Adjunctive Systemic Hyperbaric Oxygen Therapy in Treatment of Severe Prevalently Ischemic Diabetic Foot Ulcer

A randomized study

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OBJECTIVE — To evaluate the effectiveness of systemic hyperbaric oxygen therapy (s-HBOT) in addition to a comprehensive protocol in decreasing major amputation rate in diabetic patients hospitalized for severe foot ulcer.

RESEARCH DESIGN AND METHODS — From August 1993 to August 1995, 70 diabetic subjects were consecutively admitted into our diabetologic unit for foot ulcers. All the subjects underwent our diagnostic-therapeutic protocol and were randomized to undergo s-HBOT. Two subjects, one in the arm of the treated group and one in the arm of nontreated group, did not complete the protocol and were therefore excluded from the analysis of the results. Finally, 35 subjects received s-HBOT and another 33 did not.

RESULTS — Of the treated group (mean session = 38.8 ± 8), three subjects (8.6%) underwent major amputation: two below the knee and one above the knee. In the nontreated group, 11 subjects (33.3%) underwent major amputation: 7 below the knee and 4 above the knee. The difference is statistically significant ($P = 0.016$). The relative risk for the treated group was 0.26 (95% CI 0.08–0.84). The transcutaneous oxygen tension measured on the dorsum of the foot significantly increased in subjects treated with hyperbaric oxygen therapy: 14.0 ± 11.8 mmHg in treated group, 5.0 ± 5.4 mmHg in nontreated group ($P = 0.0002$). Multivariate analysis of major amputation on all the considered variables confirmed the protective role of s-HBOT (odds ratio 0.084, $P = 0.033$, 95% CI 0.008–0.821) and indicated as negative prognostic determinants low ankle-brachial index values (odds ratio 1.715, $P = 0.013$, 95% CI 1.121–2.626) and high Wagner grade (odds ratio 11.199, $P = 0.022$, 95% CI 1.406–89.146).

CONCLUSIONS — s-HBOT, in conjunction with an aggressive multidisciplinary therapeutic protocol, is effective in decreasing major amputations in diabetic patients with severe prevalently ischemic foot ulcers.

Systemic hyperbaric oxygen therapy (s-HBOT) has been used in the treatment of diabetic wounds (1) but opinions differ regarding its effectiveness (2,3). The aim of this study has been to evaluate the effectiveness of s-HBOT in decreasing major amputation (thigh or ankle) rate in a randomized record of cases of diabetic patients hospitalized for foot ulcer.

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Received for publication 1 April 1996 and accepted in revised form 8 August 1996.

ABI, ankle-brachial index; ATA, absolute atmosphere; BPG, bypass graft; PTA, percutaneous transluminal angioplasty; ROC, receiver operating curve; s-HBOT, systemic hyperbaric oxygen therapy; TcPo2, transcutaneous oxygen tension.

RESEARCH DESIGN AND METHODS

Study Design

From August 1993 to August 1995, 70 diabetic subjects, consecutively hospitalized in our diabetologic unit for foot ulcer, underwent our diagnostic and therapeutic protocol. All the subjects were randomized for s-HBOT treatment. All patients gave their informed consent. One subject randomized for s-HBOT refused the treatment; one subject, randomized for the non–s-HBOT, died of an acute stroke 6 days after admission. Both these subjects were excluded from the analysis of the results. Of the subjects, 35 underwent s-HBOT and 33 did not. Clinical characteristics of the study population are shown in Table 1. After randomization, none of the variables listed in Table 1 showed a significant imbalance between the treatment and control arm.

Diagnostic and therapeutic protocol

On admission to the hospital, lesions were classified according to Wagner (4). In our clinical practice, diabetic subjects with full-thickness gangrene (Wagner grade IV) or abscess (Wagner grade III) were admitted to hospital. Subjects with less-deep ulcers (Wagner grade II) were also admitted if the ulcer was large and infected and showed a defective healing in 30 days of outpatient therapy. All patients were examined for diabetic retinopathy (fundus oculi by ophthalmologist), albumin excretion rate (mg/24 h, the average of three 24-h collections, nephelometry-Behring), renal impairment (creatinine >133 μmol/l, Jaffe-Boehringer Mannheim), arterial hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg or antihypertensive therapy), coronary artery disease (CAD) (CAD-resting electrocardiogram and B-mode echocardiography), obesity (BMI >24 kg/m² for women, >25 kg/m² for men), dyslipidemia (total cholesterol >6.20 mmol/l, colorimetry, Boehringer

Table 1—*Clinical characteristics of s-HBOT and non–s-HBOT groups*

	s-HBOT group	non–s-HBOT group	*P* value
n	35	33	
Men	27 (77.1)	21 (63.6)	0.29
Women	8 (22.9)	12 (34.4)	
Age (years)	61.7 ± 10.4	65.6 ± 9.1	0.10
Insulin therapy	21 (60)	22 (66.7)	0.62
Oral therapy	14 (40)	11 (33.3)	
Diabetes duration (years)	16 ± 10	19 ± 9	0.20
Wagner grade			
II	4 (11.5)	5 (15.2)	0.94
III	9 (25.7)	8 (24.2)	
IV	22 (62.8)	20 (60.6)	
Prior major amputation	0 (—)	0 (—)	—
Prior minor amputation	6 (17.1)	10 (30.3)	0.25
Prior lesion	9 (25.7)	12 (36.4)	0.43
Background retinopathy	12 (34.2)	13 (39.4)	0.80
Proliferant retinopathy	13 (37.1)	9 (27.3)	0.44
Microalbuminuria	12 (34.3)	9 (27.3)	0.60
Proteinuria	8 (22.8)	7 (21.2)	1.00
Renal impairment	4 (11.4)	9 (27.3)	0.13
Hypertension	19 (54.2)	17 (51.6)	1.00
Hyperlipidemia	11 (31.4)	8 (24.2)	0.13
Obesity	9 (25.7)	9 (27.3)	1.00
Smoking habit	11 (31.4)	12 (36.4)	0.50
Coronary artery disease	14 (40)	15 (45.4)	1.00
Prior stroke	3 (8.6)	4 (12.1)	0.79
Infection	32 (91.4)	28 (84.8)	0.80
Polimicrobial infection	20 (57)	17 (51.6)	0.80
Infection recovery	26 (74.2)	17 (51.6)	0.08
Bone lisis	11 (31.4)	9 (27.3)	0.79
Ostepenia	15 (42.8)	21 (63.6)	0.09
Monckeberg sclerosis	21 (60)	20 (60.6)	1.00
Peripheral angiography	31 (88.5)	26 (78.8)	0.33
HbA_{1c} at admission (%)	9.3 ± 2.5	8.5 ± 2.3	0.17
HbA_{1c} at discharge (%)	7.1 ± 1.5	6.6 ± 1.2	0.13
Total hospital stay (days)	43.2 ± 31	50.8 ± 32	0.37

Data are means ± SD or *n* (%). *P* values were determined by a two-tailed Fisher's exact test for discrete variables and by an unpaired Student's *t* test for continuous variables. Microalbuminuria was defined as an albumin excretion rate ≥20 and <200 mg/24 h; proteinuria was defined as an albumin excretion rate >200 mg/24 h; hypertension was defined according to World Health Organization criteria or antihypertensive treatment; hyperlipidemia was defined by total cholesterol >6.20 mmol/l and/or HDL cholesterol <0.90 mmol/l for men and <1.16 mmol/l for women and/or triglyceride count >200 mg/dl and/or hypolipidemic treatment; obesity was defined as a BMI >24 and >25 kg/m² for women and men, respectively; and bone lisis and ostepenia were determined by radiographic findings.

Mannheim; and/or HDL cholesterol <0.90 mmol/l for men and <1.16 mmol/l for women, Polyethylene glycol 6000, Clinicals; and/or triglycerides >2.25 mmol/l, colorimetry, Ames; or hypolipidemic therapy). On admission and at discharge, glycosylated hemoglobin levels (HbA_{1c} high-pressure liquid chromatography, normal values 4.4–6%) were measured. Specimens of the foot lesion, after decontamination and debridement followed by curettage (5), were collected for aerobic and anaerobic culture and for antimicrobial susceptibility testing to antibiotics. Susceptibility testing to topical antimicrobial agents was also performed according to a standardized protocol set up in our microbiology laboratory (6). X-rays were taken of both feet and legs to discover medial arterial calcifications and bone abnormalities. The sensorimotor neuropathy (7) was investigated with electromyography in all subjects (considered present when showing abnormalities of nerve conduction velocity and sensory action potential in at least two nerves). The autonomic neuropathy (8) (present if the score was >4 in the five standard autonomic cardiovascular tests) and the vibration sense (9) (impaired if the vibration perception threshold measured on the malleolus with biothesiometer was >25 V) were investigated when technically possible in collaborative patients. The ankle-brachial blood pressure ratio (ankle-brachial index [ABI]) was measured by Doppler continuous wave technique. The transcutaneous oxygen tension ($TcPo_2$) was measured on the dorsum of the foot on admission to hospital and on discharge for the subjects with salvaged limbs and before amputation in subjects undergoing amputation. In Table 2, the assessment of neuropathy and vasculopathy in the study population is reported. The values of these parameters showed no significant imbalance between the two arms. In the subjects undergoing s-HBOT, $TcPo_2$ was also measured during treatment in the hyperbaric chamber.

In all subjects an aggressive and radical debridement was performed by a consultant surgeon. After surgical curettage the wound was cleaned with uncolored topical antimicrobial agents and wadded with occlusive dressing (10). Dressing, with debridement if necessary, was carried out not less than twice a day when necrosis or exudate were present, daily when the ulcer was clean, and every two days during the granulation period. On admission to hospital all patients—after collecting a specimen of the ulcer for culture examination—were given empirical broad-spectrum antibiotic therapy, subsequently modified if necessary, according to susceptibility testing results. The antibiotic therapy was continued during the hospital stay until the culture exam, repeated each week, was negative. After discontinuation of the antibiotic therapy, reculturing to assess the cure was performed every two days a total of three times. An optimized metabolic control was pursued either with subcutaneous insulin administrations or oral hypoglycemic agents, according to blood glucose determinations, 7 times/day. For blood glucose levels >22 μmol/l, a procedure of intravenous insulin infusion was administered according to an algorithm based on the assessment of blood glucose levels every 2 h, until blood glucose value < 9.9 μmol/l was reached. In the subjects with ABI <0.9 and/or $TcPo_2$ <50 mmHg, a therapy with prostacyclin was established and an arteriography by

Table 2—*Assessment of peripheral vasculopathy and neuropathy in s-HBOT and non–s-HBOT groups*

	s-HBOT group	non–s-HBOT group	*P* value
n	35	33	
Claudication	4 (11.4)	10 (30.3%)	0.07
ABI	0.65 ± 0.28	0.64 ± 0.25	0.87
$TcPo_2$ (mmHg)	23.25 ± 10.6	21.29 ± 10.7	0.45
Sensorimotor neuropathy	35 (100)	31 (93.9)	0.23
Impaired vibration sense	24 (85.7)	23 (85.2)	1.00
Autonomic neuropathy	17 (73.9)	15 (71.4)	0.70

Data are means ± SD or *n* (%). *P* values were determined by a two-tailed Fisher's exact test for discrete variables and by an unpaired Student's *t* test for continuous variables. ABI is the ankle-brachial blood pressure ratio measured by the Doppler technique; sensorimotor neuropathy was defined by abnormalities in ≥2 nerves at electromiography; impaired vibration sense was defined by a vibration perception threshold >25 V at biotesiometry; and autonomic neuropathy was defined by a score >4 at five cardiovascular tests.

intra-arterial digital subtraction technique was performed if there were no contraindications (creatinine >221 μm/l, or paraproteinemia) (11). In these subjects the opportunity and possibility of carrying out a percutaneous transluminal angioplasty (PTA) or a bypass graft (BPG) was assessed. The presence of focal stenoses involving >50% of vessel lumen was considered an indication of PTA. The stenoses completely occluding the lumen or with length >10 cm were respectively considered as an impossibility or a contraindication for PTA. When there was an impossibility of performing PTA, the arteriogram was evaluated by vascular surgeons to carry out a BPG. Based on angiographic criteria bypasses were performed when a patent vessel in continuity with the foot was present. During hospitalization, all patients were provided with orthopedic devices to remove mechanical stress and pressure at the site of the ulcer, while maintaining ambulation. The orthesis was made up of an Alkaform insole molded in plastic cast and an extra deep special shoe with a rigid sole (Buratto, Italy) allowing the insertion of a bandaged foot.

The limb was considered salvaged when the plantar support was preserved and the ulcer healed despite minor amputations (toe or forefoot amputation), as they are lost in presence of major amputation (above or below the knee). The decision to carry out a major amputation was taken by the consultant surgeon who was unaware of whether the s-HBOT was administered or not.

s-HBOT

In the group randomized for s-HBOT, the patients breathed pure oxygen in a multiplace hyperbaric chamber, pressurized with air, with a soft helmet. The chosen pressure, in our study, was 2.5 absolute atmosphere (ATA) in the first phase, to enhance the antibacterial effect and to quickly restore a sufficient tissue partial pressure of oxygen. In the second phase, to stimulate a fibroblastic activity for reparative effect, we applied 2.4–2.2 ATA. Our scheme considers a daily session (90 min for each session) in the first phase, and an hebdomadal (5/7) session in the reparative phase.

Statistical analysis

The sample size (12) of 34 patients/arm was decided to detect a reduction of 1/3 of major amputation rate with type-one error, $\alpha = 0.05$, and power 1, $\beta = 0.80$ (two-sided test). The randomization schedule adopted requires that a patient should be allocated to the treatment arm after hospital admission by consulting a table of random numbers at the hospital data elaboration center. Fisher's exact test was used for comparison of discrete variables between the two arms. The relative risk and the Wolf CIs were estimated for the comparison of major amputation rates. The student's *t* test for unpaired data was used for the comparison of continuous variables (13). Multivariate logistic regression (Stata Statistical Software, Stata, 1995) of major amputation has been performed considering the covariates listed in Tables 1, 2, and 5 and found to be significant in the univariate analysis. The relative odds ratio and 95% CIs were calculated. The global validity of the multivariate statistical model was tested using logistic receiver operating curve (ROC).

RESULTS — The subjects who underwent s-HBOT attended an average of 38 ± 8 sessions. Two subjects showed symptoms of barotraumatic otitis, which did not cause the interruption of treatment. In the s-HBOT group, three subjects underwent a major amputation (8.6%): one above the knee and two below the knee. In the non–s-HBOT group, 11 subjects underwent major amputation (33.3%): 4 above the knee and 7 below the knee. The difference is statistically significant ($P = 0.016$) (Table 3).The relative risk for the subjects treated with s-HBOT is 0.26 (95% CI 0.08–0.84). Major amputation was performed in the patients of the s-HBOT group after 57.6 ± 24 days (range: 31–78) from hospital admission, and after 72.8 ± 59 days (range: 26–176) in the patients of the non–s-HBOT group. In Table 3 the major amputation rate related to Wagner grade, and the minor amputa-

Table 3—*Major and minor amputation rates of s-HBOT and non–s-HBOT groups*

	s-HBOT group	non–s-HBOT group	*P* value
n	35	33	
Major amputations			
Amputated limbs	3 (8.6)	11 (33.3)	0.016
Salvaged limbs	32 (91.4)	22 (66.7)	
Major amputation/Wagner grade			
II	0/4 (—)	0/5 (—)	—
III	1/4 (25)	0/8 (—)	0.33
IV	2/22 (9.1)	11/20 (55)	0.002
Minor amputations			
Forefoot	5 (14.3)	4 (12.1)	0.61
Toe	16 (45.7)	8 (24.2)	
No amputation	11 (31.4)	10 (30.3)	

Data are *n* (%). *P* values were determined by a two-tailed Fisher's exact test.

Table 4—*$TcPO_2$ values of s-HBOT and non–s-HBOT groups at admission and at discharge; comparison of increase between the two groups*

	s-HBOT group	non–s-HBOT group	*P* value
n	35	33	
At admission	23.2 ± 10.7	21.3 ± 10.7	0.46
At discharge	37.3 ± 16.1	26.3 ± 13.5	—
Variation	14.0 ± 11.8	5.0 ± 5.4	0.0002

Data are means ± SD and are given as $TcPO_2$ (mmHg). *P* values were determined by an unpaired Student's *t* test [Satterthwaite (13) degrees of freedom: 48.25].

tion rate between the two arms was also compared.

The comparison between the values of $TcPO_2$ on admission and at discharge or before amputation are shown in Table 4. The increase of $TcPO_2$ in the s-HBOT group is 9.05 mmHg higher than that of the non–s-HBOT group ($P = 0.0002$). The mean "jump" of $TcPO_2$ level in hyperbaric chamber was 493.5 ± 152.1 mmHg. Vascular procedures were performed in 26 subjects (17 PTAs, 9 BPGs). The PTA was performed as follows: 6 in the iliac or femoral arteries (3 in the s-HBOT and 3 in the non–s-HBOT group) and 11 in the popliteal or infrapopliteal arteries (6 in the s-HBOT and 5 in non–s-HBOT group). All the BPG were carried out using saphenous vein in situ: in four the site of distal anastomosis was the popliteal or infrapopliteal arteries (two in the s-HBOT and three in the non–s-HBOT group) and in five the dorsalis pedis artery (two in the s-HBOT and two in the non–s-HBOT group). The outcome of vascular procedures is shown in Table 5.

Multivariate analysis, carried out on the variables found associated with major amputation in the univariate analysis, showed the protective role of s-HBOT (odds ratio 0.084, $P = 0.033$, 95% CI 0.008–0.821) and indicated as negative prognostic determinants Wagner grade (odds ratio 11.199, $P = 0.022$, 95% CI 1.406–89.146) and ABI value reduction (odds ratio 1.715, $P = 0.013$, 95% CI 1.121–2.626). The calculated odds ratio for ABI values express the increased risk of major amputation per 0.1 unit decrease of the variable itself. The area under the ROC curve calculated from the multivariate logistic model is 0.9501 (Fig. 1).

CONCLUSIONS — Diabetic ulcers frequently do not heal because of a combination of hypoxia and infection (14). Systemic hyperbaric oxygen greatly increases tissue oxygen levels even though treatment is only partial throughout the day: oxygen tension values by $TcPO_2$ remain elevated for several hours after exposure (15). The outcome of breathing O_2 hyperbarism is an increase in the diffusion of oxygen physically dissolved in plasma. The supplying of oxygen to the cells carried out by the counter-gradient diffusion of concentration sustains, in a hypoxic situation, mitochondrial breathing and cell survival, so preventing necrotic development of the tissue. Experiments on animals have proved this theory (16). The index of oxygenation and hematic level of the lactates, after occlusion of the femoral artery or the abdominal aorta, show that an inhalation of 100% oxygen under pressure of 3 ATA maintains an elevated level of oxygen partial pressure and reduces the lactates, in contrast with what happens in a normobaric condition (17). s-HBOT generates a vascular constriction as a reflex mechanism defending against hyperoxia (18). In the hypoxic tissue this mechanism is able to oppose the compensative vasodilatation of the hypoxia, and it leads to a reduction of the edema, which is often present in the diabetic foot (19), with a subsequent improvement of the microcirculatory flow (20).

s-HBOT has significant direct and indirect effects on the infection. s-HBOT has a direct antibacterial effect on the anaerobic microorganisms. Both the production of toxins and the growth of these bacteria are completely inhibited by a high level of oxygen (21). Moreover, by maintaining an O_2 partial pressure higher than 30 mmHg at tissue level, the hyperbarism allows the macrophagic killing activity O_2 dependent (22). The defective wound healing appears to be an important factor contributing toward limb loss in diabetic subjects (23): a restoration of tissue oxygen tension by s-HBOT also assists angiogenesis and advancement into the wound space (24).

On this basis, for some time now we have introduced s-HBOT in our therapeutic protocol (25). However, although other studies have reported increased limb salvage with s-HBOT (26,27), support for this treatment has been judged questionable (3), and even in a recent review the s-HBOT was not included in the armamentarium of diabetic foot care (28). This was due to the fact that the results on the effectiveness of s-HBOT were based on nonrandomized studies (3). This study shows that s-HBOT is effective in decreasing major amputations in diabetic subjects with foot ulcers. This result was obtained in patients with severe foot ulcer; Wagner grade IV was the most frequent in our study population. Although in our study population there were many subjects with neuropathy and infection, it is likely that the arterial insufficiency, which was considered the most typical characteristic of Wagner grade IV, is the predominant factor leading to major amputation. This consideration is confirmed by the indication of low ABI value and high Wagner grade as negative independent prognostic determinants for major amputation in the multivariate analysis. The ROC curve shows an excellent predictive value of this model in accordance with a wide range of odds ratio. Our data suggest that s-HBOT is

Table 5—*Outcome of vascular procedures in s-HBOT and non–s-HBOT groups*

	s-HBOT group	non–s-HBOT group	*P* value
n	35	33	
Peripheral BPG	4/35 (11.4)	5/33 (15.1)	0.73
Major amputation	1/4 (25)	1/5 (20)	1.00
Percutaneous angioplasty	9/35 (25.7)	8/33 (24.2)	1.00
Major amputation	1/9 (11.1)	3/8 (37.5)	0.29
Total vascular procedures	13/35 (37.1)	13/33 (39.4)	1.00
Total major amputations	2/13 (15.4)	4/13 (30.8)	0.60

Data are *n* (%). *P* values were determined by a two-tailed Fisher's exact test.

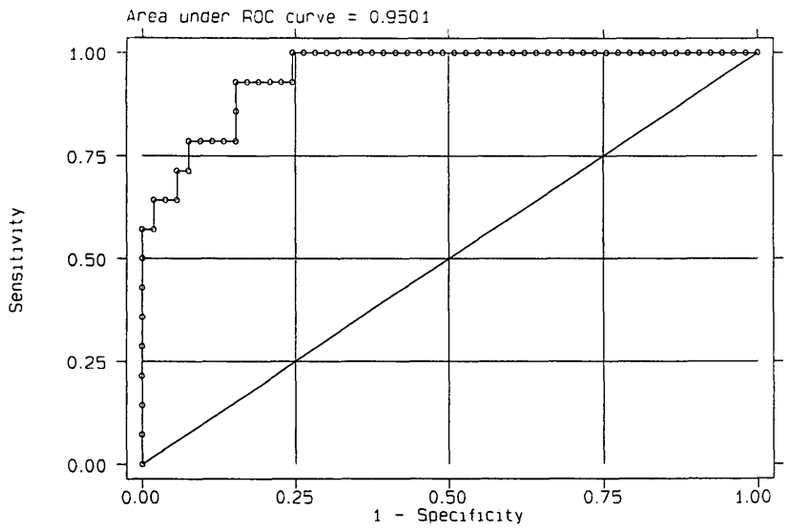

Figure 1—*ROC curve calculated from the multivariate logistic model estimated on the study population. The axes of the graph are the specificity and sensibility of the set of variables significant in predicting major amputation (the perfect prediction corresponds to an area = 1).*

indicated in subjects with severe foot ulcer and with a peripheral arterial obstructive disease. In our clinical experience, patients with neuropathic ulcer without arterial obstructive disease heal as outpatients and oxygen supply is not requested. However, we have no experimental data to confirm this observation; s-HBOT might be helpful in these subjects especially in the presence of severe infection, which itself can cause a tissue hypoxia, and is definitely advisable in the presence of gas gangrene.

s-HBOT improves cutaneous oxygenation: low $TcPo_2$ is a strong risk factor for major amputation (29). In our study, the non–s-HBOT group also showed an increase in $TcPo_2$ values that was not statistically significant. This mild improvement is attributable to the effect of the whole medical therapy, especially with vasoactive drugs. The higher lasting and statistically significant increase of $TcPo_2$ values in the s-HBOT group suggests that this treatment adds a further positive effect. This durable increase of $TcPo_2$ values can be attributable to the capacity of s-HBOT to induce a new microvessel gemmation, which improves the tissue perfusion (24,30). The positive effect of s-HBOT is always attributable to an improvement of the microcirculatory flow. The objective to restore the blood flow in the large vessels, when critical stenoses are present, is entrusted to vascular procedures: the s-HBOT is not an alternative therapy because the action processes are very different. We are convinced that only the joint use of all the various available therapeutic procedures can give the best results in limb salvage in diabetic foot care. We believe that s-HBOT is effective in conjunction with a comprehensive multidisciplinary protocol and should not be substitutive but additional to other therapeutic procedures. In conclusion, the s-HBOT is a useful tool in the armamentarium of diabetic foot care, especially in the treatment of prevalently ischemic severe foot ulcers.

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