensus on the Diabetic Foot. • Absent vibration sensation, (determined with a 128-Hz tuning fork) or vibration threshold >25 V (using semi-quantitative techniques), both tested on the hallux.

STANDARD MANAGEMENT AND OUTCOME

Several relevant clinical practice guidelines for the diagnosis and treatment of Diabetic Foot Ulceration (DFU)[7,8,9] and the literature from 2004 to March 2016 were reviewed. An algorithm that summarizes the prevention and care of the DFU, developed by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine, is depicted in the Figure 2. [7]

Specific practice recommendations[7] were made by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine using the Grades of Recommendation Assessment, Development, and Evaluation system. Specific areas of focus included (1) prevention of diabetic foot ulceration, (2) off-loading, (3) diagnosis of osteomyelitis, (4) wound care and (5) peripheral arterial disease.

1. **Prevention of Diabetic Foot Ulceration** (**DFU)**

Patients with diabetes should undergo annual interval foot inspections by physicians or advanced practice providers with training in foot care (GRADE 1C). It is suggested that foot examination includes testing for peripheral neuropathy using the Semmes-Weinstein test (GRADE 1B). Education of the patients and their families about preventive foot care is recommended (GRADE 1C). There is evidence against the routine use of specialized

therapeutic footwear in average-risk diabetic patients (GRADE 2C). Custom therapeutic footwear in high-risk diabetic patients are advised, including those with significant neuropathy, foot deformities or previous amputation (GRADE 1B). Adequate glycaemic control (haemoglobin A1c < 7% with strategies to minimize hypoglycaemia) is suggested to reduce the incidence of diabetic foot ulcerations (DFUs) and infections, with subsequent risk of amputation (GRADE 2B). There is recommendation against prophylactic arterial revascularization to prevent DFU (GRADE 1C).

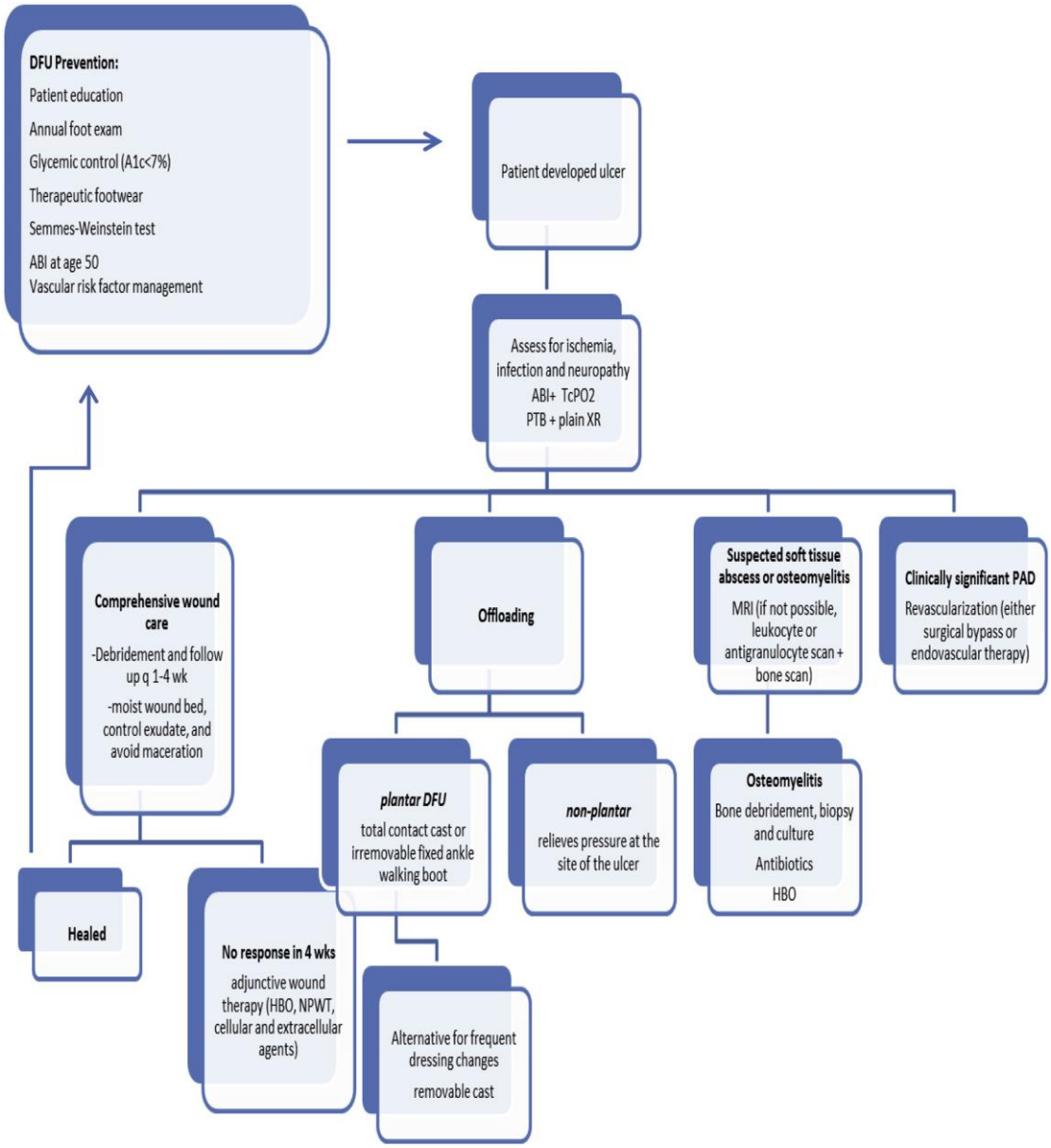
2. **Off-loading DFUs**

In patients with plantar DFU, offloading with a total contact cast (TCC) or irremovable fixed ankle walking boot are recommended (GRADE 1B). In patients with DFU requiring frequent dressing changes, off-loading using a removable cast walker as an alternative to TCC and irremovable fixed ankle walking boot are suggested (GRADE 2C). There is evidence against using postoperative shoes or standard or customary footwear for off-loading plantar DFUs (GRADE 2C). In patients with nonplantar wounds, it is recommended using any modality that relieves pressure at the site of the ulcer, such as a surgical sandal or heel relief shoe (GRADE 1C). In high-risk patients with healed DFU (including those with a prior history of DFU, partial foot amputation, or Charcot foot), wearing specific therapeutic footwear with pressure-relieving insoles is advised to aid in prevention of new or recurrent foot ulcers (GRADE 1C).

3. **Diagnosis of diabetic foot osteomyelitis (DFO)**

In patients with a Diabetic Foot Infection (DFI) with an open wound, a probe to bone (PTB) test is suggested to aid in diagnosis (GRADE 2C). In all patients presenting with a new DFI, serial plain radiographs of the affected foot are suggested to identify bone abnormalities (deformity, destruction) as well as soft tissue gas and radiopaque foreign bodies (GRADE 2C). For those patients who require additional (i.e., more sensitive or specific) imaging, particularly when soft tissue abscess is suspected or the diagnosis of osteomyelitis remains uncertain, Magnetic Resonance Imaging (MRI) is recommended as the study of choice. MRI is a valuable tool for diagnosis of osteomyelitis if the PTB test is inconclusive of if the plain film is not useful (GRADE 1B). In patients with suspected DFO for whom MRI is contraindicated or unavailable, a leukocyte or antigranulocyte scan is suggested as the best alternative, preferably combined with a bone scan (GRADE 2B). In patients at high risk for DFO, the diagnosis is most definitively established by the combined findings on bone culture and histology (GRADE 1C). When bone is debrided to treat osteomyelitis, sending a sample for culture and histology is recommended (GRADE 1C). For patients not undergoing bone debridement, it is suggested that clinicians consider obtaining a diagnostic bone biopsy when faced with diagnostic uncertainty, inadequate culture information, or failure of response to empirical treatment (GRADE 2C). Note: the Society for Vascular Surgery algorithm on DFU (Figure 2) indicates the following therapeutic strategy to manage osteomyelitis: bone debridement, biopsy an cultures, antibiotics, HBOT (the level of evidence is not reported).

Figure 2 Algorithm for prevention and care of diabetic foot. ABI = Ankle-Brachial Index; DFU = Diabetic Foot Ulceration; HBO = Hyperbaric Oxygen; MRI = Magnetic Resonance Imaging; NPWT = Negative Pressure Wound Therapy; PAD = Peripheral Arterial Disease; PTB = Probe To Bone; TcPO2 = Transcutaneous Oxygen Pressure; XR = radiography. (Copyright © 2016 by the Society for Vascular Surgery. Published by Elsevier Inc. http://dx.doi.org/10.1016/j.jvs.2015.10.003)[7]



4. Wound care for DFUs

Frequent evaluation is suggested at 1- to 4-week intervals with measurements of DFU to monitor reduction of wound size and healing progress (GRADE 1C). The evaluation for infection is recommended on initial presentation of all DFU, with initial sharp debridement of all infected DFU and urgent surgical intervention for foot infections involving abscess, gas or necrotizing fasciitis (GRADE 1B). Treatment of DFIs should follow the most current guidelines published by the Infectious Diseases Society of America (IDSA)[9] (Ungraded). The use of dressing products that maintain a moist wound bed, control exudate and avoid maceration of surrounding intact skin for DFU is recommended (GRADE 1B). Sharp debridement of all devitalized tissue and surrounding callus material from DFUs at 1- to 4-week intervals is recommended (GRADE 1B). Considering lack of evidence for superiority of

any given debridement technique, initial sharp debridement is suggested with subsequent choice of debridement method based on clinical context, availability of expertise and supplies, patient tolerance and preference and cost-effectiveness (GRADE 2C). For DFUs that fail to demonstrate improvement (>50% wound area reduction) after a minimum of 4 weeks of standard wound therapy, adjunctive wound therapy options are recommended. These include Negative Pressure Wound Therapy (NPWT), biologics *(platelet-derived growth factor [PDGF], living cellular therapy, extracellular matrix products, amnionic membrane products)* and hyperbaric oxygen therapy (HBOT). Choice of adjuvant therapy is based on clinical findings, availability of therapy and cost-effectiveness; there is no recommendation on ordering of therapy choice. Re-evaluation of vascular status, infection control, and off-loading is recommended to ensure optimization before initiation of adjunctive wound therapy (GRADE 1B). The use of NPWT is suggested for chronic DFU that do not demonstrate expected healing progression with standard or advanced wound dressings after 4 to 8 weeks of therapy (GRADE 2B). Consideration of the use of PDGF (becaplermin) is suggested for the treatment of DFUs that are recalcitrant to standard therapy (GRADE 2B). Consideration of living cellular therapy using a bilayered keratinocyte/fibroblast construct or a fibroblast-seeded matrix is suggested for treatment of DFUs when recalcitrant to standard therapy (GRADE 2B). Consideration of the use of extracellular matrix products employing acellular human dermis or porcine small intestinal submucosal tissue is suggested as an adjunctive therapy for DFUs when recalcitrant to standard therapy (GRADE 2C). In patients with DFU who have adequate perfusion that fails to respond (>50% wound area reduction) after a minimum of 4 weeks of standard wound therapy (conservative management), HBOT is recommended (GRADE 2B).

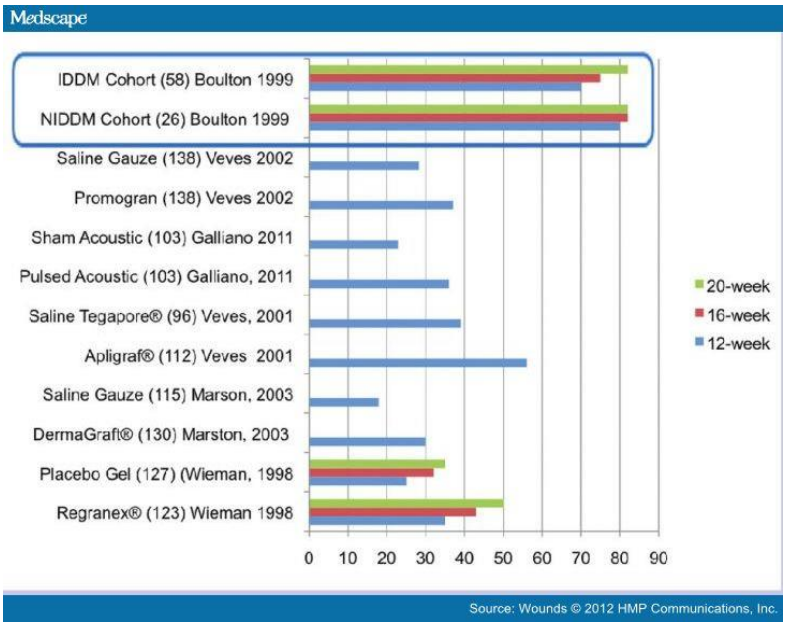
5. Peripheral arterial disease (PAD) and the DFU

Patients with diabetes should have Ankle-Brachial Index (ABI) measurements performed when they reach 50 years of age (GRADE 2C). Patients with diabetes who have a prior history of DFU, prior abnormal vascular examination, prior intervention for peripheral vascular disease or known atherosclerotic cardiovascular disease (eg, coronary, cerebral, or renal) should have an annual vascular examination of the lower extremities and feet including ABI and toe pressures (GRADE 2C). Patients with DFU should have pedal perfusion assessed by ABI, ankle and pedal Doppler arterial waveforms and either toe systolic pressure or transcutaneous oxygen pressure measurement (TCOM) annually (GRADE 1B). In patients with DFU who have PAOD, revascularization is recommended by either surgical bypass or endovascular therapy (GRADE 1B). Technical and implementation remarks: a) Prediction of patients most likely to require and to benefit from revascularization could be based on the Society for Vascular Surgery (SVS) Wound, Ischemia, and foot Infection (WIfI) lower extremity threatened limb classification. b) A combination of clinical judgment and careful interpretation of objective assessments of perfusion along with consideration of the wound and infection extent is required to select patients appropriately for revascularization. c) In functional patients with long-segment occlusive disease and a good autologous conduit, bypass is likely to be preferable. d) In the setting of tissue loss and diabetes, prosthetic bypass is inferior to bypass with vein conduit. e) The choice of intervention depends on the degree of ischemia, the extent of arterial disease, the extent of the wound, the presence or absence of infection and the available expertise.

HBOT is indicated in DFUs as an adjunctive therapy in a multidisciplinary approach. In this perspective, it is useful to compare the efficacy of HBOT over the other therapies used in the wound care (benchmark). "Best practice benchmarking" or "process benchmarking" is used to measure performance using a specific indicator resulting in a metric of performance that is

then compared to others, in order to make improvements or adapt specific best practices, usually with the aim of increasing some aspect of performance. Benchmarking should be treated as a continuous process in which organizations continually seek to improve their practices.[26,27] Bolton L.[27] published eight RCTs and one meta-analysis qualified as benchmark resources for DFU, pressure ulcer (PU) and Venous Leg Ulcer (VLU). Figure 3

Figure 3. Healing outcomes reported in RCTs for Diabetic Foot Ulcers: bar length represents percent completely healed after at least 12 weeks of care for topical interventions with at least 100 subjects. Outline contains clinical cohorts of patients with Non-Insulin Dependent Diabetes Mellitus (NIDDM) and Insulin Dependent Diabetes Mellitus (IDDM). Healing outcomes were recorded from Kaplan-Meier healing curves of DFU managed with evidence-based multi-disciplinary care in a DFU clinic. (Bolton L., Wounds© 2012 HMP Communications, Inc.)[27]

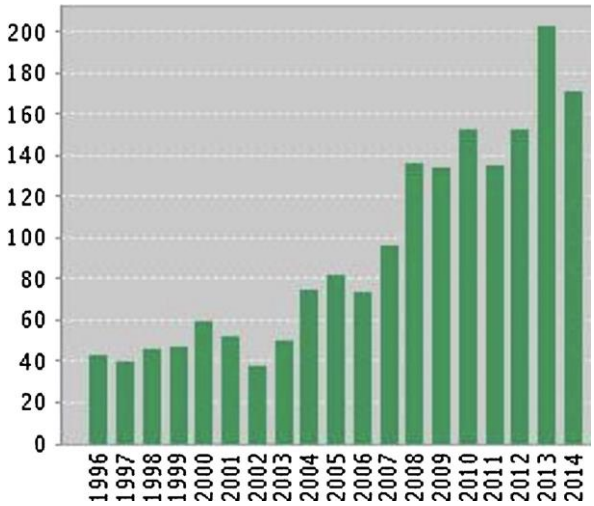


PATHOPHYSIOLOGY OF DIABETIC FOOT ULCERS (DFUs)

Our understanding of the aetiology and patho-mechanics of Diabetic Foot Ulcers (DFUs) has evolved in recent years. It is now understood that the mechanical derangements of the foot and ankle due to contracted soft tissue and osseous deformities as well as abnormal gait and motor imbalance are all essential factors in the development of ulcers in patients with longstanding diabetes and peripheral neuropathy. In these patients, however, numerous other factors, including impaired biologic condition, obesity, visual impairment and vasculopathy, affect ulcer wound care and healing. Over time, all patients with diabetes may develop some element of neuropathy expressed with sensory, motor or autonomic dysfunction. Reduced sensation leaves the foot unable to detect trauma, resulting in a 7-fold surge in the risk of ulceration[28]. Motor neuropathy results in intrinsic muscle atrophy, leading to an imbalance between flexors and extensors which promotes clawing of the toes. Consequently, the metatarsal fat pad can become displaced distally and underlying connective tissue changes render it deficient in providing protection from shear stresses over the prominent metatarsal heads during gait. Structural connective tissue changes resulting from non-enzymatic glycosylation result in stiffening of the Achilles tendon and limits dorsiflexion[29,30]. These developments, along with other morphologic changes affecting joint mobility, tend to shift the distribution of plantar pressure towards the forefoot. Forefoot overloading further promotes ulceration and diabetic patients with equinus contracture are at a 4-fold increased risk of plantar ulceration[31]. In addition to mechanical factors, biological impairment and

vasculopathy also play a significant role in the formation of DFUs and their ability to heal. Diabetes promotes an atherogenic state via systemic changes in inflammation as well as endothelium structure and function. As a result, this complex metabolic disorder negatively affects multiple organ systems, with the most frequent comorbidities being renal failure, cardiovascular disease, visual impairment, and peripheral neuropathy. Over time, blood flow to the vessels of the feet is impaired. Frequently, microvascular pathology is present even if macrovascular blood flow is acceptable. Depending on the severity, this may encourage ulcer development while inhibiting the natural healing response. Impaired activity of white blood cells involving both B and T cell types in diabetic patients may complicate the healing and treatment of these wounds, making any diabetic foot infection a potentially serious event. Breaches to the skin barrier often result from peripheral neuropathy, either from trauma or the cracking of dry skin due to the loss of normal skin moisture. These injuries allow pathogens to invade, whereas abnormalities in neutrophil phagocytosis and decreased vascular perfusion leave diabetic individuals particularly susceptible to infection from otherwise non-pathogenic bacteria. The common infecting microorganisms were Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus and mixed infection[32-34,35]. The management of Diabetic Foot Infections is a matter of increasing concern for health professionals. (Figure 4)

Figure 4 Published items per year located with the search term "diabetic foot infection" in Web of Science. *(© 2015 Uckay I et al[34]. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license)*



HBOT RATIONAL IN DIABETIC FOOT ULCERS

The possible physiologic effects of HBOT are the reduction of the regional and local ischemia, the stimulation of oxygen-dependent components of wound repair, the release of bone marrow stem cells, enhancing host antimicrobial responses and the stimulation of angiogenic healing responses to the point of local host competency[36,37,38,39,40].

Impairments in the endothelial isoform of Nitric Oxide Synthase (eNOS) function are related to hyperglycemia, insulin resistance, impaired enzyme synthesis, disordered caveolin associations and enhanced protein kinase C activity. Production of superoxide free radical ($O2^{-}$), is augmented in diabetes and this will reduce bioavailability of ·NO because the two radicals react rapidly to generate alternative Reactive nitrogen species (RNS). Disordered balance between ($O2^{-}$) and ·NO is reflected by elevated levels of nitrotyrosine in plasma of type II diabetics[41]. Data from diabetic animals and humans indicate that HBOT can overcome some aspects of eNOS inhibition[42]

A persistent increases in Nitric Oxide (NO) in wound fluid in diabetic ulcers associated with increased granulation tissue formation and wound closure when patients are exposed to 20 HBOTs at 2.0 ATA for 90 minutes has been demonstrated[43] Subsequent to HBOT treatments of diabetic patients, most wound margin HIFs and thioredoxin appear to be derived from localized Stem/Progenitor cells (SPCs)[44]. This suggests that SPCs may play an important role in supplying critical factors during wound healing in diabetic patients. The assays of SPCs during the first weeks of care in patients with DFUs can provide insight into how well wounds will respond and may aid with decisions on the use of adjunctive measures[45]
HBOT could be useful especially when the patient has different impairment factors (as the cigarette smoking; rheumatic diseases; anemia; diabetes; respiratory, liver and/or kidney disease diseases). In part this is because the effect of hyperoxia on catalytic activity is reflected by values for the apparent Michaelis-Menten constant (apparent K_m) for oxygen and it differs among the three Nitric Oxide Synthase (NOS) isoforms This depends on the fact that the enzyme activity is constrained by ferric-ferrous conversion at the active site[46,47]. (Table 9). In the presence of impairment factors, nitric oxide synthesis is slowed or inhibited because of the decreased « apparent » affinity of the substrate (oxygen) to the binding site (Nitric Oxide Synthetase). The change in Michaelis-Menten constant (apparent K_m) can be overcome by increasing the substrate concentration (that is the ppO_2), in which case the substrate will outcompete the inhibitor in binding to the enzyme (NOS).

Table 9 The effect of hyperoxia on catalytic activity is reflected by values for the apparent Michaelis-Menten constant (apparent K_m) for oxygen and it differs among the three Nitric Oxide Synthase (NOS) isoforms[46,47]

Nitric Oxide Synthase (NOS) isoforms	**ppO_2 needed to normalize the apparent Michaelis-Menten constant (apparent K_m)**	
Neuronal NOS (nNOS or NOS1)	~ 490 mmHg	350 µM
Inducible NOS (iNOS or NOS2)	~ 130 mmHg	190 µM
Endothelial NOS (eNOS or NOS3 or cNOS)	~ 38 mmHg	53 µM

A study[35] showed that adjunctive HBOT has a positive effect on wound healing in diabetic foot with infection, with the presence of a healed wound and preservation of the affected foot for at least 6 months after the completion of HBOT. The effect of HBOT seems dose dependent because the amputation rate is decreased in patients who receive adequate HBOT (HBOT > 10 sessions versus HBOT < 10 sessions, $p<0.05$). Forty-two patients with 44 infected diabetic feet receiving HBOT were divided into two groups. The common infecting microorganisms were Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus and mixed infection (table 10). One group of 21 patients with 21 DFUs received <10 sessions of HBOT. The other 21 patients with 23 feet received >10 sessions of HBOT. In patients who received <10 sessions of HBOT, seven patients achieved satisfactory wound healing. Feet were preserved in 33.3%. In patients with >10 sessions of HBOT, 16 patients with 18 feet achieved good wound healing. Of these patients, 78.3% preserved their feet. This group of patients received an average of 23 HBOT treatments, at 2.5 atmospheres absolute (ATA) for a duration of 120 minutes with an intermittent schedule of 25 min of 100% oxygen breathing and 5 min of air breathing, daily, 5 days per week.

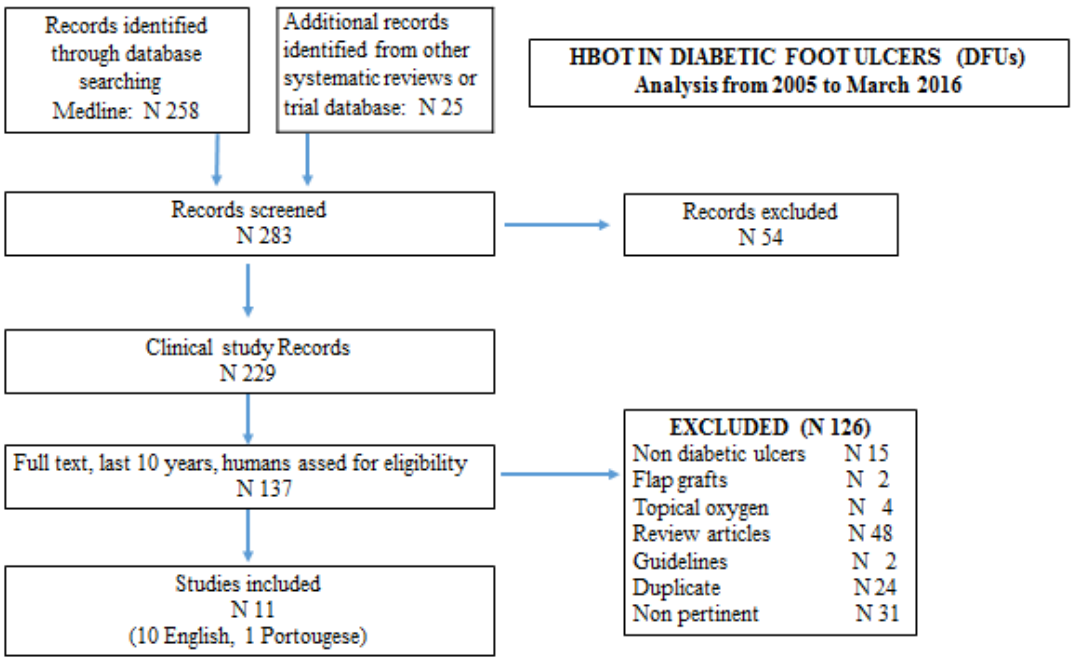
Table 10 Comparison of pathogens in diabetic foot ulcer wounds in patients with successful and failed hyperbaric oxygen therapy. (Chin-En Chen et al. © 2009 European Foot and Ankle Society. Published by Elsevier Ltd. All rights reserved)

Bacterial cultures	Successful HBOT (25 diabetic feet)	Failed HBOT (19 diabetic feet)
Aerobic		
Enterococcus species	6	3
Pseudomonas aeruginosa	6	5
Escherichia coli	4	2
Staphylococcus aureus	9	1
Klebsiella pneumonia	3	
Staphylococcus epidermidis	2	3
Acinetobacter baumannii	1	2
β-*Streptococcus, group B*	2	
Corynebacterium species	2	
Citrobacter freundii	2	1
Serratia marcescens	1	1
Morganella morganii	1	1
Proteus mirabilis		2
Proteus vulgaris	1	1
Streptococcus species		1
Citrobacter brakii	1	
Enterobacter cloacae	1	
Anaerobic		
Bacteroid fragilis	4	
Prevotella bivia		1
Peptostreptococcus	1	
Clostridium species	1	
Fungus		
Yeast-like	3	1

EVIDENCE – BASED REVIEW OF HBOT USE

In the present review the literature has been assessed from the 7th European Consensus Conference on Hyperbaric Medicine of Lille (France), 2004 to March 2016. The MEDLINE was searched with the query ("Wound Healing"[Mesh] OR "Skin Ulcer"[Mesh]) NOT "Skin Transplantation" [Mesh]) AND "Hyperbaric Oxygenation" [Mesh] AND ("2004/01/01" [PDAT]: "3000/12/31" [PDAT]). The output included 258 records, which have been extended by 25 records not included in the Medline and found in the reference list of the above mentioned papers (total 283 records included). A first selection was made applying exclusion criteria as [systematic reviews] and [medical genetics]. In the remaining 229 records a second selection was made applying the inclusion criteria as [humans], [full text] and [last 10 years]. From the 137 remaining records we excluded 126 papers as they presented studies about not diabetic foot (N 15), skin flap/grafts (N 2), topical oxygen (N 4). Furthermore, we excluded the duplicate papers (N 24), reviews articles (N 48), guidelines (2) and papers non-pertinent concerning HBOT in chronic ulcers (N 31). For analysis, 11 papers reporting 11 studies have been included in this report, enlisted in the Table 12: 8 randomized trials, 1 retrospective controlled study, 1 observational cohort study (for a total of 6995 participants) and 1 decision model. The list includes papers in English (N 10) and in Portuguese (N 1). Figure 5.

Figure 5: Literature analysis for Hyperbaric Oxygen Therapy in Diabetic Foot Ulcer from 2005 and March 2016



Fedorko et al.(2016)[6] study found that HBOT does not offer an additional advantage to comprehensive wound care in reducing the indication for amputation or facilitating wound healing in patients with chronic DFUs. Patients with diabetes and foot lesions (Wagner grade 2–4) of at least 4 weeks' duration participated in this study. One hundred fifty-seven patients were assessed for eligibility, with 107 randomly assigned and 103 available for end point adjudication. In addition to comprehensive wound care, participants were randomly assigned to receive HBOT (244 kPa or 2,4 ATA, 90 min, daily, 30 sessions) or sham (breathing air at 125 kPa or 1,2 ATA). Patients, physicians and researchers were blinded to group assignment. At 12 weeks post-randomization, the primary outcome was freedom from meeting the criteria for amputation as assessed by a vascular surgeon. Secondary outcomes were measures of wound healing. Criteria for major amputation were met in 13 of 54 patients in the sham group and 11 of 49 in the HBOT group (odds ratio 0.91 [95% CI 0.37-2.28], P = 0.846). Twelve (22%) patients in the sham group and 10 (20%) in the HBOT group were healed (0R 0.90 [95% CI 0.35-2.31], P = 0.823). All other indices of wound healing were also not statistically significantly different between groups.

These results should be interpreted with caution because, despite the randomized trial, the authors state that the two groups are not completely comparable because of some variables (years of diabetes, type of diabetes and gender), but they do not provide comparing tests against the baseline between the two groups.

Besides, they do not describe in details who performed the random allocation sequence, who enrolled the patients and who determined the patients' assignment to each intervention group.

Even though they assume a superiority of HBOT in the research protocol, when defining the sample size they refer to a two-tailed equality test. Regardless of the inappropriateness of this method, they enrolled a higher number of patients. In addition, the statistical methodology does not provide the p-value threshold.

In the protocol they state that the end of the follow-up period of 6 weeks, the patient (though not his/her medical documentation) was sent for evaluation to the vascular surgeon to determine the need for amputation. On the contrary, at page 394 of the study, Fedorko et al.[6]

state that the patient's clinical data and the digital images of wound progression were sent to the vascular surgeon, who then, case by case, would decide whether to visit the patient or not.

In Ma et al (2013)[48] a 2-week, prospective, randomized, controlled clinical study was conducted to assess the therapeutic effect and oxidative stress of HBOT in DFU. 36 patient were included. Average patient age was 60.08 ± 5.97 years and average Diabetes Mellitus (DM) duration was 16.4 ± 11.3 years; 86.1% had type 2 DM and 47.2% had Wagner grade-III foot ulcers. There was a statistically significant reduction in ulcer size in the HBOT group (that received standard care and HBOT at 2.5 ATA, for 90 minutes, twice-daily, 5 days a week for 2 weeks) versus the control group that received standard care including offloading, wound debridement, and glucose control (42.4% ± 20.0% versus 18.1% ± 6.5%, P <0.05). Malondialdehyde (MDA) and antioxidant enzyme (superoxide dismutase [SOD] and catalase [CAT]) levels were all significantly higher in the HBOT group than in the control group on day 14 (P<0.05). The authors expressed concern regarding the long-term use of HBOT because they believe that the chronic oxidative stress could induce apoptosis or even necrosis, damaging cell protein, membrane lipid and DNA in the wound bed. In their opinion, this could be an important feature in the pathogenesis of chronic, nonhealing wounds and might temper the long-term treatment effectiveness of HBOT[49]. They concluded that prolonged and/or inappropriate HBO treatment should be avoided, until needed additional research has been conducted.
Subsequently to this study, Fosen et al. (2014)[50] have shown that oxidative stress is beneficial for tissue repair. Oxidants appear to be, on the contrary, among the most important signals to control the healing process and this may be another mechanism for the benefits of HBOT in hypoxic wounds. It has been gradually established that oxidative stress plays a positive role during angiogenesis. The main mechanism of oxidative stress-induced angiogenesis involves Hypoxia-Inducible Factor (HIF)/Vascular Endothelial Growth Factor (VEGF) signaling, recent studies have identified several pathways that are VEGF-independent[50-51]. Figure 6
In addition it was also shown that HBOT and Lipoid Acid supplementation downregulates the chronic inflammatory state, changing the protease/anti-protease levels within the wound microenvironment. There is a decrease in the MMP9 expression and MMP2 upregulation, together with increased levels of Platelet derived growth factor (PDGF-BB), that contribute significantly to the acceleration of the dermal wound repair process[52].

The Khandelwal et al (2013) RCT[53] study aims to compare the efficacy of antiseptic dressings, HBOT and recombinant human Platelet Derived Growth Factor (rhPDGF) for two reasons: i) to reduce the incidence of lower limb amputations in diabetic foot ulcer (DFU); ii) to limit the duration of stay in the hospital. The prospective randomized trial was conducted on 60 patients with stage III and IV diabetic foot ulcers (International Association of Enterostomal Therapy classification, Table 11) and patients were divided randomly in three different therapy groups - antiseptics, HBOT (2.5 ATA, 60 min per sitting, for a total of 30 sessions or till the ulcer healed. These sittings were distributed over a period of 10 weeks. Patients were given either daily or alternate day therapy depending on the availability of slot in the facility), rhPDGF, with 20 patients in each group. Patients were managed initially on inpatient and then on outpatient basis till the ulcer healed completely. Results among three groups were compared using unpaired T test and the level of significance was set at P<0.05 using ANOVA. P value (0.0348) was significant ehen the groups were compared using % of patients with complete wound contraction. Complete healing % of rhPDGF (80%) was significantly higher than HBO therapy (60%) which is again significantly higher than those of antiseptic dressings (40%). While p value healing time (0.6534) and ulcer size (0.0593) in the groups was not significant (the Authors believe that a greater size of the study group would have given a clearer picture).

Khandelwal et al concluded that DFU management requires multidisciplinary and aggressive approach. PDGF should be recommended for all grade III and IV diabetic foot ulcer at least 8 weeks old. HBOT is equally good an option but could have limitations and side effects.
Note: The fact that the sessions of HBOT were distributed over a period of 10 weeks and the patients received either daily or alternate day HBOT, depending on the availability of slots at the facility, represents a bias that may adversely affect the result.

Figure 6: Schematic representation of ROS generation and its effect on angiogenesis[51]. Two main mechanisms are shown: ROS effect on known components of HIF-VEGF/VEGFR2 signalling pathway and VEGF-independent mechanism involving generation of lipid oxidation products. Abbreviations: NADH = Nicotinamide Adenine Dinucleotide Phosphate; MPO = myeloperoxidase; SOD = SuperOxide Dismutase; ROS = Reactive Oxygen Species; HIF = Hypoxia-Inducible Factor; VEGF = Vascular Endothelial Growth Factor; VEGFR2 = Vascular endothelial growth factor receptor 2; TLR2 = Toll-like receptor 2 (membrane protein); Rac1 = small (~21 kDa) signalling protein that appear to regulate cellular events, including the control of cell growth, cytoskeletal reorganization and the activation of protein kinases (proposed to be necessary for maintaining epidermal stem cells); NF-kB = nuclear factor kappa-light-chain-enhancer of activated B cells. (Kim YW, Byzova TV ©2014 by American Society of Hematology)

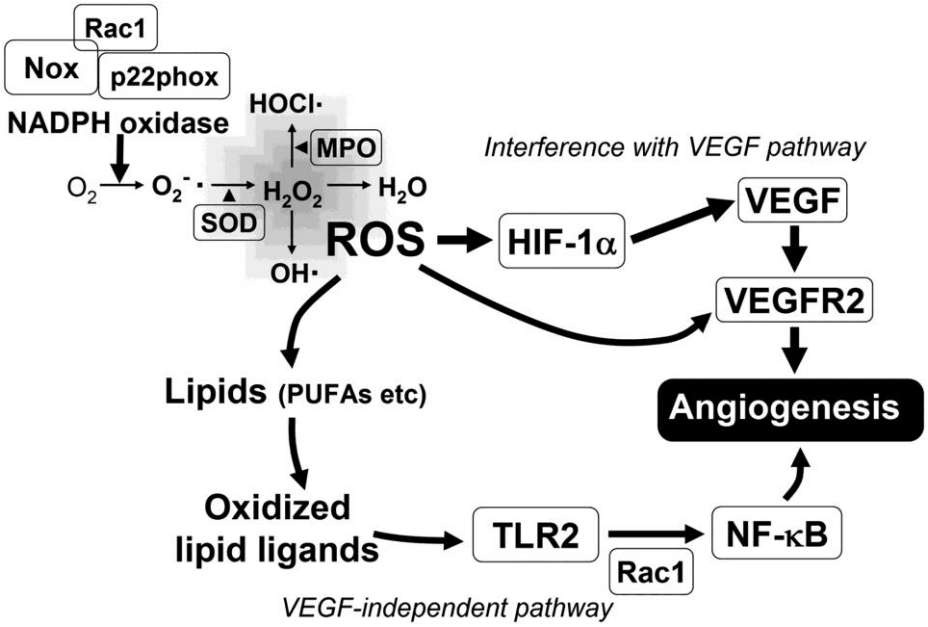


Table 11: International Association of Enterostomal Therapy classification (© Copyright S. Khandelwal et al., 2013 Licensee PAGEPress, Italy Clinics and Practice 2013; 3:e9 doi:10.4081/cp.2013.e9)

Stage I	Non-blanchable erythema of intact skin; the heralding lesion of skin ulceration.
Stage II	Partial thickness skin loss involving epidermis and/or dermis. Ulcer is superficial and presents clinically as an abrasion, blister, or shallow crater.
Stage III	Full thickness skin loss involving damage or necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia. The ulcer presents clinically as a deep crater with or without undermining of adjacent tissue.
Stage IV	Full thickness skin loss with extensive destruction, tissue necrosis or damage to muscle, bone, or supporting structures (*viz.* tendon or joint capsule).

The Margolis et al[4] (2013) longitudinal observational cohort study had the goal to compare the effectiveness of HBOT with other conventional therapies administered in a wound care network for the treatment of a DFU and prevention of lower-extremity amputation. 6,259 individuals with diabetes, adequate lower limb arterial perfusion and foot ulcer extending through the dermis were studied, representing 767,060 person-days of wound care. In the propensity score–adjusted models, individuals receiving HBOT [2.0 ATA (88.5% of treatments), 90-min sessions (99.5%), daily, 5 days per week (88%), for a median of 29 (25–75%; range of HBOT sessions15–48] were less likely to have healing of their foot ulcer (hazard ratio 0.68 [95% CI 0.63–0.73]) and more likely to have an amputation (2.37 [1.84–3.04]). Additional analyses, including the use of an instrumental variable, were conducted to assess the robustness of our results to unmeasured confounding. Margolis et al[4] found that HBOT not improves the likelihood that a wound might heal or to decrease the likelihood of amputation in any of these analyses. They concluded that the use of HBOT neither improved the likelihood that a wound would heal nor prevented amputation in a cohort of patients defined by Centres for Medicare and Medicaid Services (CMS) eligibility criteria.

According to Elraiyah et al[38], in Margolis et al[4] study there is insufficient exposure (lower pressure than the other studies); high loss to follow-up (57%); transcutaneous oxygen measurements (TCOM) or other vascular assessment were not used to select patients for HBOT and there are selection bias (higher Wagner scores in patients receiving HBOT). When the Margolis et al[4] study was added in the Elraiyah et al[38] sensitivity analysis, the beneficial effect of the HBOT on the DFUs healing rate becomes reversed (OR 2.88; 95% CI, 1.14-7.25) and on amputation becomes imprecise (OR, 0.58; 95% CI, 0.24-1.40). Therefore, according Elraiyah et al[48], the true effect of HBOT in DFU should be derived from robust RCTs because they only provide higher-quality evidence.

According Carter et al[54] the retrospective study published by Margolis et al[4] raises many questions. First, it appears that the excluded cohort had a lower extremity amputation (LEA) rate of 4.5% in the first 28 days. This seems high. Previous studies have shown overall amputation rates (major and minor) after 1 year of 12.5–22.6% in two smaller cohort studies of sicker patients[55-56]. Given that the excluded cohort was defined as having "adequate lower extremity arterial flow" (diagnostic method unknown), these results suggest that the excluded cohort was either at inherently higher risk for an LEA or that basic wound care was poorly conducted. Second, since the detailed selection criteria for HBOT at the former National Healing Corporation were not reported, it remains unclear if they were medically appropriate. Third, the method of analysis in Margolis et al[4] study has shortcomings. Although propensity scoring as a means of adjusting for the severity of wounds and patient comorbidities may be a viable approach, if conducted inappropriately it can lead to increased rather than decreased bias[57]. Many other confounders can affect outcomes directly, such as renal failure, smoking, chronic heart failure, level of tissue exposed, offloading, debridement, infection severity, management of infection, ambulation and immunosuppression (e.g., long-term steroid use/concurrent chemotherapy). Sensitivity analysis for the assumed distribution of an individual potential confounder, as reported in Margolis et al[4], is inadequate to account for the potential effects of such a long list of known confounders — and can make no allowance for any further confounding factors of which we are unaware. In summary, Carter et al[54] are not confident that the Margolis et al[4] retrospective cohort study of practice in a single wound-care organization sheds light on the difference between efficacy and effectiveness of HBOT in DFU patients as implemented in well-designed clinical practice guidelines. They agree with Margolis et al[4] that "it is entirely likely that HBO therapy enhances a specific aspect of wound repair and should not be used as a single agent to completely heal a wound."

Wang et al (2011)[58] study compared the effectiveness of extracorporeal shockwave therapy (ESWT) and HBOT in chronic DFUs. The ESWT group (39 patients/44 feet) received shockwave therapy twice per week for total six treatments. The HBOT group (38 patients/40 feet) was treated at 2.5 ATA, 90 min per treatment, daily, five times a week, for a total of 20 sessions. Evaluations included clinical assessment, blood flow perfusion scan and histopathological examination. The overall clinical results showed completely healed ulcers in 57% and 25% ($P = 0.003$); ≥ 50% improved ulcers in 32% and 15% ($P = 0.071$); unchanged ulcers in 11% and 60% ($P < 0.001$) and none worsened for the ESWT and the HBOT group respectively. The blood flow perfusion rates were comparable between the two groups before treatment ($P = 0.245$), however, significant differences were noted after treatment favouring the ESWT group ($P = 0.002$). Histopathological examination revealed considerable increases in cell proliferation and decreases in cell apoptosis in the ESWT group as compared to the HBOT group. Wang et al (2011)[58] concluded that ESWT is more effective than HBOT in chronic DFUs.

The aim of Löndahl et al. (2011)[59] randomised, double-blind, placebo-controlled HBOT in DFU study was to evaluate whether circulatory variables could help in predicting beneficial outcome of HBOT. All DFU study participants who completed therapy, predefined as receiving at least 36 out of 40 scheduled HBOT/placebo sessions (treatment schedule is not indicated in the study), were included in this study ($n = 75$). Baseline transcutaneous oximetry ($TcPO_2$), toe blood pressure (TBP) and ankle-brachial index (ABI) were measured. Ulcer healing rate was registered at the 9-month follow-up visit. An ulcer was considered healed when it was completely epithelialized and remained so at the 12-month follow-up. The authors found that in the HBOT group $TcPO_2$ were significantly lower for patients whose ulcer did not heal as compared with those whose ulcers healed. A significantly increased healing frequency was seen with increasing $TcPO_2$ levels in the HBOT group ($TcPO_2$/healing rate: <25 mmHg/0%; 26-50 mmHg/50%; 51-75 mmHg/73% and >75 mmHg/100%). No statistically significant relation between the level of TBP or ABI and healing frequency was seen. These results indicate that $TcPO_2$ in contrast to ABI and TBP correlates to ulcer healing following HBOT. Löndahl et al. suggest HBOT as a feasible adjunctive treatment modality in diabetic patients with chronic non-healing foot ulcers when basal $TcPO_2$ at the dorsum of the foot is above 25 mmHg.

In another prospective randomized placebo-controlled double-blinded study study, Löndahl et al. (2011)[60], found that HBOT improves long-term health related quality of life (evaluated using SF-36) in patients with chronic DFUs, possibly attributable to better ulcer healing. A total of 75 patients were included in the study; 38 were randomized to HBOT and 37 to placebo (hyperbaric air). The overall mean physical and mental summary scores for the entire study population at baseline were 29.6 ± 8.8 and 47.5 ± 12.4, respectively. There was a significant difference between baseline and 1 year follow-up responses to the mental summary score and two of the eight (SF-36) domains in the HBOT group, whereas no significant improvement of health related quality of life was seen in the placebo group. Comparing quality of life in patients who healed their ulcer (healers) with those who did not (non-healers), post-treatment levels of the mental health summary score, social functioning and role limitations due to physical and emotional health were significantly improved in healers. No differences were seen in any SF-36 domain in non-healers.

Löndahl et al. (2010)[37] in a well-designed randomized, single-center, double-blinded, placebo-controlled clinical trial showed that adjunctive treatment with HBOT facilitates healing in patients with DFUs. Ninety-four patients with Wagner grade II, III, or IV ulcers, which had

been present for greater than 3 months, were studied in an ambulatory setting. Study treatment was given as an adjunct to regular treatment at the multidisciplinary diabetes foot clinic, which included treatment of infection, revascularization, debridement, off-loading, and metabolic control according to high international standards. All patients were assessed by a vascular surgeon at the time of inclusion and only patients with adequate distal perfusion or non reconstructible peripheral vascular disease were included in the study. Patients having an acute foot infection were included when the acute phase was resolved. Oral or local antibiotic treatment did not exclude patients from study. Both groups were treated in a multiplace hyperbaric chamber. The HBOT group breathing 100% oxygen at 2.5 ATA, for 85 minutes, daily, 5 days a week for 8 weeks (40 treatment sessions). The control group received air in a blinded fashion. The outcomes for the HBOT group were compared with those of the hyperbaric air (control) group. In the intention-to-treat analysis, complete healing of the index ulcer was achieved in 37 patients at 1 year of follow-up: 25/48 (52%) in the HBOT compared to 12/42 (29%) who received hyperbaric air (P = .03)

Duzgun et al (2008)[61] undertook a prospective, randomized investigation of the use of HBOT versus standard therapy for the treatment of DFUs. A number of demographic variables were analysed in regard to wound healing. It was found that DFUs in patients in the HBOT group were more likely to heal and were more likely to undergo amputation distal to the metatarsophalangeal joint compared with those patients receiving standard therapy without HBOT. The authors believe that HBOT should be considered a useful adjunct in the management of DFUs

Chuck et al (2008)[22], as part of a Canadian assessment, estimated the cost-effectiveness and budget impact of HBOT in DFUs. A decision model was developed comparing adjunctive HBOT with standard care alone. The population was a 65-year-old cohort with DFU. The time horizon was 12 years taken from a Ministry of Health perspective. The health states were a healed wound with or without a minor lower extremity amputations (LEA), an unhealed wound with no related surgery and a major LEA. Efficacy data were based on outcomes reported in studies included in a literature review. Cost and capacity needs for treating DFU patients in Canada were estimated using prevalence data from the literature and cost and utilization data from government records. The 12-year cost for patients receiving HBOT was Canadian dollars/CND 40,695 (= 27,217 Euros*) compared with CND 49,786 (= 33,297 Euros*) for standard care alone. Outcomes were 3.64 quality-adjusted life-years (QALYs) for those receiving HBOT and 3.01 QALYs for controls. Estimated cost to treat all prevalent DFU cases in Canada was CND 14.4-19.7 million (= 9,6-13 million Euros*)/year over 4 years. If seven-person HBOT chambers were used, a further nineteen to thirty-five machines (multiplace hyperbaric systems) would be required nationally. The Authors found that adjunctive HBOT for DFUs is cost-effective compared with standard care. Additional HBOT capacity would be needed if it were to be adopted as the standard of care throughout Canada. (*Note: at the exchange rate CND/Euros of the April 6, 2016)

The Albuquerque and Sousa (2005)[62] retrospective controlled study provides evidence that HBOT increased significantly (13 times more) the mean healing rate of chronic DFUs, over a mean follow-up period of 45 months. It also provides evidence that this adjunctive therapy decreased significantly (2 times less) the need for amputation in these patients, over the same period of time. The need for major amputation was also less (about two times) in the HBOT group, but statistically no significant. The mean time required for amputation was higher in the HBOT group, but statistically no significant. From 1990 to 2003, 96 patients demonstrating chronic Wagner grades II-IV DFUs, with no improvement over a 6 month

average period (range 1-48 m) of full standard treatment, were studied. 55 patients received HBOT as study group (2.5 ATA, 90 minutes, daily, 5 days a week, for an average number of 54 sessions per patient (range 20-151)). 41 patients refused HBOT or left the Hyperbaric Medical Centre after first consultation. So, they did not receive HBOT (control group). HBOT and control patients did not differ in their baseline characteristics (i.e. age, sex, type and duration of diabetes, type and duration of lower limb ulcers). 78% of the control group were followed over a mean period of 55 months; 61.8% of the HBOT group patients were evaluated over a mean period of 45 months. The patients were assessed for wound healing and need for amputation. The mean healing rate of chronic ulcers was significantly higher in the HBOT group. The need for amputation was significantly less in the HBOT group. The mean rate of major amputation was less (about one half) in the HBOT group, but it was statistically no significant. The need for minor amputation was less (about two thirds) in the HBOT group, but it was statistically no significant. The authors suggest that the long-standing beneficial effects of HBOT may be explained by the sustained improvement of fibroblast collagen production and of the microvascular supply inside the leg ulcers, enhanced by adjunctive HBOT.

Table 12 Literature analysis for Hyperbaric Oxygen Therapy in Diabetic Foot Ulcer between 2005 and 2016 (assessed in March 2016)

HYPERBARIC OXYGEN THERAPY (HBOT) IN DIABETIC FOOT ULCERS (DFUs)
Updated from 2004 to March 2016

Study (authors year)	Type	Nb patients	Aim(s) / Evaluation criteria	Inclusion / Exclusion criteria	HBOT protocol (pressure, time, nb of session)	Results	Conclusion / comment
Fedorko L[6] 2016	Prospective, Double-Blind, Randomized Controlled Clinical Trial	107 (51 HBOT vs 56 control)	At 12 weeks postrandomization: Primary outcome: freedom from meeting the criteria for amputation surgeon. Secondary outcomes: wound healing	Patients with diabetes and foot lesions (Wagner grade 2–4) of at least 4 weeks	HBOT: 244 kPa/2,4 ATA, 90 min, daily, 30 sessions. SHAM: breathing air at 125 kPa / 1,23 ATA	Criteria for major amputation were met in 13 of 54 patients in the sham group and 11 of 49 in the HBOT group (odds ratio 0.91 [95% CI 0.37, 2.28], P = 0.846). Twelve (22%) patients in the sham group and 10 (20%) in the HBOT group were healed (0.90 [0.35, 2.31], P = 0.823). A	Not favours HBOT
Ma L[48] 2013	prospective, randomized, controlled study	36 (18pts HBOT vs 18 pts without HBOT)	Ulcer reduction and oxidative stress of HBOT	Wagner grade-III	2.5 ATA, 90 minutes, twice-daily, 5 days a week, for 2 weeks (20 sessions).	Ulcer size reduction in the HBOT group was greater than that of the control group (42.4% ± 20.0% versus 18.1% ± 6.5%, P <0.05). Levels of malondialdehyde (MDA) and antioxidant enzyme (superoxide dismutase [SOD], catalase [CAT], and glutathione peroxidase [GPx]) higher in the HBOT than in the control group on day 14 (P<0.05)	Favours HBOT for 2 weeks. Not favours for prolonged and/or inappropriate HBOT Note (by Longobardi): a controlled oxidative stress is beneficial for wound healing[81-82]
Khandelwal[53] 2013	Randomised controlled trial.	60 patients in total randomised in Group 1 (antiseptics dressing): 20; Group 2 (HBOT): 20 ; Group 3 (Platelet-Derived Growth Factor Therapy): 20	Healed at final follow-up (10 weeks) Time to complete healing Mean wound size	diabetic foot ulcer of at least 8weeks duration, patients with only stage III and IV diabetic foot ulcer, absence of vascular insufficiency involving large and medium sized arteries proximal to the ulcer demonstrated by Doppler study, age ≥18 years with type 1 or 2 diabetes	2.5 ATA, 60 min per sitting, for a total of 30 sessions or till the ulcer healed. These sittings were distributed over a period of 10 weeks. Patients were given either daily or alternate day therapy depending on the availability of slot in the facility	Proportion of ulcers healed at week 6: 75.1 % 1.40 [0.67, 2.91]	Favours HBOT
Margolis DJ[4] 2013	longitudinal observational cohort study.	6259 (793 HBO vs 5466 without HBO)	wound healing amputation	diabetes, adequate lower limb arterial perfusion, and foot ulcer Wagner grade >= 3, failure to heal during	2.0 ATA (88.5% of treatments), 90-min sessions (99.5%), daily, 5 days per week (88%), for a median of 29 sessions	individuals receiving HBO were less likely to have healing of their foot ulcer (hazard ratio 0.68 [95% CI 0.63–0.73]) and more likely to have an amputation (2.37 [1.84–3.04]	Not favours HBOT

				the first 4 weeks	(25–75%; range of HBOT sessions15–48)		
Wang CJ[58] 2011	controlled study	93 (46 extracorporeal shockwave therapy group vs 47 HBOT group)	Ulcer healing blood flow perfusion scan and histopathological examination.		HBOT group: 2.5 ATA, 90 min per treatment, daily, five times a week, for a total of 20 sessions SHOCKWAVE THERAPY: twice per week for total 6 treatments.	completely healed ulcers in 57% and 25% (P = 0.003); ≥ 50% improved ulcers in 32% and 15% (P = 0.071); unchanged ulcers in 11% and 60% (P < 0.001) none worsened for ESWT and HBOT group respectively. The blood flow perfusion rates, histopathological examination comparable between the two groups	ESWT is more effective than HBOT
Löndahl M[59] 2011	Prospective randomized placebo-controlled double-blinded study	75 patients; 38 were randomized to hyperbaric oxygen therapy and 37 to placebo (hyperbaric air).	Ulcer healing relation with increasing TcPO2	Patients with foot diabetic ulcer (Wagner grade 2, 3 and 4)	at least 36 out of 40 scheduled HBOT/placebo sessions (5 days a week for 8 weeks) (HBOT/hyperbaric air treatment schedule is not indicated in the study)	increased healing frequency was seen with increasing TcPO2 levels in the HBOT group (TcPO2/healing rate: 75 mmHg/ 100%).	Favours HBOT
Löndahl M[60] 2011	Prospective randomized placebo-controlled double-blinded study	75 patients; 38 were randomized to hyperbaric oxygen therapy and 37 to placebo (hyperbaric air).	quality of life in patients, ulcer healing	Patients with foot diabetic ulcer (Wagner grade 2, 3 and 4)	at least 36 out of 40 scheduled HBOT/placebo sessions (5 days a week for 8 weeks) (HBOT/hyperbaric air treatment schedule is not indicated in the study)	There was a significant difference between baseline and 1 year follow-up responses to the mental summary score and two of the eight (SF-36) domains in the -hyperbaric oxygen group, whereas no significant improvement of health related quality of life was seen in the placebo group.	Favours HBOT
Löndahl M[37] 2010	The randomised, double-blind, placebo-controlled	94 (Group sham 45 /randomised Group HBOT	Complete healing of the index ulcer Case-fatality rate 1 year Major and minor amputation rate	adults with diabetes and a foot ulcer (below the ankle) for at least 3 months. Wound clinic treatment for at least 2 months and revascularisation not possible or not indicated on vascular assessment	2.5 ATA, for 85 minutes, daily, 5 days a week over 8 to 10 weeks (40 treatment sessions)	Proportion of ulcers healed: a) at week 6: 6.3%, 6.44 [0.34, 121.33] b) at 6 months 82.4%, 1.53 [0.74, 3.15] c) at 1 year 39.5%, 1.91 [1.10, 3.34] d) Major amputation 17.0%, 2.76 [0.30, 25.54] e) Minor amputation 28.4%, 0.92 [0.24, 3.46]	a) Favours HBOT b) Favours HBOT c) Not Favours HBOT d) not significant e) High
Duzgun[61] 2008	Randomised controlled trial	100 adult (50 HBO vs 50 control)	Ulcer healing Major amputation Minor amputation	adults with diabetes requiring admission to hospital with "infected	2.0 ATA, for 90 minutes, twice one day and once the	Proportion of ulcers healed at a) 1 year 30.2%, 67.00 [4.22, 1064.23] b) Major amputation 12.7%, 0.03 [0.00,	a) Favours HBOT

				foot ulceration" for at least 4 weeks and who had received "appropriate local and systemic wound care"	next day, alternating for 20 to 30 days.	0.46] c) Minor amputation 32.1%, 0.17 [0.06, 0.45]	b) Favours HBOT c) Long course of HBOT
Chuck AW[22] 2008	Decision model	adjunctive HBOT versus standard care alone	cost-effectiveness	65-year-old cohort with DFU. The time horizon was 12 years	Not clear	The 12-year cost for patients receiving HBOT was CND 40,695 (=27,217 Euros) compared with CND 49,786 (=33,297 Euros) for standard care alone	Favour HBOT
Albuquerque and Sousa[62] 2005	retrospective controlled study	96 (55 HBOT / 41 control group)	wound healing and need for amputation	Wagner grades II-IV lower limb ulcers, with no improvement over a 6 months	2.5 ATA, 90 mins, daily, 5 days a week, for an average number of 54 sessions per patient (range 20-151)	a) The mean healing rate of chronic ulcers was significantly higher in the HBOT group (13 times more). b) The need for amputation decreased significantly (2 times less) c) The need for major amputation was also less (about two times) in the HBOT group, but statistically no significant. d) The mean time required for amputation was higher in the HBOT group, but statistically no significant	a) Favours HBOT b) Favour HBOT c) Favours but not significant d) Favours but not significant

The above described studies (Table 12) have been used in several reviews of the literature on HBOT in DFUs with results often different. In these report a critical evaluation of the most recent reviews of the literature on HBOT in DFUs is included. Table 13

Table 13 Comparison of some reviews, published between 2013 and 2016, on Hyperbaric Oxygen Therapy (HBOT) in Diabetic Foot Ulcers (DFUs). Abbreviations: RCT= Randomised Clinical Trials; IWGDF = International Working Group of the Diabetic Foot; UHMS = Undersea and Hyperbaric Medical Society. (Longobardi P, 2016)

Review	**Favourable to HBOT (level of evidence)**	**RCT number / (range of years of publication)**	**Observational, Prospective and nonrandomized number / (range of years of publication)**	**Number of Case series and case control**
IWGDF / Game et al. (2016)	NO (low)	1 (Margolis, 2013)[4]	-	-
Cychosz (2016)	NO (low)	2 (Löndahl, 2010)[37] (Ma, 2013)[48]		
Society for Vascular Surgery / Hingorani et al. (2016)	YES (moderate)	Multidisciplinary committee used the Grades of Recommendation Assessment, Development, and Evaluation (GRADE) system to rate the quality of evidence (confidence in the estimates) and to grade the strength of recommendations		
Elraiyah et al (2016)	YES (moderate)	7 (1987-2005)	5 (1992-2010)	
UHMS / Huang et al. (2015)	YES (moderate)	5 (1992-2010)	5 (1990-2013)	-
Cochrane / Kranke et al (2015)	YES (moderate)	10 (1992-2013) 531 patients	-	-
Liu et al (2013)	YES (low-moderate)	7 (1966-2012)	4 (1987-2010)	2

In 2016, the International Working Group of the Diabetic Foot (IWGDF) working group on wound healing published an updated systematic review of the evidence to inform protocols for routine care and to highlight areas, which should be considered for further study[63]. The review was by considering papers on the interventions to improve the healing of chronic ulcers published between June 2010 and June 2014. Methodological quality of selected studies was independently assessed by two reviewers using Scottish Intercollegiate Guidelines Network criteria[64]. Selected studies fell into ten categories *(sharp debridement and wound bed preparation with larvae or hydrotherapy; wound bed preparation using antiseptics, applications and dressing products; resection of the chronic wound; oxygen and other gases; compression or negative pressure therapy; products designed to correct aspects of wound biochemistry and cell biology associated with impaired wound healing; application of cells, including platelets and stem cells; bioengineered skin and skin grafts; electrical, electromagnetic, lasers, shockwaves and ultrasound and other systemic therapies, which did not fit in the aforementioned categories)*. Heterogeneity of studies prevented pooled analysis of

results. Of the 2161 papers identified, 30 were selected for grading following full text review. Based on limited information, the IWGDF working group on wound healing stated that the question of which DFU patient group would most benefit from HBOT remains unanswered. According to the working group the previous positive randomized studies of the HBOT on DFU (IWGDF had considered four 4 RCT published between 1996 and 2003) would be made weaker by the review of Margolis et al (2013)[4] that, in fact, has many bias (see the comments reported earlier in this report).

Cychosz et al (2016)[70] review had the aim to summarize the current concepts on DFU and to provide evidence-based recommendations on preventive and therapeutic management of DFUs, based on analysis of recent literature (Table 14).
The Authors believe that further evidence is necessary to determine the efficacy, cost-effectiveness and the appropriate indications of the HBOT in DFUs patients (Grade I recommendation: insufficient evidence exists to make a recommendation). This opinion was based on the double blinded RCT of Löndahl et al (2010)[37] and Ma et al (2013)[48]. These two studies have already been analysed earlier in this report (see above in the text)

Table 14 Summary of Grades of Recommendation. (© Cychosz et al (2015)[70] Foot & Ankle International® 2016, Vol. 37(3) 334–343).

Achilles tendon lengthening	Grade A
Gastrocnemius recession	Grade B
Plantar fascia release	Grade I
Toe flexor tenotomy	Grade B
Correction of static forefoot deformity	Grade I
Footwear modification	Grade A
Total contact casting	Grade A
Debridement and specialized dressings	Grade I
Hyperbaric oxygen therapy	Grade I
Negative pressure wound therapy	Grade I
Advanced biological therapy	Grade A
Electrophysical therapy	Grade I

To improve the care of patients with diabetic foot and to provide an evidence-based multidisciplinary management approach, the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine developed a clinical practice guideline (Hingorani et al., 2016)[7]. The committee made specific practice recommendations using the Grades of Recommendation Assessment, Development, and Evaluation (GRADE) system. This was based on five systematic reviews of the literature. Specific areas of focus included (1) prevention of diabetic foot ulceration, (2) off-loading, (3) diagnosis of osteomyelitis, (4) wound care and (5) peripheral arterial disease. Although only limited high-quality evidence were identified for many of the critical questions, the best available evidence, the patients' values and preferences, the clinical context were considered to develop these guidelines. HBOT is recommended in patients with DFU who have adequate perfusion that fails to respond to 4 to 6 weeks of conservative management (Grade 2B). Furthermore, the Society for Vascular Surgery algorithm on DFU (Figure 2) indicates the following therapeutic strategy to manage osteomyelitis: bone debridement, biopsy an cultures, antibiotics, HBOT (the level of evidence is not reported)
The Society for Vascular Surgery commissioned a systematic review and meta-analysis (Elraiyah et al, 2016)[38]. This review[38] was conducted to evaluate the comparative effectiveness of different adjunctive therapies *(HBOT, arterial pump devices and pharmacologic agents:*

pentoxifylline, cilostazol and iloprost) for diabetic foot ulcerations. In nine randomized trials (published between 1996 and 2011), the addition of HBOT to conventional therapy (wound care and offloading) was associated with increased healing rate (Peto Odds Ratio 14.25; 95% confidence interval 7.08- 28.68 – Figure 7) and reduced major amputation rate (Peto Odds Ratio 0.30; 95% confidence interval 0.10-0.89 – Figure 8), compared with conventional therapy alone. In one small trial, arterial pump devices had a favourable effect on complete healing compared with HBOT and in another small trial compared with placebo devices. Neither iloprost nor pentoxifylline had a significant effect on amputation rate compared with conventional therapy. No comparative studies were identified for cilostazol in diabetic foot ulcers. The review concluded that there is low to moderate quality evidence supporting the use of HBOT as an adjunctive therapy to enhance DFU healing and potentially prevent amputation. Moreover, there are only sparse data regarding the efficacy of arterial pump devices and pharmacologic interventions[38]

The results of Elraiyah et al[38] are consistent with other systematic reviews[62,65,66]. Elraiyah et al[38] suggested that the effect of HBOT on amputation was imprecise in some reviews[62,65,66] when estimated using a Relative Rik (RR) measure, whereas using Peto odds ratio (OR) method showed more precise estimates. The sensitivity of conclusions to the choice of the measure of effect used is a sign of imprecision that can lower confidence warranted by this evidence. Although conventional therapy included comprehensive wound care (debridement, wound dressing and offloading), the way this care was provided was clearly heterogeneous across studies. The benefit of HBOT in DFU, reported by Elraiyah et al[38], is consistent with another review[67] that evaluated its potential role in a variety of other types of chronic wounds. Considering the Elraiyah et al review[38], other than the cost and the burden of prolonged daily treatments, the Society for Vascular Surgery believes that HBOT is appropriate as long as the patients are selected for the therapy carefully[7]. Using transcutaneous oximetry values can help stratify patients and predict those who are most likely to benefit.[68,69]

Figure 7 Meta-analysis of healing rate (Elraiyah et al)[38]. The solid squares indicate the odds ratios and are proportional to the weights used in the meta-analysis. The diamond indicates the pooled odds ratio, and the lateral tips of the diamond indicate the associated 95% confidence interval (CI). The horizontal lines represent the 95% CIs. *Abbreviations: HBOT = Hyperbaric Oxygen Therapy; RCT = Randomized Controlled Trial*. (Copyright © 2016 by the Society for Vascular Surgery. Published by Elsevier Inc.)

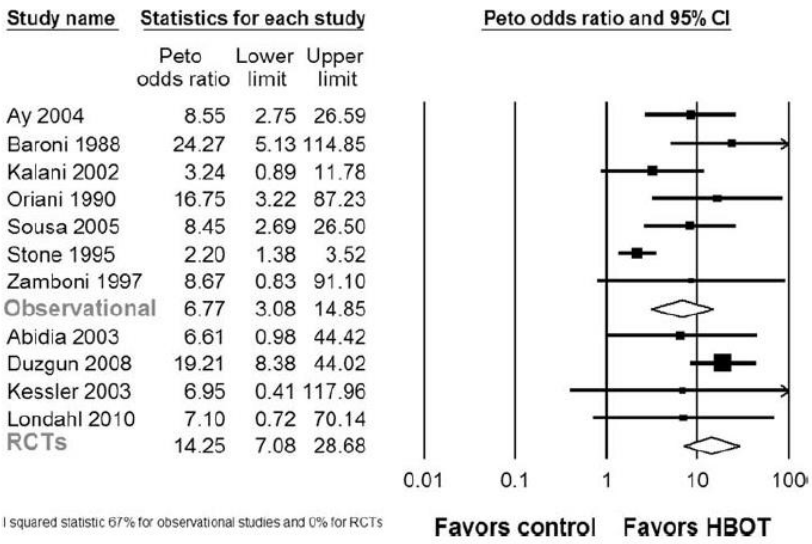
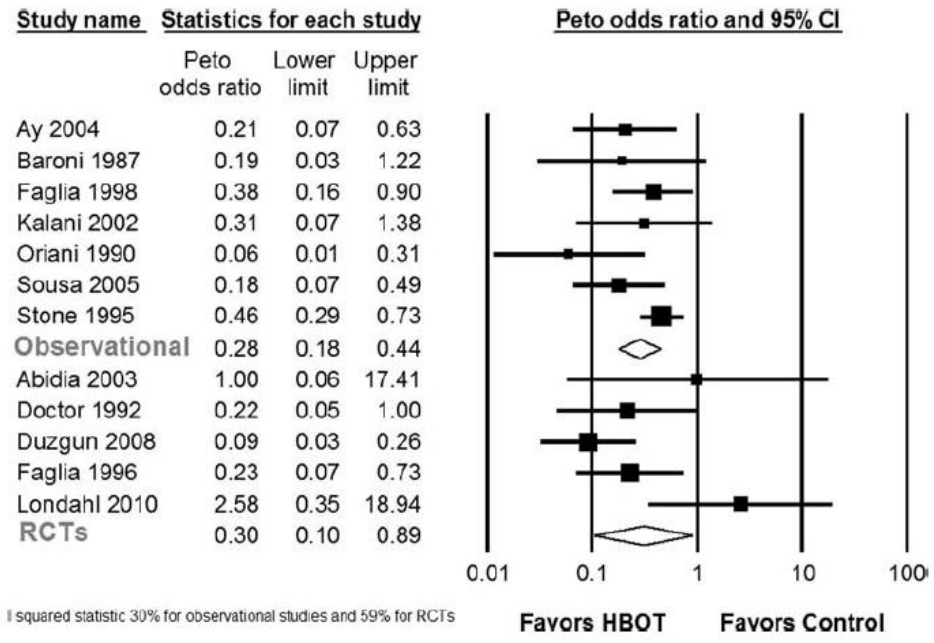


Figure 8 Meta-analysis of major amputation rate (Elraiyah et al)[38]. The solid squares indicate the odds ratios and are proportional to the weights used in the meta-analysis. The diamond indicates the pooled odds ratio, and the lateral tips of the diamond indicate the associated 95% confidence interval (CI). The horizontal lines represent the 95% CIs. Abbreviations: HBOT = Hyperbaric Oxygen Therapy; RCT = Randomized Controlled Trial. (Copyright © 2016 by the Society for Vascular Surgery. Published by Elsevier Inc.)

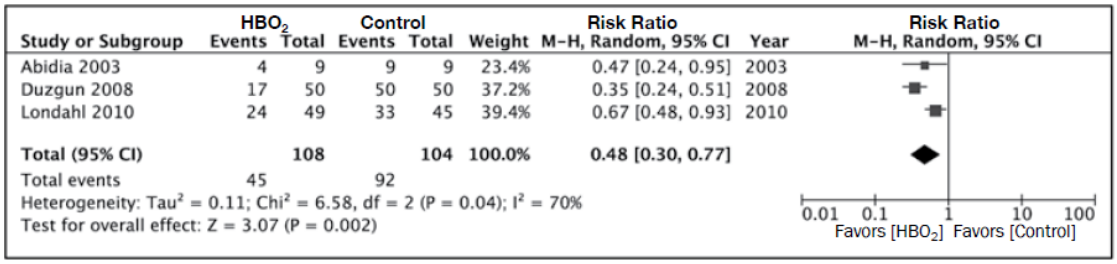


The Agency for Healthcare Research and Quality has published the Undersea and Hyperbaric Medical Society (UHMS) clinical practice guideline for the use of HBOT in the treatment of DFUs (Huang et al, 2015)[10]. Five RCTs (1992 and 2010) and five observational studies (published between 1990 and 2013) were included for this analysis. HBOT is considered beneficial (GRADE level evidence: moderate, conditional recommendation*) in promoting complete healing (Figure 9) and preventing major amputation (Figure 10) in patients with Wagner Grade 3 or greater DFUs that have shown no significant improvement after 30 or more days of treatment. In patients with Wagner Grade 3 or higher diabetic foot ulcers who have just had a surgical debridement of an infected foot *(e.g., partial toe or ray amputation; debridement of ulcer with underlying bursa, cicatrix or bone; foot amputation; incision and drainage [I&D] of deep space abscess; or necrotizing soft tissue infection),* adding acute post-operative HBOT to the standard of care is suggested to reduce the risk of incomplete healing and major amputation (GRADE level evidence: moderate, conditional recommendation*). In patients with Wagner Grade 2 or lower DFUs, there was very low-level evidence to justify the use of HBOT as an adjunctive treatment.

(*Note: this means that further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate of effect)

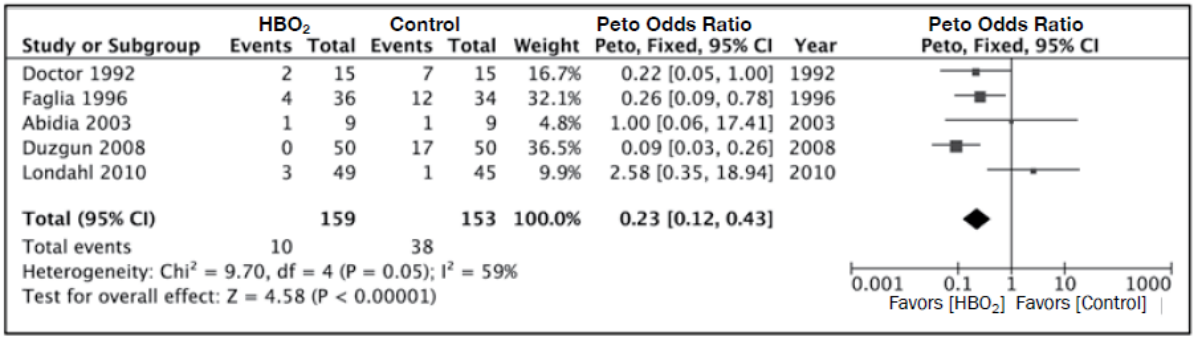
The UHMS algorithm for the use of HBOT in DFU is proposed in the Figure 11.

Figure 9 HBOT for the DFU incomplete healing. Forest plots using random effect Risk Ratio as well as Peto Odds Ratio were compared (Huang et al, 2015[10]. Copyright © 2015 Undersea & Hyperbaric Medical Society, Inc.)



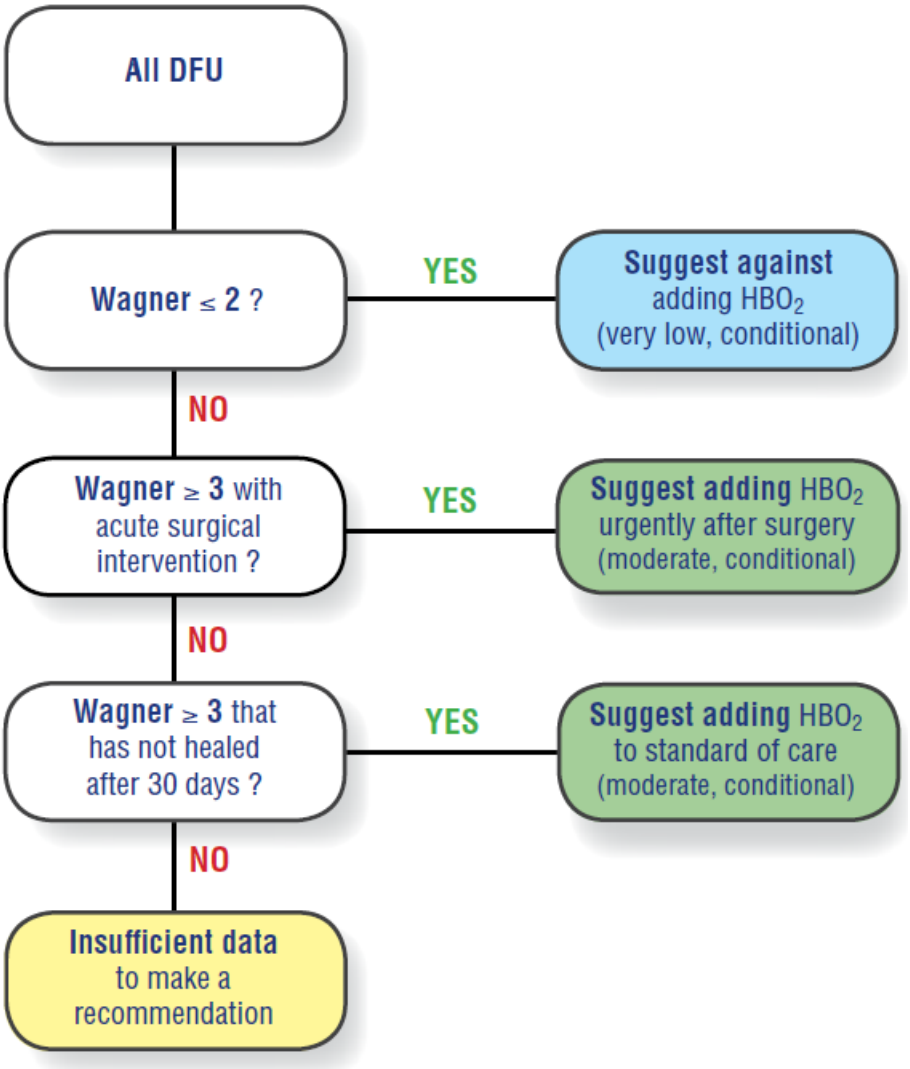
All DFU (Incomplete Healing)	Starting Score	4	Rationale
	Risk of Bias	-1	Rated down because of unclear methodology for allocation concealment in 2/3 studies, patients lost to follow-up in 1/3 studies, no blinding in 1/3 studies, and unclear intention to treat analysis in 1/3 studies.
	Inconsistency	-1	The data for Incomplete Healing shows some inconsistency as the I^2 was >50%, resulting in rating down 1 point
	Indirectness	0	No evidence of indirectness
	Imprecision	0	No evidence of imprecision
	Publication Bias	0	Could not be assessed with a small number of studies
	Large Magnitude of Effect	+1	Large magnitude of effect (direct evidence, relative risk [RR] = 2-5 or RR = 0.5-0.2 with no plausible confounders); Very large with RR > 5 or RR < 0.2 and no serious problems with risk of bias or precision (sufficiently narrow confidence intervals)
	Dose Response Relationship	0	No evidence of dose response relationship
	Confounders Strengthen Effect	0	No evidence of confounders strengthening confidence of the magnitude of effect
	Final Score	**3**	**Moderate Level of Evidence**

Figure 10 HBOT for the DFU major amputation. Forest plots using random effect Risk Ratio as well as Peto Odds Ratio were compared (Huang et al, 2015[10]. Copyright © 2015 Undersea & Hyperbaric Medical Society, Inc.)



All DFU (Major Amputation)	Starting Score	4	Rationale
	Risk of Bias	-1	Rated down because of unclear methodology for allocation concealment in 4/5 studies, patients lost to follow-up in 2/5 studies, unblinded study in 2/5 studies and unclear blinding in 1/5 studies, lack of intention to treat analysis in 1/5 studies and unclear intention to treat analysis in 2/5 studies.
	Inconsistency	-1	The point estimates from the studies varied, the confidence intervals do not overlap to some extent and the I^2 is >50%
	Indirectness	0	No evidence of indirectness
	Imprecision	0	No evidence of imprecision as Estimate of Effect had narrow Confidence Intervals. Peto analysis showed tighter confidence interval, but Random Effects Risk Ratio analysis was also deemed acceptable.
	Publication Bias	0	Could not be assessed with a small number of studies
	Large Magnitude of Effect	+1	Large magnitude of effect (direct evidence, relative risk [RR] = 2-5 or RR = 0.5-0.2 with no plausible confounders); Very large with RR > 5 or RR < 0.2 and no serious problems with risk of bias or precision (sufficiently narrow confidence intervals)
	Dose Response Relationship	0	No evidence of dose response relationship
	Confounders Strengthen Effect	0	No evidence of confounders strengthening confidence of the magnitude of effect
	Final Score	**3**	**Moderate Level of Evidence**

Figure 11 Algorithm for the use of HBOT (Huang et al, 2015[10]. Copyright © 2015 Undersea & Hyperbaric Medical Society, Inc.)



Kranke et al (2015) review[36] has included data from twelve trials (published between 1996 and 2013), ten of which recruited people with diabetic foot ulcers. In this update, the Authors presented a risk ratio (RR) of healing with hyperbaric oxygen therapy (HBOT), as opposed to a RR of failing to heal without HBOT (i.e. control) as presented in the original review[65]. This was undertaken in order to facilitate ease of interpretation of the healing outcomes for users of the update. The interpretation of the RR was that a summary estimate in which HBOT increased the occurrence of healing would have a RR > 1.00. (Table 15)

There was a statistically significant increase in the proportion of ulcers healed by six weeks following HBOT compared with control (P = 0.01; risk ratio (RR) 2.35, 95%confidence interval (CI) 1.19 to 4.62 ; I^{37} = 4%) (*Analysis 1.1 in the original study*). The pre-planned sensitivity analysis examining the effect of allocation of drop-outs suggested a benefit with HBOT in the best-case scenario but not the worst-case scenario (best-case RR 4.61, 95% CI 2.35 to 9.08; P <0.00001, worst-case RR 0.84, 95% CI 0.51 to 1.37, P = 0.48) (*Analysis 1.2; Analysis 1.3 in the original study*).

Table 15 Summary of findings for the main comparison. By Kranke et al[36] "Hyperbaric oxygen therapy for chronic wounds (Review)" Copyright © 2015 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

Hyperbaric Oxygen Therapy for chronic wounds

Patient or population: patients with chronic wounds
Settings: inpatients and outpatients in a hyperbaric facility
Intervention: Hyperbaric Oxygen Therapy

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Hyperbaric Oxygen Therapy				
Diabetic ulcers healed at 1 year. Follow-up: 1 years	Study population		RR 9.53 (0.44 to 207.76)	212 (3 studies)	⊕⊕⊕◯ moderate[1,2,3]	
	115 per 1000	1000 per 1000 (51 to 1000)				
	Low					
	0 per 1000	0 per 1000 (0 to 0)				
	High					
	0 per 1000	0 per 1000 (0 to 0)				
Diabetic ulcers - major amputations	Study population		RR 0.36 (0.11 to 1.18)	312 (5 studies)	⊕⊕⊕◯ moderate[2]	
	247 per 1000	89 per 1000 (27 to 284)				
	Low					
	0 per 1000	0 per 1000 (0 to 0)				
	High					
	0 per 1000	0 per 1000 (0 to 0)				

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Analysis comprises small studies, some with zero events in control arm
[2] small sample size
[3] very large effect: RR >5

This benefit seems not evident at longer-term follow-up. The pooled random-effects model showed no statistically significant difference between the groups (RR 9.53, 95% CI 0.44 to 207.76; P = 0.15) (Analysis 1.7). This change in the result is mainly due to the fact that there are a small number of trials with small sample sizes, two of which have no events in the control arm. Kranke et al[36] took statistical advice which indicated that this made the random-effects model for RR of healing unstable in these circumstances and repeated the analysis using a Peto odds ratio (OR) (OR 7.58, 95% CI 4.33 to 13.29; P <0.00001) (*Analysis 1.8 in the original study*). However, all these results must be approached with caution. The sensitivity analysis examining the effect of allocation of dropouts shows no statistically significant difference between the two groups in either best-case or worst-case scenario (*Analysis 1.9; Analysis 1.10 in the original study*). The trial by Duzgun (2008)[61] was judged to be at overall unclear risk of bias. This finding (RR of healing at 12 months) should be interpreted with caution given that the analyses included trials of varying sizes, some of which had no healing events in the control arm. As such, the pooled estimation may be unreliable.

Although Kranke et al.[36] found some indication amongst the included trials that HBOT may decrease the major amputation rate in people with diabetic foot lesions, there was no

statistically significant reduction in amputation rate with the application of HBOT (the RR of major amputation with HBOT was 0.36, 95% CI 0.11 to 1.18, P = 0.08, I^{37} = 50%) (*Analysis 1.11 in the original study*). This result was sensitive to the assumptions made about drop-outs (best-case RR of amputation 0.20, 95% CI 0.10 to 0.38, P < 0.00001, worst-case 0.62, 95% CI 0.13 to 2.98, P = 0.55) (*Analysis 1.12; Analysis 1.13 in the original study*). Subgroup analysis by number of treatments did not significantly affect this outcome, with a RR for amputation after 30 or more treatments of 0.40 (95% CI 0.07 to 2.23, P = 0.29). For < 30 treatments the RR was 0.29, 95% CI 0.07 to 1.16, P = 0.08 (Analysis 1.11). A post hoc subgroup analysis, according to the use of sham therapy compared with no sham, indicated a significant effect of treatment effect only amongst trials with no sham procedure as control (RR of amputation, HBOT compared with sham 0.47, 95% CI 0.09 to 2.44, P = 0.37; RR HBOT compared to control without sham 0.15, 95% CI 0.06 to 0.36, P < 0.0001) (*Analysis 1.14 in the original study*). Again, the study by Duzgun (2008)[61] were considered as high risk of performance bias as the control arm did not receive a sham treatment.

Some limitations that should be considered include the variability in the participant inclusion criteria across trials and the nature and timing of outcome assessments. In particular, there is a possibility of clinical heterogeneity due to differential wound size or severity across trials at participant enrolment. The trial by Löndahl (2010)[71], for example, excluded all participants at high risk of major amputation. Excluding this trial from the analysis resulted in a significant effect of HBOT on decreasing the risk of major amputation (P = 0.009).

Overall patient inclusion criteria were not standard across trials and were poorly reported in some trials. While subgroup analysis by treatment number suggests the benefit of HBOT on major amputation rate was significant with either the short course or long course (> 30 treatment course: RR 0.40, P = 0.29; < 30 treatment course: RR 0.29, P = 0.08,), this result should be interpreted with caution given the contribution of the trial by Löndahl (2010)[71] previously discussed. While all trials included in the meta-analysis compared HBOT with some form of 'standard' wound care, these comparator therapies were generally poorly described and could not form the basis for a meaningful subgroup analysis with the exception of the analysis of the use of a HBOT sham or no sham as comparator.

The analysis of the rate of major amputation was heterogeneous (I^{37} = 50%), suggesting a between-study variance that could not be explained by random variability. The risk of bias of the included trials was variable. The limited reporting of trial methodology in some reports (as Duzgun, 2008)[61] resulted in an unclear risk of bias associated with the effect estimates these trials contributed to the pooled analyses. There were likely to be clinical differences in the individuals recruited to the included trials. The trial by Löndahl (2010)[71] excluded participants where major amputation was likely, while the other trials included a wider range of severity. Subgroup analysis by the number of treatment sessions delivered did not assist in the interpretation of this heterogeneity. Furthermore, it is not clear if the surgical decision to amputate was made while blinded to treatment allocation. This is an important potential source of bias and thus a threat to validity.

With regard to long-term outcomes following HBOT, Kranke et al.[36] have located no relevant data. Only one trial reported a quality of life assessment (evaluated using SF-36) in a parallel publication (Löndahl, 2011)[60]. Comparing quality of life in patients who healed their ulcer (healers) with those who did not (non-healers), post-treatment levels of the mental health summary score, social functioning and role limitations due to physical and emotional health were significantly improved in healers.

Kranke et al.[36] conclude their review by stating that there is some evidence that the addition of HBOT to a standard wound care regimen in people with DFUs results in a significant improvement in wound healing by six weeks, but this benefit is not evident at longer-term follow-up at one year or longer. In terms of amputation, HBOT does not appear to

significantly improve the minor amputation rate in people with foot ulcers due to diabetes, while a potentially important effect on major amputation cannot be confirmed on this analysis. These findings are limited by trials recruiting small numbers of participants with diverse wound characteristics and by trial reporting and methodological discrepancies that present the potential to bias results. As such, these findings require a cautious interpretation. Further high quality RCTs were recommended by Kranke et al. to examine short and long-term risks and benefits.

Liu et al (2013)[72] summarized 7 RCTs showing that HBOT may increase healing of DFUs (relative risk 2.33; 95% CI 1.51-3.60 - Figure 12) and reduce major amputations when compared with therapy without HBOT (13.63% vs 30.07%; RR, 0.29; 95% CI, 0.13-0.71 - Figure 13). Confidence in this evidence is low to moderate, balanced between the inconsistency of results across studies and the small number of events and the very large (7-fold) effect found for healing rate (Peto Odds Ratio 7.57, 95% CI 4.35 to 13.19). The RR ratio revealed a significant effect in favour of adjunctive HBO therapy in patients with short-term follow-up (≤ 6 months). Although there was more heterogeneity in the subanalysis for patients with follow-up of more than 12 months, a tendency toward even larger positive effects of HBOT compared with those seen within 6 months was found (Table 16). Publication bias is a concern when all trials are small and positive.
The Liu et al[72] results are consistent with a report by Kalani et al[64] in which 76% of the patients treated with HBOT had healed ulcers after 3 years, compared with only 48% of patients treated without HBOT. Furthermore, Albuquerque and Sousa[62] reported that long HBOT (mean: 45 months) increased significantly (approximately 13-fold) the healing rate of chronic lower limb wounds in diabetic patients.
The Liu et al[72] results differed from those of a previous (2012) Cochrane analysis by Kranke et al.[65] maybe for the different characteristics and sample sizes. Only 7 RCTs were used in the meta-analysis by Kranke et al, [65] whereas 7 RCTs, 4 prospective trials and 2 case-control studies were represented in Liu et al. meta-analysis[72]. In the latter report, larger sample size likely decreased publication bias and strengthened the power of analysis. Furthermore, in the study by Kranke et al[65] only 2 trials assessed ulcer healing within 1 year. This causes problems with forest plots and assessing publication bias. More importantly, comparing healing rates of ulcers between HBOT and conventional therapy, there was significant heterogeneity in the study by Kranke et al[65] (I^2=50%).

Figure 12. Forest plots for meta-analyses of RCTs, by Liu and colleagues[72], comparing the healing rate of foot ulcer treated with or without HBOT. A) Subgroup analyses with short-term (6 months) or long-term (≥1 year) follow-ups. B) Subgroup analyses only including randomized controlled trials (RCTs). Ulcer healing was defined as complete epithelial regeneration. RR ¼ relative risk. (© 2013 Mayo Foundation for Medical Education and Research)

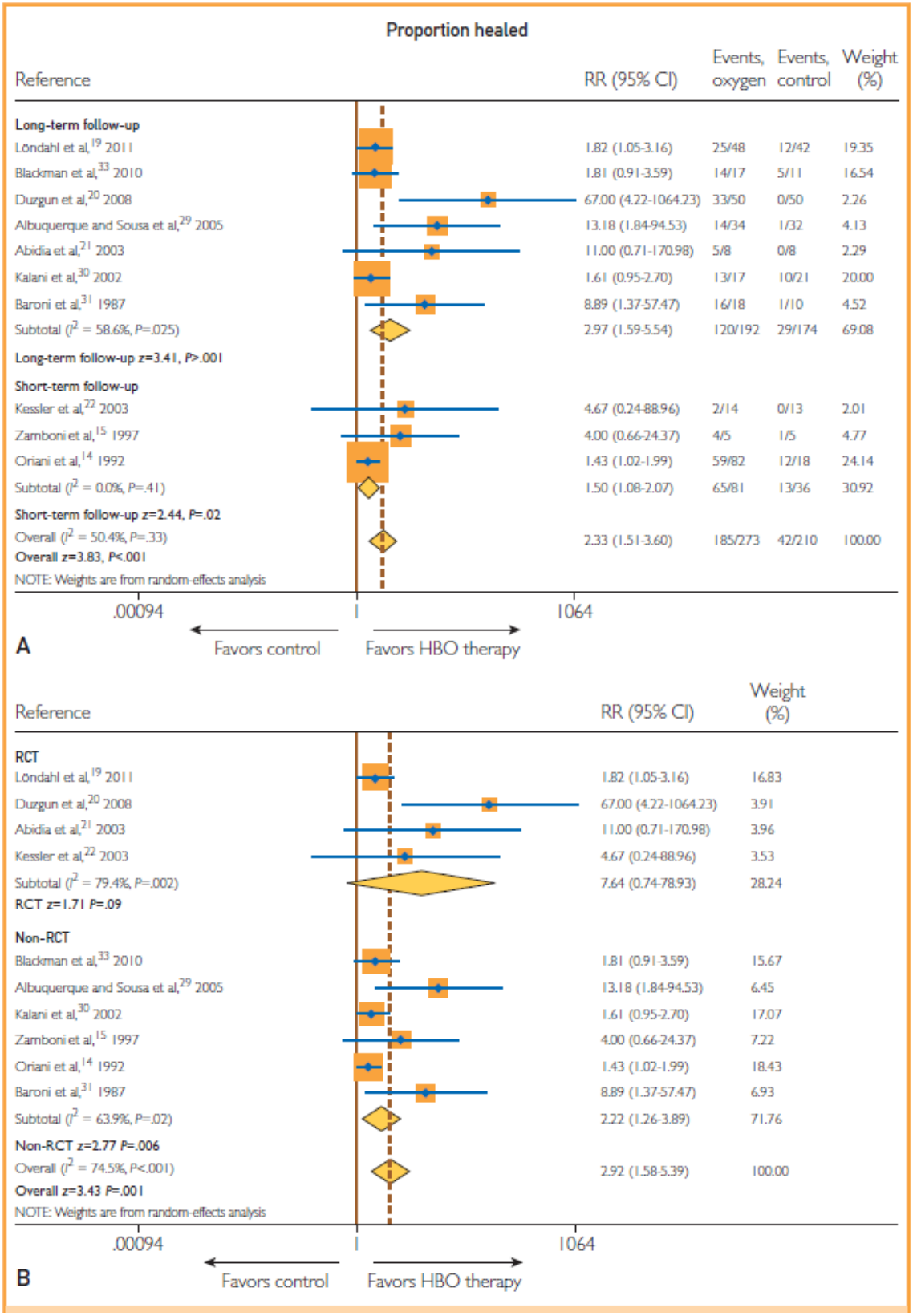


Figure 13 Forest plot for meta-analyses, by Liu and colleagues[72], comparing major amputations in diabetic foot ulcer treated with or without HBOT. Subgroup analysis including only randomized controlled trials (RCTs). Major amputation was defined as amputation above the ankle joint (AKA). RR ¼ relative risk. © 2013 Mayo Foundation for Medical Education and Research

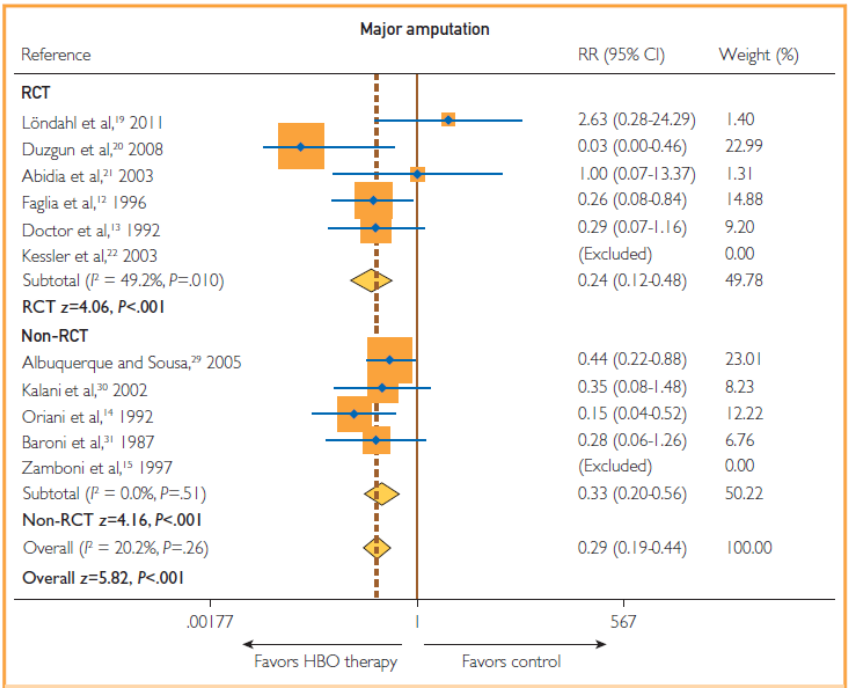


Table 16 Meta-analyses of RCTs by Liu and colleagues[72] show that adding HBOT to usual care reduced major amputations compared with usual care alone. Minor amputations and adverse events did not differ between groups. (© 2013 Mayo Foundation for Medical Education and Research)

Usual care plus hyperbaric oxygen therapy (UC + HBO) vs UC alone in patients with chronic diabetic foot ulcers*

Outcomes	Number of trials (*n*)	Weighted event rates		At 2 to 92 wk	
		UC + HBO	UC alone	RBI (95% CI)	NNT (CI)
Healed ulcers†	4 (233)	81%	11%	664% (–26 to 7793)	NS
				RRR (CI)	NNT (CI)
Major amputation‡	6 (331)	NR	NR	76% (52 to 88)	Not calculable
				RRI (CI)	NNH (CI)
Minor amputation§	4 (204)	28%	18%	55% (–3 to 147)	NS
Adverse events	4 (201)	12%	8.3%	41% (–34 to 198)	NS

**NR = not reported; NS = not significant; RBI = relative benefit increase; other abbreviations defined in Glossary. Weighted event rates, RBI, RRR, RRI, NNT, NNH, and CI calculated from control event rates and relative risks in article.*

†Complete epithelialization of the wound. Analysis based on a random-effects model since heterogeneity was 79%.

‡Amputation above the ankle joint. Analysis based on a fixed-effect model.

§Amputation below the ankle joint. Analysis based on a fixed-effect model.

Some studies (Margolis et al[4] and Fedorko et al.[6]) that raised many concerns, even though they have several weaknesses, have already been analysed earlier in this report (see above in the text).

We conclude this review of the literature reporting, only for knowledge, a RCT[73] that has shown a beneficial effect of HBOT in heart rate corrected QT (QTc) interval prolongation, a common heart condition in diabetic patients with severe foot ulcers (DFUs) and represents a risk factor associated with increased mortality.
The Fagher K et al. study[73] found that HBOT might protect against QTc prolongation in this high-risk diabetic population with hard-to-heal DFUs. In a prospective, double-blinded placebo-controlled study, patients were randomized to 40 treatment sessions with either HBOT or air (placebo), at 2.5 ATA. Patients fulfilling >35 completed treatment sessions were included in the evaluation. Of the initial 75 patients (38 HBO/37 placebo), two were excluded due to pacemaker use. Baseline characteristics were similar between groups. At the 2-year follow-up, QTc time was significantly shorter in the HBOT group compared to the placebo group (438 vs. 453ms, $p<0.05$). Further, fewer HBOT patients had a QTc time >450ms (22 vs. 53 %, $p<0.02$). This difference seemed to be caused by a significant prolongation of the QTc interval in the placebo group (427 (419-459) at baseline vs. 456ms (424-469) after 2years), whereas no significant change was seen in HBO treated patients.

PATIENTS SELECTION FOR HBOT

Failure to address the best practice treatment of DFUs obviates any discussion about the utility of HBOT for this disease. The International Working Group on the Diabetic Foot (IWGDF) guidelines[63] for the best practice treatment of DFUs includes four tenets: treatment of underlying infection; revascularization if appropriate and feasible; offloading to minimize trauma to the ulcer site and management of the wound bed to promote healing.
HBOT will not accelerate tissue repair in wounds with normal oxygen tensions and will only do so effectively in diabetic ulcers in which oxygen tension can be elevated to therapeutic levels. It is essential in clinical practice to demonstrate and evaluate critical tissue ischemia before considering the use of HBOT[74]. Hypoxia (i.e. wound PO_2 < 40 mmHg) generally best defines wounds appropriate for HBOT— or rather, lack of hypoxia (i.e. wound PO_2 >40-50 mmHg) defines wounds potentially not appropriate for HBOT. Patients should be taken with a Wagner Grade 3 or higher DFU and $PtcO_2$ less than 40 mm Hg.
Patient with $PtcO_2$ less than 30 mm Hg should be then stratified on whether a single in-chamber $PtcO_2$ at 2.0 ATA rises over 100 mm Hg or more, the patient will likely benefit from HBOT[75]. It has also been suggested that if the abnormally low $PtcO_2$ values rise to 200 mm Hg there will be a better prognosis, while it the value fails to rise over 200 mm Hg there will be a worse prognosis[10,68]. Even if the $PtcO_2$ values rise to 100 mmHg or more, during HBOT, this does not excuse the patient from a vascular workup, as many patients have multifactorial components to tissue hypoxia[68].
There may be a future role for other methods of measuring the perfusion and oxygenation of the DFU wound bed as the Laser Doppler Flow[76], the Near Infrared Spectroscopy (NIRS)[77,78] and the Fluorescence Angiography[79,80].
In USA, the Centres for Medicare & Medicaid Services (CMS) approved the use of HBOT for the management of the Diabetic Foot Ulcers[11]. Strict criteria have been established. The patient must have diabetes (Type I or Type II) and a lower extremity wound (Wagner grade III or higher) due to diabetic disease. The use of HBOT is covered by CMS as adjunctive therapy only after there are no measurable signs of healing for at least 30 –days of treatment with standard wound therapy and must be used in addition to standard wound care. Standard wound care in patients with diabetic wounds includes: assessment of a patient's vascular

status and correction of any vascular problems in the affected limb if possible, optimization of nutritional status, optimization of glucose control, debridement by any means to remove devitalized tissue, maintenance of a clean, moist bed of granulation tissue with appropriate moist dressings, appropriate off-loading and necessary treatment to resolve any infection that might be present. Failure to respond to standard wound care occurs when there are no measurable signs of healing for at least 30 consecutive days. Wounds must be evaluated at least every 30 days during administration of HBOT. Continued treatment with HBOT is not covered if measurable signs of healing have not been demonstrated within any 30-day period of treatment.

CURRENT HBOT PROTOCOL

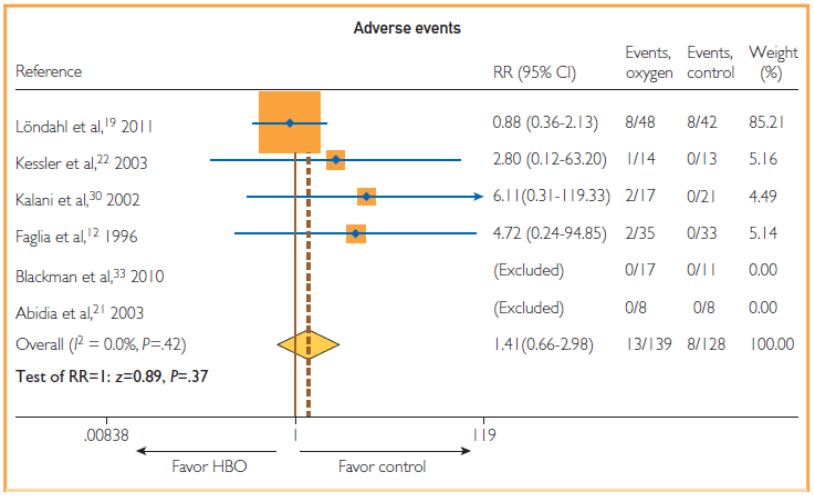
Treatment involves placing the patient in a monoplace or multiplace compression chamber, increasing the environmental pressure within the chamber and administering 100% oxygen for respiration. In this way, it is possible to deliver a greatly increased partial pressure of oxygen to the tissues. Different protocols are used for delivery of oxygen between several trials[36,38]. In Diabetic Foot Ulcers (DFU) treatments involve pressurisation to between 2.0 and 2.5 atmospheres absolute (ATA) for 90 minutes, once or twice daily, 5 or 6 days/week, between 20 and 40 sessions. In multiplace chamber we suggest that the fraction of inspired oxygen (FiO_2) should be measured to manage the adequate oxygenation in patients.

ADVERSE EVENTS

The risk of adverse events from HBOT (i.e. ear and sinus pain or barotrauma, hypoglycemic events, shortness of breath and chest pain, confinement anxiety, central nervous system oxygen toxicity, hyperoxic myopia) can be considered to be very low and self-limited when they do occur[81].

In the meta-analyses of RCT, by Liu and colleagues[72], four studies (including 3 RCTs and 1 prospective study) reported adverse events related to HBOT, including barotrauma lesions, oxygen toxicity, confinement anxiety and ocular effects. Overall, no statistically significant difference was found in adverse event rates between the HBOT and the control groups (RR, 1.41; 95% CI, 0.66-2.98; P¼.37) (Figure 14). Moreover, the pooling analysis of 4 RCTs also found no significant difference in adverse events between the 2 groups (RR 1.41; 95% CI, 0.66-2.98; P¼.37).

Figure 14 Forest plot for meta-analyses, by Liu and colleagues[72], comparing adverse events, including barotraumatic lesions, oxygen toxicity, confinement anxiety, and ocular effects, in diabetic foot ulcer treated with or without hyperbaric oxygenation (HBO). RR ¼ relative risk. (© 2013 Mayo Foundation for Medical Education and Research)

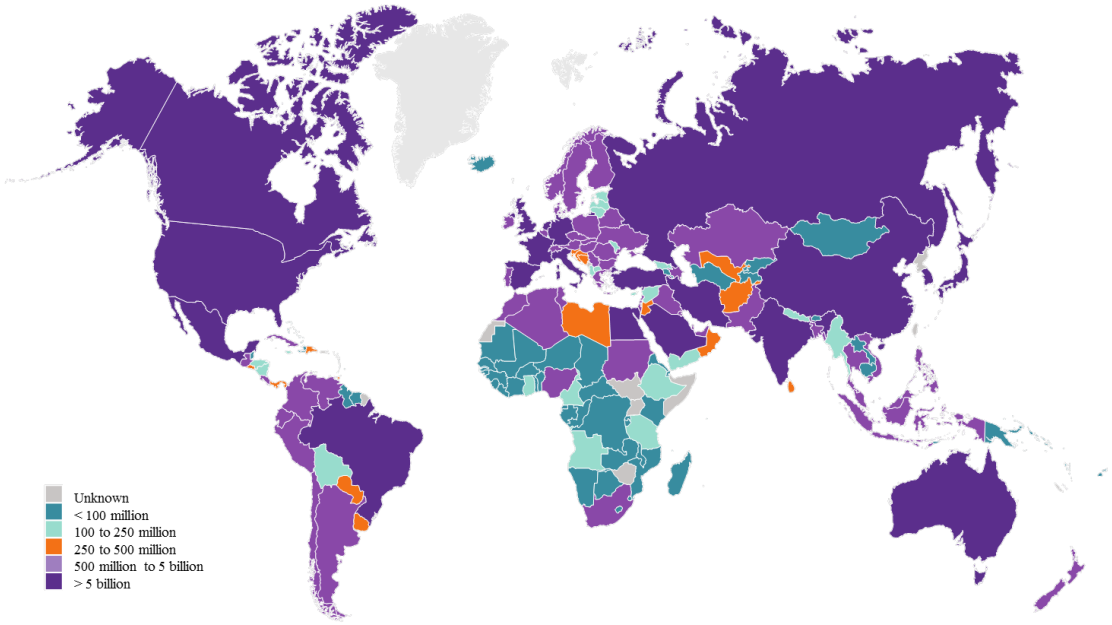


The frequency of adverse events was low in reviews on HBOT for chronic wounds by Kranke P et al (2015)[36] and Huang et al (2015)[10].

COST IMPACT

12% of worldwide global health expenditure is spent on diabetes (590 billion Euros or 673 billion USD, at the exchange rate USD/EUR 0.88 on March 31, 2016) Figure 15

Figure 15: Total annual diabetes-related healthcare expenditures (20-79 years, 2015, in International Dollars, R=2*)[37] (©International Diabetes Federation http://www.diabetesatlas.org/, accessed by web on April 6, 2016)



Estimates indicate that diabetes was responsible for about 9% of total health expenditure in the Europe Region for 2015, which means between 137 and 255 billion Euros (USD 169 - 311 billion*). This translates to 2,293 – 4,264 Euros (USD 2,821 - 5,202*) per person with diabetes per year. Just as there are wide variations in the prevalence of diabetes across the region, the range between countries of average diabetes related healthcare spending was also large – from 8,858 Euros / USD 8,235* per person with diabetes in Luxembourg to just 107 Euros / USD 296* per person with diabetes in Tajikistan[13] (*exchange rate USD/EUR 0.88 on April 7, 2016)

The treatment cost per DFU episode is between 7,700 and 25,200 Euros[17]. The estimated average cost per DFU episode in Europe is 10,000 Euros[17]. Applying these findings to the estimated 3,6 million of DFU patients in 2015 (*59,8 million of diabetic patients for an average prevalence of 6% of DFU*) [13], the indicative annual cost for the DFU treatment, in the whole Europe, is 598 billion Euros. Limiting the analysis to the European 27 leading Nations the indicative annual cost for DFU treatment is 4-6 billion Euros[13] (Table 17).

Approximately 12% of people with DFU progress to lower extremity amputation[2,16,22]. The annual average incidence of amputation is 2.5 to 18 per 1,000 diabetics[2,16]. The cost per episode of major amputation (DRG 113) varies between 9.927 and 11.803 Euros (on average 10.865 Euros)[17,95]

Table 17: prevalence, annual incidence and cost of Diabetic Foot Ulcers in Europe[13]

	Range	Total EU-27 Countries
Population with diabetes (2015)		20,2 million[16]
Diabetic Foot Ulcers treatment:		
• Prevalence	50-70/1000 diabetics[17]	1,0-1,4 million
• Incidence	20-30/1000 diabetics [18]	400,000-600,000
• Cost per episode	7,700-25,200 Euros[17]	10,000 Euros[17]
• Indicative annual cost		4-6 billion Euros
DFU amputations:		
• Annual average incidence	2,5-18/1000 diabetics[2,16]	207,050 (average)
• Cost per episode	9,927-11,803 Euros[17,95]	10,865 Euros (average)
• Indicative annual cost		2,25 billion Euros

In the United States of America (USA) alone, approximately 29.1 million people have diabetes, and about 60% of non-traumatic amputations of the lower limbs are performed on adults with the condition. This fraction equated to about 73,000 adults in 2010[82] and the economic burdens, according to a Medicare analysis in 2008, was 30,829 Euros/ 35,099 USD* for DFU treatments and 47,517 Euros/ USD 54,098* for amputations[1]. In 2010 the treatment and amputation for DFUs costed, in the USA, respectively 7,9 and 11,4 billion Euros / USD 9 and 13 billion* [3,8]. (* exchange rate USD/EUR 0.88 on April 7, 2016)

The addition of HBOT may improve the proportion of DFU that achieve healing and thereby enhance the quality of life in such selected participants. One review[22] suggests the addition of HBOT may reduce the overall costs associated with the treatment of DFU. This finding is consistent with the experience of the Problem Wound Care and Hyperbaric Centre in Ravenna (Italy)[83-84]: the multidisciplinary approach for the enhancement of healing in selected problem wounds (DFUs included) has allowed, between 2005 and 2007, to halve the incidence of amputation (total of major and minor) in DFUs patients residents in the Ravenna province compared to the average incidence for the Emilia Romagna region (data published by the Health and Social Care Regional Agency / Agenzia Sanitaria e Sociale Regionale of Emilia Romagna, Italy) [85] (Figure 16). Besides, between 2009 and 2014, in Emilia Romagna Region there has been a reduction in major amputations for DFUs (0.85 per thousand diabetic patients in 2009 compared to 0.67 per thousand in 2014) thank to a conservative approach (which includes the HBOT, too) measured by, as numerical indicator, the increase of revascularizations (2.95 per thousand diabetic patients in 2009 compared 3.38 per thousand in 2014).
Between 2009 and 2014, the total rate of major amputation (both for DFU and PAOD) in the Romagna area (which is the operational area of the Problem Wound Care and Hyperbaric Centre in Ravenna) was half the average rate for the Emilia Romagna Region (an average of 0.21 amputations per thousand inhabitants in the Romagna area compared to an average of 0.4 per thousand for the Emilia Romagna region) (data published by the Health and Social Care Regional Agency / Agenzia Sanitaria e Sociale Regionale of Emilia Romagna, Italy) (Figure 17)

Figure 16 standardized rate of amputation in people with diabetes, distributed to Local Health Authorities (Azienda Sanitaria Locale – ASL) of patient's residence (analysis between 2005 and 2007)[85] The green arrow indicates the ASL of Ravenna, where is the headquarter of the Problem Wound Care and Hyperbaric Centre in Ravenna (Italy): the standardized rate of amputation is half over the average incidence for the Emilia Romagna region. (data published by the Health and Social Care Regional Agency of Emilia Romagna / Agenzia Sanitaria e Sociale Regionale, Italy)[85]

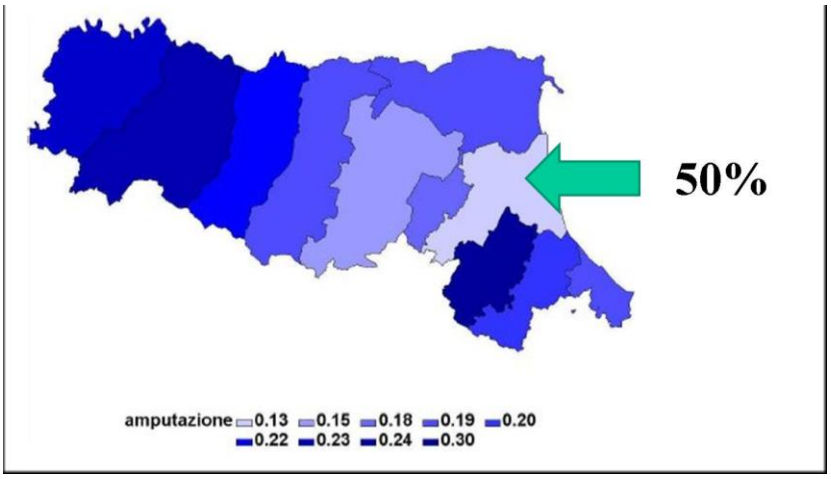
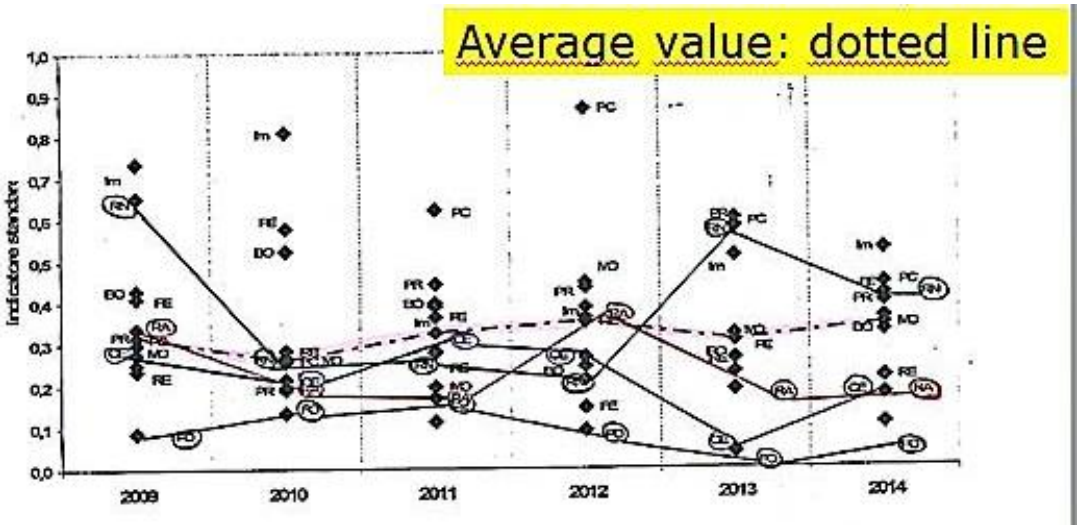


Figure 17 Standardized indicator (by sex and age) of the rate of major amputations / 1000 inhabitants living in the Emilia Romagna region (Italy), aged ≥ 18 years. The dotted line shows the average value for the Emilia Romagna region. The several solid lines show the change, between 2009 and 2014, for each Local Health Authorities (Azienda Sanitaria Locale – ASL) of patient's residence, located in the operational area of the Problem Wound Care and Hyperbaric Centre in Ravenna (Romagna area). In this area, the rate of major amputation is lower than the average rate for the Emilia Romagna Region. The other points are the other ASLs of the Emilia Romagna Region (Emilia area) that does not gravitate into the operational area of the Hyperbaric Centre of Ravenna (data published by the Health and Social Care Regional Agency of Emilia Romagna / Agenzia Sanitaria e Sociale Regionale, Italy)[85]



Unfortunately, despite these considerations favourable to the HBOT in DFUs, relatively few DFU patients received HBOT (at least as regards the USA between 2007 and 2011), with 1.2% of matched Medicare and 2.3% of matched privately insured DFU patients receiving HBOT[3] (Table 18)

Table 18 Health care resource utilization and costs during the 12 months following the index date among matched DFU patients and controls (Medicare and privately insured patients data analysis from January 2007-September 2011)[3]. Only 1.2% of matched Medicare and 2.3% of matched privately insured DFU patients received HBOT. (© 2014 by the American Diabetes Association)

	Medicare			Privately insured		
	DFU *n* ▪ 27,878	Controls *n* ▪ 27,878	*P**	DFU *n* ▪ 4,536	Controls *n* ▪ 4,536	*P**
Medical resource use, mean (SD)						
Inpatient days	2.5 (8.3)	1.1 (4.6)	<0.0001	19.5 (36.3)	7.1 (18.5)	<0.0001
ED visits	1.2 (2.0)	0.8 (1.9)	<0.0001	1.3 (2.8)	0.6 (1.7)	<0.0001
Outpatient/physician days	59.6 (48.3)	44.1 (36.7)	<0.0001	20.2 (17.5)	14.2 (12.9)	<0.0001
Home health care days	1.2 (2.4)	0.7 (1.9)	<0.0001	4.5 (12.3)	1.4 (4.7)	<0.0001
Other	7.5 (9.7)	5.7 (8.6)	<0.0001	9.0 (12.3)	5.1 (9.2)	<0.0001
Patients with select DFU-related procedures, %						
Lower-limb amputation	3.8	—	N/A	5.0	—	N/A
HBO	1.2	—	N/A	2.3	—	N/A
Skin substitute use	1.8	—	N/A	1.3	—	N/A
Direct health care costs, mean (SD)						
Total all-cause medical	$28,031 ($39,502)	$16,320 ($26,223)	<0.0001	$26,881 ($58,856)	$10,991 ($30,461)	<0.0001
Total all-cause medical and prescription drug†	—	—	—	$31,419 ($59,633)	$14,536 ($31,359)	<0.0001
Inpatient	$4,719 ($14,881)	$2,294 ($9,140)	<0.0001	$17,061 ($43,721)	$6,501 ($25,287)	<0.0001
ED	$5,346 ($13,557)	$2,924 ($8,942)	<0.0001	$1,020 ($3,483)	$484 ($1,779)	<0.0001
Outpatient/physician	$8,418 ($11,801)	$6,040 ($9,430)	<0.0001	$4,888 ($19,840)	$2,806 ($9,658)	<0.0001
Home health care	$4,390 ($10,071)	$2,283 ($7,611)	<0.0001	$1,639 ($8,307)	$371 ($2,110)	<0.0001
Other	$5,159 ($11,583)	$2,779 ($8,277)	<0.0001	$2,274 ($10,210)	$829 ($3,857)	<0.0001
Prescription drug	—	—	—	$4,538 ($6,833)	$3,545 ($4,983)	<0.0001
Total DFU-related medical costs, mean (SD)‡	$5,285 ($16,534)	—	N/A	$9,590 ($34,059)	—	N/A
Indirect costs, mean (SD)§						
Total	—	—	—	$6,311 ($9,288)	$3,052 ($5,772)	<0.0001
Absenteeism	—	—	—	$3,599 ($5,897)	$2,010 ($2,982)	<0.0001
Disability	—	—	—	$2,713 ($8,034)	$1,042 ($5,238)	<0.0001

In the Liu and colleagues[72] meta-analyses of RCT, only 106 patients in 2 trials provided information on quality of life on the basis of self-reported questionnaires. A trial[60] provides evidence to suggest that HBOT might improve long-term quality of life. Although another trial[86] implied that it did not produce significant improvements in quality of life.

CONCLUSION

RCTs and controlled cohorts, when analysed pooled or separately, demonstrate a significant beneficial effect of Hyperbaric Oxygen Therapy (HBOT), compared with standard care, in increasing the likelihood of healing in diabetic foot ulcerations (DFU) and reducing the need for major amputations (the GRADE level of evidence is of moderate quality, according the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine, 2016)[7] Unfortunately, despite this beneficial effect, relatively few DFU patients received HBOT[3]. This is a shame considering that DFU is a heavy economic burden for society[1]

The use of HBOT for DFU is founded on the assumption that practitioners have, previously, aggressively addressed revascularization of the ischemic foot, debridement of devitalized tissue, offloading of the neuropathic foot lesion, and appropriate anti-infective therapies. HBOT should be included as part of a comprehensive diabetic foot ulcer program and used as an adjunctive procedure. Proper evaluation of the patient, to establish an individualized diagnostic and treatment strategy is recommended. The decision to start HBOT should be made after ischemia status is evaluated. HBOT is unlikely to be helpful in patients with severe uncorrectable ischemia because oxygen will not reach the ischemic area in a sufficient tension to provoke benefits.

Since this recommendations were not applied in all the patients enrolled in the studies analysed, the beneficial effects of HBOT (in increased healing and reduction of major amputations in DFU) should therefore be viewed, at present, as an average expected effect in a heterogeneous group of patients with DFUs. In the future studies should be included in the

reviews only whether such principles have always been followed or a stratified analysis based on the vascular status must be conducted.
Adverse events are rare and acceptable.
It seems that the long-term quality of life of patients treated with HBOT could be improved by its judicious application.
More studies are needed to provide adequate data.

RECOMMENDATION

- We suggest the application of HBOT as standard of care in patients with Diabetic Foot Ulcers (DFUs) as part of a multidisciplinary treatment plan with ongoing wound care on a regular basis and not simply as stand-alone therapy (recommendation type 1, level A evidence)
- We suggest HBOT may significantly improve the DFU wounds healed in the short term (up to six weeks) and this is important for improving wound healing trajectory. (recommendation type 1, level A evidence)
- We believe there is low-level evidence to confirm or to exclude that HBOT improves DFUs wound healing within longer term follow-up. (recommendation type 1, level C evidence)
- We suggest HBOT may reduce the number of major amputations in people with diabetes who have chronic DFUs (recommendation type 1, level A evidence)
- We believe there is low-level evidence to say that HBOT significantly improve the minor amputation rate in people with DFUs (recommendation type 1, level C evidence)
- We recommend HBOT in DFUs that have failed to respond to adequate basic wound care after 4 weeks / 30 days *(including appropriate debridement, vascular screening for significant peripheral arterial disease and/or local wound hypoxia, adequate offloading and infection management).* (recommendation type 1, level A evidence)
- We recommend HBOT for grade 3 and above on Wagner classification. (recommendation type 1, level A evidence)
- We recommend HBOT for stage B, grade 3 and above on University of Texas classification. (recommendation type 1, level A evidence)
- We recommend HBOT for moderate severity infection and above on Infectious Disease Society of America (IDSA) classification. (recommendation type 1, level A evidence)
- We recommend HBOT for PEDIS 3 or above on International Working Group on the Diabetic Foot (IWGDF) classification. (recommendation type 1, level A evidence)
- We recommend HBOT for grade 2 or above on Society for Vascular Surgery Wound Ischemia Foot Infections (WIfI) System. (recommendation type 1, level A evidence)
- We recommend HBOT for stage III and IV on International Association of Enterostomal Therapy classification for DFUs. (recommendation type 1, level A evidence)
- We believe there is low-level evidence to justify the use of HBOT as an adjunctive treatment in grade 2 on Wagner classification (or similar level on the other DFUs classifications). We suggest more RCTs to better clarify the provision of HBOT to Wagner grade 2 lesions (or similar level on the other DFUs classifications) (recommendation type 1, level C evidence)
- We recommend the patients are selected for HBOT carefully[87]. Using transcutaneous oximetry values can help stratify patients to allow more judicious use of HBOT and predict those who are most likely to fail.[68,69] (recommendation type 1, level B evidence)
- We recommend trancutaneous oximetry measurement (TCOM - $TcPO_2$) as the golden standard. TCOM is better correlated to DFUs healing following HBOT than Ankle

Brachial Index (ABI) and Toe Blood Pressure (TBP). (recommendation type 1, level C evidence)

- We believe that hypoxia (i.e. wound PO_2 < 40 mmHg) generally best defines DFUs appropriate for HBOT— or rather, lack of hypoxia (i.e. wound PO_2 >40-50 mmHg) defines DFUs potentially not appropriate for HBOT. (recommendation type 1, level B evidence)
- We suggest HBOT as a feasible adjunctive treatment modality in diabetic patients with DFUs when basal $TcPO_2$ at the dorsum of the foot is above 25 mmHg, especially when systemic comorbidities or local factor of risk are present. (recommendation type 1, level B evidence)
- We suggest that when normobaric oximetry values are less than 35 to 40 mm Hg, a 100% normobaric oxygen challenge should be given via a non-rebreathing face mask. If the abnormally low $TcPO_2$ values rise to 100 mm Hg or more, the patient could likely benefit from HBOT (better prognosis). (recommendation type 1, level B evidence)
- We believe that hyperoxia (HBOT) has an effect on catalytic activity in DFUs, reflected by values for the apparent Michaelis-Menten constant for oxygen that, especially when several systemic comorbidities or local factor of risk are present, differs among the three Nitric Oxide Synthetase (NOS) isoforms[46,47]. (recommendation type 1, level C evidence)
- We suggest that even if the $TcPO_2$ values rise to 100 mmHg or more, during HBOT, this does not excuse the patient from a vascular workup, as many patients have multifactorial components to tissue hypoxia[46]. (recommendation type 1, level B evidence)
- We believe that there could be a future role for other methods to better measuring the perfusion and oxygenation of the DFU wound bed as the Laser Doppler Flow[76], the Near Infrared Spectroscopy (NIRS)[77-78] and the Fluorescence Angiography[79-80]. (recommendation type 3, level C evidence)
- We suggest that in DFU the HBOT schedule should be between 2.0 and 2.5 atmospheres absolute (ATA) for 90 minutes, once or twice daily, 5 or 6 days/week, between 20 and 40 sessions. (recommendation type 1, level B evidence)
- In multiplace chamber we suggest that the fraction of inspired oxygen (FiO2) should be measured to manage the adequate oxygenation in patients. (recommendation type 1, level C evidence)
- We recommend further high quality RCTs to examine short and long-term risks and benefits of HBOT in DFUs. The true effect of HBOT in DFU should be derived from robust RCTs because they only provide higher-quality evidence. (recommendation type 1, level A evidence)

AKNOWLEDGMENTS

The author thanks Marta Milandri (*Unit of Organizational Development, Training and Evaluation of AUSL Romagna; clinician librarian of the hospitals "M. Bufalini" of Cesena, "S. Solieri" of Forlì and scientific medical library of Rimini*), Gladiol Zenunaj MD, (*consultant in Vascular Surgery of the Unit of Vascular and Endovascular Surgery, University Hospital of Ferrara - Vice Director: professor Vincenzo Gasbarro*) and Elisa Casadei (*Centro iperbarico Ravenna*) who provided invaluable assistance in identifying, reviewing information and correcting the report.

ABBREVIATIONS AND ACRONYMS

- ABI = Ankle Brachial Index
- AKA = Above Knee Amputation
- ATA = Absolute pressure (is the total ambient pressure on the system).
- BKA = Below Knee Amputation

- CMS = Centers for Medicare & Medicaid Services (USA)
- CND = Canadian dollar
- CTPs = Cellular and/or Tissue-based Products
- DFI = Diabetic Foot Infection
- DFO = Diabetic Foot Osteomyelitis
- DFU = Diabetic Foot Ulceration
- ECHM = European Committee for Hyperbaric Medicine
- eNOS = Endothelial Nitric Oxide Synthase
- EPC = Endothelial progenitor cell
- FiO_2 = Fraction of Inspired Oxygen
- GRADE = the Grades of Recommendation Assessment, Development, and Evaluation system
- HBO = Hyperbaric Oxygenation
- HBOT or HBO2T = Hyperbaric Oxygen Treatment
- HIF = Hypoxia-Inducible Factor
- IDDM = Insulin Dependent Diabetes Mellitus
- IDSA = Infectious Disease Society of America
- IWGDF = International Working Group of the Diabetic Foot
- LEA = Lower Extremity Amputation
- MRI = Magnetic Resonance Imaging
- NIDDM = Non-Insulin Dependent Diabetes Mellitus
- NIRS = Near Infrared Spectroscopy
- NBO = Normobaric Oxygen
- NO = Nitric Oxide
- NPWT = Negative Pressure Wound Therapy
- PAD / PAOD = Peripheral Arterial (Occlusive) Disease
- PO2 or ppO2= partial pressure of oxygen
- PtcO2 = Transcoutaneous Oxygen Tension
- PTB = Probe To Bone
- PU = Pressure ulcer
- RCT = Randomised controlled trials
- RNS = Reactive nitrogen species
- rhPDGF = recombinant human Platelet Derived Growth Factor
- RR = Relative Risk
- SIGN = Scottish Intercollegiate Guidelines Network
- SPCs = Stem/Progenitor cells
- TCOM = TransCutaneous Oximetry Measurement
- TBP = Toe Blood Pressure
- UHMS = Undersea and Hyperbaric Medical Society
- VEGF = Vascular Endothelial Grow Factor
- VLU = Venous Leg Ulcer

REFERENCES

1. Margolis D, Malay DS, Hoffstad OJ, et al. Economic burden of diabetic foot ulcers and amputation: diabetic foot ulcers. Data Points #3. Rockville, MD, Agency for Healthcare Research and Quality, U.S. Dept. of Health and Human Services, January 2011 (AHRQ Publ. No. 10[11]-EHC009-2-EF).

2. Crawford F, Anandan C, Chappell FM, et al. “Protocol for a systematic review and individual patient data meta-analysis of prognostic factors of foot ulceration in people with diabetes: the international research collaboration for the prediction of diabetic foot ulcerations (PODUS)”. BMC Medical Research Methodology. 2013;13:22. doi:10.1186/1471-2288-13-22.

3. Rice J. B., Desai U., Cummings A. K. G., Birnbaum H. G., Skornicki M., Parsons N. B. “Burden of diabetic foot ulcers for Medicare and private insurers” Diabetes Care, p. 651-658, 2013.

4. David J. Margolis, Jayanta Gupta, Ole Hoffstad, Maryte Papdopoulos, Henry A. Glick, Stephen R. Thom, and Nandita Mitra “Lack of Effectiveness of Hyperbaric Oxygen Therapy for the Treatment of Diabetic Foot Ulcer and the Prevention of Amputation: A cohort study” Diabetes Care July 2013 36:7 1961-1966; doi:10.2337/dc12-2160. PMCID: PMC 3687310

5. Berendt AR. “Counterpoint: hyperbaric oxygen for diabetic foot wounds is not effective”. Clin Infect Dis. 2006;43(2): 193-198.

6. Fedorko L, Bowen JM, Jones W, Oreopoulos G, Goeree R, Hopkins RB, O'Reilly DJ “Hyperbaric Oxygen Therapy Does Not Reduce Indications for Amputation in Patients With Diabetes With Nonhealing Ulcers of the Lower Limb: A Prospective, Double-Blind, Randomized Controlled Clinical Trial”. Diabetes Care. 2016 Mar;39(3):392-9. doi: 10.2337/dc15-2001. Epub 2016 Jan 6. PMID: 26740639.

7. Hingorani, Anil et al. “The management of diabetic foot: A clinical practice guideline by the Society for Vascular Surgery in collaboration with the American Podiatric Medical Association and the Society for Vascular Medicine” Journal of Vascular Surgery , J Vasc Surg. 2016 Feb;63(2 Suppl):3S-21S. doi: 10.1016/j.jvs.2015.10.003. PMID: 26804367

8. Frykberg RG, Zgonis T, Armstrong DG, Driver VR, Giurini JM, Kravitz SR, et al. Diabetic foot disorders. A clinical practice guideline (2006 revision). J Foot Ankle Surg 2006;45(Suppl):S1-66.

9. 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. doi:10.1093/cid/cis346

10. Huang ET, Mansouri J, Murad MH, Joseph WS, Strauss MB, Tettelbach W, Worth ER; UHMS CPG Oversight Committee. A clinical practice guideline for the use of hyperbaric oxygen therapy in the treatment of diabetic foot ulcers. Undersea Hyperb Med. 2015 May-Jun;42(3):205-47. PMID: 26152105

11. National Coverage Determination (NCD) for Hyperbaric Oxygen Therapy (20.29) Publication Number 100-3; Manual Section Number 20.29; Version Number 3; Effective Date of this Version 19 June 2006 https://www.cms.gov/medicare-coverage-database/details/ncd-details.aspx?NCDId=12&ncdver=3&NCAId=37&ver=7&NcaName=Hyperbaric+Oxygen+Therapy+for+Hypoxic+Wounds+and+Diabetic+Wounds+of+the+Lower+Extremities&fromdb=true&IsPopup=y&bc=AAAAAAAAEAgA (assessed by web on April 02, 2016)

12. Yu, Catherine H. et al. "Impact of an Interprofessional Shared Decision-Making and Goal-Setting Decision Aid for Patients with Diabetes on Decisional Conflict – Study Protocol for a Randomized Controlled Trial." Trials 16 (2015): 286.PMC. Web. 2 Apr. 2016

13. International Diabetes Federation « Diabetes atlas, 7th edition ». Available at: http://www.diabetesatlas.org/ . Accessed March 31, 2016.

14. Eurostat (July 2015) http://ec.europa.eu/eurostat/statistics-explained/index.php/Population_structure_and_ageing

15. Posnett J, Gottrup F, Lundgren H, Saal G. "The resource impact of wounds on health-care providers in Europe" J Wound Care. 2009 Apr;18(4):154-161. PMID: 19349935

16. Wild S, Roglic G, Green A et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-1053. PMID: 15111519

17. Prompers L, Huijberts M, Schaper N, Apelqvist J, Bakker K, Edmonds M, Holstein P, Jude E, Jirkovska A, Mauricio D, Piaggesi A, Reike H, Spraul M, Van Acker K, Van Baal S, Van Merode F, Uccioli L, Urbancic V, Ragnarson Tennvall G. Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale Study. Diabetologia. 2008 Oct;51(10):1826-34. doi: 10.1007/s00125-008-1089-6. PMID: 18648766

18. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of Diabetes and Diabetes-Related Complications. *Physical Therapy*. 2008; 88(11):1254-1264. doi:10.2522/ptj.20080020. PMC 3870323

19. Boulton AJ. The diabetic foot: from art to science. The 18th Camillo Golgi lecture. Diabetologia 2004;47:1343-53.

20. Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, Mauricio D, et al. Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. Diabetologia 2008;51:747-55.

21. Jeffcoate WJ, Chipchase SY, Ince P, Game FL. Assessing the outcome of the management of diabetic foot ulcers using ulcer-related and person-related measures. Diabetes Care 2006;29:1784-7.

22. Chuck AW, Hailey D, Jacobs P, Perry DC. Cost-effectiveness and budget impact of adjunctive hyperbaric oxygen therapy for diabetic foot ulcers. International Journal of Technology Assessment in Health Care 2008;24(2):178–83. PMID: 18400121

23. Center for Disease Control. Hospital discharges for nontraumatic lower extremity amputation with diabetes as a listed diagnosis, United States, 1988-2009. Published March 6, 2012. http://www.cdc.gov/diabetes/statistics/lea/byRacetable1_2.htm

24. Widatalla AH, et al. Implementation of diabetic foot ulcer classification system for research purposes to predict lower extremity amputation. Int J Diabetes Dev Ctries, 2009. 29(1): 1-5

25. Mills JL Sr, 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(1): 220-34 e1-2.

26. Mays L Diabetes Mellitus Standards of Care. Nurs Clin North Am. 2015 Dec;50(4):703-11. doi: 10.1016/j.cnur.2015.08.001. PMID: 26596658

27. Bolton L. "Benchmarking Chronic Wound Healing Outcomes" Wounds. 2012;24(1) http://www.medscape.com/viewarticle/758217

28. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA. 2005;293:217-228. http://dx.doi.org/10.1001/jama.293.2.217.

29. Greenhagen RM, Johnson AR, Bevilacqua NJ. Gastrocnemius recession or tendo-achilles lengthening for equinus deformity in the diabetic foot? Clin Podiatr Med Surg. 2012;29:413-424. http://dx.doi.org/10.1016/j.cpm.2012.04.005

30. Orendurff MS, Rohr ES, Sangeorzan BJ, Weaver K, Czerniecki JM. An equinus deformity of the ankle accounts for only a small amount of the increased forefoot plantar pressure in patients with diabetes. J Bone Joint Surg Br. 2006;88:65-68. http://dx.doi.org/10.1302/0301-620x.88b1.16807

31. Frykberg RG, Bowen J, Hall J, et al. Prevalence of equinus in diabetic versus nondiabetic patients. J Am Podiatr Med Assoc. 2012;102:84-88.

32. Lavery LA, Fontaine JL, Bhavan K, et al. Risk factors for methicillin-resistant Staphylococcus aureus in diabetic foot infections. Diabet Foot Ankle. 2014;5. http://dx.doi.org/10.3402/dfa.v5.23575.

33. Hatipoglu M et al. "Causative pathogens and antibiotic resistance in diabetic foot infections: A prospective multi-center study" J Diabetes Complications. 2016 Feb 21. pii: S1056-8727(16)00071-4. doi: 10.1016/j.jdiacomp.2016.02.013. PMID: 26965794

34. Uckay I., Aragon-Sanchez J., Lew D., Lipsky B.A. “Diabetic foot infections: What have we learned in the last 30 years?” (2015) International Journal of Infectious Diseases, 40, pp. 81-91.

35. Chen CE1, Ko JY, Fong CY, Juhn RJ. Treatment of diabetic foot infection with hyperbaric oxygen therapy. Foot Ankle Surg. 2010 Jun; 16(2):91-5. doi: 10.1016/j.fas.2009.06.002. Epub 2009 Aug 18. PMID: 20483142

36. Kranke P, Bennett MH, Martyn-St James M, Schnabel A, Debus SE, Weibel S. “Hyperbaric oxygen therapy for chronic wounds”. Cochrane Database of Systematic Reviews 2015, Issue 6. Art. No.: CD004123. DOI: 10.1002/14651858.CD004123.pub4.

37. Löndahl M, Nilsson A, Katzman P, et al. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. Diabetes Care. 2010;33:998–1003.

38. Elraiyah T, Tsapas A, Prutsky G, Domecq JP, Hasan R, Firwana B, et al. A systematic review and meta-analysis of adjunctive therapies in diabetic foot ulcers. J Vasc Surg 2016;63(Suppl):46S-58S. PMID: 26804368

39. Stoekenbroek RM, Santema TB, Legemate DA, Ubbink DT, van den Brink A, Koelemay MJ. Hyperbaric oxygen for the treatment of diabetic foot ulcers: a systematic review. Eur J Vasc Endovasc Surg 2014;47:647–55

40. Oliveira N, Rosa P, Borges L, Dias E, Oliveira F, Cassio I. Treatment of diabetic foot complications with hyperbaric oxygen therapy: a retrospective experience. Foot Ankle Surg 2014;20:140–3. 126. Berendt AR. Counterpoint: hyperbaric oxygen for diabetic foot wounds is not effective. Clin Infect Dis 2006;43:193–8

41. Ceriello A, Mercuri F, Quagliaro L, et al. Detection of nitrotyrosine in the diabetic plasma:evidence of oxidative stress Diabetologia. 2001; 44:834–838.

42. Gallagher KA, Goldstein LJ, Buerk DG, et al. Diabetic impairments in NO-mediated endothelial progenitor-cell mobilization and homing are reversed by hyperoxia and SDF-1a. J Clin Invest.2007;117:1249–1259

43. Boykin JV Jr1, Baylis C. Hyperbaric oxygen therapy mediates increased nitric oxide production associated with wound healing: a preliminary study. Adv Skin Wound Care. 2007 Jul;20(7):382-8. PMID: 17620739

44. Thom SR, Milovanova TN, Yang M, Bhopale VM, Sorokina EM, Uzun G, Malay DS, Troiano MA, Hardy KR, Lambert DS, Logue CJ, and Margolis DJ. Vasculogenic stem cell mobilization and wound recruitment in diabetic patients: Increased cell number and intracellular protein content associated with hyperbaric oxygen therapy. Wound Rep Reg 19: 149–161, 2011

45. Thom SR, Hampton M, Troiano MA, Mirza Z, Malay DS, Shannon S, Jennato NB, Donohue CM, Hoffstad O, Woltereck D, Yang M, Yu K, Bhopale VM, Kovtun S, Margolis DJ. "Measurements of CD34+/CD45-dim Stem Cells Predict Healing of Diabetic Neuropathic Wounds". Diabetes. 2016 Feb;65(2):486-97. doi: 10.2337/db15-0517. PMID: 26487786. PMCID: PMC4747459 [Available on 2017-02-01]

46. Thom S.R. Hyperbaric oxygen – its mechanisms and efficacy. Plast Reconstr Surg. 2011 January ; 127(Suppl 1): 131S–141S. [PMID: 21200283]

47. Heyboer M, Milovanova TN, Wojcik S, et al. CD34+/CD45-dim stem cell mobilization by hyperbaric oxygen – changes with oxygen dosage. Stem cell research. 2014;12(3):638-645. doi:10.1016/j.scr.2014.02.005. PMC4037447

48. Ma L, Li P, Shi Z, et al. A prospective, randomized, controlled study of hyperbaric oxygen therapy: effects on healing and oxidative stress of ulcer tissue in patients with a diabetic foot ulcer. Ostomy Wound Manage. 2013;59:18-24. PMID: 23475448

49. Zimanova J, Batora I, Dusinska M, Burghardtova K, Blazicek P, Vojtech I et al. Short term oxidative DNA damage by hyperbaric oxygenation in patients with chronic leg ulcers. Bratisl Lek Listy. 2011;112(8):447–452

50. Fosen KM, Thom SR. Hyperbaric Oxygen, Vasculogenic Stem Cells, and Wound Healing. *Antioxidants & Redox Signaling*. 2014;21(11):1634-1647. doi:10.1089/ars.2014.5940. PMID: 24730726

51. Kim YW, Byzova TV. Oxidative stress in angiogenesis and vascular disease. Blood. 2014;123(5):625-631. doi:10.1182/blood-2013-09-512749. PMID: 24300855

52. Alleva R, Tomasetti M, Sartini D, et al. α-Lipoic Acid Modulates Extracellular Matrix and Angiogenesis Gene Expression in Non-Healing Wounds Treated with Hyperbaric Oxygen Therapy. Molecular Medicine. 2008;14(3-4):175-183. doi:10.2119/2007-00095.

53. Khandelwal S, Chaudhary P, Poddar DD, Saxena N, Singh RA, Biswal UC. Comparative Study of Different Treatment Options of Grade III and IV Diabetic Foot Ulcers to Reduce the Incidence of Amputations. Clin Pract. 2013 Feb 21;3(1):e9. PMID: 24765502

54. Carter MJ, Fife CE, Bennett M. "Comment on: Margolis et al. lack of Effectiveness of hyperbaric oxygen therapy for the treatment of diabetic foot ulcer and the prevention of amputation: a cohort study. Diabetes Care 2013;36:1961-1966". Diabetes Care. 2013 Aug;36(8):e131. doi: 10.2337/dc13-0566. PMID: 23881984

55. Beckert S, Witte M, Wicke C, Königsrainer A, Coerper S. A new wound-based severity score for diabetic foot ulcers: a prospective analysis of 1,000 patients. Diabetes Care 2006;29:988–992

56. van Battum P, Schaper N, Prompers L, et al. Differences in minor amputation rate in diabetic foot disease throughout Europe are in part explained by differences in disease severity at presentation. Diabet Med 2011;28:199–205

57. Pearl J. Remarks on the method of propensity score. Stat Med 2009;28:1415–1416; author reply 1420–1423

58. Wang CJ, Wu RW, Yang YJ Treatment of diabetic foot ulcers: a comparative study of extracorporeal shockwave therapy and hyperbaric oxygen therapy. Diabetes Res Clin Pract. 2011 May;92(2):187-93. PMID: 21310502

59. Löndahl M1, Katzman P, Hammarlund C, Nilsson A, Landin-Olsson M. Relationship between ulcer healing after hyperbaric oxygen therapy and transcutaneous oximetry, toe blood pressure and ankle-brachial index in patients with diabetes and chronic foot ulcers. Diabetologia. 2011 Jan;54(1):65-8. PMID: 20957342

60. Löndahl M1, Landin-Olsson M, Katzman P Hyperbaric oxygen therapy improves health-related quality of life in patients with diabetes and chronic foot ulcer. Diabet Med. 2011 Feb;28(2):186-90. PMID: 21219427.

61. Duzgun AP, Satir HZ, Ozozan O, Saylam B, Kulah B, Coskun F. Effect of hyperbaric oxygen therapy on healing of diabetic foot ulcers. *Journal of Foot and Ankle Surgery* 2008; **47**(6):515–9. PMID: 19239860

62. Albuquerque E, Sousa J. Long-term evaluation of chronic diabetic foot ulcers, non-healed after hyperbaric oxygen therapy. Rev Port Cir Cardiotorac Vasc. 2005; 12(4):227-237. PMID: 16474863

63. Game FL, Apelqvist J, Attinger C, Hartemann A, Hinchliffe RJ, Löndahl M, Price PE, Jeffcoate WJ: International Working Group on the Diabetic Foot (IWGDF). "Effectiveness of interventions to enhance healing of chronic ulcers of the foot in diabetes: a systematic review." Diabetes Metab Res Rev. 2016 Jan;32 Suppl 1:154-68. doi: 10.1002/dmrr.2707. PMID: 26344936

64. Scottish Intercollegiate Guidelines Network (SIGN). SIGN 50: a guideline developer's handbook. Edinburgh: SIGN; 2015. (SIGN publication no. 50). Available from URL: http://www.sign.ac.uk. Accessed by web on April 4, 2016

65. Kranke P, Bennett MH, Martyn-St James M, Schnabel A, Debus SE. Hyperbaric oxygen therapy for chronic wounds. Cochrane Database Syst Rev. 2012;4:CD004123

66. Game FL, Hinchliffe RJ, Apelqvist J, Armstrong DG, Bakker K, Hartemann A, et al. A systematic review of interventions to enhance the healing of chronic ulcers of the foot in diabetes. Diabetes Metab Res Rev 2012;28(Suppl 1):119-41.

67. Goldman RJ. Hyperbaric oxygen therapy for wound healing and limb salvage: a systematic review. PM R 2009; 1:471-89.

68. Kaur S, Pawar M, Banerjee N, Garg R. Evaluation of the efficacy of hyperbaric oxygen therapy in the management of chronic nonhealing ulcer and role of periwound transcutaneous oximetry as a predictor of wound healing response: A randomized prospective controlled trial. J Anaesthesiol Clin Pharmacol 2012;28:70–5.

69. Fife CE, Smart DR, Sheffield PJ, Hopf HW, Hawkins G, Clarke D. Transcutaneous oximetry in clinical practice: consensus statements from an expert panel based on evidence. Undersea Hyperb Med 2009;36:43-53

70. Cychosz CC, Phisitkul P, Belatti DA, Wukich DK "Preventive and Therapeutic Strategies for Diabetic Foot Ulcers" Foot Ankle Int. 2016 Mar;37(3):334-43. doi: 10.1177/1071100715611951. Epub 2015 Oct 16. PMID: 26475457.

71. Löndahl M, Nilsson A, Katzman P, Hammarlund C. Hyperbaric oxygen therapy as adjunctive treatment of chronic diabetic foot ulcers. Diabetes Care 2010;33(5):998–1003.

72. Liu R, Li L, Yang M, Boden G, Yang G. Systematic review of the effectiveness of hyperbaric oxygenation therapy in the management of chronic diabetic foot ulcers. Mayo Clin Proc 2013; 88:166-75.

73. Fagher K, Katzman P, Löndahl M. "Hyperbaric oxygen therapy reduces the risk of QTc interval prolongation in patients with diabetes and hard-to-heal foot ulcers". J Diabetes Complications. 2015 Nov-Dec;29(8):1198-202. doi: 10.1016/j.jdiacomp.2015.07.023. PMID: 26321368

74. Undersea and Hyperbaric Medical Society (UHMS). "Arterial Inefficiencies: Enhancement of Healing in Selected Problem Wounds". in Hyperbaric Oxygen Therapy Indications: 13th Edition (April 2014). Editor Lindell Weaver, MD. ISBN: 978-1930536-73-9

75. Blake DF, Young DA, Brown LH. Transcutaneous oximetry: normal values for the lower limb. Diving and Hyperbaric Medicine. 2014 September;44(3):146-153

76. Lal C, Unni SN. Correlation analysis of laser Doppler flowmetry signals: a potential non-invasive tool to assess microcirculatory changes in diabetes mellitus. Med Biol Eng Comput. 2015 Jun;53(6):557-66. doi: 10.1007/s11517-015-1266-y. PMID: 25752769

77. Weingarten MS, Samuels JA, Neidrauer M, Mao X, Diaz D, McGuire J, McDaniel J, Jenkins L, Zubkov L, Papazoglou ES. "Diffuse near-infrared spectroscopy prediction of healing in diabetic foot ulcers: a human study and cost analysis" Wound Repair Regen. 2012 Nov-Dec;20(6):911-7. doi: 10.1111/j.1524-475X.2012.00843.x. PMID: 23110417

78. Neidrauer M, Zubkov L, Weingarten MS, Pourrezaei K, Papazoglou ES. Near infrared wound monitor helps clinical assessment of diabetic foot ulcers. J Diabetes Sci Technol. 2010 Jul 1;4(4):792-8. PMID: 20663439

79. Igari K, Kudo T, Uchiyama H, Toyofuku T, Inoue Y. Quantitative evaluation of microvascular dysfunction in peripheral neuropathy with diabetes by indocyanine green angiography. Diabetes Res Clin Pract. 2014 Apr;104(1):121-5. doi: 10.1016/j.diabres.2014.01.022. PMID:24552681

80. Forsythe RO, Hinchliffe RJ. Assessment of foot perfusion in patients with a diabetic foot ulcer. Diabetes Metab Res Rev. 2016 Jan;32 Suppl 1:232-8. doi: 10.1002/dmrr.2756. PMID: 26813616

81. Camporesi EM. Side effects. In: Hyperbaric Oxygen Therapy Indications, L.K. Weaver, Editor. 2014, Best Publishing: North Palm Beach, Florida. 247-252.

82. Estimates of the burden of diabetes on the United States (2014). Accessed March 2016. http://cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf

83. Baroni P., Bondioli E, Carboni A, Fasano A., Melandri D., Longobardi P., Tomasini I. Accelerated wound healing using Platelet Gel, Skin Graft and Hyperbaric Oxygenation. Proceedings EUBS Annual Meeting, Saint-Gilles-les-Bains (la Réunion), 23-28 Sep 2013.

84. Hoxha K., Baroni P., Bondioli E., Melandri D., Longobardi P., Tomasini I. Multidisciplinary approach for the enhancement of healing in selected problem wounds. Proceedings EUBS Annual Meeting, Wiesbaden (Germany), 24-28 Sep 2014

85. De Palma R, Nobilio L, Mall S, Trombetti S, Vizioli M, Melotti MR, Paganelli A, Grilli R. "Dossier n. 179/2009 [Abstract] Care profiles and costs for diabetic people in Emilia-Romagna". Agenzia Sanitaria e Sociale Regionale / Health and Social Care Regional Agency (Emilia Romagna). The document (in Italian with English abstract) can be downloaded in open access to the website http://assr.regione.emilia-romagna.it/it/servizi/pubblicazioni/dossier/doss179-abs (assessed by web on April 06, 2016)

86. Abidia A, Laden G, Kuhan G, et al. The role of hyperbaric oxygen therapy in ischaemic diabetic lower extremity ulcers: a double-blind randomised-controlled trial. Eur J Vasc Endovasc Surg. 2003; 25(6):513-518

87. Wattel FE, Mathieu DM, Fossati P, et al. Hyperbaric oxygen in the treatment of diabetic foot lesions. J Hyperbaric Med. 1991;6: 263–268.

88. Stoekenbroek RM1, Santema TB, Koelemay MJ, van Hulst RA, Legemate DA, Reekers JA, Ubbink DT "Is additional hyperbaric oxygen therapy cost-effective for treating ischemic diabetic ulcers? Study protocol for the Dutch DAMOCLES multicenter randomized clinical trial?" J Diabetes. 2015 Jan;7(1):125-32. doi: 10.1111/1753-0407.12155. Epub 2014 Apr 28. PMID: 24674297

89. Boulton AJ "The diabetic foot: grand overview, epidemiology and pathogenesis" Diabetes Metab Res Rev. 2008 May-Jun;24 Suppl 1:S3-6. doi: 10.1002/dmrr.833. PMID: 18442166

90. Fife C. E., Buyukcakir C., Otto G., Sheffield P., Love T., Warriner R., III Factors influencing the outcome of lower-extremity diabetic ulcers treated with hyperbaric oxygen therapy. *Wound Repair and Regeneration*. 2007;15(3):322–331. doi: 10.1111/j.1524-475X.2007.00234.x

91. Posnett J, Franks PJ. The burden of chronic wounds in the UK. Nurs Times 2008;104(3): 44–5

92. Age-adjusted hospital discharge rates for nontraumatic lower extremity amputation (LEA) per 1,000 diabetic population, by level of amputation, United States, 1993e2009. Atlanta, Ga: Centers for Disease Control and Prevention (CDC), National Center for Health Statistics, Division of Health Interview Statistics; 2012

93. Gandhi GY, Murad MH, Fujiyoshi A, Mullan RJ, Flynn DN, Elamin MB, et al. Patient-important outcomes in registered diabetes trials. JAMA 2008; 299:2543-9

94. Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. *World Journal of Diabetes*. 2015;6(1):37-53. doi:10.4239/wjd.v6.i1.37.

95. Deliberation of the Emilia Romagna Regional Council of 20 October 2014, number 1673 "Determination of fees for hospital care in public and accredited private structures of the Emilia Romagna Region applicable from 1.1.2014" Official Bulletin of the Emilia Romagna Region, Part II, n . 137 of 30 October 2014, page 33 (in Italian)