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The Effect of Transcutaneous Application of Gaseous CO_2 on Diabetic Symmetrical Peripheral Neuropathy—A Double-Blind Randomized Clinical Trial

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Abstract: Aim: Diabetic symmetrical peripheral neuropathy is a common complication of diabetes mellitus. Patients treated with transcutaneous CO_2 application for chronic wounds reported an improvement in peripheral sensations. This study aimed to evaluate the effect of transcutaneous application of gaseous CO_2 on diabetic symmetrical peripheral neuropathy. Methods: A prospective randomized, double-blind study was performed at the University Medical Center Ljubljana between September 2019 and September 2020. Sixty consecutive patients with diabetes with a unilateral chronic wound were randomized into either a study group that received transcutaneous CO_2 therapy or a control group that received placebo treatment with air. Results: Vibration, monofilament sensation, and temperature of the big toe improved significantly in the study group ($p < 0.001$, for vibration sensation, monofilament test and temperature of the big toe), but not in the control group (p = ns for all evaluated outcomes). Conclusion: According to our results, a transcutaneous application of gaseous CO_2 shows promising results in treating diabetic symmetrical peripheral neuropathy. Considering the major consequences of sensory loss leading to foot ulceration and possibly amputation, we believe this treatment approach deserves future attention and investigation as a treatment modality of diabetic symmetrical peripheral neuropathy.

Keywords: diabetic peripheral neuropathy; carboxytherapy; CO_2 therapy; diabetic foot; transcutaneous CO_2 therapy

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1. Introduction

Diabetic symmetrical peripheral neuropathy (DSPN) is a well-known complication of diabetes mellitus types 1 and 2 [1–4]. Symptoms may be diverse, depending on the affected nerve fibers [5]. The first symptoms usually include impaired thermal sensation, pain, or unpleasant sensation in the feet, especially at night. As the impairment of nerve fibers progresses, patients typically complain of numbness, tingling, pain, weakness, and hypo- and hyperesthesia; the latter two begin in the feet and spread in a stocking distribution. Symptoms are typically symmetric; however, sensory symptoms are more pronounced than motor symptoms [2–4,6,7]. The major underlying cause of DSPN is hyperglycemia, although dyslipidemia also plays an important role in diabetes mellitus type 2 [7]. Nerve blood flow impaired due to hyperglycemia increases oxidative stress and inflammation, usually first affecting small-diameter sensory nerves responsible for temperature sensation and painless injury [5,6,8]. The impairment of large fiber sensory nerves responsible for kinesthetic senses of joints and vibration is exhibited in decreased ankle reflexes and a loss

of balance while walking [3,4,7]. Patients reporting a loss of sensation are at a higher risk of developing foot ulcers [5].

Maintaining normoglycemia seems to be the only preventive measure to avoid or postpone DSPN. According to clinical trials, enhanced glucose control proved much more effective in type 1 diabetes than type 2 [7]. Despite careful treatment, the probability of developing DSPN over the years is approximately 30% and 50% for diabetes type 1 and type 2, respectively [8].

Current treatment protocols include pain relief with opioids, antidepressants, anticonvulsants and many other medications still being evaluated and investigated [7–10]. Because of limited effectiveness and known adverse effects, non-pharmacological treatment approaches like transcutaneous electrical nerve stimulation and monochromatic infrared energy therapy have been investigated [10–15].

Recently, our group investigated the effect of transcutaneous application of gaseous CO_2 on diabetic chronic wound healing [16]. After only a few treatment sessions, patients reported finally sleeping through the night because the burning and tingling sensations in their feet had diminished or had even disappeared, which led us to perform further investigations. As described in our previous article, transcutaneous application of CO_2 seems to have an immediate effect on vasodilatation and elevates the oxygen release from Hb via the Bohr effect [16–19]. It seems that, as the therapy is repeated, neoangiogenesis is induced [20,21]. Since microvascular dysfunction plays an important role in DSPN, we expected transcutaneous CO_2 application to improve sensation in patients with DSPN [6,22]. Therefore, this study aimed to evaluate the effect of transcutaneous application of gaseous CO_2 on diabetic symmetrical peripheral neuropathy.

2. Materials and Methods

The research was designed as a prospective randomized, double-blind study performed at the University Medical Center Ljubljana between September 2019 and September 2020 (Clinicaltrial.gov ID: NCT04561609). The study included patients with diabetes with a unilateral chronic wound and symptoms of DSPN with scores above three on the Michigan neuropathy scoring instrument (MNSI) [23]. This study included only patients without previous amputations to fully evaluate the vibration sensation and monofilament test on all typical points. Patients with asymmetrical neuropathy due to other causes were excluded. Additionally, patients with comorbidities where CO_2-induced vasodilatation could theoretically worsen their condition (deep vein thrombosis, chronic kidney diseases grade III and IV, chronic heart diseases NYHA III and IV, patients with known malignant diseases, patients with progressive infection, signs of systemic infection with elevated inflammatory markers, or osteomyelitis) and patients with alcohol abuse were also excluded.

Sixty patients who met the defined period's inclusion criteria were randomized into a study or a control group. The detailed description of the transcutaneous CO_2 therapy using a peripheral vascular rehabilitation system (PVR System; Derma Art, Brežice, Slovenia) is described in a previous publication [16]. Patients in the study group received transcutaneous CO_2 therapy for 45 min per session every day (from Monday to Friday) for four weeks (20 sessions altogether). Instead of gaseous CO_2, the air was used as a placebo treatment on patients in the control group. Before inclusion, patients were familiarized with the study protocol, received all the relevant information on the study from the medical staff, and afterward gave their written informed consent. Both patients and the doctor who evaluated their sensation status before and after the completing treatments were blind for group assignment (study or control). The nurse performing the transcutaneous CO_2 therapy used a random number generator to randomize patients into one of the two groups. After completing the study, data were compared for the two groups, which were subsequently disclosed as study or control groups.

The basic patient characteristics are presented in Table 1.

Table 1. Basic characteristics of the study and the control groups.

	Study Group	Control Group	*p*
No. of patients	30 (24 M, 6 F)	30 (24 M, 6 F)	1.000
Average age of patients (years ± SD)	65.3 ± 11.9	66.76± 10.6	0.640
BMI (mean kg/m^2 ± SD)	29.85 ± 5.0	30.2 ± 4.9	0.756
Tobacco smokers yes/no	4/26	3/27	1.000
HbA1c before therapy (mean% ± SD)	7.08 ± 0.85	7.00 ± 0.90	0.736
Diabetes mellitus type (type 1/type 2)	1/29	2/28	0.999
Average score on Michigan neuropathy scoring instrument (score ± SD)	8.23 ± 2.61	8.67 ± 2.55	0.518

Legend: BMI, body mass index; F, females; M, males.

The main outcome measure was an improvement of DSPN based on vibration sensation, monofilament test. Additionally, we measured the toe skin temperature.

Vibration sensation (measured before the first and after the last CO_2 or placebo therapies) was performed using a 128 Hz tuning fork on five standard points on each foot (on the skin above the bony prominence of the first metatarsophalangeal joint (MPT), the base of the hallux, malleolus, diaphysis of the tibia, and tibia tuberosity). Results are presented as the number of points or scores (out of ten) with no vibration sensation [24]. Monofilament testing was performed before the first and after the last CO_2 or placebo therapies using Semmes–Weinstein monofilament (SWM) 10 g on eight standard points of each foot (on the plantar surface of the big toe, the third and fifth toes, on the plantar surface of the first, third and fifth metatarsal heads, on the plantar arch and the plantar surface of the calcaneus). Results are presented as the number of points or scores (out of 16) with no sensation [25]. An additional outcome measure was the temperature measured on the plantar aspect of the big toe using an infrared thermometer (Voltcraft IR 260-8S, Voltcraft, London, United Kingdom). Temperature measurement was standardized—before the first treatment and before the 20th treatment (to exclude increased temperature during CO_2 therapy due to vasodilatation). Patients were left to lie on the examination table barefoot for 20 min to adjust to the room temperature.

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (approval ID: KME 84/02/16).

For statistical analysis, a chi-squared test was used for binominal variables (sex, diabetes mellitus type and smoking status), and a paired T-test was performed to compare the continuous variables before and after treatment and between the groups. The mean differences and 95% confidence intervals (95% CI) were calculated with two-sided probability (*p*) values. The level of significance was set at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics, v. 24 (IBM Corp, Armonk, NY, USA).

3. Results

Table 2 shows the vibration sensation and monofilament test values and the temperature of the big toe before and after CO_2 or placebo treatments. Results show that before the treatment, the mean values of vibration sensation, monofilament test and big toe temperature were comparable between the groups ($p_{\text{ vibration }}^{\text{ ac}} = 0.841$; $p_{\text{ monofilament }}^{\text{ ac}} = 0.127$; $p_{\text{ temperature }}^{\text{ ac}} = 0.415$).

Table 2. Vibration sensation, monofilament test and temperature of the big toe before and after treatment in both legs for the study and control groups ([a] study group before therapy, [b] study group after therapy, [c] control group before therapy, [d] control group after therapy).

	Study Group (N = 30)		Control Group (N = 30)	
	Before CO_2 Therapies [a]	After CO_2 Therapies [b]	Before placebo treatment [c]	After placebo Treatment [d]
Vibration sensation score	5.467 ± 2.573	2.583 ± 2.173	5.333 ± 2.537	4.867 ± 2.596
	p vibration $^{ab} < 0.001$		p vibration $^{cd} = 0.242$	
Monofilament test score	11.700 ± 4.417	5.183 ± 3.244	10.083 ± 3.618	8.917 ± 4.027
	p monofilament $^{ab} < 0.001$		p monofilament $^{cd} = 0.484$	
Temperature in °C on the big toe	26.277 ± 2.875	30.651 ± 1.611	26.882 ± 2.828	27.263 ± 2.968
	p temperature $^{ab} < 0.001$		p temperature $^{cd} = 0.612$	

Legend: results are presented as (mean ± SD).

Vibration, monofilament sensation, and the big toe temperature improved significantly in the study group (see p vibration ^{ab}, p monofilament ^{ab} and p temperature ^{ab}), but not in the control group (see p vibration ^{cd}, p monofilament ^{cd} and p temperature ^{cd}).

After the treatment, the vibration sensation, monofilament test and toe temperature between the groups differed significantly (p vibration $^{bd} = 0.001$; p monofilament $^{bd} < 0.001$; p temperature $^{bd} < 0.001$).

No significant differences were noted in measured parameters between the leg with a chronic wound and the contralateral leg before or after the CO_2 therapy. Improvement in vibration sensation, monofilament test, and temperature of the big toe after CO_2 therapy was comparable in the leg with a chronic wound and in the contralateral leg without a wound (Figures 1–3). There was no difference in the observed parameters in any leg in the control group (Figures 1–3). Figures 1–3 present monofilament test, vibration sensation and temperature of the big toe before and after CO_2 treatment in the study and control groups—a comparison between the leg with a chronic wound and the contralateral leg without a wound.

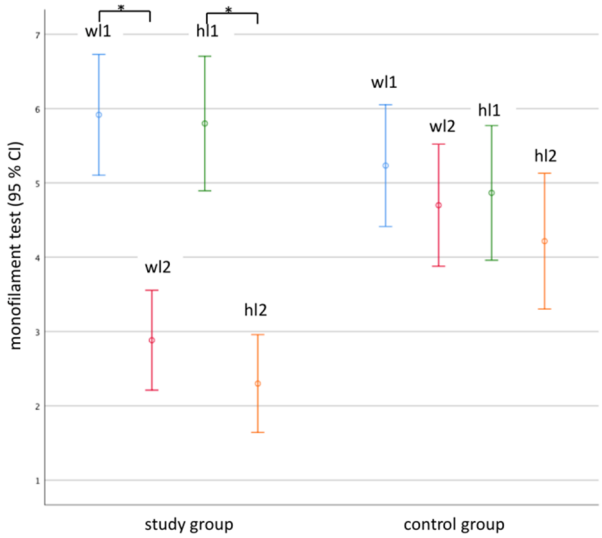

Figure 1. Monofilament test before and after the treatment. * $p < 0.001$.

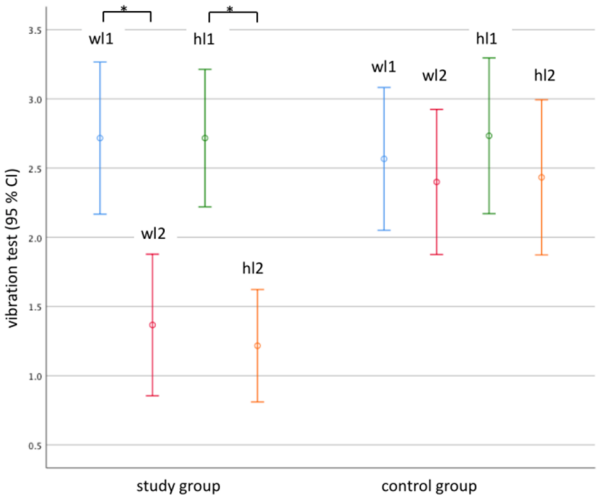

Figure 2. Vibration sensation test before and after the treatment. * $p < 0.001$.

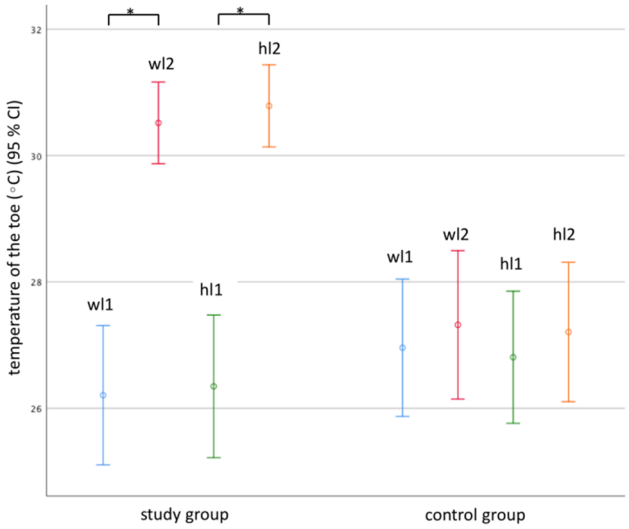

Figure 3. Temperature of the big toe before and after the treatment. Legend: wl1—leg with a wound before treatment, wl2—leg with a wound after treatment, hl1—contralateral leg without a wound before treatment, hl2—contralateral leg without a wound after treatment, * $p < 0.001$.

4. Discussion

Our study showed a positive effect of transcutaneous gaseous CO_2 application on feet sensations tested with monofilament and vibration. The vibration sensation and monofilament test were performed before treatment and after four weeks of CO_2 therapy or placebo treatment. Before the therapy, patients in both groups had no vibration sensation in five of the ten standard points. The monofilament test showed a loss of sensation in 11 (study group) or ten (placebo group) out of 16 standard points (Table 2). The pretreatment temperature of the big toe was comparable in both groups.

After four weeks of therapy, sensation was significantly improved in the study group ($p < 0.001$ for vibration and monofilament test), while no improvement was noted in the control group. Similarly, the temperature of the big toe was significantly higher in the study group, while there was no difference in the control group. These results are of great clinical importance because neuropathy is a strong cofactor in the etiology of chronic wounds in patients with diabetes and no effective therapy yet exists for this condition [6]. The only remaining approach is enhanced glycemic control, which seems to be effective predominantly in type 1 diabetes [6].

The pathogenesis of DSPN is not completely understood, but it seems that disturbances in blood flow play an important role [26]. The two theories explaining pathogenesis (metabolic and vascular) are obviously both involved in the pathogenesis of DSPN [27]. Metabolic changes are being intensively investigated to understand and treat the condition that affects millions of patients worldwide [6].

The immediate effects of transcutaneous gaseous CO_2 application are vasodilatation and elevation of oxygen release from Hb via the Bohr effect. As the therapy is repeated, neoangiogenesis is induced [16,20,21]. There is no simple explanation for the observed positive effect of transcutaneous application of gaseous CO_2 on DSPN in patients with diabetes. The improved microcirculation could be the underlying mechanism. The vasodilatory effect in transcutaneous CO_2 therapy is mediated via the NO-dependent pathway [28–30]. The role of the NO-dependent pathway in DSPN is not clear. Several studies to date have evaluated the role of endothelium-dependent and endothelium-independent microvascular dysfunction in peripheral neuropathy with no clear explanation of pathogenic pathway so far [6,22,31–35]. The improved microcirculation after transcutaneous CO_2 therapy was confirmed in a previous study [36]. In this study, we measured the temperature of the great toe as an additional parameter depending on microcirculation.

The role of natural CO_2-rich water is known for its positive effects on wound healing. Therefore, its medical application has been modified over the past decades. CO_2-enriched water is used more often than gaseous CO_2. The primary goal in our first published research was chronic wound healing, and we decided to use gaseous CO_2 to avoid the moisturizing of the wound and inhaling of evaporated CO_2 from water [16]. Since one of the positive observations was also recovery of sensory loss, we decided to adhere to the same mode of CO_2 application.

According to our knowledge, this is the first double-blind, randomized research investigating the influence of the transcutaneous application of gaseous CO_2 on DSPN. However, a positive effect was previously observed in two publications with patients treated for chronic wounds on feet [37,38].

Shalan et al. included 22 patients with diabetes who immersed their feet in CO_2-enriched water at 37 degrees for 30 min each day for 15 days [37]. The sensation of the area was evaluated on a scale from one (normal sensation) to five (no sensation) using a pressure pin. Results showed improved sensation from 4.36 before CO_2 therapy to 3.45 after therapy. There was no control group, and statistical evaluation is not presented.

Three years later, Abdulhamza et al. used the same application mode as Shalan [38]. One hundred patients with diabetes and chronic wounds immersed their feet in CO_2-enriched water, only this time the treatment protocol was somewhat modified—their feet were immersed for 30 min three times a week for 12 weeks. The control group received standard treatment for chronic wounds. Sensation was evaluated similarly to Shalan's method, using a pressure pin. The results showed significant improvement in sensation in the CO_2-treated group after the therapies compared to pretreatment values and compared to the values of the control group ($p < 0.01$).

Duration of treatment and repetition of the CO_2 applications seems to be of importance. Abdulhamza achieved statistically significant improvement in sensation as he increased the number of therapies compared to Shalan. In previously mentioned publications, the duration of the therapy is 30 min [37,38]. We decided to extend the exposure time considering our previous experiences. In healthy subjects, the temperature of the skin reached a plateau in 30 min [19]. In patients with diabetes where circulation is impaired, we added 15 min to ensure the desired effect. The number of repetitions was 20 (4 weeks, 5 times a week), which was enough to heal most of the wounds and achieve improvement in the microcirculation [16,19,36]. Considering previous results, we maintained the same protocol to evaluate treatment results for DSPN.

Another non-pharmacological treatment approach, which has been investigated to treat DSPN, is a monochromatic infrared energy therapy. The first publications were promising, but additional research findings did not corroborate previous results [11–14]. A possible explanation stated that patients treated with monochromatic infrared energy therapy also had foot infections that could aggravate the neuropathy [13]. The recovered sensations could have been falsely attributed to monochromatic infrared energy therapy as the infection was treated.

To overcome possible bias, we included only patients with a single unilateral chronic wound with no signs of progressive or systemic infections (no elevated inflammatory parameters) to separately evaluate the monofilament and vibration sensation on wound-affected and unaffected legs. In the PVR System, transcutaneous gaseous CO_2 therapy is performed on the entire lower part of the body, meaning that the affected and unaffected legs were equally treated. Results showed comparable improvements in sensation in both legs in patients treated with gaseous CO_2 (Figures 1 and 2).

Another limitation of our study is the subjectivity of the methods for sensory evaluation since they are based on patients' responses. The vibration sensation and monofilament tests are recommended for screening and evaluating peripheral neuropathy in diabetic patients [23–25]. To overcome this limitation, the study was designed as a double-blind, randomized trial in which neither the patient nor the doctor evaluating the results did not know the patient's group. The results of both subjective outcomes—monofilament

test and vibration sensation test were following results of the temperature of the great toe, which was objectively evaluated with an infrared thermometer. Previously confirmed improved microcirculation [36] could represent the underlying mechanism that improves skin temperature and blood supply to the nerves and, therefore, sensory improvement.

Despite careful consideration of possible bias, our study is the first one evaluating the effect of transcutaneous gaseous CO_2 application on DSPN and, therefore, needs further evaluation.

5. Conclusions

According to our results, transcutaneous application of gaseous CO_2 shows promise in treating DSPN. There are some reports in existing literature confirming our results. Considering the major consequences of sensory loss leading to foot ulceration and possible amputation, we believe that this treatment approach deserves future attention and investigation as a treatment modality of DSPN.

Author Contributions: I.F. designed the study, contributed data, critically reviewed the manuscript and supervised the research. H.B.F. drafted the manuscript, organized data and performed the statistical analysis, J.M. prepared the data and contributed to performing the therapies, Z.R. contributed to the study performance and critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the National Medical Ethics Committee of the Republic of Slovenia (approval ID: KME 84/02/16).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chao, C.C.; Hsieh, S.C.; Yang, W.S.; Lin, Y.H.; Lin, W.M.; Tai, T.Y.; Hsieh, S.T. Glycemic control is related to the severity of impaired thermal sensations in type 2 diabetes. *Diabetes Met. Res. Rev.* **2007**, *23*, 612–620. [CrossRef]
2. Sveen, K.A.; Karimé, B.; Jørum, E.; Mellgren, S.I.; Fagerland, M.W.; Monnier, V.M.; Dahl-Jørgensen, K.; Hanssen, K.F. Small- and large-fiber neuropathy after 40 years of type 1 diabetes: Associations with glycemic control and advanced protein glycation: The Oslo Study. *Diabetes Care* **2013**, *36*, 3712–3717. [CrossRef]
3. Bansal, V.; Kalita, J.; Misra, U.K. Diabetic neuropathy. *Postgrad Med. J.* **2006**, *82*, 95–100. [CrossRef]
4. Tesfaye, S.; Boulton, A.J.; Dyck, P.J.; Freeman, R.; Horowitz, M.; Kempler, P.; Lauria, G.; Malik, R.A.; Spallone, V.; Vinik, A.; et al. Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* **2010**, *33*, 2285–2293. [CrossRef]
5. American Diabetes Association. 5. Lifestyle Management: Standards of Medical Care in Diabetes-2019. *Diabetes Care* **2019**, *42*, S46–S60. [CrossRef]
6. Feldman, E.L.; Nave, K.A.; Jensen, T.S.; Bennett, D.L.H. New Horizons in Diabetic Neuropathy: Mechanisms, Bioenergetics, and Pain. *Neuron* **2017**, *93*, 1296–1313. [CrossRef]
7. Callaghan, B.C.; Cheng, H.T.; Stables, C.L.; Smith, A.L.; Feldman, E.L. Diabetic neuropathy: Clinical manifestations and current treatments. *Lancet Neurol.* **2012**, *11*, 521–534. [CrossRef]
8. Hicks, C.W.; Selvin, E. Epidemiology of Peripheral Neuropathy and Lower Extremity Disease in Diabetes. *Curr. Diab. Rep.* **2019**, *19*, 86. [CrossRef]
9. Iqbal, Z.; Azmi, S.; Yadav, R.; Ferdousi, M.; Kumar, M.; Cuthbertson, D.J.; Lim, J.; Malik, R.A.; Alam, U. Diabetic Peripheral Neuropathy: Epidemiology, Diagnosis, and Pharmacotherapy. *Clin. Ther.* **2018**, *40*, 828–849. [CrossRef]
10. Snyder, M.J.; Gibbs, L.M.; Lindsay, T.J. Treating Painful Diabetic Peripheral Neuropathy: An Update. *Am. Fam. Phys.* **2016**, *94*, 227–234.
11. Naderi Nabi, B.; Sedighinejad, A.; Haghighi, M.; Biazar, G.; Hashemi, M.; Haddadi, S.; Fathi, A. Comparison of Transcutaneous Electrical Nerve Stimulation and Pulsed Radiofrequency Sympathectomy for Treating Painful Diabetic Neuropathy. *Anesth. Pain Med.* **2015**, *5*, e29280. [CrossRef]

12. Lavery, L.A.; Murdoch, D.P.; Williams, J.; Lavery, D.C. Does anodyne light therapy improve peripheral neuropathy in diabetes? A double-blind, sham-controlled, randomized trial to evaluate monochromatic infrared photoenergy. *Diabetes Care* **2008**, *31*, 316–321. [CrossRef]
13. Nawfar, S.A.; Yacob, N.B. Effects of monochromatic infrared energy therapy on diabetic feet with peripheral sensory neuropathy: A randomised controlled trial. *Singap. Med. J.* **2011**, *52*, 669–672.
14. Robinson, C.C.; Klahr, P.D.S.; Stein, C.; Falavigna, M.; Sbruzzi, G.; Plentz, R.D.M. Effects of monochromatic infrared phototherapy in patients with diabetic peripheral neuropathy: A systematic review and meta-analysis of randomized controlled trials. *Braz. J. Phys.* **2017**, *21*, 233–243. [CrossRef]
15. Kerstman, E.; Ahn, S.; Battu, S.; Tariq, S.; Grabois, M. Neuropathic pain. *Handb Clin. Neurol.* **2013**, *110*, 175–187. [CrossRef]
16. Macura, M.; Ban Frangez, H.; Cankar, K.; Finžgar, M.; Frangez, I. The effect of transcutaneous application of gaseous CO_2 on diabetic chronic wound healing-A double-blind randomized clinical trial. *Int. Wound J.* **2020**, *17*, 1607–1614. [CrossRef]
17. Sakai, Y.; Miwa, M.; Oe, K.; Ueha, T.; Koh, A.; Niikura, T.; Iwakura, T.; Lee, S.Y.; Tanaka, M.; Kurosaka, M. A novel system for transcutaneous application of carbon dioxide causing an "artificial Bohr effect" in the human body. *PLoS ONE* **2011**, *6*, e24137. [CrossRef]
18. Hartmann, B.R.; Bassenge, E.; Hartmann, M. Effects of serial percutaneous application of carbon dioxide in intermittent claudication: Results of a controlled trial. *Angiology* **1997**, *48*, 957–963. [CrossRef]
19. Finzgar, M.; Melik, Z.; Cankar, K. Effect of transcutaneous application of gaseous carbon dioxide on cutaneous microcirculation. *Clin. Hemorheol. Microcirc.* **2015**, *60*, 423–435. [CrossRef]
20. Oda, T.; Iwakura, T.; Fukui, T.; Oe, K.; Mifune, Y.; Hayashi, S.; Matsumoto, T.; Matsushita, T.; Kawamoto, T.; Sakai, Y.; et al. Effects of the duration of transcutaneous CO_2 application on the facilitatory effect in rat fracture repair. *J. Orthop. Sci.* **2020**, *25*, 886–891. [CrossRef]
21. Ueha, T.; Oe, K.; Miwa, M.; Hasegawa, T.; Koh, A.; Nishimoto, H.; Lee, S.Y.; Niikura, T.; Kurosaka, M.; Kuroda, R.; et al. Increase in carbon dioxide accelerates the performance of endurance exercise in rats. *J. Physiol. Sci.* **2018**, *68*, 463–470. [CrossRef]
22. Stirban, A. Microvascular dysfunction in the context of diabetic neuropathy. *Curr. Diab. Rep.* **2014**, *14*, 541. [CrossRef]
23. Moghtaderi, A.; Bakhshipour, A.; Rashidi, H. Validation of Michigan neuropathy screening instrument for diabetic peripheral neuropathy. *Clin. Neurol. Neurosurg.* **2006**, *108*, 477–481. [CrossRef] [PubMed]
24. Pop-Busui, R.; Boulton, A.J.; Feldman, E.L.; Bril, V.; Freeman, R.; Malik, R.A.; Sosenko, J.M.; Ziegler, D. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* **2017**, *40*, 136–154. [CrossRef] [PubMed]
25. Sosenko, J.M.; Sparling, Y.H.; Hu, D.; Welty, T.; Howard, B.V.; Lee, E.; Robbins, D.C. Use of the Semmes-Weinstein monofilament in the strong heart study. Risk factors for clinical neuropathy. *Diabetes Care* **1999**, *22*, 1715–1721. [CrossRef] [PubMed]
26. Østergaard, L.; Finnerup, N.B.; Terkelsen, A.J.; Olesen, R.A.; Drasbek, K.R.; Knudsen, L.; Jespersen, S.N.; Frystyk, J.; Charles, M.; Thomsen, R.W.; et al. The effects of capillary dysfunction on oxygen and glucose extraction in diabetic neuropathy. *Diabetologia* **2015**, *58*, 666–677. [CrossRef]
27. Tesfaye, S.; Selvarajah, D. Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy. *Diabetes Metab Res. Rev.* **2012**, *28*, 8–14. [CrossRef]
28. Kreska, Z.; Németh, B.; Kiss, I.; Péter, I.; Ajtay, Z.; Hejjel, L. Transcutaneous Carbon Dioxide Treatment Affects Heart Rate Variability—A Pilot Study. *In Vivo* **2018**, *32*, 1259–1264. [CrossRef]
29. Németh, B.; Kiss, I.; Ajtay, B.; Péter, I.; Kreska, Z.; Cziráki, A.; Horváth, I.G.; Ajtay, Z. Transcutaneous Carbon Dioxide Treatment Is Capable of Reducing Peripheral Vascular Resistance in Hypertensive Patients. *In Vivo* **2018**, *32*, 1555–1559. [CrossRef]
30. Nemeth, B.; Kiss, I.; Jencsik, T.; Peter, I.; Kreska, Z.; Koszegi, T.; Miseta, A.; Kustan, P.; Boncz, I.; Laczo, A.; et al. Angiotensin-converting Enzyme Inhibition Improves the Effectiveness of Transcutaneous Carbon Dioxide Treatment. *In Vivo* **2017**, *31*, 425–428. [CrossRef]
31. Green, A.Q.; Krishnan, S.; Finucane, F.M.; Rayman, G. Altered C-fiber function as an indicator of early peripheral neuropathy in individuals with impaired glucose tolerance. *Diabetes Care* **2010**, *33*, 174–176. [CrossRef]
32. Smith, A.G.; Ramachandran, P.; Tripp, S.; Singleton, J.R. Epidermal nerve innervation in impaired glucose tolerance and diabetes-associated neuropathy. *Neurology* **2001**, *57*, 1701–1704. [CrossRef]
33. Vas, P.R.; Green, A.Q.; Rayman, G. Small fibre dysfunction, microvascular complications and glycaemic control in type 1 diabetes: A case-control study. *Diabetologia* **2012**, *55*, 795–800. [CrossRef] [PubMed]
34. Jörneskog, G.; Brismar, K.; Fagrell, B. Skin capillary circulation severely impaired in toes of patients with IDDM, with and without late diabetic complications. *Diabetologia* **1995**, *38*, 474–480. [CrossRef] [PubMed]
35. Stansberry, K.B.; Peppard, H.R.; Babyak, L.M.; Popp, G.; McNitt, P.M.; Vinik, A.I. Primary nociceptive afferents mediate the blood flow dysfunction in non-glabrous (hairy) skin of type 2 diabetes: A new model for the pathogenesis of microvascular dysfunction. *Diabetes Care* **1999**, *22*, 1549–1554. [CrossRef] [PubMed]
36. Finžgar, M.; Frangež, H.B.; Cankar, K.; Frangež, I. Transcutaneous application of the gaseous CO_2 for improvement of the microvascular function in patients with diabetic foot ulcers. *Microvasc Res.* **2021**, *133*, 104100. [CrossRef]
37. Shalan, N.; Al-Bazzaz, A.; Al-Ani, I.; Najem, F.; Al-Masri, M. Effect of Carbon Dioxide Therapy on Diabetic Foot Ulcer. *J. Diabetes Mellit.* **2015**, *5*, 284–289. [CrossRef]
38. Abdulhamza, G.R.; Al-Omary, H.I. Physiological Effects of Carbon Dioxide Treatment on Diabetic Foot Ulcer Patients. *J. Pharm. Biol. Sci.* **2018**, *13*, 1–7.